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REVIEW

Cancer and comorbidity: The role of leptin in breast cancer and associated pathologies

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Abstract

Obesity is an important risk factor for postmenopausal breast cancer and also a poor prognostic factor among cancer patients. Moreover, obesity is associated with a number of health disorders such as insulin resistance/type-2 diabetes mellitus, hypertension, and other cardiovascular diseases. Frequently, these health disorders exhibit as components/complications of the metabolic syndrome. Nevertheless, obesity-related diseases may coexist with postmenopausal breast cancer; and these comorbid conditions could be substantial. Therefore, it may be assumed that different diseases including breast cancer could originate from a common pathological background in excessive adipose tissue. Adipocyte-released hormone-like cytokine (or adipokine) leptin behaves differently in a normal healthy state and obesity. A growing body of evidence suggests an important role of leptin in our major obesity-related health issues such as insulin resistance, hypertension, and neoplasia. In this context, this review describes the relationships of the abovementioned pathologies with leptin.

Key words: Hypertension; Obesity; Postmenopausal breast cancer; Comorbidity; Diabetes

Core tip: Obesity and associated pathologies such as insulin resistance and metabolic syndrome are interrelated health disorders wherein a chronic low-grade inflammation persists. Perhaps this inflammatory condition is associated with the etiology and disease course of postmenopausal breast cancer, like other obesity-related diseases such as type-2 diabetes mellitus and hypertension. Often these diseases may coexist, and comorbidity worsens the prognosis of cancer patients. Leptin is an important adipokine (mainly released by fat cells), which may play a crucial role in these obesity-related diseases.

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INTRODUCTION

Obesity has been emerging as an important public health problem since about 1980, and currently almost all nations are affected by this health disorder. As per the World Health Organization, global estimates in 2016 revealed that more than 1.9 billion adults (39%, 18 years and older) were overweight; of these, over 650 million (13%) were obese. Logically, this health disorder is closely linked with lifestyle changes such as inappropriate diets and widespread physical inactivity: Both are currently prevalent in many societies. It may be worth mentioning that obesity promotes several pathological conditions, including dyslipidemia, insulin resistance or type-2 diabetes mellitus, hypertension and other cardiovascular diseases, and certain cancers.

Evidence shows that obesity is associated with an increased risk of postmenopausal breast cancer, which occurs more frequently compared to premenopausal cases[1,2]. Furthermore, obesity is a poor prognostic factor among cancer survivors. Obesity in postmenopausal women may influence the disease process in several ways. For instance, aromatase enzyme present in adipose tissue converts androgens to estrogens; therefore, more aromatase activities and estrogens are expected in subjects having excessive adipose tissue. Moreover, decreased levels of sex hormone-binding globulins (SHBG) in obese postmenopausal women may increase free/bioavailable estrogens and breast cancer risk. It has also been suggested that an excess of local adipose tissue may play a critical role in disease progression by providing various substances such as fatty acids and pro-inflammatory cytokines[3].

Systemic low-grade inflammation and insulin resistance are two related mechanisms assumed to play a role in the association between obesity and relevant pathologies[4,5]. On the other hand, it is now fairly understandable that adipose tissue acts as an endocrine organ and releases several hormone-like substances/cytokines (adipokines) such as leptin, resistin, adiponectin[6]. The majority of these adipokines, including leptin, participates in the pro-inflammatory processes in obesity and perpetuates the state of insulin resistance. Adult obesity is commonly associated with higher blood levels of leptin[7,8]. In regard to carcinogenesis, several studies have indicated that leptin potentiates the growth of breast cancer cells[3,9,10].

Leptin, a 16 kDa protein, is primarily released from adipocytes and maintains energy homeostasis by influencing the central/hypothalamic anorexigenic pathway. Nevertheless, in obesity, leptin possibly acts differently and helps create a pro-inflammatory situation. Leptin exerts its effects through at least six alternatively spliced isoforms of leptin receptor (Ob-R), including the long form Ob-Rb and secretory form Ob-Re/sOb-R. Perhaps Ob-Rb plays a key role in both physiologic and pathologic conditions[2].

LEPTIN IN BREAST CANCER

A number of studies documented higher blood levels of leptin among patients with breast cancer, particularly postmenopausal cases[11-17]. Furthermore, circulating leptin concentration has been shown to correlate with different prognostic indicators, such as tumor grade, TNM stage, and receptor status[18-20]. In postmenopausal patients, studies also detected a correlation between blood levels of leptin and aromatase activity[21,22]. Leptin's association with aromatase, which catalyzes the conversion of androgen to estrogen, reasonably suggests an involvement with estrogen biosynthesis. Interestingly, Jardé *et al*[23] observed that Ob-R expression in breast cancer tissue was positively correlated with estrogen receptor (ER) expression. On the other hand, high tissue expression of leptin has been reported to be associated with tumors that were ER(-), progesterone receptor (PR)+, and human epidermal growth factor receptor-2 (HER2)(-)[24]. In another study, investigators found that increased Ob-R mRNA expression was associated with the triple negative phenotype, *i.e.*, ER(-), PR(-), and HER2(-)[25]. They also noticed that higher serum leptin levels were linked with prognosis, such as recurrence and mortality.

In many studies, high levels of leptin and/or Ob-R were detected in malignant tissue compared to non-cancerous breast tissue samples[26-29]. In an initial study that immunohistochemically analyzed the tumor specimens, Ishikawa *et al*[30] observed that distant metastasis was detected more frequently in Ob-R and leptin overexpressing tumors, but in none of the tumors that lacked Ob-R or leptin overexpression. Similarly, Miyoshi *et al*[31] reported that high intra-tumoral mRNA levels of both Ob-Rb and short isoform Ob-R were significantly associated with a poor prognosis for patients with high serum leptin or high intra-tumoral leptin mRNA levels, but not in the subset of patients with low serum leptin or low intra-tumoral leptin mRNA levels. In addition, in a study conducted by Révillion *et al*[32], high Ob-R mRNA expression in breast tumor samples was associated with a shorter relapse-free survival. In an interesting study, mRNA expression of leptin in mammary adipose tissue and Ob-R in tumor tissue was significantly higher in patients with metabolic syndrome compared to obese only or normal weight cancer patients[33]. It is worth mentioning that metabolic syndrome or its components may affect the pathologic course of breast cancer in different phases, such as the risk for disease development, comorbidities, and prognosis.

METABOLIC SYNDROME AND COMORBIDITY IN BREAST CANCER

In general, characteristics of metabolic syndrome include abdominal obesity, hyperglycemia/insulin resistance, dyslipidemia, and hypertension, which result in an increased risk for the development of type-2 diabetes and cardiovascular disease. Many studies revealed that the presence of metabolic syndrome increased the risk of postmenopausal breast cancer[34-36]. Remarkably, an important environmental factor for both hypertension and type-2 diabetes is obesity. Furthermore, it has been observed that obesity, in combination with the metabolically unhealthy condition, was associated with the highest risk of postmenopausal breast cancer[37]. Mechanistically, in an environment of metabolic syndrome, pathological phenomena such as insulin resistance, pro-inflammatory cytokines and subacute chronic inflammation may influence the risk and prognosis of breast cancer[38,39].

On the other hand, comorbid conditions could be substantial in breast cancer patients, and prevalent comorbidities usually include various disorders, *e.g.*, diabetes, hypertension, arthritis, osteoporosis, and psychological difficulties[40-43]. Reports from different geographical areas demonstrated that type-2 diabetes increased breast cancer risk and can affect patients' prognosis (Table 1)[44-56]. Interestingly, studies have demonstrated different impacts of type-1 and type-2 diabetes on breast cancer risk. Liaw *et al*[57] analyzed the entire adult female population in Taiwan and found that the breast cancer incidence rate was significantly higher in patients with type-2 diabetes compared to type-1 diabetes patients and persons without diabetes. Conversely, some investigators reported a decreased risk of breast cancer in women with type-1 diabetes[58,59]. Regarding the quality of life among breast cancer survivors, a worse condition was revealed in patients with type-2 diabetes than those with type-1[60]. However, obesity and diabetes probably act synergistically for a worse outcome in breast cancer[61-63].

Another important comorbid condition among cancer patients is hypertension. In general, it is the most common cardiovascular disease and a risk factor for several other cardiovascular problems, such as atherosclerosis, coronary artery disease, and cerebrovascular accident. Nevertheless, a significant proportion of postmenopausal breast cancer patients with hypertension have been detected in different studies[64-66]. In addition, certain antihypertensive drugs have been shown to increase the risk of breast cancer[67-69]. Biological mechanisms linking hypertension and breast cancer risk are clearly intricate. However, a number of factors may play a key role in this link, such as obesity, adipokines like leptin, angiogenic factors like vascular endothelial growth factor (VEGF), macrophages, and insulin resistance[67,70-74].

ROLE OF LEPTIN IN DIABETES AND HYPERTENSION

A number of investigators documented higher blood levels of leptin in patients with type-2 diabetes[75-78]. Furthermore, higher leptin concentrations were detected in saliva samples from type-2 diabetes patients compared to healthy controls[79]. It has been demonstrated that leptin positively correlated with different cardiometabolic risk factors, *e.g.*, body mass index (BMI), waist circumference, blood pressure, dyslipidemia, and insulin resistance index[80-83]. Therefore, hyperleptinemia can be considered a critical link between obesity and insulin resistance[84]. It is thought that leptin upregulates pro-inflammatory cytokines such as interleukin-6 and tumor necrosis factor-α, and these are associated with insulin resistance and type-2 diabetes[85].

In human subjects, different studies observed that hyperleptinemia was associated with hypertension[86-89]. Furthermore, hyperleptinemia could be involved in arterial stiffness[90] and cardiac autonomic dysfunction[81]. Interestingly, human subjects or animal models with loss-of-function mutations in leptin/Ob-R or melanocortin receptor genes exhibit lower blood pressure despite severe obesity[91,92]. Of note, in the hypothalamic anorexigenic pathway, leptin binds to Ob-R on the pro-opiomelanocortin-expressing neurons, which leads to the release of alpha melanocyte-stimulating hormone that subsequently binds to melanocortin receptors[93]. Overall, leptin activates the sympathetic nervous system *via* the melanocortin system, and this effect particularly involves the renal sympathetic outflow in order to increase blood pressure[94-96]. Apart from the sympatho-excitatory actions, leptin may influence the blood pressure *via* a number of mechanisms, such as the renin-angiotensin and aldosterone system[95-97]. Furthermore, leptin is thought to be associated with other hypertension-related phenomena, *e.g.*, endothelial dysfunction, impairment of nitric oxide-mediated vasodilation, atherosclerosis, cardiomyocyte hypertrophy, cardiac disorders, and kidney damage[98-104]. However, the precise mechanisms by which the hyperleptinemia state influences hypertension remains poorly understood.

APPROACHES FOR OBESITY MANAGEMENT

Clinical laboratories play a significant role in the metabolic assessment and early diagnosis of complications associated with obesity. Due to the fact that obesity acts like a chronic low-grade inflammatory process, an alteration can be expected in the circulating levels of various metabolic components and biomolecules, including leptin (Table 2)[105-115]. Nonetheless, laboratory values of different nutritional parameters are useful in all levels of prevention[116]. In order to prevent various

Table 1 Selected recent reports on diabetes and epidemiological/clinical characteristics of breast cancer

Authors and report time	Study types, geographical areas and patients	Findings in brief
Bronsveld *et al*[44] 2017	Population-based cohort study among British population, 2371 breast cancer cases during approximately 1.6 million person-years	Approximately 2880 women with T2D are diagnosed with breast cancer per year in the United Kingdom
Charlot *et al*[45] 2017	1621 African-American women with invasive breast cancer (232 had T2D) were followed	A positive association of T2D with breast cancer mortality
Dankner *et al*[46] 2016	Israel, 2186196 individuals (prevalent diabetes: 159104 and incident diabetes: 408243) were followed for various cancers	Diabetes posed an increased risk of breast cancer in postmenopausal women
Gini *et al*[47] 2016	Retrospective population-based cohort study, Italy, 32247 T2D patients	T2D patients are at increased risk of several cancers, and of premature death in women with breast cancer
Lipscombe *et al*[48] 2015	Retrospective cohort study, Ontario, Canada, 38407 women with breast cancer (6115 had diabetes)	Diabetes was associated with more advanced-stage breast cancer
Luo *et al*[49] 2014	Women's Health Initiative, United States, 8108 women with breast cancer	T2D increased risk of total mortality among women with breast cancer
Ma *et al*[50] 2014	China, 865 early stage triple-negative breast cancer cases	T2D exhibited a significantly lower disease-free survival; increased likelihood of recurrence and metastasis
Maskarinec *et al*[51] 2017	Multiethnic cohort, Among 103721 women with 14558 T2D cases, 6692 women developed breast cancer	T2D status was primarily associated with higher breast cancer risk in Latinas
Palmer *et al*[52] 2017	Prospective cohort of African-American women, 1851 breast cancer cases during 847934 person-years of follow-up	Women with T2D were at increased risk of developing ER(-) breast cancer
Pan *et al*[53] 2018	Prospective study in China, 17463 incident cases (various cancers) among 508892 participants	Participants with T2D had increased risks of breast cancer
Samson *et al*[54] 2016	Retrospective cohort study, South Carolina, 7310 participants (3835 European-origin and 3475 African-American women)	Negative association between T2D and breast cancer was stronger in African-American women
Wu *et al*[55] 2015	Multiethnic cohort, California, 8952 breast cancer cases	Risk of mortality increased among cases with diabetes
Xu *et al*[56] 2015	Population-based retrospective cohort study, China, 36379 T2D patients	Elevated risk of breast cancer

T2D: Type-2 diabetes; ER(-): Estrogen receptor negative; Person-years: Amount of total time in years contributed by all participants.

obesity-related complications, a number of reports have advised different strategies, which are primarily connected with physical activity and healthy eating practice[117,118]. Apart from caloric restriction, regular intake of certain dietary constituents such as garlic and fenugreek are perhaps helpful[119-123]. It is clearly understood that there is an urgent need to develop appropriate therapeutic strategies for the treatment of obesity. It may be worth noting that the currently available anti-obesity pharmaceutical agents include monotherapy options, such as orlistat and lorcaserin, as well as combination products, such as phentermine/topiramate and naltrexone/bupropion[124].

On the other hand, surgical options may help extremely obese individuals. Bariatric or obesity surgery encompasses many types of weight-reduction procedures, such as gastric bypass, gastric banding or sleeve gastrectomy, and involves structural and physiologic alterations of the gastrointestinal tract. A number of studies have been performed to document the quality of life after weight-loss surgery. In a few reports, bariatric procedures were performed after the diagnosis of cancer[125-127]. In a retrospective cohort study, the investigators concluded that long-term mortality after gastric bypass surgery was significantly reduced, particularly deaths from diabetes, heart disease, and cancer[128]. Similarly, other studies found that bariatric surgery resulted in a decreased risk for the development of cancers, including breast cancer[129,130]. However, a national population-based cohort study from the United Kingdom noticed that individuals who had undergone a bariatric procedure exhibited a decreased risk of hormone-related cancers such as breast and endometrial cancers, while gastric bypass was associated with an increased risk of colorectal cancer[131]. In contrast, a similar study from the United Kingdom recorded that prior obesity surgery was not associated with an increased colorectal cancer risk[132]. In their study, the risk of breast cancer was reduced, while the risk of endometrial and kidney cancers remained elevated. In line with the conflicting trends, a nationwide population-based cohort study in Sweden found increased mortality from rectal cancer following obesity surgery[133]. Conversely, in a Dutch population-based study, which collected information on colorectal cancer cases, no differences were observed between hospitals performing bariatric surgery and hospitals that did not[134].

In general, studies have demonstrated a significant decrease in blood levels of leptin after bariatric surgery[135,136]. One study has shown that Ob-R expression

Table 2 Levels of circulating leptin in various pathophysiological conditions

Investigators	Subjects and salient findings
Al-Daghri *et al*[105] 2007 (Saudi Arabia)	308 adults participated [type-2 diabetes = 142 (female- 45), prediabetes = 86 (female- 37), normal controls = 80 (female- 35)]. Serum leptin levels among male subjects with type-2 diabetes (BMI- 27.3 ± 4.1 kg/m^2) were 12.4 (3.2-72) ng/mL; among pre-diabetes (BMI- 28.5 ± 4.3 kg/m^2) - 7.6 (1.2-72) ng/mL; and in controls (BMI- 29.2 ± 7.3 kg/m^2) - 3.9 (0.8-20) ng/mL. Leptin levels among female subjects with type-2 diabetes (BMI- 32.5 ± 10.3 kg/m^2) were 13.3 (3.6-49.1) ng/mL; among pre-diabetes (BMI- 32.5 ± 8.4 kg/m^2) - 14.09 (2.8-44.4) ng/mL; and in controls (BMI- 30.4 ± 6.4 kg/m^2) - 10.2 (0.25-34.8) ng/mL
Al-Harithy[106] 2004 (Saudi Arabia)	Females (n = 57) had higher serum leptin concentration (6.04 ± 4.71 ng/mL *vs* 1.72 ± 0.95 ng/mL) than males (n = 65). BMI values showed a strong association with leptin levels in both genders
Al Maskari and Alnaqdy[107] 2006 (Oman)	Overall, there was a significant difference in serum leptin between the obese group (n = 35, 34.78 ± 13.96 ng/mL) and the control non-obese subjects (n = 20, 10.6 ± 4.2 ng/mL). Obese females (n = 25): age- 29.2 ± 1.6 yr, BMI- 39.6 ± 1.5 kg/m^2, leptin- 38.2 ± 2.5 ng/mL; Obese males (n = 10): age- 30.0 ± 3.1 yr, BMI- 39.0 ± 2.9 kg/m^2, leptin- 27.0 ± 4.9 ng/mL
Kazmi *et al*[108] 2013 (Pakistan)	Obese and overweight group: n = 40, female- 33, age- 34.8 ± 4.6 yr, BMI- 31.7 ± 3.1 kg/m^2; and non-obese group: n = 50, female- 32, age- 32.7 ± 6.1 yr, BMI- 21.2 ± 1.5 kg/m^2. Serum leptin concentrations were higher in obese subjects (52.8 ± 24.6 ng/mL) than in non-obese subjects (6.3 ± 3.1 ng/mL)
Laimer *et al*[109] 2002 (Austria)	18 morbidly obese women were studied before and one year after SAGB. In addition, eight lean women were examined as a control group. Serum leptin levels decreased from 44.6 ± 18.0 ng/mL in pre-SAGB subjects (age- 40.3 ± 9.8 yr, BMI- 42.9 ± 5.6 kg/m^2) to 20.0 ± 13.1 ng/mL in post-SAGB state (BMI- 32.9 ± 6.0 kg/m^2). Control subjects: age- 38.3 ± 9.8 yr, BMI- 22.9 ± 2.2 kg/m^2, leptin- 6.3 ± 3.3 ng/mL
Miyawaki *et al*[110] 2002 (Japan)	During four weeks, ten obese subjects (five men and five premenopausal women: age- 33 ± 13 yr, BMI- 35.4 ± 2.4 kg/m^2, plasma leptin level- 46.2 ± 14.6 ng/mL) underwent 800 kcal/day LCD. In addition, ten obese subjects (five men and five premenopausal women: age- 31 ± 11 yr, BMI- 32.3 ± 2.1 kg/m^2, leptin- 14.9 ± 3.5 ng/mL) consumed a 1400 kcal/day BDD for the same period. Plasma leptin levels in the LCD group markedly decreased (13.2 ± 3.6 ng/mL) with the decrement in BMI (33.1 ± 2.2 kg/m^2); while in the BDD group, BMI and leptin concentrations were 31.0 ± 2.5 kg/m^2 and 13.4 ± 2.8 ng/mL, respectively
Osegbe *et al*[111] 2016 (Nigeria)	80 obese females (BMI- 39.1 ± 7.2 kg/m^2) were examined. Prevalence of hyperleptinemia was 92.5% and serum leptin levels- 48.4 ± 24.4 ng/mL
Sinorita *et al*[112] 2010 (Indonesia)	57 obese persons (female- 33) were divided into obese class Ⅰ (BMI > 25 kg/m^2 to < 30 kg/m^2) and obese class Ⅱ (BMI > 30 kg/m^2). Leptin concentration in obese class Ⅰ was 13.998 ± 13.486 ng/mL, and in obese class Ⅱ was 31.074 ± 26.158 ng/mL
Tasaka *et al*[113] 1997 (Japan)	In BMI < 25 kg/m^2, plasma leptin was 2.24 ± 0.25 ng/mL in males (n = 29) and 3.01 ± 0.39 ng/mL in females (n = 13); in BMI 25-30 kg/m^2, levels were 3.14 ± 0.31 ng/mL in males (n = 10) and 10.66 ± 2.86 ng/mL in females (n = 7) and in BMI > 30 kg/m^2, levels were 8.98 ± 1.5 ng/mL in males (n = 11) and 11.74 ± 2.2 ng/mL in females (n = 6)
Tong *et al*[114] 2005 (United States)	The subjects consisted of nondiabetic Japanese-American population (n = 518, male- 51%) enrolled in the Japanese-American Community Diabetes Study. The mean plasma leptin level for men (BMI- 25.2 ± 3.0 kg/m^2) was 4.0 ± 2.7 pmol/L and 11.6 ± 7.3 pmol/L for women (BMI- 22.9 ± 3.1 kg/m^2) (1 pmol/L = 0.445 ng/mL)
van Rossum *et al*[115] 2000 (United States)	54 postmenopausal obese women before and after a 6-mo hypocaloric diet - the women lost an average of 7.1% of body weight and 14.5% serum leptin levels during the 6-mo weight loss intervention (initial BMI- 32.0 ± 4.5 kg/m^2, leptin- 30.9 ± 20.2 ng/mL; and after weight loss BMI- 29.8 ± 4.7 kg/m^2, leptin- 24.3 ± 14.8 ng/mL)

BMI: Body mass index; SAGB: Swedish adjustable gastric banding; LCD: Low-calorie diet; BDD: Balanced deficit diet.

was increased, while adipocyte size was decreased following surgical obesity reduction[137]. After a direct comparison of the effect of caloric restriction and bariatric surgery on circulating levels of different inflammatory cytokines including leptin, the investigators concluded that caloric restriction seemed to have more favorable effects[138]. In the same way, another study found that caloric restriction plus exercise resulted in weight loss of similar magnitude to a matched group of subjects following bariatric surgery[139]. On the other hand, anti-obesity pharmacotherapy such as orlistat (or in combination with other conservative methods) has been shown to exert beneficial effects on weight loss and inflammatory cytokines including leptin[140-142].

CONCLUSION

There are substantial comorbidities among postmenopausal breast cancer patients, which include obesity-related diseases such as type-2 diabetes mellitus, hypertension, and other cardiovascular disorders. The abovementioned health issues possibly originate from a state of chronic low-grade inflammation that is associated with a dysregulation of pro-inflammatory adipokines like leptin. A growing body of evidence has shown that leptin can impact different obesity-related pathologies and patients' prognosis. Overall, there is an urgent need to understand the precise functions of leptin, its interactions with various adipokines and classical hormones, and methods to develop a nontoxic and clinically effective leptin antagonist.

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REVIEW

One more chance of fistula healing in inflammatory bowel disease: Stem cell therapy

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Abstract

Patients with fistulizing inflammatory bowel disease are traditionally difficult to treat. This patient population often experiences delayed or insufficient healing of fistulas using current standard regimens including antibiotics, immunomodulators, anti-tumor necrosis factor-α drug, placement of setons, and surgical repair. Several studies over the last ten to fifteen years have been conducted using stem cell therapies with promising results in this patient population. These studies show stem cell therapy in fistulizing disease to be successful in healing between 60%-88% compared to currently 50% with infliximab. Moreover, remission was seen 24 wk to 52 wk in these studies. Further research with a multi-approach treatment using medications, stem cell therapy, and surgical interventions will likely be the future of this innovative treatment approach.

Key words: Crohn's disease; Stem cells; Mesenchymal; Fistulizing; Fistula; Inflammatory bowel disease

Core tip: There appear to be limited adverse events as well as significant benefit to multi-approach therapy using stem cells to treat fistulizing inflammatory bowel disease. Comparing studies to current treatment rates of fistula healing, which has a less than 50% success rate, stem cell therapy for fistulizing Crohn's disease appears to be beneficial, as the majority of studies claim 60%-88% fistula healing and maintenance of remission at 24-52 wk. Further large-scale studies analyzing a multi-approach therapy including stem cells should be conducted, especially in a randomized double-blind approach.

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INTRODUCTION

Inflammatory bowel disease (IBD), including Crohn's disease (CD), ulcerative colitis (UC), and IBD-unclassified, is a very complicated unique spectrum of disease processes, ranging from relatively asymptomatic to daily complications of significant pain and fistulizing disease. While fistulizing disease primarily occurs in CD, sometimes patients are initially diagnosed with UC, which later is realized to actually be CD. Furthermore, UC patients sometimes develop fistulas for other reasons besides their IBD. Unfortunately, little is known on which afflicted individuals will progress despite signs and symptoms with approximately 25% of CD patients developing fistulas within 20 years of diagnosis[1]. Some patients will do well and achieve complete remission with newer biological agents (*i.e.,* vedolizumab) and placement of setons, while others will continue to be refractory in their disease course. Antibiotics, immunomodulators, and anti-tumor necrosis factor-alpha (TNF-α) drugs all have been utilized for fistulizing disease with less than ideal response rates: 90% recurrence with antibiotics and 50% recurrence with infliximab[2]. Furthermore, one-third of patients do not respond to anti-TNF-α medications and 10% of patients are non-responders to existing medications[3]. Stem cell therapy is an emerging treatment for these difficult to treat patients with fistulizing CD.

In this review, the authors aim to highlight the progression of stem cell therapies in patients with refractory CD with fistulizing disease. A literature search of clinical trials in humans was performed with PubMed through April 2018 using keywords including stem cell therapy, fistulas, fistulizing, IBD, and CD. The searches were limited to English language, and excluded comments, editorials, or letters. The outcomes of safety and efficacy using this innovative treatment are presented throughout and are outlined after each section of type of stem cell modality in Tables 1-5.

Four main groups of stem cell therapies exist which include embryonic, tissue-specific, mesenchymal, and induced pluripotent stem cells. Most studies evaluating treatment of fistulizing disease for IBD patients utilize mesenchymal stem cell (MSC) therapy, whether autologous or allogeneic in nature. MSC are stromal cells surrounding other tissues and organs that are able to undergo angiogenesis of the cells they are derived from and are available to help with immunomodulatory effects. This includes adipose tissue and bone marrow cells. Three criteria for MSC *in vitro* must be met per the International Society for Cellular Therapy, which include differentiation potential, *i.e.,* adipogenic lineages, expression of surface antigens including human leukocyte antigen DR-, CD79a, CD19-, CD14-, CD11b-, CD45-, CD34-, CD105+, CD90+, CD73+, and ability to adhere to plastic[4]. Limited studies exist in animal models and human trials, yet there has been an emergence of research in this area within the last ten years. The majority of these studies had the same exclusion criteria unless otherwise specified below. These included evidence of any infections, the need for antibiotics or immediate surgery, unwilling to use contraceptives, pregnant or breast-feeding, presence of complex fistulas with more than two openings or malignancy within the past five years, and any evidence of end-organ failure. Fistulas in most studies were a mix of transsphincteric, suprasphincteric, and extrasphincteric and sometimes rectovaginal. No studies using stem cell therapy specifically for fistulizing disease commented on development of graft versus host disease. Most side effects were limited for stem cell therapy with fistulizing disease as local injection was used. However, with hematological stem cell therapy infusions, the most common adverse effect seen was systemic infection.

AUTOLOGOUS ADIPOSE TISSUE DERIVED STEM CELL THERAPY TRIALS

Autologous adipose tissue derived stem cell (ASC) therapy is a type of MSC therapy derived from one's own adipose tissue. In 2003, one of the first case reports for fistulizing CD using ASC for CD-related rectovaginal fistulas (CRRVF) was reported[5]. This utilized ASC for a patient with refractory disease to infliximab and placement of setons, with resultant resolution of symptoms in one week after local injection with no reoccurrence after three months.

Lee *et al*[6] studied 33 patients with fistulizing disease using autologous ASC proportional to fistula surface area by conducting a non-randomized, single group assignment open-label phase 1 study. Using photography, patients were documented on weeks 4, 6, and 8 and if complete healing was not found at week 8, re-injected with ASC. The authors defined complete healing as "complete closure of fistula tract and internal and external openings, without drainage or any sign of inflammation"[6]. Here, promising results of ASC therapy for fistulizing disease were seen with 79% of patients showing complete closure after a first dose, and 88.5% of patients not having recurrence at the one-year mark. This study had a wide variety of patients regarding their duration of CD and duration of fistula.

Next, Cho *et al*[7] studied autologous ASC in a phase 1 non-randomized, open-label dose escalation trial with ten patients enrolled. Three dosing groups with three patients in each were evaluated with dosing given at four-week intervals and patients evaluated at eight weeks, and four, six, and eight months. Fifty percent of patients after a single injection observed complete healing, compared to 16% with prior studies of fibrin glue. These patients who showed healing at eight we-

Table 1 Composite of autologous adipose tissue derived stem cell therapy trials

Author	Yerr of study	Fistula site	Type of study	Study population	Method of administration	Healing type of fistula	Safety	Outcome
García-Olmo *et al*[5]	2003	RV	Case Report	1	Injection of cells into rectal mucosa	Fully healed	No AE or SAE	Complete resolution at 1 wk with closure still at 3 mo
Lee *et al*[6]	2013	TS SS IS ES	Clinical Trial, Phase Ⅱ Multi-center	33	Fistula tract was curetted and irrigated and then ASCs were injected into the submucosa of tract and opening	27 of 33 patients with complete fistula healing at 8 wk 1 of 7 without complete healing had healing after 2nd dose 5 of 33 patients with > 50% closure	60% postoperative pain 19% anal pain -7% anal bleeding 1 patient with exacerbation of disease 1 patient with peritonitis from enteritis from CD	79% patients with complete closure after first dose
Cho *et al*[7]	2013	TS SS ES	Clinical Trial, Phase Ⅰ Multi-center	10	Tract curettage was performed and internal opening was closed. Then, subcutaneous adipose tissue collected by liposuction was injected into the fistula tract wall and the surrounding internal opening	Group 1: Three patients with partial closure Group 2: Two patients with complete healing Group 3: One patient with complete healing, one with partial healing	13 AE in 7 patients which were not related to study drug: pain, diarrhea 2 patients SAE: enterocolitis, infliximab administration for new fistulas unrelated to target fistula	All patients with complete closure at 8 wk had sustained complete healing at 8 mo 50% patients after single injection with complete healing
Cho *et al*[8]	2015	TS SS ES	Clinical Trial, Phase Ⅱ Multi-center	43	Tract curettage was performed and internal opening was closed. Then, subcutaneous adipose tissue collected by liposuction was injected into the fistula tract wall and the surrounding internal opening. This was done on a primary endpoint of 8 wk; then a retrospective clinical study was conducted looking at patient outcomes after 2 yr	41 of 43 patients were enrolled in the retrospective clinical study After excluded patient: 27 of 33 patients with complete closure	53 AE in 30 patients: abdominal pain (17.1%), eczema (9.8%) exacerbation of disease (9.8%), anal inflammation (7.3%), diarrhea (7.3%), fever (7.3%)	At 12 and 24 mo, respectively, 80% ($P \leqslant 0.0001$) and 75% ($P \leqslant 0.001$) of patients continued to have complete closure
Dietz *et al*[9]	2017	TS SS IS	Clinical Trial, Phase Ⅰ Multi-center	12	Delivered ASC to the fistula through attachment of bioabsorbable matrix for surgical placement (MSC-MATRIX) through intraoperative placement	9 of 12 patients with complete healing at 3 mo 10 of 12 patients with compete healing at 6 mo	1 SAE from CD not study (debridement of granulation tissue of fistula tract) 2 AE: seromas at site of fat collection 11 AE: due to underlying CD	83.3% patients at 6 mo with complete healing after MSC-MATRIX placed

CD: Crohn's disease; ASC: Autologous stem cells; TS: Transsphincteric; SS: Suprasphincteric; IS: Intersphincteric; ES: Extrasphincteric; RV: Rectovaginal; AE: Adverse events; SAE: Serious adverse events.

Table 2 Composite of autologous bone marrow derived stem cell therapy trials

Author	Year of study	Fistula site	Study population	Method of administration	Healing type of fistula	Safety	Outcome
Ciccocioppo *et al*[10]	2015	Perianal Enterocutaneous	10	Serial intrafistula injections of autologous bone marrow MSCs	2 patients with no recurrence of fistula at 5 yr	No adverse events	Fistula relapse free: 88% at 1 yr, 50% at 2 yr, and 37% during the following 4 yr

MSC: Mesenchymal stem cell.

Table 3 Composite of allogeneic adipose tissue derived stem cell therapy trials

Author	Year of study	Fistula site	Study population	Method of administration	Healing type of fistula (unhealed, partially, fully)	Safety	Outcome
García-Arranz *et al*[11]	2016	RV	10	Tract curettage was performed and vaginal or rectal flap added with intralesional injection of 20 million allogeneic adipose stem cells injected into the fistula tract and vaginal submucosa. If complete healing was not seen at 12 wk, patients were re-administered stem cells	2 patients with complete healing at 12 wk 2 patients with complete healing from the 8 patients with second administration of stem cells 9 patients at some point during the study had fistula healing	No SAE or AE	3 of 5 patients included in total (others excluded during study) remained healed at 52 wk, showing 60% efficacy
de la Portilla *et al*[12]	2013	Perianal	24	Intralesional fistula tract injection with stem cells with repeat administration at 12 wk with dose escalation if incomplete closure	38.1% patients achieved complete closure at week 12 65.3% patients achieved complete closure at week 24	13 patients with 32 AE and of these 5 were treatment related: anal abscess (3 patients), pyrexia (1 patient), uterine leiomyoma (1 patient)	69.2% patients had fistula reduction at 24 wk
Panés *et al*[13]	2016	TS SS IS ES	212	Patient randomized into two groups: Placebo with 24 ml saline Intralesional injection of Cx601 cells Study conducted over 24 wk	50% patients with Cx601 *vs* 34% placebo achieved complete fistula healing and remained closed at week 24 (P = 0.024)	TEAE: proctalgia, anal abscess, and nasopharyngitis 5% in treatment group and 6% in placebo group withdrew	Cx601 is effective and safe for treatment of refractory fistulizing CD
Panés *et al*[14]	2017	TS SS IS ES	212	This was a continuation of the above study from 24 to 52 wks Patient randomized into two groups: Placebo with 24 ml saline Intralesional injection of Cx601 cells	35%-40% patients withdrew before end of study 59.2% patients with Cx601 *vs* 41.6% patients with placebo (P = 0.013) achieved clinical remission 56.3% patients with Cx601 versus 38.6% patients with placebo (P = 0.010) achieved combined remission	TEAE: 76.7% in treatment group and 72.5% in control group: anal abscess/ fistula 8.7% treatment group and 8.8% control group withdrew	Cx601 is safe and effective for treatment refractory complex perianal fistulas in patients with CD

Wainstein *et al*[15]	2018	TS IS Pouch-vaginal	9 (2 of 9 patients had 2 fistulas, so total fistula count was 11)	Two part study including: Examination under anesthesia, fistula mapping, drainage and seton placement Setons were removed 4-6 wks afterwards with ASC injected with biological plug formation	Complete healing in 10 of 11 fistulas Partial healing in 1 of 11 fistulas	No AE or SAE	Fistulizing disease can be treated successfully with a multi-approach treatment including ASCs, platelet rich plasma, and endorectal advancement flaps

CD: Crohn's disease; ASC: Autologous stem cells; TS: Transsphincteric; SS: Suprasphincteric; IS: Intersphincteric; ES: Extrasphincteric; RV: Rectovaginal; AE: Adverse events; SAE: Serious adverse events; TEAE: Treatment emergent adverse event.

Table 4 Composite of allogeneic bone marrow derived stem cell therapy trials

Author	Year of study	Fistula site	Study population	Method of administration	Healing type of fistula (unhealed, partially, fully)	Safety	Outcome
Molendijk *et al*[1]	2015	Perianal	21	Patients assigned to four groups with curettage then intralesional fistula tract injection with stem cells or placebo (1) 1×10^7 (2) 3×10^7 (3) 9×10^7 (4) placebo	Week 24 fistula healing for groups (1) 66.7% ($n = 5$) (2) 85.7% ($n = 5$) (3) 28.6% ($n = 5$) (4) 33.3% ($n = 6$)	All patients reported pain and pus and/or discharge from fistula for 1 wk postoperatively One patient in each group (1, 2, 3, and placebo) developed perianal abscess	Use of intralesional injections of 3×10^7 was successful in fistula healing.

Table 5 Summary of all clinical trials evaluating stem cell therapy for fistulizing inflammatory bowel disease

Author	Year of study	Fistula site	Study population	Stem cell therapy	Method of administration	Healing type of fistula	Safety	Outcome
Sanz-Baro *et al*[16]	2015	RV Perianal	5	2 patients with Autologous ASC injected into fistula 3 patients with Allogeneic ASC injected into fistula	All 5 patients treated with either autologous or allogeneic ASCs and achieved remission who became pregnant were given data collection forms assessing age of treatment with ASCs, gestation age, gestational complications, any medication used during pregnancy for CD, type of delivery, fetal weight, and newborn malformations	2 of 5 patients with gestational complications of first term abortions, fetal growth restriction, and small for gestational age 1 of 4 patients who delivered with newborn malformations of syndactyly and clinodactyly	Two of the five patients experienced gestational complications: first trimester miscarriages, fetal growth restriction, and small for gestational age	No evidence that allogeneic or autologous ASC affects fertility in women

CD: Crohn's disease; RV: Rectovaginal; ASC: Autologous stem cell.

eks sustained healing at eight months. The authors compared this to a fistula recurrence rate of 43% of patients with CD treated with infliximab[7].

In addition, Cho *et al*[8] went on to analyze 41 of 43 patients in their previous phase 2 trial with dosage proportional autologous ASC administration for an additional year in a retrospective chart review of these patients. They evaluated sustainability and efficacy of ASC applied and further documented safety 24 mo after ASC administration. Patients were excluded if they had operations during that timeframe and three patients met this criterion; four patients were excluded due to lack of data. Results showed 82% of patients had resolution of their fistulas and durability was 80% ($P \leqslant 0.0001$) at 12 mo and 75% ($P \leqslant 0.001$) at 24 mo.

Furthering stem cell therapy studies, Dietz *et al*[9] conducted a phase 1 single center non-randomized trial evaluating stem cell treatment for patients remaining

on biologic therapy of infliximab, adalimumab, and certolizumab. Twelve patients were given a stem cell loaded plug (MSC-MATRIX) with complete clinical healing in 75% of the population at three months, and 83.3% within six months. MRI was used to define characteristics of treated fistula tracts at baseline and six months to further confirm healing.

AUTOLOGOUS BONE MARROW DERIVED STEM CELL THERAPY TRIALS

Between 2007 and 2014, Ciccocioppo *et al*[10] looked at fistulizing CD assessing patients with autologous bone marrow-derived MSC (BM-MSC) for safety and efficacy. The authors found that fistula relapse-free survival was 88%, 50% and 37% at one-, two-, and five-year follow-up with no adverse events (AE). Thus, they concluded that BM-MSC was safe and efficacious for fistulizing CD.

ALLOGENEIC ADIPOSE TISSUE DERIVED STEM CELL THERAPY TRIALS

García-Arranz *et al*[11] conducted a phase 1-2 non-randomized, open-label trial with ten patients using allogeneic ASC for rectovaginal fistulas. Primary endpoint was safety and feasibility to treat CRRVF, and patients were followed at 1, 4, 8, 12, 24, and 52 wk after ASC administration. If complete re-epithelialization was not obtained by week 12, a second dose of ASC was administered. CRRVF was defined as healed "when the vaginal and rectal walls showed complete re-epithelialization and absence of vaginal drainage, including feces, flatus or suppuration". Nine patients had their fistula cured during the study, yet fistula re-occurrence occurred in seven of these patients. Due to patients being excluded for reasons such as need for biologic therapy or surgeries, the final efficacy rate for sustained fistula healing at 52 wk was 60% (three of five patients did not have reoccurrence). It was concluded that the primary endpoint was met as the study was found to be safe and feasible as a treatment option.

de la Portilla *et al*[12] analyzed in a phase 1-2 open-label single-arm non-randomized multi-center study 24 patients, who were given allogeneic expanded adipose-derived ASC (eASC) for complex perianal fistulas in CD. The endpoint was to determine safety and efficacy in this population. Patients underwent initial magnetic resonance imaging (MRI), and then eASC injection with a second injection if incomplete closure was found at 12 wk, with conclusion of the study at 24 wk. The same definition of closure as the Lee *et al*[6] study was used with evaluation at weeks 10, 12, 22, and 24 by both the treating physician and a blinded gastroenterologist/surgeon. The study found that 69.2% of patients had reduction in their fistula, with 38.1% of patients achieving complete closure at week 12 and 65.3% at week 24. Thus, it was concluded that eASC were safe and efficacious in the treatment of complex perianal fistulas.

Panés *et al*[13] authored a phase 3 randomized, double-blind, parallel-group, placebo-controlled, multicenter trial utilizing eASC as treatment in complex perianal fistula CD patients known as the ADMIRE CD study. Patients with stenosis, CRRVF, diverting stoma, or abscesses > 2 cm were additionally excluded to the above criteria. Inclusion criteria for the study were patients with refractory disease to immunologic, antibiotics, or biologics such as anti-TNF drugs. Closure was a similar definition as the above studies. Two hundred and twelve patients were randomly assigned, with 88 *vs* 83 patients at completion of the 24 wk. Overall, 50% of patients treated with eASC either solo or in combination with medical treatment achieved remission compared to 34% in the placebo group (P = 0.024)[13]. Treatment was also documented to be safe and efficacious with similar adverse reactions occurring more in the placebo group, thus being secondary to the nature of disease course in CD.

Panés *et al*[14] extended the ADMIRE CD Study from 24 wk to 52 wk and documented both clinical remission and combined remission. They defined this as "clinical assessment of closure of all treated external openings that were draining at baseline and the absence of collections > 2 cm." The trial concluded that eASC is still superior to placebo with clinical remission in 59.2% Cx601 *vs* 41.6% placebo (95%CI: 4.1-31.1; P = 0.013) and 56.3% *vs* 38.6% (95%CI: 4.2-31.2; P = 0.010) in combined remission.

Wainstein *et al*[15] also published a single center prospective observational pilot study conducted between 2013-2016 and included nine patients. Two stages were included in this study which was (1) "examination under anesthesia, fistula mapping, drainage and seton placement" and (2) setons removed four to six weeks with subsequent debridement and ASC then injected with biological plug formation. There were three classes of treatment results: complete healing, partial healing, and no healing. Partial healing was defined as external fistula opening remaining but with decrease of > 50% in size. This study found complete healing in 10/11 patients' fistulas and partial healing in 1/11[15]. Conclusions were made that excellent success rates can be made for fistulizing CD with a multi-approach treatment method including ASCs, platelet rich plasma, and endorectal advancement flaps.

ALLOGENEIC BONE MARROW DERIVED STEM CELL THERAPY TRIALS

Molendijk's team conducted a randomized, double-blind, placebo-controlled, dose-escalating study using allogeneic bone-marrow MSCs with surgical treatment for 21 patients with refractory perianal fistulizing CD[1]. The study used either MSCs from five different donors

or normal saline-5% albumin solution as placebo with surgery performed by two surgeons with expertise in IBD. Fistula healing was documented by photography at weeks 0, 12, and 24, in addition to finger pressure at external openings and MRI at week 12. Endpoints were absence of discharge and absence of collections of > 2 cm on MRI. Results were 66.7%, 85.7%, and 28.6% fistula healing for the three groups at week 24 compared to placebo (P = 0.06 group 2 *vs* placebo)[1]. The study concluded that allogeneic bone-marrow MSCs are superior in promoting fistula healing compared to placebo for patients with refractory perianal fistulizing CD.

MIXED STEM CELL TREATMENT MODALITIES

Interestingly, there was a case study published in 2015 that included five pregnant females with fistulizing CD analyzing their reproductive outcomes[16]. Of this patient population, three had CRRVF and two had perianal fistulas and had undergone ASC injection with resolution of their fistulas and subsequent ability for pregnancy (between 17 mo to 2 years). Thus, three patients received autologous and two patients had received allogeneic ASC prior to conception. All five patients were in their 30s during administration of ASC, and mid-thirties to early forties for age at gestation[16]. All but one patient had 18-24 mo between ASC and gestation. After their pregnancies, the patients were given data collection sheets. Two of the five patients experienced gestational complications, namely being first trimester miscarriages (no treatment during pregnancy) and fetal growth restriction and small for gestational age (azathioprine during pregnancy). Of the patients who gave birth, all four patients underwent cesarean section with only one newborn malformation occurring, which was syndactyly with clinodactyly[16].

DISCUSSION

Suggestions and Practical Guidance

Patients who present with continued fistulas from their IBD despite other medical and surgical therapies should be referred to centers that are utilizing stem cell therapies. Such patients can have relief and resolution of anxiety and frustration of their disease process with this novel treatment. Physicians caring for this patient population who consider such therapies should make sure to counsel patients on the risks versus benefits including the commonly seen AE and significant adverse effects (SAE) mentioned above. Additionally, patients should know that if they undergo allogeneic transplantation they may fail to harvest enough stem cells for treatment. Yet, the authors of this paper and the authors of the literature reviewed here are excited for future studies and a novel treatment for a complicated disease.

CONCLUSION

In this review, we highlight the progression of utilization of stem cell therapy in fistulizing IBD, specifically CD. While still early along in this evaluation process, these therapies do offer a lot of potential for a difficult to treat population. Likely because of its immunomodulatory ability with differentiation and suppression of proliferation, stem cell therapies appear to be a promising treatment option for a sizeable population of CD patients with fistulizing disease.

FUTURE PERSPECTIVES

Currently on the horizon, there are four clinical trials registered for fistulizing CD. Three of these studies are recruiting and one is still pending recruitment. Three of these will be non-randomized, one of these will be randomized single-blind, and the majority will be utilizing autologous stem cells[17]. Studies need to be streamlined in the amount of stem cells used and the type of cells harvested such as allogeneic versus autologous hosts and bone marrow versus adipose tissue. Since there does not appear to be a benefit to bone marrow harvesting thus far, we believe that studies should focus on adipose-derived stem cells, either autologous or allogeneic. Comparing studies to current treatment rates of fistula healing, which is less than 50%, stem cell therapy for fistulizing CD appears to be beneficial as the majority of studies claim 60%-88% fistula healing and remission observed at 24-52 wk. Studies even showed benefit of remission five years out from administration[10]. Moreover, these studies show that stem cell treatment for fistulizing disease is safe with very few AE or SAE, with the majority including pain, bleeding, or abscesses. Most AE or SAE observed were due to the underlying nature of IBD itself.

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REVIEW

Treat-to-target in Crohn's disease: Will transmural healing become a therapeutic endpoint?

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Abstract

Crohn's disease (CD) represents a chronic transmural inflammatory condition of the gastrointestinal tract, which usually leads to structural damage and significant disability. Deep remission - defined by both clinical and endoscopic remission, signifying mucosal healing - represents the current endpoint in the treat-to-target strategy, significantly improving patients' long-term outcomes. Transmural healing (TH) could be a more effective target, but this possibility remains unclear. This narrative review aims to critically review and summarize the available literature relating TH to long-term outcomes, being the first of its kind and to the best of the author's knowledge. A systematic literature search (from inception to March 31 2018) was performed, using multiple databases, and identifying seven full-text manuscripts. In those studies, long-term favorable outcomes ($\geqslant$ 52 wk) included sustained clinical remission, as well as fewer therapeutic changes, CD-related hospitalizations, and surgeries. Despite heterogeneous design and methodological limitations, six of the studies demonstrated that TH or intestinal healing (TH plus mucosal healing) were predictive for the aforementioned favorable outcomes. Therefore, TH may become a reasonable therapeutic target and be included in the concept of deep remission. Further prospective, well-designed, multicenter trials aiming to better define the role of TH in personalized therapy for CD and to determine the long-term influence of TH on bowel damage and disability are warranted.

Key words: Treat to target; Cross sectional imaging; Deep remission; Transmural healing; Intestinal healing; Long-term outcomes; Bowel damage; Crohn's disease

Core tip: Crohn's disease (CD) represents a chronic transmural inflammatory condition of the gastrointes-

tinal tract, potentially leading to structural damage and disability. Deep remission (clinical and endoscopic remission), the current therapeutic goal, significantly improves patients' long-term outcomes. Transmural healing (TH) could be a more effective target. Therefore, this narrative review (the first of its kind, to the best of the author's knowledge) aims to provide the currently available scientific evidence on the predictive role of TH for long-term outcomes (clinical remission, therapeutic changes, CD-related hospitalizations and surgeries) and to establish whether TH should become a therapeutic endpoint in the treat-to-target strategy.

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INTRODUCTION

Crohn's disease (CD) represents a chronic transmural inflammatory condition of the gastrointestinal tract, that could lead to structural damage and significant disability[1-5]. Therefore, it is crucial to initiate the most effective therapy in a timely manner (in the so-called "window of opportunity"), in order to prevent bowel damage (BD; *i.e.,* complications like strictures, fistulae, and abscesses) requiring surgery, leading to further disability and poorer quality of life[6,7]. According to the concept of "treat-to-target" strategy (STRIDE), deep remission (DR) has become the new therapeutic goal[8]. DR includes clinical (steroid-free)/patient-reported outcome remission (defined as resolution of abdominal pain and diarrhea/altered bowel habit) and endoscopic remission [*i.e.*, mucosal healing (MH), defined as resolution of ulceration at ileocolonoscopy (IC)], or resolution of findings of inflammation on cross-sectional imaging (CSI) in patients who cannot be adequately assessed with IC[8].

Systematic reviews and meta-analyses of both cohort studies and randomized controlled trials have demonstrated that MH (part of DR) assessed by IC was a strong predictor for better outcomes, including sustained long-term steroid-free clinical remission (CR) and MH as well as lower rates of CD-related hospitalizations and surgeries[9,10]. However, over the last few years, researchers have questioned whether MH is a sufficient endpoint, given that CD represents a transmural disease and active intramural inflammation and that damage can persist despite the presence of MH at endoscopy[11-17]. It was suggested that the more inclusive "bowel healing" or "deep healing" (referred to as "IH" and describing healing of the whole intestinal wall) may be a more appropriate therapeutic goal than MH[9,13,18].

The concept of transmural healing (TH) evaluated by CSI was developed several years ago and regarded as a logical goal of treatment, both in adult[19] and pediatric patients[20,21] with CD; however, reliable definitions of TH were not available at that time. Castiglione *et al*[22] were among the first to define and highlight the concept of TH as a bowel wall thickness (BWT) of < 3 mm assessed by bowel ultrasonography (US). The authors also reported that TH was reached in 25% of adults with CD who were treated with anti-tumor necrosis factor alpha (TNF) agents for 2 year[22]. Since then, the achievement of TH under certain therapeutic approaches has become a subject of growing interest, in both children and adults. More than 20 recent studies have revealed that therapeutic response is associated with improvements, including TH, detected at CSI. Imaging techniques used in these studies included magnetic resonance enterography or enteroclysis (MRE)[23-32], US [Doppler[22,32-35] or with contrast agents (contrast-enhanced US, commonly known as CEUS[36], or small intestine contrast US, commonly known as SICUS[11,12]) and computed tomography enterography or enteroclysis (CTE)][37-40]. The demonstrated rates of TH under various medications range from 0% (after 2 wk[24] and even 12 mo[11,39]) to 14% (after 1 year)[12] and as high as 25% (after 2 year)[32]. However, no unanimous definition of TH was used in these studies.

Most series have found a good significant correlation between TH and MH[22,26,28-32,36], while other researchers found agreement with endoscopy only for some parameters, mainly extramural[40], and others did not find any agreement between CSI and IC improvements[11,12,37]. Among the last category, one study reported that 27% of patients with complete MH showed evidence of transmural inflammation[12]. TH, therefore, likely reflects a deeper level of healing, which could be correlated with a more stable and long lasting CR[18]. However, none of the aforementioned studies was designed to analyze the benefits of achieving TH on patients' long-term outcomes.

Recent interesting studies have included radiological response/TH under therapy as a treatment endpoint and related it to long-term outcomes[13,14,41], showing significant benefits of achieving TH *vs* persistent active transmural inflammation. TH could become a therapeutic goal, but only if it has been constantly demonstrated to improve patients' long-term outcomes (*i.e.,* sustained CR, fewer therapeutic changes, CD-related hospitalizations and surgeries, and reduction of BD and of disability). Therefore, the aim of this narrative review is to provide the currently available scientific evidence on the predictive role of TH in CD for long-term outcomes, in order to establish whether TH should become a therapeutic endpoint in the "treat-to-target" strategy.

LITERATURE SEARCH

Database searches

Published full-text manuscripts were identified from inception to March 31 2018 by a systematic literature

search of MEDLINE (PubMed), EMBASE, The Cochrane Library, Web of Science, Google Scholar, ResearchGate and Mendeley databases. Only articles published in the English language were included. The reference lists from the selected studies were manually examined to identify additional research studies. Duplicates were excluded. The search included the following items/key words: ("Crohn's disease", "inflammatory bowel disease") and ("transmural healing", "transmural remission", "deep healing", "bowel healing", "gut healing", "intestinal healing", "parietal healing", "radiological remission", "radiological healing") and ("cross-sectional imaging", "magnetic resonance imaging", "magnetic resonance enterography", "magnetic resonance enteroclysis", "computed tomography enterography", "abdominal ultrasonography", "abdominal ultrasound", "color-Doppler ultrasound", "small-intestine contrast-ultrasonography", "contrast-enhanced ultrasonography") and ("outcomes"). Only studies reporting on long-term outcomes after TH (at least 12 mo), like CR and/or TH, medication changes, CD-related surgery rate and hospitalization rate, and influence on BD and disability were included.

Data extraction

The following data were extracted from each identified article: Last name of the first author; publication year; country; study design; characteristics of the included population, specifically sample size, age at inclusion, sex, behavior and location of CD, previous CD-related surgery, duration of the disease, and medication; aim of the study; follow-up time; definition of CR and endoscopic MH; type of CSI used and included parameters; definition of TH and rate of achieved TH; correlation between TH and endoscopic MH; long-term CR and TH; change in medication; CD-related hospitalization; CD-related surgery; influence of TH on BD and disability; potential limits of the studies; and, any other relevant data regarding TH.

TRANSMURAL HEALING

Characteristics of included studies

Seven full manuscripts, all published in 2016 or 2017, were included, with heterogeneous design. The main characteristics of these studies are detailed in Table 1[13-15,41-44]. Three studies were prospective[15,41,44] and four were retrospective[13,14,42,43]. Only one study was performed in children[43]. The size of the study population ranged from 26[42] to 214[13] patients. Location of CD, defined by Montreal[45] or Paris[46] classification was as follows: ileocolon (L3)[42]; terminal ileum (± caecum) (L1) and ileocolon (L3)[13,14,44]; and, all types of location (L1, L2, L3 ± L4)[15,41,43]. Patients presented all types of CD behavior (defined by Montreal[45] or Paris[46] classification), the most prominent being inflammatory behavior (B1)[13,14,41-44]. Prior CD-related surgery was mentioned in five studies[13-15,41,44] and its rate ranged from 22.8%[15] to 61.3%[14]. All studies mentioned the duration of the disease, with the lowest median being 4 year[42] and the highest median being 9 year[14]. Three studies included only patients treated with anti-TNF agents, either as monotherapy or combined with other medication[41,42,44], while in other studies patients were on various therapies[13-15,43]. The following CSI were used: MRE[13,42,43]; MRE and CTE[14]; US and CEUS[41]; US and US elastography[44]; and, CTE[15]. Timing of CSI performance varied among the studies, representing at inclusion in the study[13,15], at a certain point after diagnosis[43], before and after anti-TNF induction[42], pre-therapy and after 6 mo or two examinations given $\geqslant$ 6 mo apart[14], and at baseline, after induction, and after 1 year of treatment[41,44].

Various definitions/scoring systems were used to describe the CR, MH and radiological remission/TH, as detailed in Table 2. CR was considered in four studies[15,41-43], two of which did not use IC[41,43]. MH was defined at IC in four studies[13-15,42]. Regarding CSI, various definitions of TH were used, according to several scores and parameters, as detailed in Table 2. Two studies also included IH[13,42], assessed by colonoscopy and MRE. In studies where TH was available only at baseline (for evaluation as a predictor for outcomes), the percentage of TH varied between 17.5%[15] and 35.6%[43]. IH at inclusion was detected in 15.4%[13]. After induction therapy with anti-TNF agents, TH was detected in 14%[41], 27%[44] and 38%[42] and IH was present in 31%[42]. After at least 6 mo of various therapies, TH was achieved in 37%[14]. After 12 mo of anti-TNF therapy, TH was detected in 30%[41,44].

In studies which compared MH (at IC) and TH (evaluated by CSI), no good agreement was detected[13,15] (Table 2). In addition, one study showed that nearly one in two patients with a normal terminal ileum (at IC) had evidence of active disease (at MRE/CTE) either in the terminal ileum or proximal to it[14]. Of those with MH, TH was detected in 27% (at CTE)[15] and 54% (at MRE/CTE)[14].

Transmural healing and long-term outcomes

The included studies are detailed in Table 3.

Long-term CR: Eder *et al*[42] found that IH (achieved after induction therapy) was significant in predicting long-term CR. Median duration of CR among long-responders was 45 mo *vs* those with relapse (18 mo, P = 0.02). Moreover, lack of IH (even if CR, MH or TH were achieved) had 90% probability of exacerbation shortly after stopping 1 year of anti-TNF therapy[42]. Ripollés *et al*[41] showed that sonographic response after 12 wk of anti-TNF (induction) was more pronounced than during maintenance treatment and predicted good response at 1 year with a sensitivity and specificity of 75.9% and 81.8%, respectively, with an odds ratio of 14.14. A good sonographic response at 52 wk significantly predicted good long-term clinical outcome[41]. In children, long-term CR was significantly higher for those with TH *vs*

Table 1 Characteristics of the included studies

First author, year, country	Study type	CD population, disease location, behavior, surgery	Duration of CD in yr	MD	Aim of study	Methods used to assess CD activity, timing	Follow-up time
Eder, 2016, Czech Republic[42]	RS	26 adults, responsive to induction doses of anti-TNF, median age (IQR) 27 yr (IQR: 21-36), 61% F, L3, B1 62%, B2 7%, B3 31%	Median (IQR): 4 (2-6)	Study MD: IFX or ADA, 1 yr Concomitant MD: CS 88%, AZA 88%, 5ASA 100%, AB 54%	Predictive role of MH, TH and IH healing on long-term CR	Clinical, endoscopic, and MRE activity: before starting anti-TNF and after induction (week 12-14 for ADA and week 9-12 for IFX)	Median 29 mo (IQR: 14-46) after finishing 1 yr of anti-TNF
Sauer, 2016, United States[43]	RS	101 children, 41.6% F, L1 28%, L2 24%, L3 54.5%, L4a 17.8%, L4b 24.7%, B1 76%, B2 18%, B3 2%, B2B3 4%, perianal 14%	Median (range): 4.7 (1.65-11.5)	IMD 33%, Biologic 67%	Predictive role of MRE remission on long-term CR, MD change and surgery	MRE, at median of 1.3 yr from diagnosis	Median 2.8 yr after MRE
Deepak, 2016, United States[14]	RS	150 adults, 66% treatment-naïve, median age (IQR) at diagnosis 23 yr (IQR: 19-33), 50% F, L1 48.7%, L3 40.7%, L4 10.6%, B1 45%, B2 35.3%, B3 19.3%, perianal 19.3%, prior CD-related surgery 61.3%	Median (IQR): 9 (3-21)	At second CTE/MRE: Anti-TNF alone: 20%, THIO alone 36%, MTX alone 5.3%, Anti-TNF + THIO 24%, Anti-TNF + MTX 5.3%, Budesonide 8%, Natalizumab 1.4%	Predictive role of radiologic response on long-term outcomes: CS use, hospitalization, and surgery	Serial CTE/MRE: first and follow-up (705 CTE/MREs): pre-therapy and after 6 mo or 2 CTE/MREs ⩾ 6 mo apart (during maintenance therapy)	Median 4.6 yr (IQR: 1.6-7)
Fernandes, 2017, Spain[13]	RS	214 adults, 49.5% F, median age (IQR) 36.8 (16–77) yr, L1 76.6%, L3 23.4%, L4 10.3%, B1 44.4%, B2 26.2%, B3 29.4%, perianal 29.9%, prior intestinal resection 40.7%	Median (IQR): 7.4 (0-40.8)	THIO 54.7%, MTX 0.5%, Anti-TNF 18.7%	Predictive roles of MH and TH for hospital admission, surgery and MD escalation (start an IMD or biologic, escalate anti-TNF or switch to a different biologic)	MRE and IC performed within a 6-mo interval (median: 2.3 mo)	Median (IQR): 3.5 (1-7.9) yr Evaluation after 12 mo
Ripollés, 2016, Spain[41]	PS multicenter	51 adults, active disease, 47% F, median age (IQR) 35 yr (27-46), L1 57%, L2 21.5%, L3 21.5%, B1 57%, B2 10%, B3 33%, perianal 27.5%, history of surgery 33%	Median (IQR): 5 (2-10.3)	Active MD: Anti -TNF (IFX or ADA) 100% (63% combined with IMD)	Predictive role of TH on clinical outcome, change in MD, surgery	Clinical and US / CEUS at baseline, 12 wk and 1 yr after treatment	Median (IQR): 16 mo (12.2-32)
Orlando, 2018, Italy[44]	PS	30 adults, 33.3% F, mean age (± SD) 38.8 (± 14.5) yr, L1 40%, L3 60%, B1 53.3%, B2 40%, B3 6.7%, prior intestinal resection 40%	Mean ± SD: 9.8 ± 7.7	Active MD: Anti-TNF (IFX 53.3%, ADA 46.7%) Concomitant MD: 5ASA 10%, CS 10%, THIO 16.7%	Predictive role of TH and intestinal fibrosis on clinical outcome (hospitalization and surgery)	US and UEI at baseline, 14 and 52 wk after therapy	Median (range): 20 mo (10-38)

Laterza, 2018, Italy[15]	PS	57 adults, mean age (± SD) 45.3 (± 17) yr, 42.2% F, L1 38.6%, L2 8.7%, L3 52.6%, B1 31.6%, B2 54.4%, B3 14%, perianal 7%, previous surgery 22.8%	Mean ± SD: 7.4 ± 1	No therapy 10.5%, CS 26.3%, Anti-TNF 10.5%, CS + IMD 15.8%, CS + anti-TNF 8.8%, IMD + anti-TNF 8.8%, CS + IMD + anti-TNF 19.2%	Predictive role of a single and/or combined (CR, MH and TH) remission on outcomes (surgery, hospitalizations, MD changes - introduction of IMD or anti-TNF, anti-TNF escalation, switch to another anti-TNF, need for CS and deaths)	Clinical, endoscopic and CTE at baseline	Up to 36 mo

5ASA: 5-Amino salicylates; AB: Antibiotics; ADA; Adalimumab; Anti-TNF agents: Anti-tumoral necrosis alpha agents; AZA: Azathioprine; B: Behavior according to Montreal or Paris classification, with B1 inflammatory, B2 stricturing, B3 perforating, B2B3 both stricturing and perforating; CD: Crohn's disease; CEUS: Contrast-enhanced ultrasound; CR: Clinical remission; CS: Corticosteroids; CTE: Computed tomography enterography; F: Female; IC: Ileocolonoscopy; IFX: Infliximab; IH: Intestinal healing; IMD: Immunomodulators; IQR: Interquartile range; L: Location according to Montreal or Paris classification, with L1 distal 1/3 ileum ± limited cecal disease, L2 colonic, L3 ileocolonic, L4 upper proximal disease with L4a upper disease proximal to the ligament of Treitz, L4b upper disease distal to the ligament of Treitz and proximal to distal 1/3 ileum; MD: Medication; MH: Mucosal healing; MRE: Magnetic resonance enterography; MTX: Methotrexate; N/A: Not available; PS: Prospective; RS: Retrospective; SD: Standard deviation; TH: Transmural healing; THIO: Thiopurines; UEI: Ultrasound elasticity imaging; US: Ultrasonography.

those with active inflammation[43].

Long-term stable medication: Eder *et al*[42] also included "no need of corticosteroids" in long-term CR, and found IH to be a good predictor for this outcome. A Spanish study showed the IH group to have significantly less therapy escalation and longer time until therapy escalation *vs* the group with only MH and *vs* the no healing group[13]. Deepak *et al*[14] showed that TH significantly decreased the risk of corticosteroid use by over 50%. Another Spanish study found that three quarters of patients who did not change medication had sonographic improvement or TH *vs* only 8% of patients who needed medication change or surgery[41]. Children with TH had a significantly lower percentage of requiring any switch in therapy *vs* those without TH[43].

CD-related hospitalization-free status: Eder *et al*[42] also included "no hospitalization" in long-term CR, and found IH to be a good predictor for this outcome. In another study, IH was shown to be significantly associated with lower hospitalization rate and longer time until hospital admission *vs* MH and *vs* NH[13]. Lastly, complete CTE/MRE remission decreased the risk of hospitalizations by over two-thirds[14].

CD-related surgery-free status: IH was associated with significantly lower surgery rates *vs* MH and *vs* NH, and significantly longer time to surgery *vs* the other groups, without any difference between MH and NH[13]. Deepak *et al*[14] found that only "complete response" decreased the risk of surgery by over two-thirds. In another study, significantly less surgery was found to be required in patients with a strain ratio of < 2 at baseline, showing that less intestinal fibrosis is predictive for a better course of CD[44]. Lastly, in pediatric patients, the rate of CD-related surgery was significantly lower in those with TH *vs* no TH[43].

Limitations of the included studies: In essence, four studies were retrospective[13,14,42,43], two had low number of patients[42,44], three did not include IC[41,43,44], and none of the studies used a validated score for CSI. In addition, only one CSI examination was performed in three studies[13,15,43], thus not allowing for a dynamic assessment of transmural changes. No study included the influence of TH on long-term sustained TH, disability, and BD. More details are presented in Table 3.

DISCUSSION

The influence of TH or IH on long-term outcomes represents a new concept, as was described in this review and evidenced by the fact that all the relevant studies have been published since 2016. Six of the seven studies demonstrated that patients with IH[13,42] or TH[14,41,43,44] had significantly higher rates of favorable long-term outcomes *vs* those with persistent inflammation, including long-term CR[41-43], fewer therapeutic changes[13,14,41-43], reduced rate of CD-hospitalization[13,14,42,44] and of CD-related surgery[13,14,41,43,44]. Also, IH (evaluated by CSI and IC) was superior to MH alone (at IC) in predicting significantly better long-term outcomes[13,42]. The poor agreement between MH and TH[13-15], showing that active inflammation beyond the mucosa could persist even in patients with MH, is in accordance with previous data[11,12,23,27]. Therefore, MH does not seem to be an adequate surrogate marker of IH. Since treating to a TH target leads to better patients' outcomes than those of patients without TH, TH should be incorporated in the

Table 2 Definitions used in the included studies

First author, year, country	CR definition; percentage	MH: definition; percentage, timing	Cross-sectional imaging method (details)	TH (± IH): definition	Percentage of TH, timing	Agreement MH-TH
Eder, 2016, Czech Republic[42]	CDAI < 150	MH: ⩾ 50% decrease in SES-CD; 62%, after induction	MRE (score: SEAS-CD)	TH: ⩾ 50% decrease in SEAS-CD IH: TH + MH: ⩾ 50% decrease in both SES-CD and SEAS-CD	TH: 38%, IH: 31%, both after induction	N/A
Sauer, 2016, United States[43]	According to PGA	No IC	MRE (no score; "all or none" approach - abnormal BWT, increased enhancement)	TH: lack of active inflammation, complete MRE healing (normal BWT and no increased enhancement)	TH: 35.6%, at inclusion	N/A
Deepak, 2016, United States[14]	N/A	Inactive IC; 17.3%, at 2nd CTE/MRE (data missing in 61% of patients)	MRE/CTE (score by[37]): BWT ⩾ 3 mm, mural hyperenhancement, or intramural hyperintense T2 signal; segments length; comb sign, peri-enteric inflammation (absent, localized edema, inflammatory mass, abscess), fistula, stricture	TH: reduction in lesion length to 0 cm and a score < 1 for all other parameters (decreased enhancement or length of disease, no worsening of parameters of active inflammation - dilated vasa recta/comb sign, perienteric inflammation (edema, phlegmon, or abscess), or fistula	Complete radiologic responders: 37%, at 2nd CTE/MRE	Of inactive ileum at IC: 46% with active disease at 2nd CTE/MRE
Fernandes, 2017, Spain[13]	N/A	Inactive IC: no mucosal ulceration; in operated patients - Rutgeerts score 0-1; Inactive IC: 39.4% MH group = inactive IC + active MRE: 24.3%	MRE (active: BWT > 3 mm, increased contrast enhancement, and complications - stricture, abscess, or fistulae; additionally: fat creeping and comb sign)	IH (TH) group: MH + inactive MRE NH: active endoscopy, irrespective of the MRE findings	Inactive MRE: 25.7% IH group: 15.4% NH group: 60.3%	Significant low correlation between inflammation assessed by MRE and IC (Spearman's rho = 0.244, P < 0.001)
Ripollés, 2016, Spain[41]	HBi < 5 and normal CRP, without CS	No IC	US/CEUS (sonographic score: transmural inflammation - BWT, color Doppler grade, mural enhancement; extramural involvement, and obstructive disease)	TH: BWT < 3 mm, besides color Doppler grade 0 and the absence of complications, regardless of the persistence of parietal enhancement	TH: 14%, at 12 weeks and 30%, at 52 wk	N/A
Orlando, 2018, Italy[44]	N/A	No IC	US/UEI (bowel wall stiffness: strain ratio between mesenteric tissue and bowel wall; strain ratio ⩾ 2 = severe ileal fibrosis	TH: BWT ⩽ 3 mm	TH at 14 and 52 wk: 27% and 30%, respectively. Baseline strain ratio: lower in those with TH (P < 0.05)	

Laterza, 2018, Italy[15]	HBi ⩽ 4; 56% at baseline	MH: SES-CD ⩽ 2; 19%, at baseline	CTE (qualitative judgment on transmural activity, based on lesions: BWT, stenosis, target sign, comb sign, lymphadenopathy, fistula, abscess, sinus tract, fibrofatty proliferation, perienteric stranding, free fluid in the abdomen)	TH: absence of typical CTE lesions	TH: 17.5%, at baseline	Agreement between CTE and IC in 47% (k = – 0.05; P = 0.694); Agreement between CTE, IC and HBi in 18% (k = 0.01; P = 0.41), TH: detected in 27% with MH

BWT: Bowel wall thickness; CD: Crohn's disease; CDAI: Crohn's disease activity index; CEUS: Contrast-enhanced ultrasonography; CR: Clinical remission; CRP: C-reactive protein; CTE: Computed tomography enterography; HBi: Harvey-Bradshaw index; IC: Ileocolonoscopy; IH: Intestinal healing; MH: Mucosal healing; MRE: Magnetic resonance enterography; N/A: Not available; NH: No healing; PCDAI: Pediatric-CD activity index; PGA: Physician global assessment; SEAS-CD: Simple enterographic activity score for CD; SES-CD: Simple endoscopic score in CD; TH: Transmural healing; UEI: Ultrasound elasticity imaging; US: Ultrasonography.

concept of DR.

As the above results showed, however, TH may be more difficult to reach than MH. It had been suggested that TH needs a longer period of therapy (*i.e.,* > 1 year). The sonographic response after 12 wk of anti–TNF therapy has also appeared to be more pronounced than in the maintenance period[41], and this result is similar to findings from a previous MRE study[23]. Therefore, TH could probably be achieved earlier but only if aggressive therapy is used and applied in a timely manner. It was also previously considered that, when significant BD was already present, the effect of therapy on transmural lesions might be less effective[12]. The collective population that comprised the seven studies in this review had a relatively long duration of disease already[13-15,41-44] and many patients had complicated behavior (stricturing and/or fistulizing) and previous surgery (BD)[13-15,41,44]. These facts could explain the relatively small percentage of patients reaching TH. Ripollés *et al*[41] demonstrated that initial stricture was the only sonographic feature predictive of negative response, while Deepak *et al*[14] found that penetrating behavior was a risk for hospitalization for active disease and showed a trend towards increased surgical risk. Laterza *et al*[15] provided evidence that TH following anti-TNF therapy was mostly achieved in the absence of significant bowel fibrosis.

CSI techniques are of paramount importance, not only in assessing TH but also for determining BD, while IC is not accurate enough. A recent study showed that surgical resections (26%) were not associated with the presence of severe lesions at IC, while stenosis or intra-abdominal fistulae (part of BD) at MRE correlated significantly with a higher risk of surgery[47]. Unfortunately, no study in this review was designed to include the CD Damage Score - the Lémann index (LI)[2,26] - at baseline or to predict the role of TH on further LI. In a prospective study by Fiorino *et al*[48], 39.4% of CD patients had BD at diagnosis. Even if treatment with anti-TNF agents was able to reverse BD in a subgroup of CD patients[49], within the first 10 year of the disease at least two thirds of the patients demonstrated significant BD[50]. These data provide support, once more, for the theory that earlier introduction of disease-modifying therapy might prevent the onset of irreversible BD[6,12].

A key strength of this narrative review is its being the first of its kind, to the best of the author's knowledge. It showed the significance of including TH as a new therapeutic goal and the importance of using CSI techniques in assessing TH. This review also has several limitations. First, since the concept of TH related to long-term outcomes is recent, the number of studies is small; however, all available full-text manuscripts in the literature were included, representing studies of pediatric and adult patients and, given the paucity of data, prospective and retrospective studies. Second, no manuscript addressed all long-term outcomes; although, three of the four mentioned outcomes were included in five studies[13,14,41-43]. Third, different CSI techniques, scores and parameters were used to define TH.

Do we know which method is the most accurate (gold standard) to assess TH? Reviews and meta-analyses[51,52] have highlighted the high accuracy (> 80%-90%) of CTE, MRE, and US for the diagnosis of CD, assessment of disease activity, or abdominal complications of CD, with no significant differences among these procedures in terms of sensitivity and specificity[22]. However, the use of CTE in children should be limited to exceptional circumstances (when US and/or MRE cannot be used)[53], as it could increase the risk of developing malignancies[54]. Castiglione *et al*[32] recently showed that TH rates achieved after anti-TNF therapy (detected by US and MRE) were approximately equal (25% *vs* 23%, k = 0.90; P < 0.01) in patients with ileocolonic CD. They concluded that these two techniques were similar in assessing TH, with the choice being largely determined by local availability, experience, cost and patient preference[55].

Nowadays, given their non-invasiveness, non-irradiation and high accuracy, MRE and US are considered the most appropriate techniques to monitor CD patients after treatment[18]. Benefits and limits of CTE, MRE and US

Table 3 Outcomes of patients achieving transmural healing and intestinal healing

First author, year, country	Long-term CR; percentage; other findings	Change in medication	Reduction in hospitalizations for active CD	Reduction in CD-related surgery	Other findings/ Comments	Limitations
Eder, 2016, Czech Republic[42]	38%; TH: not useful for predicting long-term CR IH: predicts long-term CR, $P = 0.02$ (75% Sen, 72% Spe)	N/A	N/A	N/A	MH: borderline significance ($P = 0.06$) in predicting long-term CR (50% Sen, 80% Spe)	RS, Low number of patients, Only ileocolonic CD, No MRE, No IC by the end of 1 yr therapy
Sauer, 2016, United States[43]	TH: 88.9% *vs* 44.6% of those with MRE active inflammation (no TH), $P < 0.001$	TH: 8.3% *vs* no TH: 44.6% (switching from IMD to biologic and changing type of biologic, $P < 0.001$)	N/A	TH: 2.8% *vs* No TH: 18.5%, $P = 0.024$	N/A	RS, All MRE - part of patient care, No standardized MRE score, No MRE, No IC at end of follow-up
Deepak, 2016, United States[14]	N/A	Complete or partial radiologic response decreases risk for CS use by over 50% [HR: 0.37 (95%CI: 0.21-0.64), $P < 0.001$ and 0.45 (95%CI: 0.26-0.79), $P = 0.005$ respectively]	Complete response decreases risk of hospitalizations by over two-thirds [HR: 0.28 (95%CI: 0.15-0.50), $P < 0.001$]; also partial response decreases risk [HR: 0.54; (95%CI: 0.32-0.92), $P = 0.04$]	Complete response decreases risk of surgery by over two-thirds [HR: 0.34 (95%CI: 0.18-0.63)], $P < 0.001$	First data to demonstrate the magnitude and significance of radiological response as a treatment target and endpoint; Penetrating behavior is a risk for hospitalization for active disease and shows a trend towards increased surgical risk	RS Tertiary referral center Not all IC available
Fernandes, 2017, Spain[13]	N/A	IH: less therapy escalation *vs* MH and *vs* NH (15.2% vs 36.5%, $P = 0.027$ and vs 54.3%, $P < 0.001$); IH: longer time until therapy escalation *vs* MH, $P = 0.046$ and *vs* NH, $P < 0.001$; MH better outcome than NH	IH: hospitalization rate lower *vs* MH and *vs* NH (3.0% *vs* 17.3%, $P = 0.044$ and *vs* 24.0%, $P = 0.003$); no difference MH *vs* NH IH: time until hospital admission longer *vs* MH, $P = 0.046$ and *vs* NH, $P = 0.008$	IH: surgery rates lower *vs* MH and *vs* NH (0% *vs* 11.5%, $P = 0.047$ and *vs* 11.6%, $P = 0.027$); no difference MH *vs* NH IH: longer time to surgery *vs* MH ($P = 0.045$) and *vs* NH ($P = 0.044$)	Endoscopic remission (OR: 0.331, 95%CI: 0.178-0.614, $P < 0.001$) and MRE remission (OR: 0.270, 95%CI: 0.130-0.564, $P < 0.001$): independently associated with a lower likelihood of reaching any of the studied outcomes	RS, dichotomous definition of IH and MH, No scores, No patients with stenosis, Interval between IC and MRE (up to 6 mo) Only baseline IC and MRE
Ripollés, 2016, Spain[41]	Good sonographic response at 52 wk predicts good long-term clinical outcome (2-3 yr) with a Sen of 78% and Spe of 81.3%; OR: 15.5	TH at 52 wk: 93% did not require change in medication/ surgery	N/A	TH/sonographic improvement at 52 wk: less likely to require change/ intensification in MD or surgery during follow-up *vs* no improvement (11% *vs* 65%, $P < 0.001$)	Changes in BWT: most important in assessment of the effects of therapy; 42% of patients without complications achieved TH *vs* only 5% with complicated behavior; Initial stricture: the only sonographic feature predictive for negative response ($P = 0.0001$)	No IC, No validated US-based activity score

Orlando, 2018, Italy[44]	N/A	N/A	Hospitalization rate decreases significantly with an increase in the number of parameters indicating remissions at baseline	Significant less surgery in patients with a strain ratio < 2 at baseline (P = 0.009)	No association between baseline BWT at US and therapeutic outcomes	Low number of patients, No IC, Single center study
Laterza, 2018, Italy[15]	N/A	Complete remission vs patients with one or two remissions (partial remission) *vs* no remission: differences among groups different only for the need of topical CS (P = 0.03)	Complete remission (CR, MH, TH): trend for fewer hospitalizations *vs* patients with only MH or TH or CR	N/A	Endoscopic remission: significantly less changes in therapy *vs* endoscopic activity (P = 0.02) Multiparametric (CR, MH, and TH) evaluation might have a better value to predict significant changes in therapy and hospitalization	Heterogeneous therapies CTE: qualitative non-validated score Only baseline clinical, IC and CTE evaluation

BWT: Bowel wall thickness; CD: Crohn's disease; CFREM: Clinical CS-free remission; CR: Clinical remission; CS: Corticosteroids; CTE: Computed tomography enterography; IC: Ileocolonoscopy; IH: Intestinal healing; IMD: Immunomodulators; MD: Medication; MH: Mucosal healing; MRE: Magnetic resonance enterography; N/A: Not available; NH: No healing; RS: Retrospective study; Sen: Sensitivity; Spe: Specificity; TH: Transmural healing.

have been extensively presented in recent reviews[53,55-61], systematic reviews and meta-analyses[52,62], and consensus guidelines[53,63-65]; therefore, they are beyond the scope of this review. Validated CSI scores to quantify activity in CD are mainly based on MRE[55,56,58]. A comparison between the three most used scores in adults with CD - Magnetic Resonance Index of Activity (MaRIA)[16,66], Clermont[67] and London[68] - was recently published[69]. All scores had high accuracy for evaluating CD activity, but MaRIA had better overall operational characteristics for its use in both clinical trials and clinical practice[69]. A recent paper questioned the role of MaRIA in properly assessing BD and as a prognostic factor. Fiorino *et al*[48] showed that BD and LI were independent prognostic factors for intestinal surgery [hazard ratio (HR): 3.21 and 1.11, respectively; P < 0.001] and for CD-related hospitalization during patient follow-up (HR: 1.88 and 1.08, respectively; P = 0.002 and < 0.001, respectively). Disease activity as expressed by the MaRIA score did not predict the disease course and the correlation between the LI and MaRIA score was weak (rho: +0.32; P < 0.001)[48]. Therefore, it appears that there are still some unanswered questions regarding the most appropriate score to use.

None of the studies included in this review used a validated index to quantify TH, even when a system score was used in three of the studies[14,41,42]. Some authors consider that in daily clinical practice, normalization of BWT (< 3 mm), without signs of hypervascularization, at MRE[23,25,30,32] and US[12,22,32,41,55], represent the best criteria for TH[18,32]. Only one study in this review used the above mentioned definition of TH, which was related to better outcomes[43]. However, Orlando *et al*[44] found no association between baseline BWT at US and therapeutic outcomes. Moreover, abnormal BWT detected at MRE (as a result of fibrosis and fibromuscular hyperplasia) may persist in the absence of a significant active inflammatory component[17], reflecting BD[70,71]. Some authors consider that extramural lesions should also be included in TH[40]. Even the STRIDE concept mentions that "resolution of findings of inflammation on CSI" should be achieved[8], but without clearly defining how that would be ideally quantified.

Considering all the presented data above, TH evaluated by CSI still remains an evolving concept[32] and its exact definition is not clearly established yet. Therefore, even if the studies included in this review had different definitions of TH (the third limitation listed above), they reflect the current literature. Moreover, the same criteria were used across each of the seven studies. Given all these remarks, the third limitation of this review is not as bad as it would appear.

A fourth limitation of this review is its inclusion of heterogeneous populations (adults and children) with different duration of CD, various phenotypes, and medications used.

CONCLUSION

Despite their heterogeneous design and methodological limitations, six of the seven identified studies demonstrated that achieving TH or IH was associated

with favorable long-term outcomes (≥ 52 wk), including sustained CR, less need of rescue therapy, less CD-related hospitalizations and less CD-related surgery. Since treating to a TH target leads to better patients' outcomes than those of patients without TH, TH should be incorporated in the concept of DR. Evaluation by CSI techniques appears crucial in monitoring response to therapy and assessing TH, with potential application in changing treatment paradigms, as well as our practice.

Since TH is achieved in only a minority of patients with MH, earlier and stronger therapeutic interventions should be employed to reach higher rates of TH. Further continuous tight monitoring in order to maintain TH may prevent BD and disability. As IH (TH plus MH) appeared superior to MH alone for long-term outcomes, IH may become the future therapeutic endpoint in the treat-to-target strategy and an indispensable parameter in decision algorithms, with the view of altering natural history of CD, not only in clinical trials but also in daily clinical practice.

PERSPECTIVES ON FUTURE RESEARCH

Several aspects remain to be clarified regarding the ideal definition of TH and the best scores/parameters to use. Further studies should standardize and validate these aspects. Prospective well-designed multicenter trials (including stratified populations or treatment-naïve patients, and using both CSI and IC) are required to definitively assess the benefits of TH/IH on long-term outcomes, including BD and disability. More research is also warranted to better define the role of TH/IH (and timing of their assessment) in the era of personalized treatments targeted to individual patients.

Whether highly accurate, non-irradiating, non-invasive CSI techniques are able to replace endoscopy in monitoring response to therapy and be used as surrogate to endoscopy for MH represent other challenging fields of research. Novel biomarkers assessing fibrosis by ultrasound elastography or advanced MRE techniques and their use in identifying potential patients for TH could represent another promising research field. The cost-effectiveness of this tight control algorithm guiding therapy to a TH target should also be investigated *vs* MH alone. Finally, the predictive role of long-term TH (after long periods of maintenance therapy) for discontinuing or de-escalating treatment represents yet another interesting topic.

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ORIGINAL ARTICLE

Basic Study

CYP2C19 polymorphism has no influence on rabeprazole-based hybrid therapy for *Helicobacter pylori* eradication

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Abstract

AIM

To evaluate the impact of cytochrome P450 2C19 (CYP2C19) and interleukin-1β (IL-1β) polymorphisms on the efficacy of *Helicobacter pylori* (*H. pylori*) eradication by using rabeprazole-based hybrid therapy.

METHODS

A total of 88 *H. pylori*-infected patients were recruited to receive 14-d of hybrid therapy from March 2013 to May 2014. Three patients were excluded from analysis because of incomplete compliance. Either a follow-up endoscopy or ^{13}C-urea test was performed to determine the results of *H. pylori* eradication therapy. The genotypes of CYP2C19 and IL-1β were analyzed to investigate the impact on treatment effect.

RESULTS

The total eradication rate of *H. pylori* was 92.94% (79/85). According to the CYP2C19 genotypes, the rates of *H. pylori* eradication were 89.19% in extensive metabolizers (EM) and 95.83% in non-EM. The *H. pylori* eradication rates regarding the IL-1β genotypes were 92.59% in the normal acid secretion group and 93.10% in the low acid secretion group. After multivariable logistic regression analysis, both the genotypes of CYP2C19 and IL-1β had no significant influences on the eradication rates of *H. pylori*.

CONCLUSION

The CYP2C19 and IL-1β polymorphisms are not significantly independent factors of *H. pylori* eradication using rabeprazole-based hybrid therapy.

Key words: *Helicobacter pylori*; Cytochrome P450 2C19; Interleukin-1β; Hybrid therapy; Rabeprazole

Core tip: In this study, we investigated the efficacy of hybrid therapy as a first-line treatment for *Helicobacter pylori* eradication and evaluated the independent predictor associated with eradication efficacy, including cytochrome P450 2C19, interleukin-1β (IL-1β)-511 polymorphism and antibiotic resistance. This study is pilot work investigating the impact of the IL-1β-511 polymorphism on the eradication rate of hybrid therapy.

Lin TJ, Lee HC, Lin CL, Wang CK, Chen KY, Wu DC. CYP2C19 polymorphism has no influence on rabeprazole-based hybrid therapy for *Helicobacter pylori* eradication. *World J Clin Cases* 2018; 6(12): 514-520 Available from: URL: http://www.wjgnet.com/2307-8960/full/v6/i12/514.htm DOI: http://dx.doi.org/10.12998/wjcc.v6.i12.514

INTRODUCTION

The global infection rate of *Helicobacter pylori (H. pylori)* is more than 50%. The infection of *H. pylori* is associated with gastric cancer, gastric ulcer, duodenal ulcer, non-ulcer dyspepsia, chronic gastritis, and mucosa-associated lymphoid tissue lymphoma[1-4]. The efficacy of standard proton pump inhibitor (PPI)-clarithromycin-amoxicillin triple therapy for *H. pylori* eradication has decreased to an unacceptable level (< 80%) due to rising rates of antibiotic resistance in most countries, especially clarithromycin[4-7]. Therefore, several novel first-line regimens have been proposed, including sequential, concomitant and hybrid therapies.

The hybrid therapy, first proposed by Hsu *et al*[8], is an effective treatment method, and a 14-d hybrid therapy can achieve a > 95% eradication rate of *H. pylori* in their study. Hybrid therapy is composed of PPI and amoxicillin for 14 d, and clarithromycin and metronidazole/tinidazole for the final 7 d. This is similar to the hybrid form of the sequential (the first 7 d) and concomitant therapies (the last 7 d). Two recent reports of systematic review and meta-analysis showed that hybrid therapy can achieve similar eradication rates of *H. pylori* compared with sequential or concomitant therapies[9,10].

The PPI is metabolized by the hepatic cytochrome P450 system, especially CYP2C19[11]. Three different genotypes of CYP2C19, including extensive metabolizers (EM), intermediate metabolizers and poor metabolizers (PM), will have different degrees of PPI metabolism. The PM genotype will result in higher intragastric pH levels and higher effectiveness in *H. pylori* eradication due to the low pH level of the stomach that may affect the stabilization of acid-labile antibiotics, such as clarithromycin[12]. Therefore, the EM genotype of CYP2C 19 may result in treatment failure for *H. pylori* eradication[13]. Since rabeprazole is mainly metabolized by a non-enzymatic reaction[14], the CYP2C19 polymorphism may have less influence on the efficacy of rabeprazole-based *H. pylori* eradication treatment[15].

The interleukin (IL)-1 family of cytokines comprises 11 members, including seven pro-inflammatory agonists (IL-1α, IL-1β, IL-18, IL-33, IL-36α, IL-36β, IL-36γ) and four defined or putative antagonists [IL-1R antagonist (IL-1Ra), IL-36Ra, IL-37, and IL-38] that exert anti-inflammatory activities[16]. The proinflammatory cytokine IL-1β is a strong inhibitor of gastric acid secretion and is highly expressed in the gastric mucosa of *H. pylori*-infected patients[17]. Different genotypes of IL-1β have been reported to have different influences on gastric acid secretion[18]. The IL-1β-511 C/T or T/T genotype has low gastric acid secretion and the IL-1β-511 C/C genotype has normal gastric acid secretion. Therefore, the efficacy of *H. pylori* eradication may be affected by the particular IL-1β-511 genetic polymorphism. One study reported that CYP2C19 genotype-dependent differences in eradication rates of one-week triple therapy were only observed in patients with the IL-1β-511 C/C type[19].

In the initial study of hybrid therapy, no significant factors related to treatment failure were found; however, the CYP2C19 polymorphism was not analyzed[8]. The one study addressing the influence of CYP2C19 by using hybrid therapy as a first-line treatment for *H. pylori* eradication found that resistance to clarithromycin or metronidazole and poor compliance were the independent factors of treatment failure[20]. Thus, the CYP2C19 polymorphism was not the significant predictor.

The goals of our study were to investigate the efficacy of hybrid therapy as a first-line treatment for *H. pylori* eradication and to evaluate the independent predictor associated with eradication efficacy, including CYP2C19, IL-1β-511 polymorphisms and antibiotic resistance. This study is pilot work investigating the impact of IL-1β-511 polymorphisms on the eradication rate of hybrid therapy.

MATERIALS AND METHODS

Patients

At the out-patient Department of Gastroenterology in Taipei City Hospital, Ren-Ai Branch, patients without a history of *H. pylori* eradication were recruited consecutively from March 2013 to May 2014. All of the patients received endoscopic examinations, and biopsies of the gastric mucosa was evaluated by rapid urease test, histology and tissue cultures. The infection of *H. pylori* was defined as either (1) a positive result of culture; or (2) positive results of both the rapid urease test and histological examination. The following patients were excluded: (1) previously treated for *H. pylori* infection; (2) use of antibiotics within the preceding 30 d; (3) regular use of a PPI (> 3 times per week) in the 30 d before enrollment; (4) allergy to any medication in this study; (5) known to interact with study medication; (6) use of concomitant antibiotics; (7) previous surgery of the stomach; (8) presence of Zollinger–Ellison syndrome; (9) presence of a serious medical condition; and (10) pregnancy or lactation. The study was approved by the Institutional Review Board and ethics committee of Taipei City Hospital (TCHIRB-1011111). Written informed consents were provided by all participants.

Interventions

A total 88 patients with *H. pylori* infections were included in our study and treated with 14 d of hybrid therapy (20 mg rabeprazole and 1000 mg amoxicillin twice daily for 7 d, followed by 20 mg rabeprazole, 1000 mg amoxicillin, 500 mg clarithromycin and 500 mg metronidazole twice daily for 7 d). A written handout with instructions about how to take the drugs correctly was given to the patients. Medical history and demographic data were obtained by a well-trained interviewer who interviewed the patients by using a standardized questionnaire. Patients were arranged to return to evaluate the drug compliance and adverse events 2 wk after the start of drug administration. Endoscopic examination with biopsy for histology, rapid urease test, and culture was repeated to assess the status of *H. pylori* infection 8 wk after the completion of *H. pylori* eradication. If the patient refused an endoscopy during follow-up, the ^{13}C-urea test was alternatively used at least 4 wk after the completion of therapy. *H. pylori* eradication was defined as (1) a negative result of the ^{13}C-urea test; or (2) negative results of both the rapid urease test and histological examination.

Questionnaires

The questionnaires contained questions regarding personal medical histories and demographic data, including systemic disease, age, gender, alcohol, smoking, tea and coffee consumption. Drinkers were defined as drinking more than one cup of alcoholic beverage per day, and smokers were defined as consuming more than one pack of cigarettes per week. The adverse events included bitter taste, headache, dizziness, nausea, vomiting, anorexia, abdominal pain, diarrhea, constipation, fatigue and skin rash.

Diagnosis of H. pylori infection

Rapid urease test: The results of the rapid urease test (Delta West Bently, Western, Australia) were interpreted as positive if the color of the gel turned pink or red six hours after examination at room temperature.

Histological examination and culture: We performed biopsies from the lesser curvature site of the antrum and corpus of gastric mucosa for histological examination. The biopsy specimens were smeared on the surface of a Columbia blood agar plate and then incubated at 35℃ under microaerobic conditions for 5 d. When a curvy, gram-negative bacterium was found on the smear, the Gram stain was defined as a positive result. The pathologists were blinded to the results of the laboratory or genotypic tests as well as to the therapies each patient received. If one or more colonies of Gram-negative bacilli with positive urease, oxidase, and catalase tests were found, the result of the *H. pylori* culture was defined as positive.

^{13}C-urea test: Seventy-five mg ^{13}C-Urea mixed with 100 mL of fresh water was used as the test drink. The ^{13}C-urea was manufactured by the Institute of *Wagner Analysen Technik Vertriebs GmbH,* Germany.

Analysis of CYP2C19 and IL-1β-511 genotypes

Peripheral blood was drawn in an EDTA vacutainer, and a commercially available kit (Qiagen K.K., Tokyo, Japan) was used to isolate DNA from the leukocytes. The method of polymerase chain reaction–restriction fragment length polymorphism established by de Morais *et al*[21,22] with minor modifications was performed to analyze the wild-type (wt) gene and the two mutated alleles, CYP2C19 m1 and CYP2C19 m2[21-23]. Homozygous EM (*i.e.*, wild-type) was defined as wt/wt; heterozygous EM as wt/m1 and wt/m2; and PM as m1/m1, m2/m2 and m1/m2, respectively. We also used the method of polymerase chain reaction–restriction fragment length polymorphism with allele-specific primers to identify the C-to-T single nucleotide polymorphism of IL-1β-511[24].

Analysis of antibiotics resistance

To culture *H. pylori*, we rubbed one antral gastric biopsy specimen on the surface of a Campy-BAP agar plate

Table 1 Univariable analysis of the clinical factors and genotyped polymorphisms *n* (%)

Variable	Eradication (*n* = 79)	No eradication (*n* = 6)	*P* value
Age (yr)	51.95 ± 13.48	53.17 ± 12.06	0.831
Sex (male:female)	35:44	2:4	0.693
Smoking	14 (17.72)	0 (0)	0.583
Alcohol	19 (24.05)	1 (16.67)	1.000
Betel	1 (1.27)	0 (0)	1.000
Coffee	56 (70.89)	4 (66.67)	1.000
Tea	59 (74.68)	4 (66.67)	0.646
NSAID user	7 (8.86)	1 (16.67)	0.458
Steroid user	3 (3.80)	0 (0)	1.000
Anticoagulant user	4 (5.06)	1 (16.67)	0.316
CYP2C19 genotype			0.380
HomoEM	33 (41.77)	4 (66.67)	
HeteroEM	36 (45.57)	1 (16.67)	
PM	10 (12.66)	1 (16.67)	
IL-1β-511 genotype			0.934
CC	25 (31.65)	2 (33.33)	
CT	32 (40.51)	2 (33.33)	
TT	22 (27.85)	2 (33.33)	
Resistance (*n* = 65)	(*n* = 61)	(*n* = 4)	
Amoxicillin	0/61 (0)	0/4 (0)	
Clarithromycin	8/61 (13.11)	0/4 (0)	1.000
Metronidazole	25/61 (40.98)	1/4 (25.00)	0.644

Data are expressed as mean ± SD or *n* (%). NSAID: Non-steroid anti-inflammatory drug; CYP2C19: Cytochrome P450 2C19; EM: Extensive metabolizer; PM: Poor metabolizer; IL-1β: Interleukin-1β.

(Brucella agar, Difco, Sparks Maryland) + IsoVitalex (Gibco, Grand Island, New York) + 10% whole sheep blood. The agar plate then was incubated at 37 ℃ under microaerobic conditions (5%O_2, 10% CO_2 and 85%N_2) for 4-5 d. Antibiotic susceptibility for the *H. pylori* strain was tested for clarithromycin, metronidazole and amoxicillin by using an E-test (AB Biodisck, Solna, Sweden). Resistance to clarithromycin, metronidazole, and amoxicillin was defined as a minimal inhibitory concentration value of 1 μg/mL, 8 μg/mL, and 0.5 μg/mL, respectively.

Statistical analysis

Data were summarized as mean ± SD. Data were compared between groups on the basis of *H. pylori* eradication results. Categorical variables were compared with the chi-square test or Fisher's exact test as required. Continuous variables were compared between groups by using the unpaired *t*-test. The Mann-Whitney test was used when appropriate. Multivariable logistic regression analysis was used to identify the independent predictors related to the eradication of *H. pylori*. A *P* value < 0.05 was statistically significant.

RESULTS

Baseline demographic data of patients

A total of 88 *H. pylori*-infected patients were treated with hybrid therapy. Three patients were excluded from analysis because of poor compliance. According to the treatment outcome, baseline demographic data from the 85 patients with complete therapy of *H. pylori* eradication are shown in Table 1. A total of 79 patients had successful eradication of *H. pylori* and the eradication rate was 92.94% using 14-d of hybrid therapy.

Genotypes of CYP2C19 and single nucleotide polymorphisms of the IL-1β gene (SNP-511)

Three different patterns of CYP2C19 polymorphisms were analyzed in our study, including homozygous EMs, heterozygous EMs and PMs. The prevalence of CYP2C19 homEM, hetEM, PM was 41.77%, 45.57%, 12.66% in patients with eradicated *H. pylori* and 66.67%, 16.67%, 16.67% in patients without eradicated *H. pylori*, respectively. In addition, three different allelic patterns of the IL-1β-511 gene were examined, including C/C, C/T, and T/T. The CC/CT/TT genotype frequency was 31.65%, 40.51%, 27.85% in patients with eradicated *H. pylori* and 33.33%, 33.33%, 33.33% in patients without eradicated *H. pylori*, respectively (Table 1).

Factors associated with H. pylori eradication

No significant clinical or genetic factors were found to be associated with successful eradication of *H. pylori* by univariable analysis, including age, gender, coffee/tea drinking, alcohol drinking, betel using, use of steroid, anticoagulant or non-steroid anti-inflammatory drug, antibiotic resistance, or CYP2C19 and IL-1β-511 polymorphisms (Table 1). The rates of *H. pylori* eradication were 89.19% in EM and 95.83% in non-EM (Table 2). Patients were classified into two groups according to IL-1B-511 genetic polymorphisms. The normal acid secretion group was defined as those with the alleles (C/C), and the low acid secretion group was defined as those with either the alleles (T/T) or (C/T). The cure rates of each IL-1β-511 genetic polymorphism in relation to

Table 2 Eradication rates according to cytochrome P450 2C19 and interleukin-1β genotypes *n* (%)

Hybrid therapy	(*n* = 85)	IL-1β-511 C/C (normal gastric acid) (*n* = 27)	IL-1β-511 C/T, T/T (low gastric acid) (*n* = 58)
	79/85 (92.94)	25/27 (92.59)	54/58 (93.10)
CYP2C19 EM (*n* = 37)	33/37 (89.19)	13/14 (92.86)	20/23 (86.96)
CYP2C19 PM and hetero EM (*n* = 48)	46/48 (95.83)	12/13 (92.31)	34/35 (97.14)
P value	0.649	1.000	0.556

CYP2C19: Cytochrome P450 2C19; IL-1β: Interleukin-1β; EM: Extensive metabolizer; PM: Poor metabolizer.

Table 3 Multivariable logistic regression analysis of independent predictors of *Helicobacter pylori* eradication rates

Variable	Odds ratio	95%CI	*P* value
EM *vs* PM and hetero EM	0.359	0.062–2.075	0.252
IL-1β-511 C/C *vs* IL-1β-511 C/T, T/T	1.047	0.175–6.251	0.960

EM: Extensive metabolizer; PM: Poor metabolizer; IL-1β: Interleukin-1β.

the CYP2C19 genotype are showed in Table 2. The rates of *H. pylori* eradication were 92.59% in the normal acid secretion group and 93.10% in the low acid secretion group. There was no statistically significant difference in the eradication rates of *H. pylori* between the two CYP2C19 genotype subgroups (EM and non-EM) for both normal acid (IL-1β-511 C/C) and low acid (IL-1β-511 C/T and T/T) secretion groups. After multivariable analysis, both CYP2C19 and IL-1β-511 genetic polymorphisms were not significant factors of *H. pylori* eradication by using 14-d of hybrid therapy (Table 3).

DISCUSSION

The failure of *H. pylori* eradication is mainly related to antibiotic resistance, poor compliance of patients, and duration of therapy[25]. The present study was performed to investigate the eradication rate of *H. pylori* by using 14-d of hybrid therapy. In addition, the main purpose of our study was to further explore the influence of CYP2C19 and IL-1β-511 genotypes on the outcome of hybrid therapy. Therefore, patients with poor compliance were excluded from analysis.

In one review article, a total of 1871 patients in 12 studies received hybrid therapy[26]. The eradication rate of *H. pylori* was 82.6%-99.1%, and pooled analysis showed the eradication rate was 91.2% in per-protocol analyses. The other review article with meta-analysis included 2516 patients from eight studies, and the mean cure rate of hybrid therapy was 93.3% (*n* = 1109, range: 85.7%-99.1%) by per-protocol analyses[27]. Our study found that the eradication rate was 92.94% by using 14-d of hybrid therapy, which was comparable to the results of the two review articles. Nonetheless, one study in the population with high antibiotic resistance rates found that the eradication rate of hybrid therapy was 86.0% in per-protocol analyses and graded as an unacceptable level[20]. In addition, the study showed that resistance to clarithromycin, resistance to metronidazole and poor compliance were the significant predictors of treatment failure for hybrid therapy and that the CYP2C19 genotype was not. In our study, no independent factors were found to be associated with treatment failure. The first key factor was compliance; however, we excluded patients with poor compliance from the beginning. Secondarily, because hybrid therapy achieved a high eradication rate in our study, the number of patients with treatment failure was too small to identify the significant predictors. Two other studies also explored the influence of antibiotic resistance on the treatment outcome of hybrid therapy[8,28]. Both of the studies had not demonstrated the significance of antibiotic resistance on the eradication rate of *H. pylori.* This may be related to the lack of antimicrobial susceptibility of *H. pylori* for most patients included in the studies. The study revealed that a compliance of more than 80% was the only significant factor of successful eradication[28]. However, the first study of 14-d hybrid therapy found that no risk factors, including compliance, influenced the efficacy of *H. pylori* eradication[8]. Another study of 10-d hybrid therapy also showed that no clinical factors were associated with treatment failure, however antibiotic resistance and CYP2C19 genotype were not investigated in the study[29].

The key finding of our study was that CYP2C19 and IL-1β-511 genotypes had no influence on the treatment outcome of 14-d hybrid therapy. To date, only one study had examined the influence of the CYP2C19 genotype on hybrid therapy[20]. This study showed no significant effect of CYP2C19 polymorphisms on the eradication rate of *H. pylori*. In one study of 12-d reverse hybrid

therapy, the CYP2C19 genotype also had no significant impact on the treatment outcome[30]. The limitation of our study was that the number of patients may be too small to identify the significant factors predicting eradication failure.

Both CYP2C19 and IL-1β polymorphisms had no significant impact on rabeprazole-based hybrid therapy. Our findings suggest that rabeprazole may be used as a priority in EMs of CYP2C19 to maintain the eradication rate of *H. pylori*.

ARTICLE HIGHLIGHTS

Research background

In the initial study of hybrid therapy, no significant factors related to treatment failure were found; however, the cytochrome P450 2C19 (CYP2C19) polymorphism was not analyzed. Only one study addressed the influence of CYP2C19 by using hybrid therapy as a first-line treatment for *Helicobacter pylori* (*H. pylori*) eradication.

Research objectives

The aims of this study were to investigate the efficacy of hybrid therapy as a first-line treatment for *H. pylori* eradication, and to evaluate the independent predictors associated with eradication efficacy, including CYP2C19, the interleukin (IL)-1β-511 polymorphism, and antibiotic resistance.

Research methods

About 88 *H. pylori*-infected patients were recruited to receive 14-d of hybrid therapy. Endoscopies or ^{13}C-urea tests were performed to determine the results of *H. pylori* eradication therapy. To investigate the impact on treatment effect, the genotypes of CYP2C19 and IL-1β were analyzed.

Research results

The total eradication rate of *H. pylori* was 92.94%. The rates of *H. pylori* eradiation were 89.19% in extensive metabolizers (EM) and 95.83% in non-EM, according to the CYP2C19 genotypes. Both the genotypes of CYP2C19 and IL-1β had no significant influence on the eradication rates of *H. pylori*.

Research conclusions

The CYP2C19 and IL-1β polymorphisms are not significantly independent factors on rabeprazole-based hybrid therapy for *H. pylori* eradication.

Research perspectives

The limitation of this study was that the number of patients may be too small to identify the significant factors predicting eradication failure. In addition, the findings suggest that rabeprazole may be used as a priority in EMs of CYP2C19 to maintain the eradication rate of *H. pylori*.

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ORIGINAL ARTICLE

Retrospective Study

Declining diagnostic accuracy of non-invasive fibrosis tests is associated with elevated alanine aminotransferase in chronic hepatitis B

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Author contributions: Wang L and Fan YX designed the study, enrolled the patients, analyzed the data and prepared the manuscript; Dou XG designed and supervised the study.

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Abstract

AIM

To explore the effect of alanine aminotransferase (ALT) on the performance of non-invasive fibrosis tests in chronic hepatitis B (CHB) patients.

METHODS

A total of 599 treatment-naive and biopsy-proven CHB patients were included in the study. The cohort was divided into the following three groups: Normal ALT (ALT $\leqslant$ 40), slightly elevated ALT (40 < ALT $\leqslant$ 80) and elevated ALT (ALT > 80). The diagnostic performance of five common non-invasive fibrosis tests for liver fibrosis (stages S2-4), including the aspartate aminotransferase (AST)-to-platelet (PLT) ratio index (APRI), fibrosis index based on 4 factors (FIB-4), King's score, Forns index and gamma-glutamyl transpeptidase (GGT)-to-PLT ratio (GPR), were evaluated for each group.

RESULTS

Higher ALT levels were associated with higher non-

invasive fibrosis test scores. Patients with the same fibrosis stage but higher ALT levels showed higher non-invasive test scores. The areas under the receiver operating characteristics curves (AUROCs) of the non-invasive tests for prediction of ⩾ S2 were higher for patients with ALT ⩽ 40 U/L (range 0.705-0.755) and 40 < ALT ⩽ 80 U/L (range 0.726-0.79) than for patients with ALT > 80 U/L (range 0.604-0.701). The AUROCs for predicting ⩾ S3 and S4 were higher in patients with ALT ⩽ 40 U/L (range 0.736-0.814 for ⩾ S3, 0.79-0.833 for S4) than in patients with 40 < ALT ⩽ 80 U/L (range 0.732-0.754 for ⩾ S3, range 0.626-0.723 for S4) and ALT > 80 U/L (range 0.7-0.784 for ⩾ S3, range 0.662-0.719 for S4). The diagnostic accuracy of the non-invasive tests decreased in a stepwise manner with the increase in ALT.

CONCLUSION

ALT has a significant effect on the diagnostic performance of non-invasive fibrosis tests. The ALT level should be considered before performing these non-invasive tests.

Key words: Chronic hepatitis B; Non-invasive tests; Liver fibrosis; Alanine aminotransferase; Inflammation

Core tip: Because of their high applicability and good interlaboratory reproducibility, many convenient non-invasive fibrosis tests have been established. To explore the effect of alanine aminotransferase (ALT) on the performance of non-invasive fibrosis tests in chronic hepatitis B (CHB) patients, we retrospectively analyzed 599 treatment-naive and biopsy-proven CHB patients at our hospital. The diagnostic accuracy of the non-invasive tests decreased in a stepwise manner with the increase in ALT. ALT has a significant effect on the diagnostic performance of non-invasive fibrosis tests.

Wang L, Fan YX, Dou XG. Declining diagnostic accuracy of non-invasive fibrosis tests is associated with elevated alanine aminotransferase in chronic hepatitis B. *World J Clin Cases* 2018; 6(12): 521-530 Available from: URL: http://www.wjgnet.com/2307-8960/full/v6/i12/521.htm DOI: http://dx.doi.org/10.12998/wjcc.v6.i12.521

INTRODUCTION

Liver fibrosis is one of the main characteristics of chronic hepatitis B (CHB). Accurate assessment of liver fibrosis is vital for predicting disease progression and choosing optimal treatment timing. Liver biopsy (LB) is the current gold standard for evaluating the liver fibrosis stage. However, repeatedly utilizing LB in patients is very difficult because of its invasiveness and complications[1]. Therefore, non-invasive methods have been developed to assess liver fibrosis when the risk-benefit trade-off does not favor a LB[2].

According to the EASL-ALEH Clinical Practice Guidelines, non-invasive methods were recently divided into serum biomarkers and liver stiffness (LS) measurements[3]. Because of their high applicability (> 95%) and good interlaboratory reproducibility, serum biomarkers are widely used in clinical practice[3]. Over the past few years, many convenient non-invasive fibrosis tests have been established, including the aspartate aminotransferase (AST)-to-platelet (PLT) ratio index (APRI)[4] and the fibrosis index based on 4 factors (FIB-4)[5]. Moreover, the APRI and FIB-4 were recommended by the WHO for evaluation of liver fibrosis in resource-limited regions[6]. However, most non-invasive fibrosis tests have been developed primarily based on chronic hepatitis C patients, and their use in assessing liver fibrosis in CHB patients is controversial[7-9]. Therefore, more data should be collected regarding the use of these established non-invasive tests to predict liver fibrosis in CHB patients. Recently, Lemoine *et al*[10] constructed the gamma-glutamyl transpeptidase (GGT)-to-PLT ratio (GPR) model based on CHB patients, but its diagnostic performance assessing liver fibrosis varied among different studies[11,12].

Alanine aminotransferase (ALT) is considered an accurate and valuable routine indicator of liver inflammation. Many studies have reported the influence of serum ALT levels on LS[13,14]. According to the EASL-ALEH Clinical Practice Guidelines for CHB[3], LS measurements should be interpreted in patients with an elevated ALT level and should not be used in patients with ALT levels more than 10 times the upper limit of normal (ULN). However, no report has investigated the effects of changes in CHB patients' serum ALT levels on the diagnostic performances of these non-invasive tests. For these reasons, the aim of this study was to explore the effect of ALT on the diagnostic performance of non-invasive fibrosis tests in CHB patients.

MATERIALS AND METHODS

Patients

The study included treatment-naive CHB patients who underwent LB from 2012-2017 in the Shengjing Hospital affiliated with China Medical University. CHB was defined as hepatitis B surface antigen-positive lasting at least six months. The exclusion criteria were as follows: (1) coinfection with human immunodeficiency virus; (2) other liver disease (other viral hepatitis, autoimmune liver disease, drug hepatitis and liver failure); (3) significant alcohol intake (> 20 g/d for women and > 30 g/d for men) for more than 5 years; (4) tumor; (5) liver transplantation; and (6) hematological diseases.

The study was approved by the Medical Ethics Committee of Shengjing Hospital of China Medical University and was performed in accordance with the guidelines of the 1975 Declaration of Helsinki. Written informed consent for the LB was obtained from all patients.

Laboratory data and histology

Laboratory data, including the blood test results (PLT), liver biochemistry (ALT; aspartate aminotransferase, AST; alkaline phosphatase, ALP; γ-glutamyltransferase, GGT and cholesterol), HBV serological marker levels (HBsAg; hepatitis B envelope antigen, HBeAg; antibodies against HBsAg, anti-HBs; antibodies against HBeAg, anti-HBe and antibodies against HBcAb, anti-HBc), blood coagulation (international normalized ratio, INR) and HBV DNA levels, were recorded for all included patients.

All liver specimens were a minimum of 10 mm with at least five portal tracts. A histopathological assessment was performed by two expert pathologists who were unaware of the patient's clinical characteristics. According to Scheuer's classification score, liver fibrosis was classified from 0 to 4[15].

Non-invasive fibrosis tests and calculation formulas

Non-invasive prediction methods and calculation formulas were applied for the CHB patients according to the original reported formulas with the original cut off values for the APRI, FIB-4, Forns index[16], GPR and King's score[17]. The formulas of these non-invasive tests are as follows:

APRI = AST(/ULN)/ PLT (10^9/L) × 100

FIB-4 = Age (year) × AST(ULN)/{PLT (10^9/L) × [ALT(ULN)]$^{1/2}$}

GPR = GGT(/ULN)/ PLT (10^9/L) × 100

King's score = Age × AST × INR/PLT

Forns index = 7.811 - 3.131 × ln(PLT count) + 0.781 × ln(GGT) + 3.467 × ln(age) - 0.014 × (cholesterol)

Statistical analysis

Continuous and abnormal variables are expressed as the median (interquartile range, IQR), and categorical data are expressed as the frequency and percentage; the data were compared using the Kruskal-Wallis test. Spearman's rank correlation was used to analyze the liver fibrosis stages and the non-invasive test results. The diagnostic performances of these non-invasive tests were assessed by receiver operating characteristic (ROC) curve analysis. The diagnostic performances of each test for the assessment of significant fibrosis, advanced fibrosis and cirrhosis are described as the areas under the receiver operating characteristics curve (AUROCs) with 95% confidence intervals (95%CIs) and the diagnostic accuracy. All *P*-values were 2-sided, and any value of $P < 0.05$ was considered statistically significant. The data analysis was performed using SPSS, version 22.0 (SPSS Inc., Chicago, IL, United States) and the GraphPad Software, version 7.0 (GraphPad Prism Inc., San Diego, CA, United States).

RESULTS

Study population

From January 2012 to July 2017, 1262 biopsy-proven patients with liver disease were assessed in the study. Among them, 575 patients were excluded according to the exclusion criteria, and 88 patients were excluded because of insufficient liver tissue and clinical data. Finally, 599 CHB patients were included in the cohort (Figure 1). The median (IQR) age of the patients was 37 (29-44) years, and 349 (58.3%) patients were male. In all, 96 (16%) patients had significant fibrosis (⩾ S2), 54 (9%) had advanced fibrosis (⩾ S3), and 38 (6.3%) had cirrhosis (S4). The clinical parameters and stages of fibrosis are shown in Table 1.

Effects of ALT on clinical factors in patients with CHB

To detect the effect of ALT on non-invasive fibrosis tests, the patients were divided into the following three groups: normal ALT (ALT ⩽ 40), slightly elevated ALT (40 < ALT ⩽ 80) and elevated ALT (ALT > 80). The baselines for these three groups are shown in Table 1.

The ALT levels were significantly correlated with AST (r = 0.878), GGT (r = 0.565), HBV DNA (r = 0.363) and HBsAg (r = 0.137) ($P < 0.05$ for all). Significant negative associations were also found between the ALT level and age (r = -0.206) and male sex (r = -0.195) ($P < 0.05$ for all). Other clinical factors, including cholesterol, INR and PLT, had no association with the ALT level ($P > 0.05$ for all).

Effects of ALT on the fibrosis scores of the non-invasive fibrosis tests

In addition, the ALT levels were positively correlated with the fibrosis stage (r = 0.141), APRI (r = 0.762), GPR (r = 0.545), King's score (r = 0.615), FIB-4 (r = 0.125) and Forns index (r = 0.107) ($P < 0.05$ for all). Increasing ALT levels were significantly associated with an increased fibrosis stage ($P < 0.05$).

The patients with ALT > 80 had the highest fibrosis scores, whereas the lowest fibrosis scores were observed for patients with ALT ⩽ 40. In general, CHB patients with higher ALT levels had significantly higher fibrosis scores on the non-invasive tests ($P < 0.05$ for all) except for the Forns index ($P = 0.081$) (Table 1). Moreover, the patients with higher ALT levels showed significantly higher fibrosis scores on the non-invasive tests than those with lower ALT levels at the same stage of liver fibrosis ($P < 0.05$ for all) except for Forns index and FIB-4 at S1, S2 and S4 (Table 2).

Effects of ALT on the diagnostic performance of the non-invasive fibrosis tests

A summary of the diagnostic performances of these non-invasive tests, including the AUROCs, 95%CIs and *P*-values in the different ALT groups for the prediction of significant fibrosis, advanced fibrosis and cirrhosis, are shown in Table 3 and Figure 2.

Generally, the AUROCs of the non-invasive tests for the prediction of ⩾ S2 in CHB patients with ALT ⩽ 40 U/L (range 0.705-0.755) and 40 < ALT ⩽ 80 U/L (range 0.726-0.79) were higher than those in patients with ALT > 80 U/L (range 0.604-0.701). For the ⩾ S3 and

Table 1 Baseline characteristics of the subjects

Characteristics	Total group (n = 599)	ALT ⩽ 40 group (n = 272)	40 < ALT ⩽ 80 group (n = 190)	ALT > 80 group (n = 137)	P-value
Age (yr)	37 (29, 44)	39 (31.25, 46)	37.5 (28, 43.25)	34 (28, 40.5)	< 0.001
Male (%)	349 (58.3)	131 (48.2)	130 (68.4)	88 (64.2)	< 0.001
ALT (U/L)	44 (28, 76)	26 (31, 33)	56 (48, 66)	127 (95, 218.5)	< 0.001
AST (U/L)	32 (24, 48)	24 (20, 28)	36 (31, 42)	70 (55, 115.5)	< 0.001
GGT (U/L)	25 (17, 41)	19 (14, 25)	30 (20, 41.25)	48 (30, 78)	< 0.001
Cholesterol (mmol/L)	4.31 (3.84, 4.96)	4.35 (3.86, 4.95)	4.24 (3.75, 4.95)	4.34 (3.84, 4.99)	0.732
PLT (10^9/L)	182 (150, 217)	184 (155, 223)	177.5 (145.5, 212)	180 (149, 215)	0.215
INR	1 (1, 1.1)	1 (1, 1.1)	1 (0.99, 1.1)	1 (0.94, 1.1)	0.4
HBV DNA (log_{10}IU/mL)	7.03 (4.24, 8.23)	4.77 (3.32, 7.78)	7.64 (5.72, 8.23)	8.03 (6.81, 8.23)	< 0.001
S (%)					
S0	264 (44.1)	128 (47.1)	80 (42.1)	56 (40.9)	
S1	147 (24.5)	73 (26.8)	53 (27.9)	21 (15.3)	
S2	96 (16)	35 (12.9)	29 (15.3)	32 (23.4)	
S3	54 (9)	19 (7.0)	18 (9.5)	17 (12.4)	
S4	38 (6.3)	17 (6.3)	10 (5.3)	11 (8.0)	
Stage S0-1 fibrosis	411 (68.6)	201 (73.9)	133 (70)	77 (56.2)	0.001
Stage S2-4 fibrosis	188 (31.4)	71 (26.1)	57 (30)	60 (43.8)	
APRI	0.53 (0.38,0.86)	0.38 (0.3, 0.49)	0.61 (0.44,0.8)	1.16 (0.88, 1.93)	< 0.001
FIB-4	1.02 (0.72,1.47)	1.02 (0.72,1.37)	0.96 (0.66,1.55)	1.22 (0.82, 1.79)	< 0.001
GPR	0.22 (0.15,0.38)	0.16 (0.11, 0.23)	0.24 (0.17,0.38)	0.42 (0.25, 0.68)	< 0.001
King's score	6.77 (4.31,11.49)	4.89 (3.54,7.03)	7.18 (4.58,11.58)	13.04 (9.81, 27.15)	< 0.001
Forns index	6.48 (5.44,7.51)	6.33 (5.37,7.37)	6.57 (5.51,7.69)	6.65 (5.57, 7.57)	0.081

Data are expressed as the n (%) or median (interquartile range, IQR). ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase; PLT: Platelet; S: Stage of fibrosis; GPR: Gamma-glutamyl transpeptidase (GGT)-to-platelet ratio; APRI: Aspartate aminotransferase (AST)-to-platelet (PLT) ratio index; FIB-4: Fibrosis index based on 4 factors; INR: International sensitivity index.

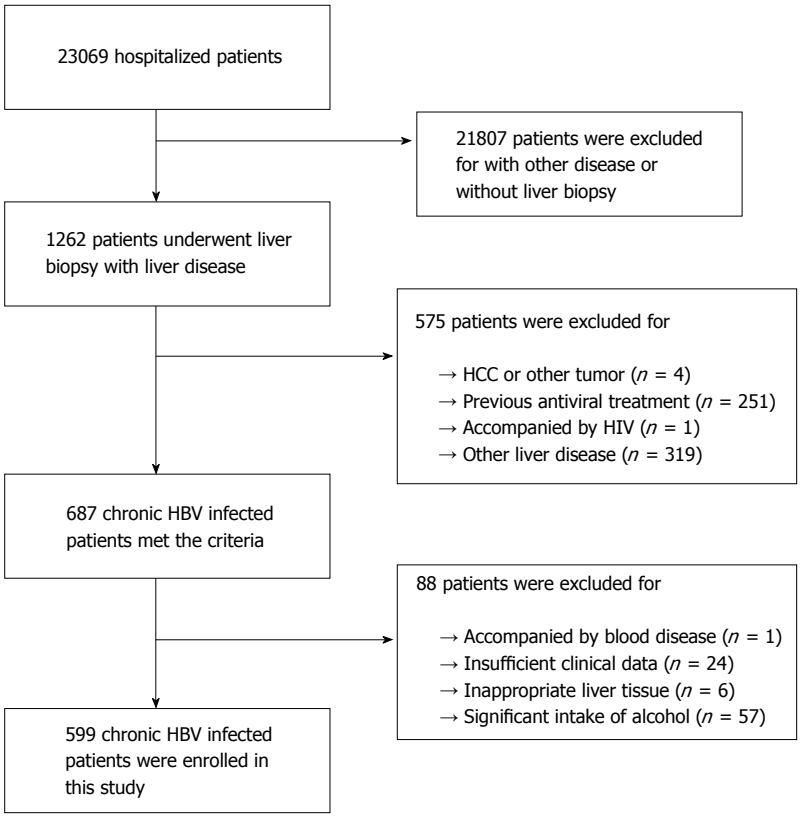

Figure 1 Flow chart of the study population selection. HCC: Hepatocellular carcinoma; HIV: Human immunodeficiency virus; HBV: Hepatitis B virus.

S4 predictions, the AUROCs of the non-invasive tests in CHB patients with ALT ⩽ 40 U/L (range 0.736-0.814 for ⩾ S3, range 0.79-0.833 for S4) were higher than those in patients with 40 < ALT ⩽ 80 U/L (range 0.732-0.754 for ⩾ S3, range 0.626-0.723 for S4) and ALT > 80 U/L (range 0.7-0.784 for ⩾ S3, range 0.662-0.719 for S4)

Table 2 Fibrosis scores of the non-invasive tests for liver fibrosis stages in the different alanine aminotransferase groups

Characteristics	APRI	FIB-4	GPR	King's score	Forns index
S0 (*n*)					
ALT ⩽ 40 (128)	0.37 (0.26-0.44)	0.88 (0.65-1.26)	0.15 (0.1-0.2)	4.3 (3.09-5.89)	6.06 (5.04-7.03)
40 < ALT ⩽ 80 (80)	0.51 (0.39-0.68)	0.77 (0.57-1.15)	0.22 (0.14-0.31)	5.91 (4.1-8.08)	6.16 (4.91-7.04)
ALT > 80 (56)	1.02 (0.85-1.5)	0.97 (0.74-1.52)	0.29 (0.22-0.48)	11.62 (7.99-18.36)	6.27 (5.36-7.26)
P-value	< 0.001	0.019	< 0.001	< 0.001	0.5
S1 (*n*)					
ALT ⩽ 40 (73)	0.36 (0.3-0.44)	0.92 (0.7-1.29)	0.13 (0.1-0.19)	4.59 (3.54-6.42)	5.9 (5.24-6.99)
40 < ALT ⩽ 80 (53)	0.52 (0.42-0.72)	0.82 (0.58-1.28)	0.23 (0.19-0.32)	6.55 (4.11-9.67)	6.04 (5.34-7.32)
ALT > 80 (21)	0.94 (0.65-1.68)	1.1 (0.85-1.36)	0.55 (0.23-0.89)	11.7 (8.58-21.25)	6.95 (6.06-7.54)
P-value	< 0.001	0.177	< 0.001	< 0.001	0.098
S2 (*n*)					
ALT ⩽ 40 (35)	0.44 (0.37-0.58)	1.2 (0.8-1.54)	0.2 (0.12-0.34)	5.88 (4.26-9.25)	6.79 (5.97-8.06)
40 < ALT ⩽ 80 (29)	0.76 (0.66-0.93)	1.34 (0.98-1.83)	0.32 (0.21-0.54)	10.9 (7.6-13.85)	7.55 (6.41-8.1)
ALT > 80 (32)	1.32 (0.87-2.15)	1.24 (0.82-1.73)	0.45 (0.25-1.09)	12.42 (9.95-28.94)	6.5 (5.41-7.67)
P-value	< 0.001	0.472	< 0.001	< 0.001	0.111
S3 (*n*)					
ALT ⩽ 40 (19)	0.49 (0.38-0.74)	1.2 (0.9-1.62)	0.34 (0.17-0.44)	7.28 (4.44-9.28)	7.48 (6.4-8.01)
40 < ALT ⩽ 80 (18)	0.81 (0.73-0.98)	1.57 (1.18-2.11)	0.46 (0.22-0.57)	11.8 (9.59-16.76)	7.99 (7.01-8.79)
ALT > 80 (17)	1.95 (1.35-5.32)	2.23 (1.47-2.81)	0.89 (0.46-1.57)	28.37 (18.46-48.99)	7.38 (6.32-9.15)
P-value	< 0.001	0.023	0.001	< 0.001	0.538
S4 (*n*)					
ALT ⩽ 40 (17)	0.57 (0.49-0.73)	1.78 (1.24-2.49)	0.35 (0.18-0.58)	9.48 (6.15-14.1)	8.4 (6.35-9.3)
40 < ALT ⩽ 80 (10)	0.67 (0.6-0.85)	1.31 (1.09-1.45)	0.4 (0.29-0.5)	8.96 (8.26-11.96)	7.67 (6.71-8.15)
ALT > 80 (11)	1.95 (1.09-3.37)	1.82 (0.92-3.3)	0.55 (0.46-0.94)	27.56 (12.86-45.31)	7.48 (5.77-9.11)
P-value	< 0.001	0.168	0.019	0.001	0.617

Data are expressed as the median (interquartile range, IQR); ALT: Alanine aminotransferase; S: Stage of fibrosis; GPR: Gamma-glutamyl transpeptidase (GGT)-to-platelet ratio; APRI: Aspartate aminotransferase (AST)-to-platelet (PLT) ratio index; FIB-4: Fibrosis index based on 4 factors.

(Table 3).

However, evaluating the effect of ALT on non-invasive fibrosis tests based only on one AUROC from one stage is difficult, and a more comprehensive and systematic assessment is greatly need. Thus, a grading system based on AUROCs to evaluate the effect of ALT on the prediction of liver fibrosis (⩾ S2, ⩾ S3 and S4) at different ALT levels was developed (Table 4). According to the AUROC grading system, non-invasive fibrosis tests were grade A in CHB patients with ALT ⩽ 40 U/L and grade B in patients with 40 < ALT ⩽ 80 U/L and ALT > 80 U/L. Although the non-invasive tests in patients with 40 < ALT ⩽ 80 U/L and ALT > 80 U/L were both grade B, the grading points in the patients with 40 < ALT ⩽ 80 U/L were higher than those in the patients with ALT > 80 U/L (Table 4). Above all, the grading points of the AUROCs decreased in a stepwise manner with the increase in the serum ALT level.

Effects of ALT on the diagnostic accuracy of the non-invasive fibrosis tests

The relationship between the diagnostic accuracy and serum ALT was also evaluated using the cut off value as the original threshold. For the ⩾ S2 prediction, the diagnostic accuracies of the APRI (lower cut off value 0.5), APRI (higher cut off value 1.5), Forns index (lower cut off value 4.2), Forns index (higher cut off value 6.9), GPR and King's score were 75.74%, 73.90%, 33.09%, 68.75%, 81.62% and 77.21%, respectively, for patients with ALT ⩽ 40 U/L; 62.63%,72.63%, 35.79%, 66.84%, 71.05% and 72.11%, respectively, for patients with 40 < ALT ⩽ 80 U/L; and 45.99%, 66.42%, 46.72%, 58.39%, 61.31% and 43.80%, respectively, for patients with ALT > 80 U/L.

For the ⩾ S3 prediction, the diagnostic accuracies of GPR, FIB-4 (lower cut off value 1.45) and FIB-4 (higher cut off value 3.25) were 87.13%, 78.68% and 87.13% for patients with ALT ⩽ 40 U/L, 70.53%, 72.63% and 85.79%, respectively, for patients with 40 < ALT ⩽ 80 U/L and were 52.55%, 72.26% and 81.02%, respectively, for patients with ALT > 80 U/L.

For the S4 prediction, the diagnostic accuracies of APRI (lower cut off value 1.0), APRI (higher cut off value 2.0) GPR and King's score wered 93.01%, 93.75%, 93.01% and 94.44%, respectively, for patients with ALT ⩽ 40 U/L; 83.68%, 94.21%, 84.74% and 87.37%, respectively, for patients with 40 < ALT ⩽ 80 U/L; and 42.34%, 77.37%, 65.69% and 61.31%, respectively, for patients with ALT > 80 U/L.

The above results indicate that the non-invasive tests for the prediction of liver fibrosis (⩾ S2, ⩾ S3 and S4) exhibited the highest diagnostic accuracy for patients in the ALT ⩽ 40 U/L group and the lowest diagnostic accuracy for patients in the ALT > 80 U/L group, except for the Forns index (using the lower cut off values) for the ⩾ S2 prediction and APRI (using the higher cut off values) for the S4 prediction (Table 3 and Figure 3). The diagnostic accuracy of the non-invasive tests decreased in a stepwise manner with the increase in the serum ALT level.

Table 3 Areas under the receiver operating characteristics curves of the non-invasive tests for prediction of significant fibrosis, advanced fibrosis and cirrhosis in the different alanine aminotransferase groups

Tests	ALT ≤ 40			40 < ALT ≤ 80			ALT > 80		
	≥ S2	≥ S3	S4	≥ S2	≥ S3	S4	≥ S2	≥ S3	S4
APRI									
AUROC	0.746	0.795	0.833	0.79	0.751	0.626	0.701	0.778	0.71
95%CI	0.676-0.815	0.716-0.873	0.744-0.922	0.727-0.854	0.678-0.824	0.519-0.732	0.612-0.789	0.688-0.868	0.568-0.851
P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.182	< 0.001	< 0.001	0.021
Diagnostic accuracy (optimized cut off)	75.74% (≤ 0.5) 73.90% (> 1.5)	-	93.01% (< 1.0) 93.75% (≥ 2.0)	62.63% (≤ 0.5) 72.63% (> 1.5)	-	83.68% (< 1.0) 94.21% (≥ 2.0)	45.99% (≤ 0.5) 66.42% (> 1.5)	-	42.34% (< 1.0) 77.37% (≥ 2.0)
FIB-4									
AUROC	0.705	0.736	0.792	0.767	0.747	0.663	0.672	0.765	0.698
95%CI	0.635-0.774	0.647-0.825	0.665-0.919	0.699-0.836	0.666-0.829	0.563-0.763	0.582-0.762	0.668-0.863	0.533-0.862
P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.083	0.001	< 0.001	0.03
Diagnostic accuracy (optimized cut off)	-	78.68% (< 1.45) 87.13% (> 3.25)	-	-	72.63% (< 1.45) 85.79% (> 3.25)	-	-	72.26% (< 1.45) 81.02% (> 3.25)	-
GPR									
AUROC	0.755	0.814	0.799	0.726	0.732	0.723	0.696	0.748	0.693
95%CI	0.684-0.826	0.737-0.892	0.69-0.907	0.648-0.803	0.633-0.83	0.607-0.839	0.607-00.785	0.654-0.841	0.58-0.807
P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.018	< 0.001	< 0.001	0.034
Diagnostic accuracy (optimized cut off)	81.62% (≥ 0.32)	87.13% (≥ 0.32)	93.01% (≥ 0.56)	71.05% (≥ 0.32)	70.53% (≥ 0.32)	84.74% (≥ 0.56)	61.31% (≥ 0.32)	52.55% (≥ 0.32)	65.69% (≥ 0.56)
King's score									
AUROC	0.75	0.773	0.831	0.781	0.751	0.659	0.69	0.784	0.719
95%CI	0.685-0.816	0.689-0.857	0.733-0.929	0.715-0.847	0.672-0.83	0.56-0.759	0.601-0.779	0.694-0.874	0.577-0.862
P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.09	0.001	< 0.001	0.016
Diagnostic accuracy (optimized cut off)	77.21% (≥ 12.3)	-	94.44% (> 16.7)	72.11% (≥ 12.3)	-	87.37% (> 16.7)	43.80% (≥ 12.3)	-	61.31% (> 16.7)
Forns index									
AUROC	0.731	0.758	0.79	0.752	0.754	0.708	0.604	0.7	0.662
95%CI	0.661-0.8	0.668-0.849	0.665-0.915	0.677-0.827	0.66-0.848	0.587-0.829	0.508-0.7	0.588-0.81	0.485-0.838
P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.027	0.038	0.001	0.076
Diagnostic accuracy (optimized cut off)	33.09% (< 4.2) 68.75% (> 6.9)	-	-	35.79% (< 4.2) 66.84% (> 6.9)	-	-	46.72% (< 4.2) 58.39% (> 6.9)	-	-

ALT: Alanine aminotransferase; S: Stage of fibrosis; GPR: Gamma-glutamyl transpeptidase (GGT)-to-platelet ratio; APRI: Aspartate aminotransferase (AST)-to-platelet (PLT) ratio index; FIB-4: Fibrosis index based on 4 factors; AUROC: Areas under the receiver operating characteristics curve.

DISCUSSION

In this study, we evaluated the diagnostic performances of five convenient non-invasive tests for liver fibrosis among a large cohort of treatment-naive CHB patients with different ALT levels. To the best of our knowledge, this study is the first to thoroughly analyze the effect of ALT on the diagnostic performance and accuracy of non-invasive tests in CHB patients. The results showed that elevated serum ALT levels negatively affected the diagnostic performance of the non-invasive fibrosis tests. These non-invasive fibrosis tests showed a better diagnostic accuracy in CHB patients with normal ALT levels than in patients with abnormal ALT levels.

One important finding of our study was that CHB patients with higher ALT levels showed significantly higher fibrosis scores in the non-invasive tests even at the same liver fibrosis stage. The result demonstrated that non-invasive tests could overestimate liver fibrosis in some CHB patients with elevated ALT. In other words, patients who had elevated ALT with no or mild fibrosis would be considered to have significant fibrosis, advanced fibrosis or cirrhosis. For example, the GPR, which had the highest AUROCs for the non-invasive test to predict cirrhosis in the study, showed that 6/272 (2.21%) CHB patients with normal ALT, 19/190 (10%) patients with slightly elevated ALT and 41/137 (29.9%) patients with elevated ALT would be misdiagnosed (*i.e.*, approximately 10% of CHB patients with slightly elevated ALT and 30% of patients with elevated ALT

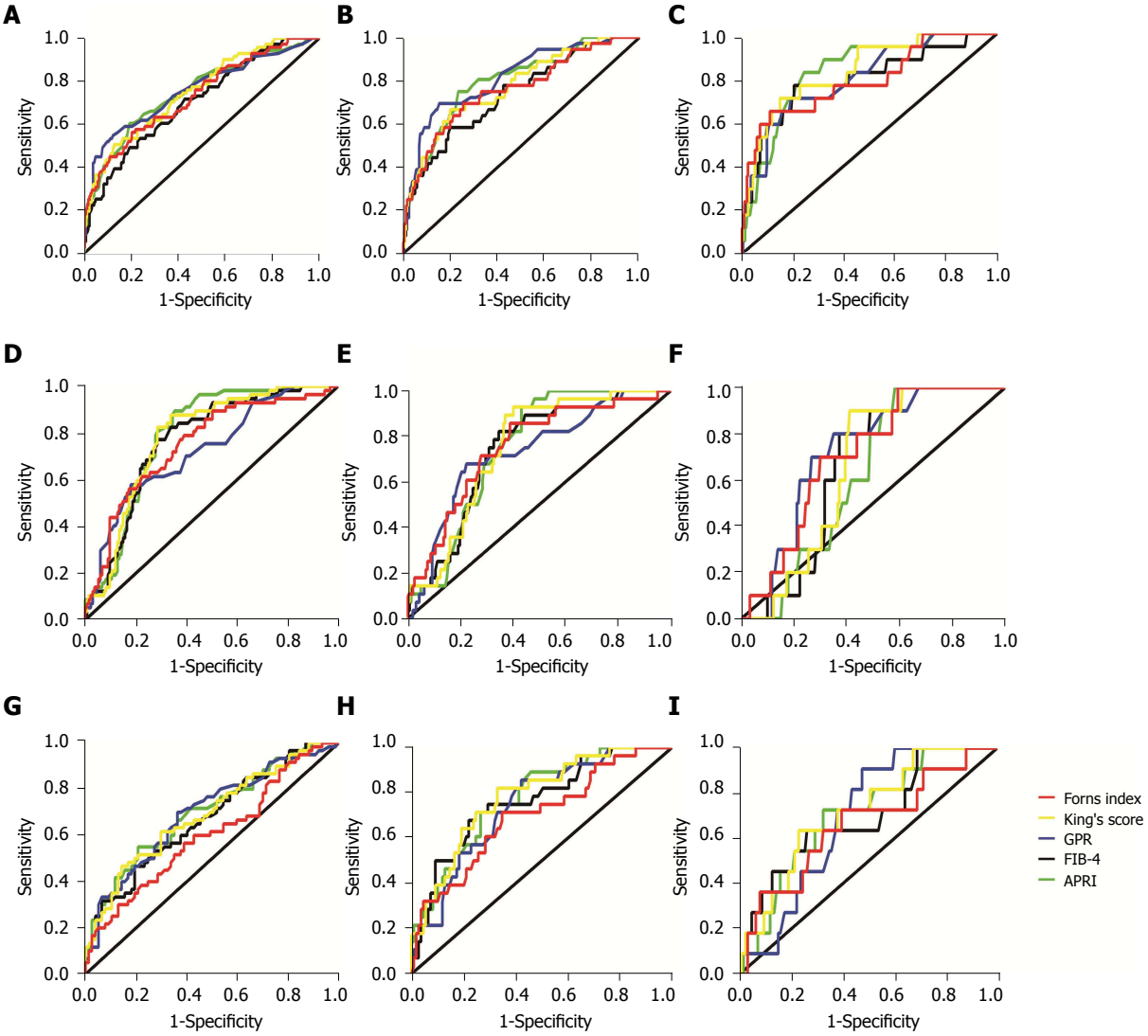

Figure 2 Receiver operating characteristic curve analysis of non-invasive tests for prediction of liver fibrosis stages (S2-4) in the different alanine aminotransferase groups. A-C: Predicting liver fibrosis in the normal alanine aminotransferase (ALT) group; A: For significant fibrosis (≥ S2); B: For advanced fibrosis (≥ S3); C: for cirrhosis (S4); D-F: Predicting liver fibrosis in the slightly elevated ALT group; D: For significant fibrosis (≥ S2); E: For advanced fibrosis (≥ S3); F: For cirrhosis (S4); G-I: Predicting liver fibrosis in the elevated ALT group; G: For significant fibrosis (≥ S2); H: For advanced fibrosis (≥ S3); I: For cirrhosis (S4). GPR: Gamma-glutamyl transpeptidase (GGT)-to-platelet ratio; APRI: Aspartate aminotransferase (AST)-to-platelet (PLT) ratio index; FIB-4: Fibrosis index based on 4 factors.

would be misclassified as having cirrhosis). ALT flares often occur during the progression of CHB, especially for patients with acute or active hepatitis. Thus, the effect of ALT should be considered when assessing liver fibrosis using non-invasive tests.

To detect the effect of ALT on non-invasive tests, we compared the diagnostic performance and accuracy of the non-invasive tests in different ALT groups. In this study, most AUROCs (11/15) for prediction of ≥ S2, ≥ S3 or S4 by the non-invasive tests were greater than 0.75 in patients with normal ALT but less than 0.75 (12/15) in patients with elevated ALT. Thus, the tests showed a slightly higher performance for the CHB patients with normal ALT than for the patients with elevated ALT.

To further examine the effect of ALT on the non-invasive tests, an overall analysis of AUROCs should be conducted in patients with different ALT levels. However, the performance of AUROCs varies among different fibrosis tests, etiologies and cohorts[10,11]. Therefore, evaluating the diagnostic performance of non-invasive fibrosis tests using one AUROC from one stage is difficult. A simple, comprehensive and systematic assessment is more reasonable and acceptable; thus, the AUROC grading system for the prediction of liver fibrosis was developed. In this study, we comprehensively evaluated the effect of ALT on five common non-invasive fibrosis tests based on the AUROC grading system. The ability of the AUROCs to predict liver fibrosis decreased with the increase in the ALT level. Additionally, studies have reported that elevated ALT can influence the diagnostic performances of non-invasive fibrosis tests, although most of these studies have focused on ≥ S2 or S4[18-20]. In our study, the effect of ALT on the AUROCs of the

Table 4 The grading system based on the receiver operating characteristic curves of the non-invasive tests for predicting significant fibrosis, advanced fibrosis and cirrhosis in the different alanine aminotransferase groups

ALT groups	AUROC					Points	Grade
	≥ 0.8	0.750-0.800	0.700-0.750	0.650-0.70	< 0.65		
ALT ≤ 40	GPR (≥ S3) APRI (S4) King's score (S4)	APRI (≥ S3) FIB-4 (S4) GPR (≥ S2) GPR (S4) King's score (≥ S2) King's score (≥ S3) Forns index (≥ S3) Forns index (S4)	APRI (≥ S2) FIB-4 (≥ S2) FIB-4 (≥ S3) Forns index (≥ S2)	-	-	22	A
40 < ALT ≤ 80	-	APRI (≥ S2) APRI (≥ S3) FIB-4 (≥ S2) King's score (≥ S2) King's score (≥ S3) Forns index (≥ S2) Forns index (≥ S3)	FIB-4 (≥ S3) GPR (≥ S2) GPR (≥ S3) GPR (S4) Forns index (S4)	FIB-4 (S4) King's score (S4)	APRI (S4)	16.5	B
ALT > 80	-	APRI (≥ S3) King's score (≥ S3) FIB-4 (≥ S3)	APRI (≥ S2) APRI (S4) GPR (≥ S3) King's score (S4) Forns index (≥ S3)	FIB-4 (≥ S2) FIB-4 (S4) GPR (≥ S2) GPR (S4) King's score (≥ S2) Forns index (S4)	Forns index (≥ S2)	12.5	B

≥ 0.8: 2 points; 0.750-0.800: 1.5 points; 0.700-0.750: 1 point; 0.650-0.700: 0.5 points; < 0.65: 0 points; Grade A: 20-30 points; Grade B: 10-20 points; Grade C: 0-10 points. ALT: Alanine aminotransferase; S: Stage of fibrosis; GPR: Gamma-glutamyl transpeptidase (GGT)-to-platelet ratio; APRI: Aspartate aminotransferase (AST)-to-platelet (PLT) ratio index; FIB-4: Fibrosis index based on 4 factors; AUROC: Areas under the receiver operating characteristics curve.

non-invasive fibrosis tests among three fibrosis stages (≥ S2, ≥ S3 and S4) were all included. The AUROCs for prediction of ≥ S2, ≥ S3 and S4 were more stable in patients with ALT ≤ 40 U/L.

Similarly, the best diagnostic accuracy of the non-invasive tests was found in patients with a normal ALT level, whereas the accuracy was lowest in patients with elevated ALT. The diagnostic accuracy of these non-invasive tests decreased in a stepwise manner with the increase in the ALT level. In patients with normal ALT, the AUROCs of these non-invasive tests for the diagnosis of cirrhosis ranged from 0.79 to 0.833 with a diagnostic accuracy range from 93.01% to 94.44% when the cut off value was the original threshold. However, for CHB patients with elevated ALT, the AUROCs of these non-invasive tests for the diagnosis of cirrhosis ranged from 0.662 to 0.719 with a diagnostic accuracy range from 42.34% to 77.37% when the cut off value was the original threshold. From the above results, we can infer that the diagnostic performances of the non-invasive tests were less reliable in CHB patients with elevated ALT levels.

The phenomenon may contribute to liver inflammation. Liver fibrosis is the process by which damaged hepatocytes are repaired and is a product of dysregulated inflammation in chronic viral hepatitis[21]. Liver inflammation can influence LS[14]. Moreover, systemic inflammation can serve as a degenerating factor for intrahepatic hypertension in cirrhosis[22]. The serum ALT level is the most direct and valuable marker that reflects liver inflammation in the clinic. To some extent, more severe liver inflammation is associated with greater ALT elevation[23], which has more impact on the non-invasive tests. This phenomenon may be the main reason why the non-invasive tests showed better diagnostic performances in patients with normal ALT.

This study had several limitations. First, this study was a single-center retrospective study. A multicenter study will be performed in the future. Second, we compared only some of the more convenient non-invasive diagnostic tests. Other non-invasive diagnostic tests and LS measurements will be evaluated in future studies.

In conclusion, the ALT level has a significant effect on the diagnostic performance and accuracy of non-invasive fibrosis tests. The ALT level should be considered before performing these non-invasive tests.

ARTICLE HIGHLIGHTS

Research background

Because of their high applicability and good interlaboratory reproducibility, many convenient non-invasive fibrosis tests have been established. Many studies have reported the influence of alanine aminotransferase (ALT) levels on liver stiffness (LS) measurements. However, no report has investigated the effects of changes in the serum ALT levels of chronic hepatitis B (CHB) patients on the diagnostic performances of these non-invasive tests.

Research objectives

To explore the effect of serum ALT on the diagnostic performances of non-invasive fibrosis tests in CHB patients.

Research methods

A total of 599 treatment-naive and biopsy-proven CHB patients were included

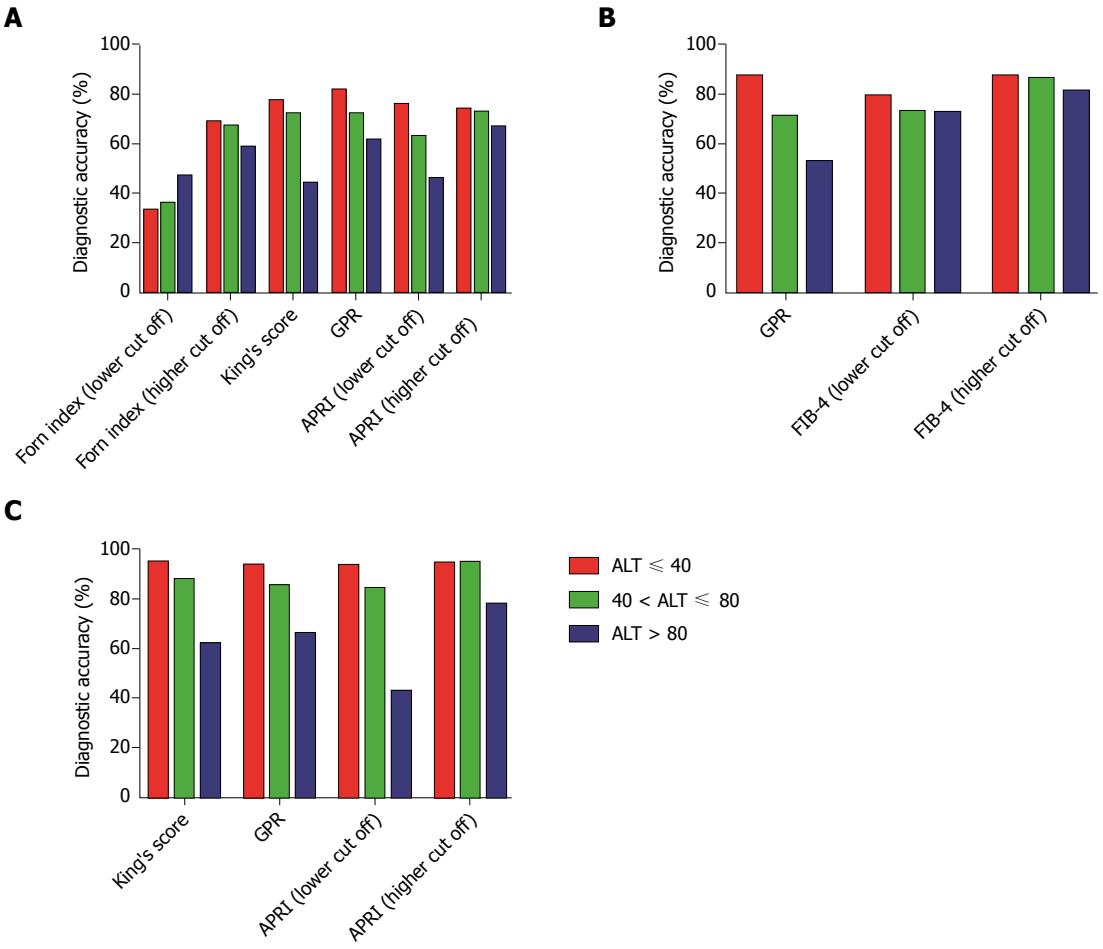

Figure 3 Relationship between serum alanine aminotransferase and the diagnostic accuracy of non-invasive fibrosis tests in chronic hepatitis B patients. A: Significant fibrosis (⩾ S2); B: Advanced fibrosis (⩾ S3); C: Cirrhosis (S4). The fibrosis stages are based on Scheuer's classification. ALT: Alanine aminotransferase; GPR: Gamma-glutamyl transpeptidase (GGT)-to-platelet ratio; APRI: Aspartate aminotransferase (AST)-to-platelet (PLT) ratio index; FIB-4: Fibrosis index based on 4 factors.

in the study. The cohort was divided into the following three groups: normal ALT (ALT ⩽ 40), slightly elevated ALT (40 < ALT ⩽ 80) and elevated ALT (ALT > 80). The diagnostic performances of five common non-invasive fibrosis tests for liver fibrosis (stages S2-4), including the aminotransferase (AST)-to-platelet (PLT) ratio index (APRI), fibrosis index based on 4 factors (FIB-4), King's score, Forns index and gamma-glutamyl transpeptidase (GGT)-to-PLT ratio (GPR), were evaluated for each group.

Research results

Higher ALT levels were associated with higher non-invasive test scores for the prediction of liver fibrosis. Patients with the same fibrosis stage but higher ALT levels showed higher non-invasive test scores. The areas under the receiver operating characteristics curves (AUROCs) of the non-invasive tests for the ⩾ S2 prediction were higher in patients with ALT ⩽ 40 U/L (range 0.705-0.755) and 40 < ALT ⩽ 80 U/L (range 0.726-0.79) than in patients with ALT > 80 U/L (range 0.604-0.701). The AUROCs for the ⩾ S3 and S4 predictions were higher in patients with ALT ⩽ 40 U/L (range 0.736-0.814 for ⩾ S3, 0.79-0.833 for S4) than in patients with 40 < ALT ⩽ 80 U/L (range 0.732-0.754 for ⩾ S3, range 0.626-0.723 for S4) and ALT > 80 U/L (range 0.7-0.784 for ⩾ S3, range 0.662-0.719 for S4). The diagnostic accuracy of the non-invasive fibrosis tests decreased in a stepwise manner with the increase ALT level.

Research conclusions

ALT has a significant effect on the diagnostic performances of non-invasive fibrosis tests.

Research perspectives

The ALT level should be considered before performing these non-invasive tests.

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CASE REPORT

Gemcitabine-induced haemolytic uremic syndrome, although infrequent, can it be prevented: A case report and review of literature

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Abstract

Gemcitabine is an antineoplastic used to treat several malignancies including pancreatic cancer. Its toxicity profile is well known with myelotoxicity, increased vascular permeability and peripheral oedema as most frequent adverse events. However, several cases of acute renal failure have been reported and haemolytic uremic syndrome (HUS) seems to be the underlying process. The cause of HUS remains unknown but its consequences can be lethal. Therefore, a high grade of suspicion is crucial to diagnose it and promptly treat it. This hopefully will reduce its morbidity. HUS is characterized by progressive renal failure associated with microangiopathic haemolytic anaemia and thrombocytopenia. The primary event is damage to endothelial cells and thrombotic microangiopathy (TMA) is the histopathological lesion. TMA affects mainly renal microvasculature. However, some cases evolve with central nervous or cardiovascular systems involvement. We present here a case of gemcitabine-induced HUS, with renal and cardiovascul-

ar system affected at the time of diagnosis which to our knowledge this is the first time of such case to be reported.

Key words: Thrombocytopenia; Haemolytic uremic syndrome; Thrombotic microangiopathy; Gemcitabine; Microangiopathic haemolytic anaemia

Core tip: Gemcitabine has a well-known toxicity profile though rare cases of acute renal failure caused by haemolytic uremic syndrome (HUS) have also been reported. The cause of HUS remains unknown but its consequences may be lethal. HUS consists of progressive renal failure with microangiopathic haemolytic anaemia and thrombocytopenia. Thrombotic microangiopathy is the histopathological lesion and this affects mainly renal microvasculature. We present a case of gemcitabine-induced HUS and review literature to make professionals fully aware of its existence, thus a high grade of suspicion might help with early diagnosis and prompt treatment which hopefully will reduce its morbidity.

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INTRODUCTION

Gemcitabine is an antimetabolite drug used in the treatment of several malignancies including pancreatic cancer[1]. Although it has got multiple adverse effects, the most relevant ones include myelotoxicity, increased vascular permeability and peripheral oedema. Unfortunately, several cases of acute renal failure have also been reported and haemolytic uremic syndrome (HUS) appears to be the underlying process. The cause of this syndrome and its treatment remain unknown[2] but its consequences may be lethal.

HUS is characterized by progressive renal failure associated with microangiopathic haemolytic anaemia and thrombocytopenia. The primary event in this syndrome's pathology is damage to endothelial cells. Thrombotic microangiopathy (TMA) is the key histopathological lesion for which the features are thickening and inflammation of the walls of arterioles and capillaries, disengagement of endothelial cells, accumulation of proteins and cellular debris in the endothelium, and the formation of platelet thrombi that obstruct the vessels lumen[3]. TMA involves mainly the renal microvasculature but involvement of the central nervous system, cardiovascular system, lungs, skin, skeletal muscle and gastrointestinal tract occurs in 20% of patients[4].

The most frequent cause of HUS is an infection by *Escherichia coli* which produces Shiga toxin. This is known as "typical HUS"[5]. However, other factors can also cause HUS, known as "secondary HUS". Among these factors, pregnancy, organ transplantation, other infections and medical treatments such as gemcitabine can be named[6].

The association of HUS with gemcitabine has been reported several times in the literature but to our knowledge this is the first case with cardiovascular system involvement at the time of diagnosis. The incidence of this complication seems to be low, but underreporting is also a possibility[7]. Although infrequent, HUS is a serious complication and a high grade of suspicion is needed to diagnose it early and initiate treatment. Uncertainty exists regarding the best treatment to apply, although discontinuation of gemcitabine is agreed as the first step. We present here a recent case seen in our Department. The patient has survived but unfortunately she remains dialysis dependent.

CASE REPORT

In April 2017, a 66-year-old Caucasian female with a history of a deep vein thrombosis after an air flight a few years back, was admitted due to extreme fatigue, peripheral oedema and general malaise. She had been previously diagnosed with an ampullary adenocarcinoma and underwent a Whipple's procedure (pancreatico-duodenectomy and splenectomy) in June 2016. Pathological results showed a pT4pN1 (3/5) R0 adenocarcinoma. Her postoperative period was a little difficult. She complained of restless legs, sleeplessness, occasional diarrhoea and vomiting not following any pattern. She required expert dietician to support. On the suspicion of pancreatic insufficiency, her pancreatic enzymes were increased. She was also started on Quinine Sulphate to help with restless legs and continued to take Omeprazole, Metoclopramide, Zopiclone and Erythromycin.

A few months after her surgery, she was started on adjuvant treatment with gemcitabine. Initially she had been planned for a combination with capecitabine but due to her diarrhoea, this plan was abandoned. The dose of gemcitabine was reduced for the first cycle in view of her long postoperative period to recover up to an acceptable level of fitness to start her adjuvant chemotherapy. The plan was to re-evaluate at the second visit.

She developed diarrhoea (3 episodes daily) and mild fatigue, phlebitis post-cannulation in arms and phlebitis in legs which were painful and hard to touch. She was then started on Rivaroxaban 10 mg daily and recommended to apply topical Hydrocortisone. She declined a PICC line. She also developed one episode of a prolonged chest infection without any neutropenia. This

was treated with Doxycycline and needed a delay of her planned 2nd cycle.

Due to all these side-effects, we decided to keep the dose reduced by 20% as performed for the first cycle. After cycle 4, she complained of sore mouth CTC (Common Terminology Criteria for Adverse Events used by oncologists to classify the intensity of side-effects (https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm) grade 2 and continued with her usual diarrhoea although only CTC grade 1. Her haemoglobin levels had been fluctuating between 123 g/L and 95 g/L and her creatinine between 69 μmol/L and 107 μmol/L. At her pre-chemotherapy appointment for cycle 6 (last cycle), she complained of extreme fatigue and significant peripheral oedema lasting for the previous 2 wk. On the day of the appointment she was feeling significantly better and the oedema had significantly resolved. Following discussion with the patient about the risks of having the final cycle *vs* discontinuation, she proceeded with day 1 and day 15th, but to avoid day 8th as she would be on holidays.

Her haemoglobin was 78 g/L and her creatinine levels had increased to 146 μmol/L. At the time these were considered to be due to bone marrow toxicity with gemcitabine itself and the increased creatinine levels as being pre-renal cause, resulting from suboptimal fluid intake.

She went ahead with day 1 and received two units of blood with clinical benefit. Two weeks later, before day 15th, she presented to the acute medical oncology department with a complaint of extreme fatigue and weakness, peripheral oedema and feeling generally unwell, with mild dizziness and mild chest pain. On examination, she was tachycardic with a pulse of 120 bpm, blood pressure of 202/83 mmHg, respiratory rate of 18 and afebrile. She looked pale, dehydrated and with significant peripheral oedemas. She did not have any skin rash or purpura. Laboratory workup showed a creatinine of 392 μmol/L (baseline of 69 μmol/L), which gradually went up to 759 μmol/L in 48 h. Full blood count (FBC) showed haemoglobin of 92 g/L, hematocrit of 0.275 L/L, reticulocytosis of 3.2% and a white cell count of 22 × 10^9/L. Her platelet count was 77 × 10^9/L. Troponin was 509 ng/L and the electrocardiogram showed a NSTEMI with widespread T-wave inversion.

An echocardiogram showed all apical regions akinetic, with an ejection fraction of 45%, moderate diastolic impairment and a chest X-ray reported a mild left pleural effusion. Urinalysis showed mild proteinuria. The patient was intensively managed according to the unit relevant protocols. Her lactate dehydrogenase (LDH) level was elevated to 2328 iU/L (90-275) and haptoglobin was < 0.10 g/L (0.5-2.40). Her ADAMTS-13 levels were 87 (64-132). Peripheral smear was examined and showed anisocytosis, poikilocytosis, microspherocytes, rouleaux formation and few platelet clumps. An ultrasound of the renal tract demonstrated normal kidneys with non-obstructing features. A CT scan showed doubts with peritoneal metastases. The working diagnosis of HUS probably induced by gemcitabine was made as the patient had not had evidence of malignant recurrence. Her cardiology issues were optimised and she was started on steroids (high dose prednisolone) and although initially the patient was very reluctant to other treatments, eventually she accepted haemodialysis. She has been under close follow up and continues free of recurrence eighteen months after this episode. Her echocardiogram has shown improvement with hypokinesis of a single mid-septal segment. The remaining wall motion appears normal with globally preserved ejection fraction (> 55%) and left ventricular diastolic function is moderately impaired. She had received a total cumulative dose of 23940 mg of gemcitabine before cycle 6 and received cycle 6 day 1 as her haemoglobin drop was put in relation to gemcitabine haematological toxicity as mentioned above. The patient now feels clinically well although this has impacted negatively on her quality of life as she remains dialysis-dependant.

DISCUSSION

Gemcitabine is an antineoplastic agent commonly used in the treatment of several cancers such as pancreatic, lung, breast and other tumours. It is an analog of deoxycytidine and works as a pro-drug. Once transported into the cell, it must be phosphorylated by deoxycytidine kinase to change it into the active form that will inhibit DNA synthesis[1,8]. Several phase I studies of gemcitabine as single agent have recommended 1000 mg/m^2 administered as a 30 min infusion[8,9].

With this regimen, the toxicity profile is low, with myelosuppression as most frequent adverse event[10]. Other studies have shown similar efficacy with prolonged infusions[11], although some have suggested that it could increase cytotoxicity and survival[12]. However, it clearly increases the rate of haematological toxicities grade 3-4. Therefore, it continues to be administered as a 30 min infusion[13] in pancreatic adenocarcinoma.

Other side-effects include mainly increased vascular permeability and peripheral oedema but several cases of HUS have been documented as well[2]. It is difficult to estimate HUS incidence as it is easily underreported but the literature have published 0.078% in clinical trials and 4% when taken from spontaneous sources[14,15]. HUS is characterized by renal failure, thrombocytopenia and microangiopathic hemolytic anemia (MAHA), proteinuria and haematuria. MAHA consists of increased levels of LDH, low haptoglobin and the presence of schistocytes on the peripheral blood smear. Unfortunately HUS diagnosis is often delayed due to the fact that anaemia and thrombocytopenia might be attributed to myelotoxicity of the drug itself[16]. However, when these toxicities are combined with renal insufficiency, a high index of suspicion is needed to prompt a laboratory workup looking for signs of haemolysis[17].

There are other reasons leading to the difficulties

in diagnosis. The lack of physicians' awareness or the patients' poor oral intake, diarrhoeas, older age or co-morbid diseases such as hypertension, diabetes, or vascular disease, may contribute. Glezerman *et al*[18] reviewed 29 patients with gemcitabine nephrotoxicity and described new onset or worsening hypertension in 26; oedema, shortness of breath and congestive heart failure in 21, 15 and 7 patients respectively[18]. All of them developed anaemia, thrombocytopenia and elevated serum LDH. Haptoglobin was low and schistocytes were present in most of them[18]. These authors concluded that gemcitabine-induced HUS presents as new-onset renal failure with hypertension, thrombocytopenia and MAHA, but agree that the final diagnosis is not easy and emphasise again the relevance of a high index of suspicion[18].

In addition to this, there could be patients showing only a small decrease in renal function. In these patients, an increase in serum creatinine might be the only sign of HUS[19]. However, physicians need to know that mild renal deterioration resolving quickly on rehydration is not related to HUS. To add even more difficulties, some patients develop livedo reticularis in lower extremities or digital necrosis as an early sign of HUS[20]. We have recently published a review of 157 patients on adjuvant gemcitabine for pancreatic adenocarcinoma. Two patients developed gemcitabine-HUS. Both had a drop in haemoglobin of 37% and 34% from the baseline levels and a drop in creatinine clearance of 41% and 31%. Logistic regression analysis showed that a drop in haemoglobin > 25% and in creatinine clearance > 30% from baseline, increased significantly the chances of ending on hemodyalisis (P = 0.0001)[21]. We proposed that in those suspicious cases, gemcitabine should be at least delayed to undertake all those extra laboratory tests and confirm or dismiss this diagnosis before a final decision regarding gemcitabine continuity is made[21].

Serke *et al*[17] recommended reticulocyte-counting if patients develop anaemia or thrombocytopenia. If strongly elevated, this supports hyperregenerative anaemia due to haemolysis, ruling out myelotoxicity. Coombs test can also be performed and it should be negative if renal insufficiency is not related to HUS[22]. Finally, in some cases, a renal biopsy could be considered to be able to confirm this complication[22]. In the case presented here, we established the diagnosis based on clinic-analytical parameters and although a renal biopsy was considered and discussed, this was finally abandoned. To throw more challenges to the diagnosis, timing and cumulative dose behind HUS are variable[7,14,15,23,24]. Whereas Fung *at al*[15] documented HUS within 1 to 2 mo of the last infusion with a median of cumulative dose of 18252 mg/m^2, Flombaum *et al*[25] reported a broad range of cumulative doses, from 2450 to 48000 mg/m^2. None of these authors found a dose-response relationship[24]. HUS may also occur many months after the last infusion[25,26]. As such this variability further clouds the its' recognition and so encourages awareness that it is a risk, possibly serious of this treatment. Unfortunately its prognosis is poor, with mortality rates ranging from 10% to 40% in most series[27] to as high as 60%-70% in others[23].

Although gemcitabine-induced HUS occurs in early and advanced disease, older literature reviews indicate that it is more frequent when the patient is free of disease or has minimal tumour burden[25]. However, HUS could be cancer associated as well but this is more frequent with metastatic disease[25,27]. The mechanism or mechanisms behind HUS are unknown, but one hypothesis propose a micro vascular endothelial injury as the key. This may be *via* a direct gemcitabine interaction or indirectly following neutrophil or platelet activation[28,29].

Others are inclined to think that the origin is immunologic, following the observation that there appears to be benefit from treatments that remove circulating immunocomplexes or from immunosuppressants[30]. Reduced complement and partial reduction in the activity of ADAMTS13 (< 60% of normal activity) have been documented in most patients with atypical HUS, respectively. This has led to the proposal that functional tests of ADAMTS13 should be considered in these patients[31]. Consistent with this observation, metastatic cancers may have reduced serum ADAMTS13 activity[32]. Another mechanism taken into consideration is the activation of the clotting pathway following gemcitabine drug-induced endothelial injury[33].

In addition platelet activation may be a secondary response to endothelial injury[34]. In TMA, the renal and cerebral vessels are commonly involved, while the pulmonary and hepatic microvasculature is usually spared. Evidence also indicates that acute myocardial infarction is an early, frequent and severe complication during TMA[35]. A study with 74 patients with TMA (not associated with gemcitabine) showed that 18% had acute myocardial infarctions, 9 non- and 5 ST-segment elevation. All these episodes happened 5 ± 3 d after the TMA diagnosis predominantly in thrombotic thrombocytopenic purpura. This caused left ventricular dysfunction in 3 of 8 survivors[36].

Cardiac complications are frequently seen in thrombotic thrombocytopenic purpura and also occur in atypical HUS. Therefore these patients should be assessed for cardiac sequelae[36]. Our patient showed signs of myocardial infarction in the context of haemolysis which we have not seen reported before in gemcitabine-induced HUS, and this was an early complication. Another issue with gemcitabine-induced HUS is the optimal management. Immediate discontinuation[19] seems appropriate as first step, although it is unknown whether this ameliorates the course of the syndrome. Other interventions include steroids, transfusions, dialysis, plasmapheresis, vincristine, rituximab and more recently eculizumab[5,29] with limited effectiveness[16,30,31]. Plasmapharesis has shown to modify the evolution of haemolytic anaemia but not in renal impairment[5,29].

Recent studies have explored the use of Rituximab

(an anti-CD20 monoclonal antibody) and Eculizumab. Eculizumab is a recombinant humanized monoclonal antibody that binds to complement C5 protein and inhibits its cleavage, preventing the generation of the inflammatory peptide C5a and the cytotoxic membrane-attack complex C5b[9,37]. The most frequent side-effects are headache, anaemia and diarrhea[38]. Neisseria meningitidis vaccination is also indicated at least two weeks prior to treatment[39]. It has shown a fast and sustained interruption of the TMA process in patients with non-atypical HUS, including those with drug-induced HUS[40] and it has been associated with significant long-term improvements in renal function, the interruption of plasmapheresis and important reductions in the need for dialysis[6]. Al Ustwani *et al*[19] have reported resolution of the haemolysis and thrombocytopenia in four patients with gemcitabine-induced HUS. Renal function improved significantly although it did not return to baseline and only one patient required temporary haemodialysis, but renal function subsequently improved[19]. Although Eculizumab has been recently approved by FDA for atypical HUS, its role in malignancy or chemotherapy induced HUS has not been defined[41,42] and its current cost limits accessibility[19].

Bharthuar *et al*[43] presented a case of gemcitabine-induced HUS which was aggressively treated with plasmapheresis, high-dose steroids, vincristine and rituximab. The patient improved clinically and the platelets recovered concurrently with administration of rituximab but needed aggressive supportive measures to manage renal failure (haemodialysis) and hypertension.

Ritchie *et al*[44] reported their experience of managing three patients with pancreatic adenocarcinoma who developed gemcitabine-induced HUS. One patient showed some benefit with plasmapheresis and rituximab resulted in durable resolution of HUS in the others. These authors concluded that immune based therapies seem to reverse haemolysis and stabilise renal function[44].

Although there is an urgent need for better therapy, it seems that immunotherapy offers promise but requires more evaluation. In the case reported here, we did not see any significant benefit with steroids but the patient was very reluctant to receive any other treatments. She was not keen on trying any other options after knowing potential side-effects and the uncertain benefits. Rituximab and Eculizumab were mentioned in the discussion but finally abandoned for these reasons. It was only after several long discussions with the patient and a significant clinical deterioration that she finally accepted haemodialysis. We can conclude here that gemcitabine-induced HUS is a rare but serious toxicity with significant morbidity and mortality that requires prompt diagnosis and intervention. We hope that this article would help all professionals, making them aware of this extremely serious syndrome. Subtle signs such as increase level of serum creatinine or a significant drop in haemoglobin should flag an alert. Gemcitabine should then be withheld to undertake all the required laboratory workup to confirm or dismiss this diagnosis. However, as previously discussed, it is unknown if this measure would be able to stop or minimize the damage already initiated.

ARTICLE HIGHLIGHTS

Case characteristics

A 66-year-old female developed a significant renal impairment and anaemia while receiving adjuvant Gemcitabine.

Clinical diagnosis

She was diagnosed with haemolytic uremic syndrome.

Laboratory diagnosis

Her laboratory tests showed haemolysis and ruled out any myelotoxicity.

Imaging diagnosis

An electrocardiogram showed a NSTEMI with widespread T-wave inversion. A renal US did not show any evidence of lesion or cortical damage.

Pathological diagnosis

Although considered a renal biopsy, this was finally declined.

Differential diagnosis

Myelotoxicity and general decline with low intake and dehydration but these were ruled out immediately after receiving results showing haemolysis. Myocardial infarction as the cause but ruled out after parameters showing haemolysis, and considered a consequence of the haemolysis as part of the thrombotic microangiopathy (TMA).

Treatment

Steroids were tried and she was also started on aspirin. Haemodialysis was needed.

Term explanation

HUS: Haemolytic uremic syndrome; TMA: Thrombotic microangiopathy.

Experiences and lessons

Subtle signs such as increase level of serum creatinine or a significant drop in haemoglobin should flag an alert in patients on Gemcitabine. Although it is unknown if by withholding Gemcitabine this would be able to stop or minimize the damage already initiated, this should be done until all the laboratory workup to confirm or dismiss the diagnosis has been performed and received.

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CASE REPORT

Colovesical fistula as the initial manifestation of advanced colon cancer: A case report and review of literature

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Abstract

Colovesical fistulas (CVFs) are rare complications of very advanced cancers of the abdominal or pelvic cavity and often cause diagnostic troubles. CVFs are found more often in males, whereas females usually suffer from rectovaginal or vesicovaginal fistulas. This article presents a case of a female patient who was admitted to the hospital because of acute diarrhea, presumably of infectious origin, and with only subtle abnormalities in blood tests and urinalysis. Owing to the ineffectiveness of the performed treatment and progressive intensification of symptoms, diagnostics were extended to include a computed tomography scan, sigmoidoscopy and cystography. The imaging results revealed a large heterogeneous conglomerate of solid and fluid structures in the pelvis, which involved reproductive organs, the bladder and sigmoid colon. The excrement leaking from the digestive tract was urine, and CVF was the first manifestation of colon cancer. Shortly after the final diagnosis, the patient deteriorated and eventually died after an urgent colostomy was performed because of a bowel obstruction.

Key words: Fecaluria; Pelvic cavity; Colovesical fistula; Colorectal cancer; Diarrhea

Core tip: Colovesical fistulas are a rare complication of very advanced cancers of the abdominal or pelvic cavity.

This article presents a case of a female patient whose first manifestation of colon cancer was a misleading watery diarrhea, which was later found to be urination through a pathologic junction between the bladder and distal colon.

Skierucha M, Barud W, Baraniak J, Krupski W. Colovesical fistula as the initial manifestation of advanced colon cancer: A case report and review of literature. *World J Clin Cases* 2018; 6(12): 538-541 Available from: URL: http://www.wjgnet.com/2307-8960/full/v6/i12/538.htm DOI: http://dx.doi.org/10.12998/wjcc.v6.i12.538

INTRODUCTION

Colovesical fistulas (CVFs) are rare medical findings and mostly represent complications of diverticulitis, cancer, or Crohn's disease. Advanced tumors, which grow abundantly in the abdominal or pelvic cavity, are responsible for approximately 20%-30% of CVFs[1]. Most frequently, CVFs originate from colon adenocarcinomas but may also occur as a consequence of neoplasms in other pelvic organs[2,3]. In any case, CVFs are observed at very advanced stages of neoplastic diseases[4,5].

This article presents a case of a patient with late manifestations and a rapid course of an advanced colon cancer. The first symptom of the disease was urination through a pathologic junction between the bladder and distal colon, which was misinterpreted as diarrhea.

CASE REPORT

A female aged 81, previously healthy, physically fit and active was admitted to the hospital because of diarrhea, which had intensified within a few preceding days, despite maintaining a complete diet. On admission, the patient presented with a mild but recurring fever, abdominal cramps, nausea and a lack of appetite. The abnormalities in laboratory blood tests, performed upon the admission, were as follows: low potassium (2.68 mmol/L) and albumin (2.1 g/dL) levels, leukocytosis with a predominance of granulocytes (18.52 × 109/L) and an increased level of C-reactive protein (191.8 mg/L; norm: < 5 mg/L). Urinalysis revealed mild bacteriuria and leukocyturia. An abdominal ultrasound showed mild hepatic steatosis with multiple solid, hyperechoic lesions in the entire liver, which were described as simple cysts (the largest measuring 20 mm). Initially, the patient was treated for diarrhea and urinary tract infection. After a week of ineffective antibiotic therapy, ruling out the initial diagnosis of infectious diarrhea, the patient was constantly deteriorating, and the symptoms of abdominal pain, massive watery diarrhea and nausea were progressively intensifying. The patient had no control urinalysis because of defecating to incontinence pads and a high possibility of contamination. She refused to be catheterized. An abdominal computed tomography scan revealed a 112 mm × 78 mm × 10 mm heterogeneous conglomerate of solid and fluid structures, which presumably originated from some gynecologic or colon cancer and involved reproductive organs, the bladder and sigmoid colon (Figure 1). Locoregional lymph nodes were irregularly enlarged to approximately 20 mm and formed large masses, suggestive of peritoneum involvement. Moreover, there were numerous hypodense solid lesions in the liver and both adrenal glands, as well as one in the right kidney cortex, all suggestive of metastases. By sigmoidoscopy, 10 cm from the anal verge, an irregular infiltrating mass and leakage of a colorless fluid were found, which were suggestive of CVF. The diagnosis was confirmed by cystography (Figure 2). While undergoing to all the diagnostic examinations, the patient collapsed, and her condition severely worsened. She started vomiting feces, and after consultation with a surgeon, she was qualified for an urgent colostomy because of a bowel obstruction. The patient died within few hours after the surgery. The postmortem pathology diagnosis was a G3 poorly differentiated colorectal adenocarcinoma.

DISCUSSION

From the literature, it seems that fistulas between the bladder and digestive tract are uncommon and often cause diagnostic troubles. The patients are mostly characterized by recurrent urinary tract infection, dysuria, fecaluria, and pneumaturia and less often by intestinal abnormalities such as diarrhea, melena and hematemesis[6]. Electrolyte and acid-base disturbances depend on the localization of the pathologic junction. Enterovesical fistulas cause pathological reuptake of electrolytes[4,7]. In cases of CVFs, cells of the colon epithelium exchange bicarbonates for ammonium and chloride ions[8]. CVFs tend to cause severe physical complications, and in most cases, patients end up with a close death[4,9-11].

Wei *et al*[6] have described a case of a patient with CVF, whose symptoms were comparable to those described in our case, *i.e.*, chronic diarrhea with only mild urinary tract symptoms. However, their patient was a male, and the outstanding feature of our case is that the patient was a female. CVFs are more likely to occur in males, whereas females usually suffer from rectovaginal or vesicovaginal fistulas.

The presented patient experienced an unexpectedly late manifestation of a very advanced cancer. Supposedly, she must have had some alarming symptoms before the admission to the hospital, but they were not troublesome enough to make her seek any help. The bothering problems started from the perforation of the tumor and the formation of a fistula that caused a misleading urine passage through the digestive tract, which appeared as diarrhea. If control urinalysis was performed, the diagnosis would have been made a few days earlier. In this particular case, an earlier diagnosis would not have probably changed the course of events. Nevertheless, the most basic test, a urinalysis, might ha-

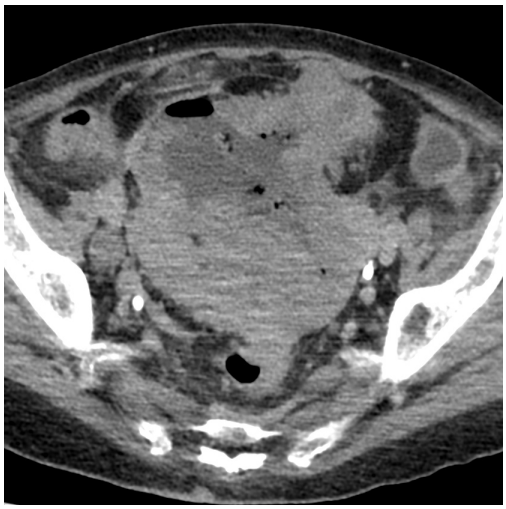

Figure 1 Computed tomography scan - axial view. A heterogeneously thickened bowel wall with an infiltrating tumor and separated fluid collection with air bubbles. The scan is suggestive of a perforated sigmoid tumor and an adjacent intraperitoneal abscess.

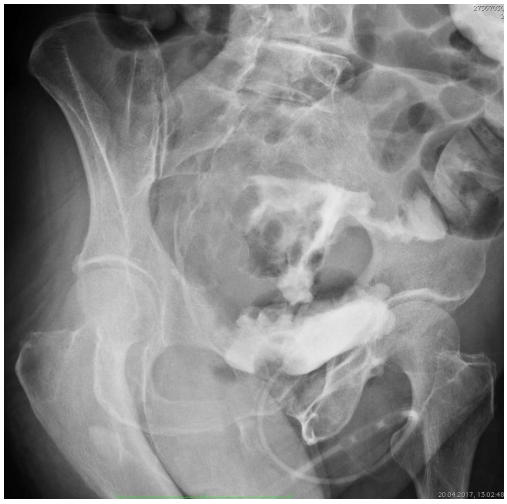

Figure 2 Cystography - an oblique projection. An aqueous solution of the contrast medium was administered through a Foley catheter. A poorly filled urinary bladder with an irregular outline, a thickened and trabeculated wall. The contrast escapes upward and forms an irregular shape, which is the origin of the strip that contrasts a colon loop. The X-ray is suggestive of a colovesical fistula.

ve been as informative as were the complicated, time-consuming and expensive radiological examinations. The concluding remark from the case is that the excrement from the digestive tract may not always be only feces, and diarrhea may be a misleading symptom of CVF.

ARTICLE HIGHLIGHTS

Case characteristics

Misinterpretation of diarrhea in a female patient with a colovesical fistula (CVF) due to an advanced colon cancer.

Clinical diagnosis

Acute diarrhea, recurring fever, abdominal cramps, nausea and a lack of appetite.

Differential diagnosis

Infectious diarrhea.

Laboratory diagnosis

Hypokalemia, hypoalbuminemia, leukocytosis with a predominance of granulocytes and elevated C-reactive protein. Bacteriuria and leukocyturia in urinalysis.

Imaging diagnosis

A computed tomography scan revealed a heterogeneous tumor, enlarged lymph nodes in the pelvis and metastases in the liver and peritoneum. Cystography showed a CVF.

Pathological diagnosis

G3 poorly differentiated colorectal adenocarcinoma.

Treatment

An urgent colostomy conducted because of a bowel obstruction.

Related reports

Wei *et al* have described a case of a male patient with CVF, whose symptoms were comparable to those described in our case. However, to our limited knowledge, this is the first case report describing CVF in a female.

Term explanation

CVF is a pathologic junction between the bladder and colon. Approximately 20%-30% of CVFs develop as complications of advanced tumors of the abdominal or pelvic cavity.

Experiences and lessons

Diarrhea may be a misleading symptom of CVF. A basic test such as urinalysis should never be neglected. In our case, repeated urinalysis may have been very informative. Patients with diarrhea of unknown reason should be catheterized to control the renal loss of fluids and the quality of urine.

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CASE REPORT

Robotic transoral vestibular parathyroidectomy: Two case reports and review of literature

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Author contributions: Ozdenkaya Y and Arslan NC designed the report; Ozdenkaya Y and Ersavas C collected the patients' clinical data; Ozdenkaya Y and Arslan NC analyzed the data and wrote the paper.

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Abstract

Advances in preoperative localization studies and demands for scarless surgery have promoted the investigation for remote techniques in parathyroid surgery. Transoral vestibular approach seems to provide the most comfortable and safest access to the neck. In this paper, we report our initial experience with robotic transoral vestibular parathyroidectomy (RTVP) in four patients with primary hyperparathyroidism. The surgery was performed with the Da Vinci system through three trocars introduced from the lower lip vestibule. The procedure was converted to open in two patients due to inappropriate preoperative localization. The mean operative time was 169 min. No postoperative complications were seen. Patients were discharged on postoperative day 1. RTVP is a feasible and safe technique, which allows better surgical exposure and manipulation of the instruments. The advantages of transoral vestibular approach can be enhanced by robotics. Further studies are needed to analyze complications and costs.

Key words: Transoral vestibular surgery; Parathyroid adenoma; Natural orifice transendoluminal surgery; Robotics; Parathyroidectomy

Core tip: In this paper we present the first national transoral parathyroidectomy cases and to our knowledge, these are the first transoral vestibular robotic parathyroidectomy cases without thyroidectomy. Our results indicate that correct preoperative localization and

experience is essential for success in minimally invasive parathyroidectomy.

Ozdenkaya Y, Ersavas C, Arslan NC. Robotic transoral vestibular parathyroidectomy: Two case reports and review of literature. *World J Clin Cases* 2018; 6(12): 542-547 Available from: URL: http://www.wjgnet.com/2307-8960/full/v6/i12/542.htm DOI: http://dx.doi.org/10.12998/wjcc.v6.i12.542

INTRODUCTION

Transverse neck incision is the standard in endocrine neck surgery. Despite satisfactory surgical outcome, surgeons have been searching for alternative approaches because the cosmetic results are disappointing, particularly in young female patients. Open minimally invasive parathyroidectomy was substituted for bilateral neck exploration with less complications and a smaller scar[1]. Nevertheless, demands for scarless surgery have prompted surgeons to continue to try alternative approaches. Several endoscopic or robotic techniques were described for thyroidectomy and/or parathyroidectomy including trans-axillary, trans-areolar and retroauricular approaches, however none of them have become widespread due to the necessity of extensive dissection, limitations in exposure, morbidities, and the presence of small but visible incisions[2-5]. The transoral approach, which allows better exposure to the surgical field, easy identification of recurrent laryngeal nerve (RLN), and comfortable extraction of the specimen has emerged to overcome these limitations[6]. Transoral parathyroidectomy was first described through the mouth floor but was not widely accepted due to complications and poor patient compliance[7-9]. Endoscopic transoral vestibular parathyroidectomy is a feasible and safe technique, which provides a direct approach to the glands with excellent cosmetic results[10-13].

Robotic endocrine neck surgery was initially introduced in South Korea[14]. Several reports suggested the safety and comfort of robotic endocrine neck surgery through axilla and/or breasts when compared with conventional laparoscopy[3,15,16]. Robotic transoral vestibular approach to the neck may combine the advantages of robotics and natural orifice surgery. There are two studies in the literature reporting robotic transoral vestibular parathyroidectomy (RTVP) including only four cases[17,18]. In this paper we present the first national transoral parathyroidectomy cases and to our knowledge, these are the first RTVP parathyroidectomy cases without thyroidectomy.

CASE REPORT

All cases underwent RTVP by a single surgeon in our institution between January and February 2018. Based on patients' demands for scarless surgery, RTVP was discussed with the patients and written informed consent was received. Etiology was primary hypoparathyroidism (PHPT) in all patients. Details of the patient characteristics are given in Table 1.

Surgical technique

Patients were placed in supine position and intubated with nerve monitoring endotracheal tube. Intravenous antibiotic prophylaxis of 1 g of amoxicillin plus clavulanic acid was administered. Hyperextended neck position was adjusted. Skin and oral antisepsis were provided with chlorhexidine. The lower lip vestibule was incised at the center. Subcutaneous tissue was dissected bluntly until the mandible was reached. One to five hundred thousand epinephrine of 40 cc was injected to create a subplatysmal plane. The surgeon introduced a 12 mm central and two 5 mm lateral trocars and CO_2 insufflation at 6 mmHg was started. Da Vinci Xi system (Intuitive Surgical, Sunnyvale, CA, United States) was docked. Blunt and sharp dissection with hook cautery was performed until reaching sternal notch in the inferior and sternocleidomastoids in the laterals. Strap muscles were lateralized and hanged to the skin with silk sutures. The related thyroid lobe was mobilized and enlarged parathyroid gland was removed using endoscopic dissector (Figure 1). The integrity of RLN was identified visually and by nerve monitoring during the surgery. The specimen was extracted through a midline incision (Figure 2). The success of the resection was confirmed with intraoperative quick parathyroid hormone (PTH) level decrease. The surgical site was irrigated, and incisions were closed with polyglactin sutures. A compression dressing was applied.

In patient 1 and patient 2, we converted to open surgery due to inconsistency between preoperative localization studies and intraoperative findings. Preoperative scintigraphy was negative in both patients. In patient 1, ultrasound indicated an enlarged left superior gland that was completely normal in intraoperative exploration. Similarly in patient 2, ultrasound reported an enlarged left superior gland. We kept exploring other glands, but the operative time and tissue dissection had been excessive. These were our first cases and the BMIs of both patients were over 30 kg/m^2. Therefore, we decided to perform open neck exploration. We found a right inferior parathyroid adenoma in patient 1 and a left inferior parathyroid adenoma in patient 2. The success of both surgeries confirmed by quick PTH decrease. Intraoperative bleeding was insignificant (< 25 mL) in all cases.

Postoperative care

All patients were discharged on postoperative day 1 after calcium and PTH levels were checked. Oral cephalexin (500 mg) twice a day was continued for five days. On postoperative day 7, patients were seen at an outpatient visit and vocal cords were examined by

Table 1 Characteristics and surgical results of the patients

Case	Age	Sex	BMI (kg/m^2)	Etiology	Preop. PTH (pq/mL)	Intraop. PTH (pq/mL)	Blood loss (mL)	Op. time (min)	Conversion to open	Hospital stay (d)	Final diagnose
1	37	F	35.3	PHPT	161	6.9	50	205	Yes	1	Right inferior parathyroid adenoma (15 mm × 10 mm × 8 mm)
2	38	M	32.4	PHPT	97	21	40	196	Yes	1	Left inferior parathyroid adenoma (18 mm × 12 mm × 10 mm)
3	43	F	26.6	PHPT	815	33	20	162	None	1	Left inferior parathyroid adenoma (32 mm × 13 mm × 7 mm)
4	66	F	27.5	PHPT	281	70	20	176	None	1	Left inferior parathyroid adenoma (18 mm × 12 mm × 10 mm)

F: Female; M: Male; PHPT: Primary hyperparathyroidism; PTH: Parathyroid hormone.

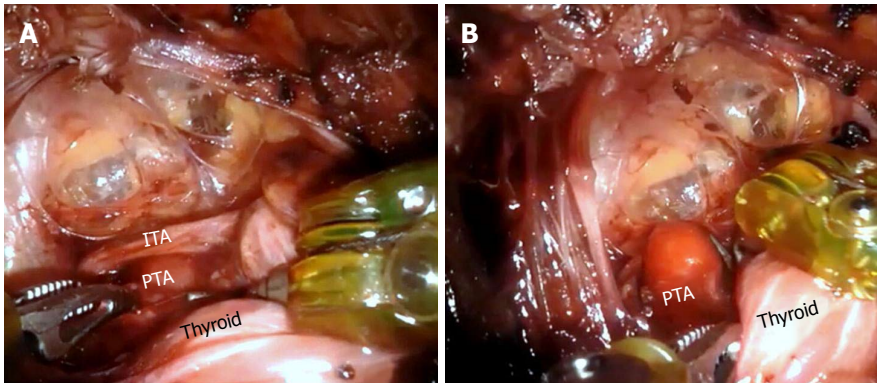

Figure 1 Intraoperative view of case 3. A: Left inferior parathyroid adenoma located under inferior thyroid artery; B: Resection of the gland. ITA: Inferior thyroid artery; PTA: Parathyroid adenoma.

flexible laryngoscopy. The minimum follow-up was six months with a range between six and eight months. No postoperative complications were seen. Cosmetic satisfaction was excellent in the two patients whose surgery was completed endoscopically.

DISCUSSION

Developments in preoperative localization studies have enabled minimally invasive techniques in PHPT treatment. Considering a substantial number of these patients are young women, remote access to the neck without visible scars has been the focal point for surgeons. Several techniques such as transaxillary or inframammary approaches have been described, however it is difficult to qualify those as minimally invasive due to the requirement of extensive dissection[2,4]. A good minimally invasive technique should provide not only invisible or short incisions, but also a short distance between the incision and surgical site, which avoids excessive dissection and direct access to the surgical site. Instrumental collision should be minimum with a good operative exposure to safely identify important anatomical structures. For this purpose, transoral neck surgery has been described.

Witzel *et al*[6] and Karakas *et al*[7] reported the first experimental trials on fresh human cadavers and pigs through transoral sublingual approach. Karakas *et al*[7] published the first transoral sublingual parathyroidectomies in humans in 2010 and 2014[9]. Of the seven patients, two required a conversion to open surgery, two had transient hypoglossal nerve palsy, and one had transient RLN palsy. Since then, sublingual thyroid and/or parathyroid surgery have been studied but have not been popularized due to difficulty and complications[19].

The transoral vestibular approach to the neck was

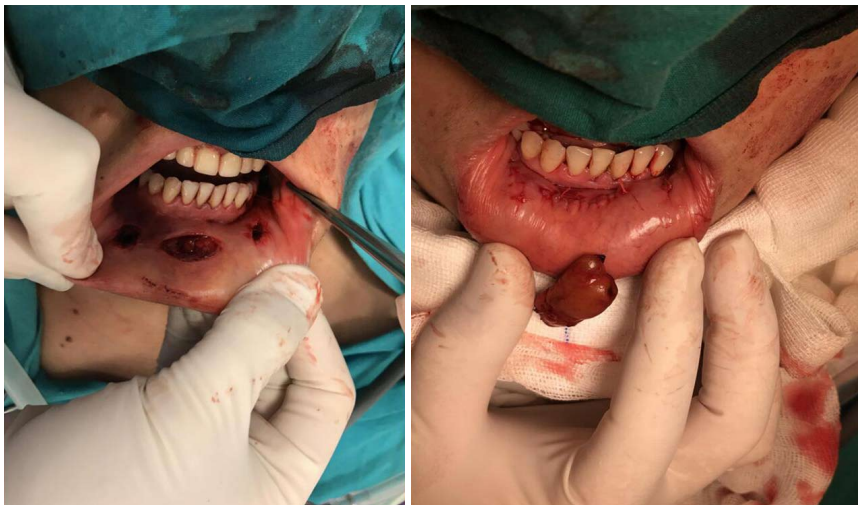

Figure 2 View of the surgical site after the specimen extraction.

first described by Richmon *et al*[20] in a cadaveric study of two robotic thyroidectomies. They concluded that introducing the camera from the oral vestibule was more comfortable than sublingual approach and robotics might overcome the limitations of conventional endoscopic neck surgery. Further studies have been published on endoscopic transoral vestibular thyroidectomy using either a gasless technique or CO_2 insufflation. The most common complication was persistent paresthesia of the chin skin (mental nerve injury)[21,22]. In 2016, Anuwong[11] published a transoral endoscopic vestibular thyroidectomy series of 60 cases. He reported no mental nerve injury. Two patients had transient hoarseness and a late postoperative hematoma. In another study, 46 patients who underwent transoral endoscopic vestibular thyroidectomy were compared with open thyroidectomy in Graves' disease. Operative time was longer and pain was less in the endoscopic group, where other results were similar[23].

The conformation of transoral vestibular approach has been evolving parallel with thyroidectomy. In 2016, Udelsman *et al*[12] reported two transoral endoscopic vestibular parathyroidectomies without any complications. In 2017, Sasanakietkul *et al*[10] published the results of 12 transoral endoscopic vestibular parathyroidectomies. This group is extremely experienced in endoscopic neck surgery. The mean operative time was 107.5 min for PHPT patients in their study. They reported RLN injury in one patient, which resolved spontaneously in one month. No mental nerve injury or infections were seen. Recently, another report from India was published[13]. This study included 12 patients with PHPT who underwent transoral endoscopic vestibular parathyroidectomy. The mean operative time was 112 min and there were no postoperative complications. In our robotic procedures, the mean operative time was 169 min. The docking of the robot and preliminary learning curve are the reasons for this difference. In our cases, we did not have any complications.

Transoral vestibular approach seems to be the best option for remote access neck surgery. The feasibility and safety of the procedure have been shown in numerous studies. We believe that the superiority of this technique can be enhanced by robotics. Conventional endoscopy has well known limitations including interposition of the instruments, inadequate 2-dimensional exposure, unfavorable surgical comfort, and a long learning curve. Robotic transoral vestibular surgery provides a 3-dimentional magnified view through a direct access to the neck, which enables superior identification of important anatomical structures. Robotics also minimized the collision with articulated instruments and a stable platform. There are scarce data in the literature focused on robotic transoral vestibular approach. Russell *et al*[18] performed six robotic thyroid lobectomy, one with parathyroidectomy, through transoral vestibular access. Another case report of RTVP by Bearelly *et al*[17] described excision of an ectopic retropharyngeal parathyroid, but the approach was through the posterior pharynx. To our knowledge, our report presents the first pure RTVP cases[24]. Transoral vestibular approach can also provide convenience in neck dissection and surgery of thyroid malignancies, however there is no data about parathyroid malignancies in the literature[24,25]. The steps of the surgery should be well defined for patient safety before adoption of this technique[26]. A recent study comparing robotic and laparoscopic transoral vestibular approach revealed similar safety and feasibility but longer operative time for robotic surgery, which indicates the role of learning curve[27].

In our institution, we have performed robotic surgery for more than five years for procedures, including bariatric and adrenal procedures. The application of robotics to endocrine neck surgery just started in 2018. Out of four cases, we converted to open surgery in two, due to incorrect preoperative localization of the glands. The limited experience and extended operative time drove us to convert to open. Relatively high BMI

of those patients might have been another contributing factor. We did not perform a cost analysis. Despite these limitations, we did not see any postoperative complications. Cosmetic results were excellent even in early postoperative period.

The transoral vestibular approach appears to be the future of minimally invasive parathyroidectomy. A robotic approach through the transoral vestibular access may overcome the limitations of initially described endoscopic transoral vestibular technique. The potential benefits of RTVP may theoretically decrease the incidence of postoperative complications. Further studies including wide series and cost analysis are needed.

ARTICLE HIGHLIGHTS

Case characteristics

The patients presented with asymptomatic hypercalcemia.

Clinical diagnosis

All the patients were diagnosed with parathyroid adenoma.

Differential diagnosis

Neck ultrasound and parathyroid scintigraphy were performed to identify the etiology of primary hyperparathyroidism.

Laboratory diagnosis

Calcium and parathormone levels were elevated and intraoperative decrease of parathormone was observed in all cases.

Imaging diagnosis

Single parathyroid adenoma was detected in all cases.

Pathological diagnosis

Diagnose of parathyroid adenoma was confirmed by postoperative histopathologic examination.

Treatment

Robotic transoral vestibular parathyroidectomy was performed.

Related reports

Udelsman R, Anuwong A, Oprea AD, Rhodes A, Prasad M, Sansone M, Brooks C, Donovan PI, Jannitto C, Carling T. Trans-oral Vestibular Endocrine Surgery: A New Technique in the United States. *Ann Surg* 2016; 264: e13-e16 [PMID: 27649533]

Experiences and lessons

Robotic transoral vestibular approach is a safe and feasible method for well-located parathyroid adenomas.

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CASE REPORT

Atypical lipomatous tumor in the ligamentum teres of liver: A case report and review of the literature

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Author contributions: Usuda D collected the case data, prepared the photos, and wrote the manuscript; all authors proofread the pathologic materials; Takeshima K, Sangen R, Nakamura K, Hayashi K, Okamura H, Kawai Y, Kasamaki Y, Iinuma Y, Saito H, Kanda T and Urashima S proofread and revised the manuscript; all authors approved the final version to be published.

Informed consent statement: Both written and verbal informed consents were obtained from the patient for publication of this case report and any accompanying images.

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Abstract

A 61-year-old male was referred to our hospital with a three-month history of persistent epigastralgia and right hypochondralgia. Initial examination revealed a fist-size mass at the epigastric fossa. Ultrasonography showed a hemangioma and a mosaic echoic lesion in the ventromedian with poor blood-flow signal and linear hyperechoic part inside, and a clear border to the surroundings. Dynamic computed tomography revealed a highly enhanced effect from the portal-venous phase

continuing to the equilibrium phase. T1-weighted gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid-enhanced image revealed a high intensity effect at the early phase that continued to the next phase. On the other hand, it contained a low intensity area by a fat suppression of that image. In addition, a T2-weighted image did not show a high intensity effect. Laparotomy was performed on the second day of hospitalization. The tumor had arisen from the ligamentum teres of the liver, and no metastasis or invasion of other organs was noted. It consisted of a lipid component of mature adipocytes and a fibrous component of deep dyeing pleomorphic or multinuclear atypical stromal cells. Immunohistochemical study of the atypical stromal cells demonstrated that they were positive for MDM2 and CDK4. A pathological diagnosis of atypical lipomatous tumor (ALT) was made, and the patient was discharged on the eighth day following the procedure. At the 6-mo follow-up dynamic CT, the patient was free of recurrence or metastasis. We experienced a patient with ALT in the ligamentum teres of the liver. This case suggests the need for a careful and detailed examination when encountering patients presenting with a mass; when neoplastic lesion is confirmed by image inspection, we should thoroughly investigate, including further image investigations and pathologic examination. The latter is the most important.

Key words: Liposarcoma; Atypical lipomatous tumor; Malignant adipose mesenchymal tumor; Ligamentum teres of liver; Operation

Core tip: Liposarcoma is one of the most common adult soft tissue sarcomas, accounting for approximately 20% of all mesenchymal malignancies. Atypical lipomatous tumor (ALT) is the most common intra-abdominal primary sarcomas. On the other hand, it is an extremely rare malignant adipose mesenchymal tumor. We report the first case of ALT occurring in the ligamentum teres of liver.

Usuda D, Takeshima K, Sangen R, Nakamura K, Hayashi K, Okamura H, Kawai Y, Kasamaki Y, Iinuma Y, Saito H, Kanda T, Urashima S. Atypical lipomatous tumor occurred in ligamentum teres of liver: A case report and review of literature. *World J Clin Cases* 2018; 6(12): 548-553 Available from: URL: http://www.wjgnet.com/2307-8960/full/v6/i12/548.htm DOI: http://dx.doi.org/10.12998/wjcc.v6.i12.548

INTRODUCTION

Liposarcoma is one of the most common adult soft tissue sarcomas, accounting for approximately 20% of all mesenchymal malignancies[1-3]. Well-differentiated and dedifferentiated liposarcoma, especially the former is called atypical lipomatous tumor (ALT). It is the most common intra-abdominal primary sarcoma[4]. On the other hand, it is an extremely rare malignant adipose mesenchymal tumor[5]. Typically, it is locally aggressive and shows a tendency toward recurrence after surgical excision, despite the fact that it does not metastasize and very rarely dedifferentiates[2]. As far as we know, there have been no reported liposarcoma cases occurring from the ligamentum teres of the liver. Herein, we report a case with a review of the existing literature.

CASE REPORT

The patient was a 61-year-old male, referred to our hospital with a three-month history of persistent epigastralgia and right hypochondralgia. Patient medical history included transient ischemic attacks, hypertension and hyperlipidemia, for which he was being treated with aspirin, antihypertensive medication, and bezafibrate. He was diagnosed with hepatic hemangioma following complete medical check-up ten years prior to his visit to our hospital. Examination revealed a fist-sized mass at the epigastric fossa. Other findings were normal. Routine blood tests showed elevated low-density lipoprotein cholesterol, decreased creatine kinase, and abnormal glucose tolerance. Blood count, C-reactive protein, liver enzyme, tumor maker including carcinoembryonic antigen, carbohydrate antigen 19-9, a fetal-specific glycoprotein antigen, and soluble interleukin-2 receptor were all within normal limits. In addition, the patient was negative for hepatitis B, C and syphilis, as well as collagen diseases. Abdominal ultrasonography (GE Healthcare, LOGIQ E9 XDclear 2.0) showed two tumors located in the right posterior superior segment of liver and ventromedian. The former showed hyperechoic lesion, namely hemangioma, the latter showed mosaic echoic lesion with poor blood flow signal, as well as linear hyperechoic part inside and a clear border to the surroundings (Figure 1). Dynamic CT (GE Healthcare, Discovery CT750 HD) revealed a highly enhanced effect from the portal-venous phase, continuing to the equilibrium phase for the latter tumor. MRI (GE Haelthcare, Signa HDxt 1.5T), including fat suppression radiography to confirm the existence of a lipid component, was performed. T1-weighted gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid-enhanced imaging revealed a high intensity effect at the early phase that continued to the next phase. In addition, it showed a partial low intensity area by a fat suppression image (yellow arrow). On the other hand, a T2-weighted image did not show a highly enhanced effect (Figure 2). Absence of metastasis was confirmed. Gallium-67 scintigraphy (Canon Medical Systems, E.CAM) showed that there was no abnormal accumulation to the abdominal tumor.

The above findings prompted a diagnosis of abdominal mesenchymoma, and we admitted the patient to the hospital. Laparotomy was performed on the

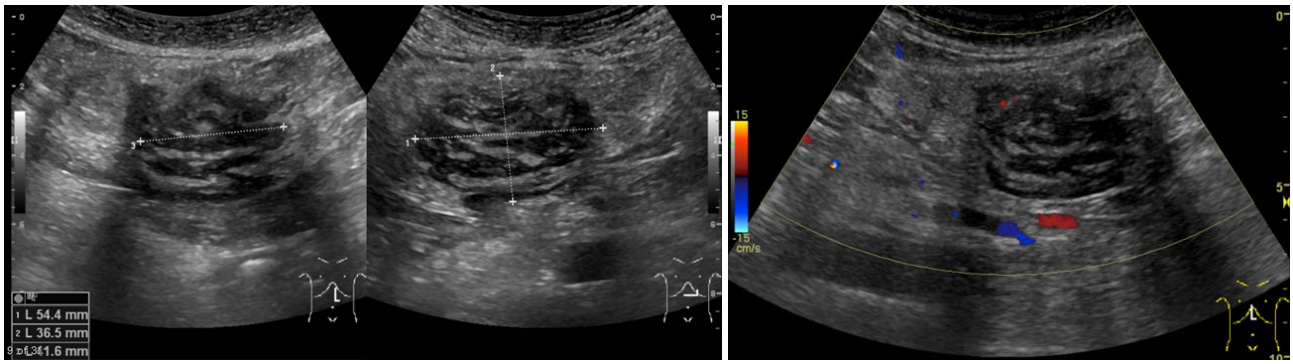

Figure 1 Abdominal ultrasonography shows mosaic echoic tumor at ventromedian. Tumor has poor blood flow signal and a linear hyperechoic part inside and a clear border to the surroundings.

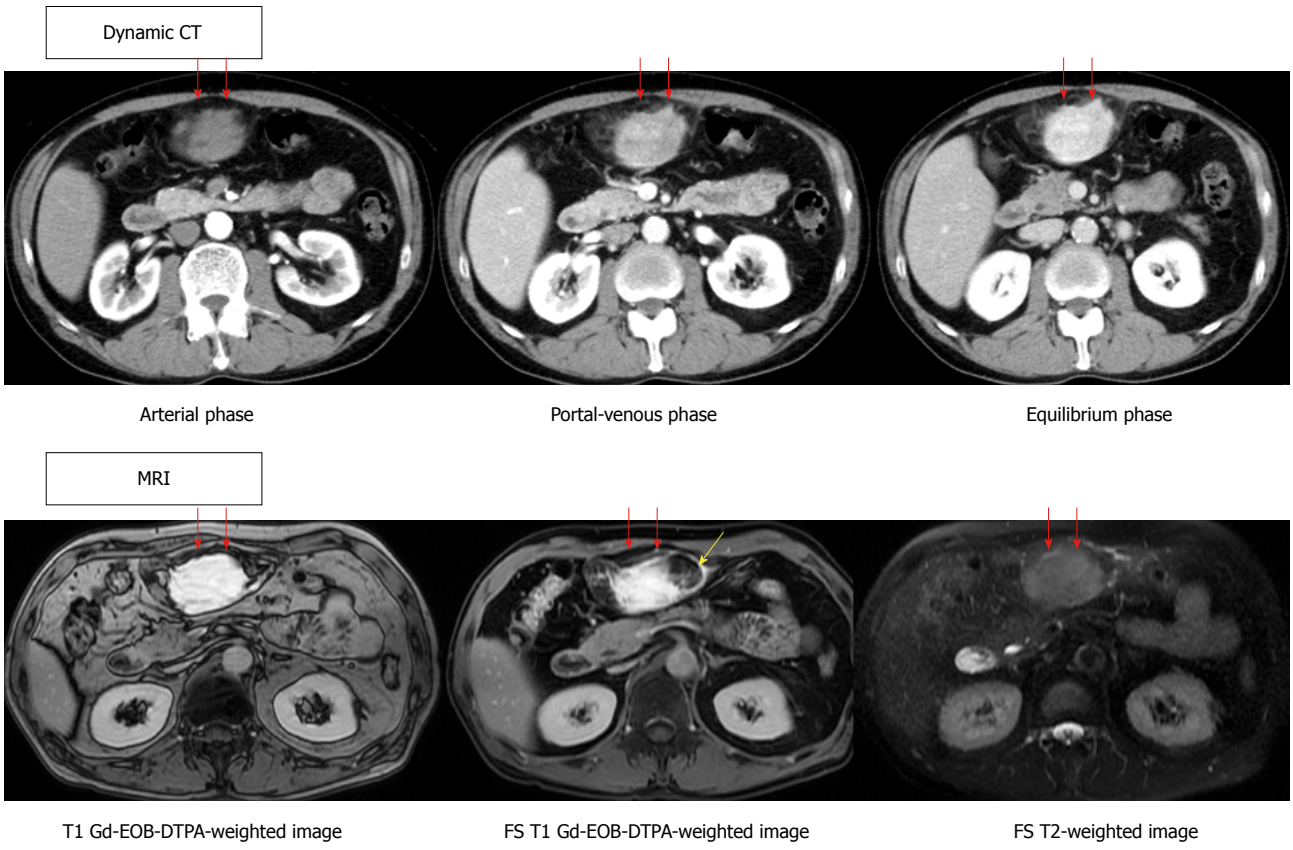

Figure 2 Dynamic CT and MRI of the abdomen show a ventromedian tumor in front of pancreas. CT reveals high enhanced effect of tumor from portal-venous phase continuing to the equilibrium phase (red arrow). T1-weighted gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid-enhanced image revealed a high intensity effect at the early phase, which continued to the next phase (red arrow). In addition, it showed a partial low intensity area by a fat suppression image (yellow arrow). On the other hand, the T2-weighted image did not show a high intensity effect. Gd: Gadolinium; EOB: Ethoxybenzyl; DTPA: Diethylenetriamine pentaacetic acid-enhanced; FS: Fat suppression.

second day post-admission. During an operation, we paid attention to two points for completing the tumor removal, avoiding bleeding and reducing the risk of recurrence; one was to apply gauze for discriminating between the tumor and surrounding tissues, and the other was to finely ligate surrounding blood vessels and cut them little by little. Intraoperative findings revealed as follows: (1) the tumor was arisen from the ligamentum teres of the liver and was highly covered with a capsule without invasion, adhesion, or infiltration to surrounding tissues. Therefore, it was easily enucleated; and (2) the absence of metastasis or other organ invasion was confirmed (Figure 3A). In the resected split specimen, the tumor size measured 13 cm x 9 cm x 5 cm and consisted of yellow lipid and a white fibrous component (Figure 3B). The lipid component was composed of mature adipocytes, and deep dyeing pleomorphic or multinuclear atypical stromal cells were confirmed in the fibrous component (Figure 4A and B). Immunohistochemistry of atypical stromal cells was po-

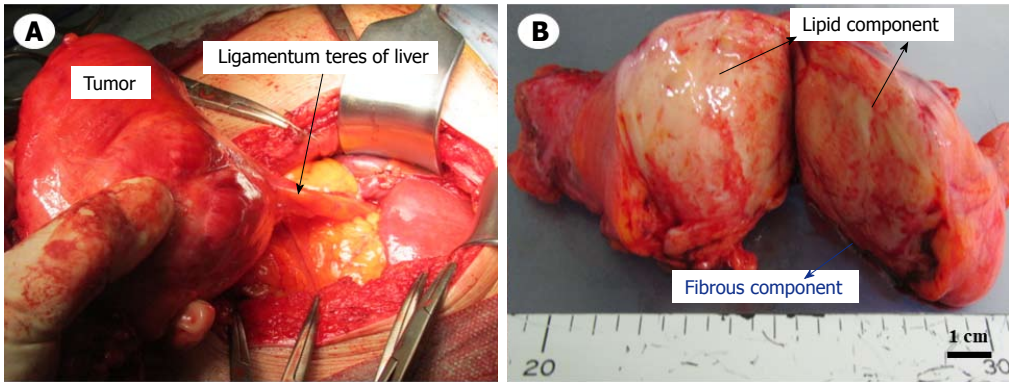

Figure 3 Intraoperative finding and macroscopic view of the resected split specimen. A: A tumor arising from the ligamentum teres of liver; B: A tumor measuring a maximum of 13 cm × 9 cm × 5 cm consisting of yellow lipid and a white fibrous component.

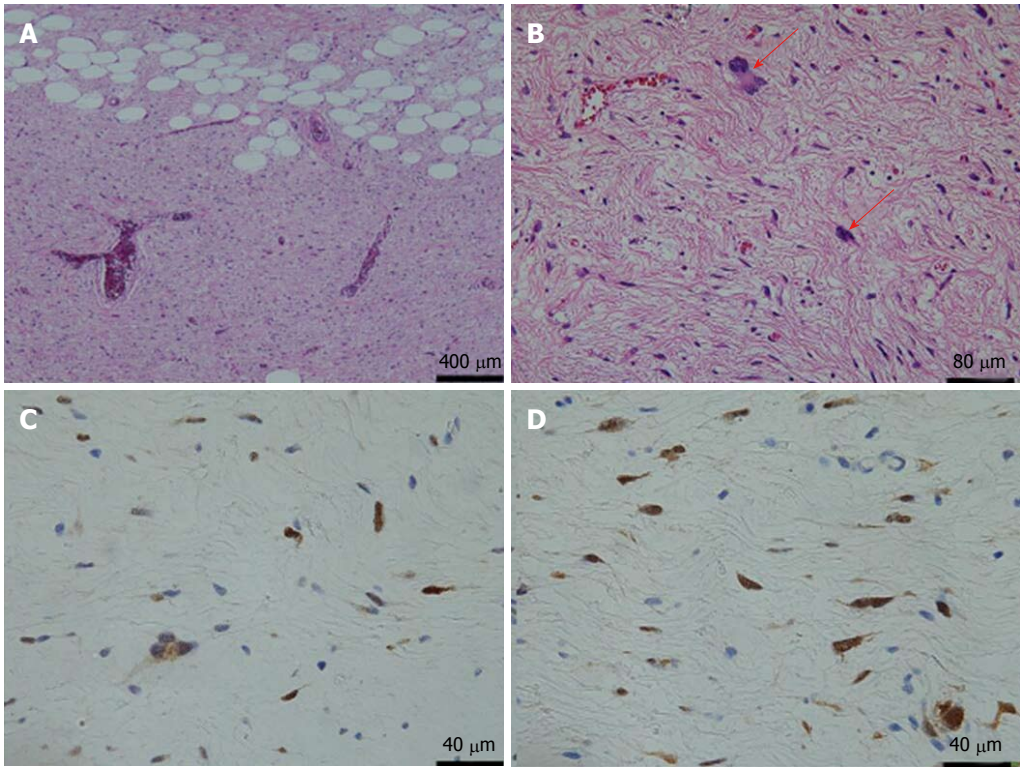

Figure 4 Microscopic view of the resected specimen. A: Lipid and fibrous component are confirmed. Lipid component consist of mature adipocytes. Hematoxylin and eosin staining (× 40); B: Deep dyeing multiforme or multinuclear atypical stromal cells are confirmed in fibrous component (red arrow). Hematoxylin and eosin staining (× 200); C: Immunostaining showed that atypical stromal cells were positive for MDM2 (× 400); D: Immunostaining showed that atypical stromal cells were positive for CDK4 (× 400).

sitive for MDM2 and CDK4 and negative for α-SMA, S-100 protein, CD34 and STAT-6 (Figure 4C and D). Based on these findings, a pathological diagnosis of atypical lipomatous tumor (ALT) was made. The patient responded well and was discharged on the eighth day post-procedure. At the 6-mo follow-up dynamic CT appointment, the patient was free of recurrence or metastasis.

DISCUSSION

This is the first case of liposarcoma occurring from the ligamentum teres of the liver. As laid out in the World Health Organization's classification of tumors of soft tissue and bone: IARC Press 2013, liposarcomas are divided into five subtypes: myxoid, pleomorphic, dedifferentiated, mixed liposarcoma, and ALT[5]. ALT is an intermediate tumor with risk of local recurrence but no potential for metastasis[1,5,6]. The points of differential diagnosis are focused on those mentioned above, especially dedifferentiated liposarcoma. However, it can be difficult to distinguish ALT from poorly differentiated sarcomas and benign adipose tumors[7]. The clinical presentation of ALT tends to be a progressive mass that

can cause symptoms that are aesthetic, functional, or compressive in nature, depending on its size and localization[2].

On the other hand, chromosomal translocations and fusion genes are very commonly found in human cancer, particularly in liposarcoma and other subtypes of sarcomas[8]. The advancement of next-generation sequencing technologies, such as whole genome sequencing, has made it possible to discover novel chromosomal translocations and fusion genes in different tumors[8]. Recently, these next-generation sequencing approaches have led to the identification of many novel chromosomal translocations and gene fusions in different types of sarcomas[8]. In addition to sarcoma fusion genes that had previously been discovered, these novel specific fusion genes and their associated molecular events represent important targets for novel therapeutic approaches to sarcoma treatment[8]. Of these, the majority of ALTs express MDM2 (97%) and CDK4 (92%) on chromosome 12q13-15, compared to benign adipose tumors (MDM2, 5%; CDK4, 2%)[7,9]. The sensitivity and specificity of MDM2 and CDK4 immunostainings used to identify ALT among other soft tissue tumors were 97% and 92%, and 83% and 95%, respectively[7]. Therefore, MDM2 and CDK4 immunostaining is especially useful in separating ALT from large groups of differentiated adipose tumors, and this is important for the differential diagnosis[7]. In this case, we finally diagnosed ALT based on the immunostaining findings, which were positive for MDM2 and CDK4. In addition, there were also caveolae, which are cholesterol-enriched invaginations of the plasma membrane that are involved in various processes, including the absorption of glucose and fatty acids, cell transduction, and mechanoprotection[10]. In vitro analysis shows that both the biogenesis and the function of caveolae are dependent on the activity of the Caveolin (Cav-1, -2 and -3) and Cavin (Cavin-1, -2, -3 and -4) protein families[10]. Cavin-2, along with Cavin-1, Cav-1, and Cav-2, is mainly expressed in ALT, while it is almost impossible to detect in the more aggressive myxoid, pleomorphic tumors, and ALT[10]. In addition, the expression of Cavin-2 increases in liposarcoma tumor cell lines during differentiation, as opposed to proliferation in immunoblotting and immunofluorescence analysis[10]. Therefore, Cavin-2 serves as a useful marker that can be used to discriminate the degree of differentiation in liposarcoma tumors[10].

More studies are still necessary to determine the treatment and therapeutic strategies needed to improve the survival rate of patients with liposarcoma, as the disease is generally associated with frequent relapse[11]. To date, surgical resection has been the mainstay of curative treatment[11]. Several authors have recommended using wide excision with free margins in order to minimize the risk of recurrence, while others have reported having good results and a low rate of recurrence when opting for more conservative or even marginal excision, thereby avoiding complications caused by surgical site morbidity[2]. In fact, marginal excision is a good alternative in cases where the tumor is located near vascular or nerve structures, and it is not associated with elevated recurrence[2]. For metastatic disease, systemic treatment options have historically been represented by standard cytotoxic chemotherapy[3]. Eribulin has recently been approved for advanced liposarcoma, after anthracycline-containing regimen demonstrated an overall survival advantage in liposarcoma in a randomized Phase Ⅲ clinical trial. However, further studies are required to better understand the precise mechanism of action of this agent and its potential role in combination schedules[12]. On the other hand, recent innovative therapies have been introduced and are currently part of the therapeutic armamentarium positively impacting disease control and patient quality of life[3]. Moreover, a better understanding of the molecular characteristics of each soft tissue sarcoma subtype over the last decade has allowed the detection of new potential targets and the development of novel, biology-driven compounds at different stages of testing[3]. Peculiar molecular features and fundamental signaling pathways now represent druggable targets for novel therapies[3]. Therapy that combines both CDK4 and RTK inhibitors may prove to be an effective option for ALT patients with RTK gene amplification[9]. In addition, liposarcoma subsets are less mutated, but they do express immunogenic self-antigens; as a result, strategies to improve antigen presentation and T-cell infiltration may potentially allow for successful immunotherapy in patients who have been given these diagnoses[13].

Long survival is correlated with the active resection of recurrence and recognition of high-grade dedifferentiated type liposarcoma at an early stage[10,11]. In addition, according to recent basic research, although limited compared to other malignancies, formalin-fixed paraffin-embedded tissue biomarkers, namely microRNA-155, may be novel independent indicators of unfavorable prognosis in liposarcoma[14]. However, there remains a need to identify predictive biomarkers for the better selection of target population and testing of combinations of drugs with the ultimate goal of improving outcomes[3].

In conclusion, we experienced a patient with ALT occurring in the ligamentum teres of the liver. This case suggests the need for a careful and detailed examination when encountering patients presenting with a mass; when neoplastic lesion is confirmed by image inspection, we should thoroughly investigate, including further image investigations and pathologic examination. The latter is especially important.

ARTICLE HIGHLIGHTS

Case characteristics

A three-month history of persistent epigastralgia and right hypochondralgia.

Clinical diagnosis

Abdominal tumor.

Differential diagnosis
Neoplastic etiology.

Laboratory diagnosis
Routine blood tests showed elevated low-density lipoprotein cholesterol, decreased creatine kinase, and abnormal glucose tolerance.

Imaging diagnosis
Abdominal mesenchymoma based on MRI, including of fat suppression radiography.

Pathological diagnosis
Immunohistochemical study of atypical stromal cells, namely tumor cells were positive for MDM2 and CDK4 and negative for α-SMA, S-100 protein, CD34 and STAT-6, which led to the pathological diagnosis of atypical lipomatous tumor (ALT).

Treatment
Surgery.

Related reports
ALT is an intermediate tumor with risk of local recurrence but no potential for metastasis. However, ALT may be difficult to distinguish from benign adipose tumors and poorly differentiated sarcomas. Up to now, MDM2 and CDK4 immunostaining have been particularly useful in separating ALT from the large group of differentiated adipose tumors, and they are important for the differential diagnosis. To date, surgical resection has been the mainstay of curative treatment. Long survival is correlated with the active resection of recurrence and recognition of high-grade dedifferentiated type liposarcoma at an early stage. As far as we know, there have been no reported ALT cases occurring from the ligamentum teres of the liver.

Term explanation
ALT: Atypical lipomatous tumor.

Experiences and lessons
When neoplastic lesion is confirmed by image inspection, we should thoroughly investigate, including further image investigations and pathologic examination. The latter is especially important.

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CASE REPORT

Computed tomography and magnetic resonance imaging findings of metastatic rectal linitis plastica from prostate cancer: A case report and review of literature

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Author contributions: You JH and Song JS wrote the manuscript. Jang KY performed the pathological examination; Lee MR performed excisional biopsy; Jang KY and Lee MR edited the manuscript.

Informed consent statement: The study was performed after obtaining the patient's informed consent. The patient was treated according to the provisions of the Helsinki criteria.

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Abstract

Linitis plastica is a rare condition showing circumferentially infiltrating intramural anaplastic carcinoma in a hollow viscus, resulting in a tissue thickening of the involved organ as constricted, inelastic, and rigid. While most secondary rectal linitis plastica (RLP) is caused by metastasis from stomach, breast, gallbladder, or bladder cancer, we report an extremely rare and unique case of secondary RLP due to prostate cancer with computed tomography (CT) and magnetic resonance imaging (MRI) findings, including diffusion weighted imaging (DWI). A 78-year-old man presented with approximately a 2-mo history of constipation and without cancer history. On sigmoidoscopy, there was a luminal narrowing and thickening of rectum with mucosa being grossly normal in its appearance. On contrast-enhanced CT,

marked contrast enhancement with wall thickening of rectum was noted. On pelvic MRI, rectal wall thickening showed a target sign on both T2-weighted imaging and DWI. A diffuse infiltrative lesion was suspected in the prostate gland based on low signal intensity on T2-weighted imaging and restricted diffusion. A transanal full-thickness excisional biopsy revealed metastasis from a prostate adenocarcinoma invading the submucosa to the muscularis propria consistent with metastatic RLP. We would like to emphasize the CT and MRI findings of metastatic RLP due to prostate cancer.

Key words: Prostate cancer; Linitis plastica; Magnetic resonance imaging; Rectum; Metastasis

Core tip: Secondary rectal linitis plastica (RLP) from prostate cancer is extremely rare. A target sign on T2-weighted imaging and diffusion weighted imaging is characteristic for RLP. The presence of elevated serum prostate specific antigen, T2 low signal intensity, and low apparent diffusion coefficient value lesion on prostate should raise the suspicion of secondary RLP from prostate adenocarcinoma. For confirmative diagnosis, full-thickness excisional biopsy is required due to its characteristic mucosal sparing.

You JH, Song JS, Jang KY, Lee MR. Computed tomography and magnetic resonance imaging findings of metastatic rectal linitis plastica from prostate cancer: A case report and review of literature. *World J Clin Cases* 2018; 6(12): 554-558 Available from: URL: http://www.wjgnet.com/2307-8960/full/v6/i12/554.htm DOI: http://dx.doi.org/10.12998/wjcc.v6.i12.554

INTRODUCTION

Linitis plastica refers to a circumferential intramural tumor infiltration of a hollow viscus, generating a desmoplastic reaction that result in a rigid shrunken viscus with thickened walls[1,2]. The stomach is the most commonly involved organ, although the small intestine, colon, and rectum also can be involved. This process can be either primary or secondary to metastasis, and shows characteristic mucosal sparing while involving only the submucosa and muscularis propria[3]. Primary rectal linitis plastica (RLP), also known as signet ring cell carcinoma, is a very rare disease affecting less than 1% of colorectal cancer patients, while secondary RLP from stomach, breast, gallbladder, or bladder cancer occurs more frequently and has been reported previously[4-8]. Due to the widespread use of pelvic magnetic resonance imaging (MRI) in rectal cancer evaluation, some characteristic findings have been suggested, such as a concentric ring pattern or target sign on T2-weighted imaging[7,9].

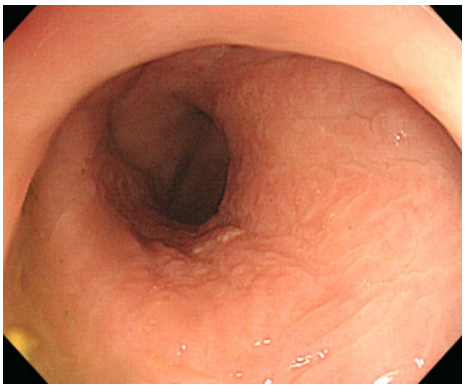

Figure 1 Sigmoidoscopic imaging showing narrowing of the rectal lumen with normal overlying mucosa.

Here we describe an extremely rare and unique case of metastatic RLP due to prostate cancer with findings of computed tomography (CT) and MRI including diffusion-weighted imaging (DWI). To the best of our knowledge, metastatic RLP as a first manifestation of prostate cancer with both CT and MRI findings has never been reported.

CASE REPORT

A 78-year-old man presented with a 2-mo history of constipation and thinning of stool caliber. He had a medical history of benign prostatic hyperplasia and hypertension. Laboratory findings were unremarkable, except the prostate-specific antigen (PSA) level was elevated to 409 ng/mL. Digital rectal examination revealed luminal narrowing of rectum with rigidity. Sigmoidoscopy revealed luminal narrowing of the mid to lower rectum, with grossly indurated, non-ulcerated mucosa (Figure 1). On contrast-enhanced CT, rectal wall thickening (mean thickness, 1.3 cm) with marked homogeneous enhancement and mild perirectal fat stranding was demonstrated (Figure 2A). Based on these observations, a submucosal spreading tumor was suspected, and pelvic MRI was performed for further evaluation. Coronal and sagittal T2-weighted imaging showed concentric thickening of the mid to lower rectum, about 6-7 cm in length, with stratification and perirectal fascial thickening. On axial T2-weighted imaging, the rectum showed three-layered wall thickening, with the middle layer showing isointensity and the inner and outer layers showing hypointensity (Figure 2B). On DWI, the lesion also revealed a concentric ring pattern or target sign, with the middle layer showing restricted diffusion while the inner and outer layers showed no restriction (Figure 2C). The prostate gland was enlarged and a diffuse infiltrative lesion was suspected showing low SI on both T1- and T2-weighted images with a low apparent diffusion coefficient (ADC) value (Figure 2D). There was also low SI on both T1- and T2-weighted images of the left seminal vesicle, and both external iliac lymph nodes were enlarged and round shaped. Positron emission

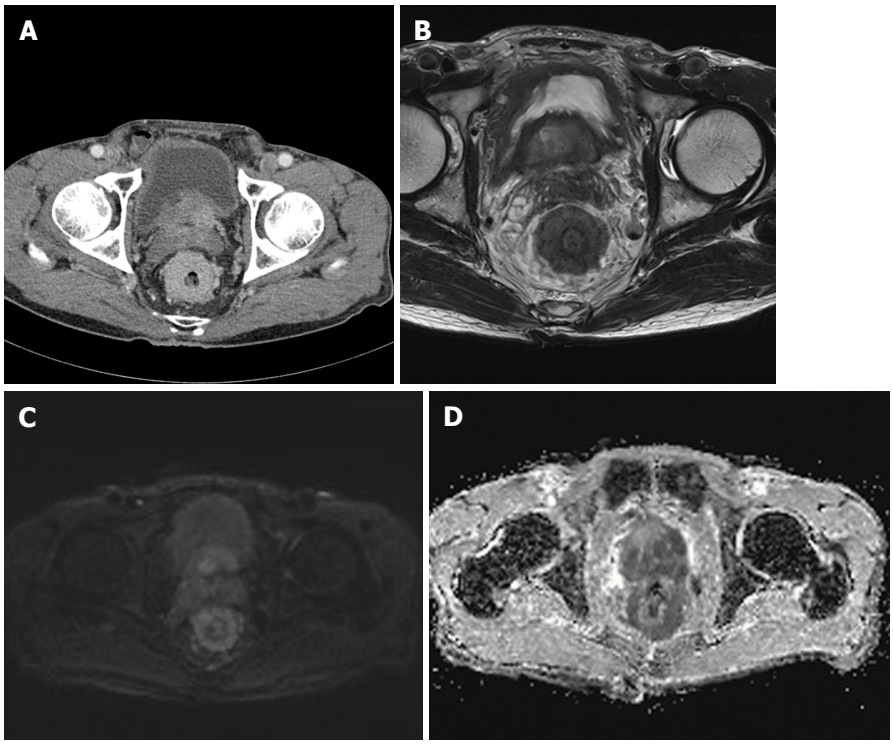

Figure 2 Contrast-enhanced computed tomography and pelvic magnetic resonance imaging of secondary rectal linitis plastica from prostate cancer. A: Contrast-enhanced CT shows circumferential rectal wall thickening with marked enhancement and minimal perirectal fat stranding; B: T2-weighted images; C: Diffusion-weighted images. On T2-weighted images and diffusion-weighted images, a target sign is present; D: Apparent diffusion coefficient map, the prostate shows infiltrative lesions with low ADC values, compatible with prostatic adenocarcinoma.

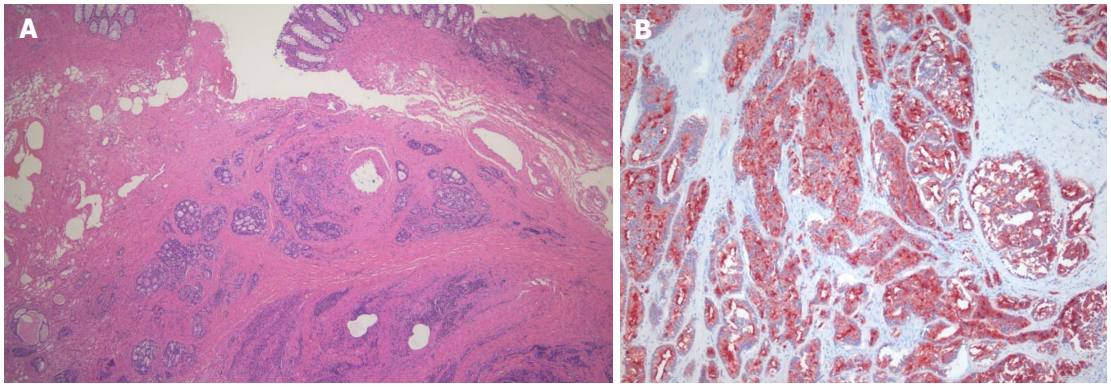

Figure 3 Histological images of metastatic prostate adenocarcinoma obtained from transanal full-thickness excisional biopsy. A: Hematoxylin and eosin (HE) staining showed a moderate to poorly differentiated adenocarcinoma characterized by tumor cells diffusely infiltrated into submucosa and muscularis propria, sparing the mucosal layer (original magnification ×100); B: Immunohistochemical stains were performed and the tumor cells were positive for prostate specific antigen (PSA, original magnification ×100).

tomography–computed tomography (PET/CT) revealed diffuse wall thickening of the mid to lower rectum without significant 2-deoxy-2-[^{18}F]fluoro-D-glucose (FDG) uptake. There was no other pathologic site with abnormal FDG uptake. For both confirmative diagnosis and symptom relief, intraoperative transanal full-thickness excisional biopsy with loop sigmoid colostomy formation was performed. The pathologic examination revealed metastatic prostate adenocarcinoma invading the submucosa to muscularis propria with a positive PSA stain and negative for CDX2, consistent with metastatic RLP due to prostate cancer (Figure 3). Gleason score of biopsy specimen was 9 (4 + 5). The patient underwent Luphere Depot (leuprolide acetate; a synthetic nonapeptide analog of naturally occurring gonadotropin-releasing hormone; used in the palliative treatment of advanced prostatic cancer) treatment and the PSA level decreased to normal without any adverse event. The patient has had no symptoms and his PSA levels have been normal for 2 years now.

DISCUSSION

Linitis plastica is characterized by diffuse proliferation of

connective tissue of a hollow organ wall by infiltrating tumor cells. This term was first introduced by Brinton W meaning the "leather bottle" shape of the stomach, and was later extended to other hollow organs to be constricted, inelastic, and rigid aspects of the wall[10]. Primary RLP is very rare, while secondary RLP is more common, with some CT and MRI findings available for secondary RLP from breast and bladder cancer[11-14]. To the best of our knowledge, there were only 2 case reports of secondary RLP from prostatic adenocarcinoma with only endoscopic ultrasonographic findings available[5,15]. Therefore, this is the first case report to describe the CT and MRI findings of secondary RLP from prostatic adenocarcinoma.

Ha *et al*[14] previously reported CT features of metastatic RLP in 22 patients. Primary cancer included 18 gastric cancer patients, 1 patient with bladder cancer (transitional cell carcinoma), 1 patient with ovarian cancer (serous cystadenocarcinoma), 1 patient with cervical cancer (squamous cell carcinoma), and 1 patient with colon cancer. Twenty patients (90%) had more than 5 cm in length of rectal involvement. In 19 patients (86%), rectal wall thickening (mean, 1.6 cm) extended to the lower rectum close to the level of the anal verge. In 6 patients (27%), the rectum showed three zones of layered wall thickening (target sign): hyperattenuated inner zone, hypoattenuated middle zone, and hyperattenuated outer zone. Of the 16 patients without the target sign, 3 showed strong contrast enhancement in the rectum. Our patient also showed marked rectal wall thickening with its length involving more than 5 cm, and showed marked contrast enhancement without a target sign on CT.

In the present case, T2-weighted imaging revealed doubled-layered concentric thickening of the rectal wall with an isointense middle layer and hypointense inner and outer layers representing a target sign. Based only on initial CT images, our first impression was infiltrative malignancy, such as lymphoma involving rectum, since smooth overlying mucosal thickening and concentric narrowing in the rectum was seen. However, there were only two lymph node enlargements at both external iliac chains, which is an unusual finding of lymphoma. Furthermore, other conditions such as chronic radiation colitis may contribute to rectal narrowing with circumferential wall thickening, but the patient did not have a history of pelvic malignancy or radiation therapy. On MRI, the involved rectum demonstrated a target sign on both T2-weighted imaging and DWI, which is considered as one of specific finding for diagnosing RLP[12]. In addition, an infiltrative lesion with a low signal intensity on both T1- and T2-weighted imaging accompanied by heterogenous enhancement and low ADC values were revealed in the enlarged prostate gland. On PET/CT, there was no evidence of other sites with abnormal FDG uptake suggestive of metastasis or another primary cancer site. Based on these imaging findings and elevated serum PSA level, we suspected secondary RLP from prostatic adenocarcinoma.

In reviewing the literature, a characteristic pathologic finding of linitis plastica is an exuberant desmoplastic response that prominently elicits tumor cells and its product in submucosa and subserosa[2,16,17]. It is difficult to diagnose on endoscopy in most cases because of its characteristic mucosal sparing. Therefore, cross sectional imaging findings may be helpful to guide early and precise diagnosis of RLP, whether primary or secondary. When there is a concentric rectal wall thickening with a layered appearance resembling a target sign, an experienced radiologist would check the patient's history to make a differential diagnosis such as inflammatory bowel disease (*e.g.*, ulcerative colitis, Crohn's disease), pelvic radiation (radiation proctitis), or primary malignancy (secondary RLP). If the patient has no such history, the possibility of either primary or secondary RLP should be raised, and workup for primary cancer detection is necessary.

In the present case, there was a target sign on MRI which also revealed a similar pathologic finding on full-thickness excisional biopsy, in which metastatic prostate adenocarcinoma invaded the submucosa and muscularis propria while sparing the mucosal layer. Although the direct comparison of whole rectum and MRI could not be made, we assume that this pathologic finding on the biopsy specimen was well correlated with T2-weighted imaging and DWI showing a concentric ring pattern or target sign, which represents a double-layered wall thickening. On DWI, diffusion restriction can be caused by highly cellular tissue (*e.g.*, tumor tissues), intracellular edema, increased viscosity, tortuous extracellular space (*e.g.*, fibrosis with or without granulation tissue) and increased densities associated with hydrophobic cellular membranes[18]. We assume that in RLP, a tumor infiltrating the submucosa and muscularis propria associated with fibrosis and desmoplastic reaction would correlate with restricted diffusion in the middle zone, depicted as a target sign on DWI. The target sign could be a result of the normal zonal anatomy becoming more pronounced due to the invasion of an infiltrative tumor with fibrosis into the submucosa and on the longitudinal and circular layers of the proper muscle. In spite of substantial mural infiltration seen with RLP, the proper muscle layer is usually conserved, which may further explain the target sign[14]. Further studies with whole rectal specimens to make precise correlations with MRI are needed.

In conclusion, when there is a long segment of circumferential rectal wall thickening with luminal narrowing, a target sign on T2-weighted imaging and DWI is suggestive of RLP. Therefore, knowledge of a patient's primary cancer history at other organs and the secondary findings in abdominal organs can suggest the possibility of secondary RLP rather than primary RLP.

ARTICLE HIGHLIGHTS

Case characteristics

A 78-year-old male patient was admitted because of a 2-mo history of constipation

and luminal narrowing of the rectum on sigmoidoscopy.

Clinical diagnosis
A submucosal spreading tumor of the rectum, such as lymphoma, was suspected.

Differential diagnosis
Chronic radiation colitis, inflammatory bowel disease.

Laboratory diagnosis
Elevated serum prostate specific antigen, suggesting prostate cancer.

Imaging diagnosis
Findings from pelvic magnetic resonance imaging led to a diagnosis of secondary linitis plastica of the rectum due to prostate cancer.

Pathological diagnosis
Metastatic rectal linitis plastica (RLP) due to prostate cancer.

Treatment
Loop sigmoid colostomy for immediate symptom relief and hormone therapy for prostate cancer.

Related reports
Metastatic RLP due to prostate is extremely rare, and this is the first case report to describe both computed tomography and magnetic resonance imaging findings.

Term explanation
There are no uncommon terms used in this manuscript.

Experiences and lessons
Although rare, careful evaluation with a high suspicion for other sites of malignancy, including the prostate, is needed when RLP is suspected on imaging studies.

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CASE REPORT

Live birth after hysteroscopy performed inadvertently during early pregnancy: A case report and review of literature

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Abstract

Generally, hysteroscopy is not appropriate for pregnant women without an indication. What if a patient undergoes hysteroscopy accidentally during the early gestational period? We here report a rare case of a woman who continued pregnancy after a diagnostic hysteroscopy was performed in early pregnancy and delivered a healthy baby. The patient had a history of infertility and oligomenorrhea, probably due to a previous induced abortion. A hysteroscopy was performed after the end of her "menstruation" for assessment of her uterine cavity. Early pregnancy, instead of the expected intrauterine adhesions, was suspected, and the procedure was immediately ceased. Subsequent tests confirmed the diagnosis of pregnancy. She had a full-term delivery by elective caesarean section. The success of this case was attributed to the use of vaginoscopic techniques in hysteroscopy and correct judgment and decision-making during the procedure. This case report provides some useful methods and experience that might be helpful when a similar situation occurs in clinical practice.

Key words: Gestation; Hysteroscopy; Livebirth; Ongoing pregnancy

Core tip: An intended pregnancy without any suspec-

ted abnormalities is a contraindication for hysteroscopy. Although hysteroscopy is often scheduled in the follicular phase, potential pregnancy is unavoidable, even in patients with a history of infertility. When the image under hysteroscopy is not so typical for a doctor to recognize and immediately confirm a state of pregnancy, gentle and careful operation along with appropriate measures can benefit the patients to the largest extent.

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INTRODUCTION

Hysteroscopy is considered the gold standard for the evaluation of the uterine cavity[1]. Although diagnostic hysteroscopy is a technique with minimal invasiveness, it remains a contraindication for viable pregnancy. Whether the technique itself, the distention medium or the pressure used during the procedure produces harmful effects on early pregnancy remains unknown. Since Assaf *et al*[2] reported live births after the removal of intrauterine devices by hysteroscopy during early pregnancy, some similar cases have been reported on this issue. However, most cases had a definitive diagnosis of pregnancy beforehand, while hysteroscopy was only used as a treatment. Some cases occurred during the implantation period, and diagnosis of pregnancy was confirmed a few weeks after hysteroscopy. Here, we report a rare case of a woman whose pregnancy was unexpectedly identified during diagnostic hysteroscopy and the procedure did not disturb the pregnancy.

CASE REPORT

A 30-year-old woman was referred to our outpatient department because of hypomenorrhea and secondary infertility after an induced abortion performed three years previously. She was suspected to have intrauterine adhesions (IUAs) according to her symptoms and clinical history. She had regular menstrual cycles, and her past medical history was unremarkable. No abnormal signs were found by pelvic examination. A transvaginal sonography (TVS) was performed 20 d prior to hysteroscopy, and the result was normal.

The patient underwent hysteroscopy 4 d after the end of her "last menstrual period" (it was actually menstrual-like bleeding). A history of abstinence during the previous month was provided by the patient (it was actually a false medical history). We used a 4.5-mm rigid hysteroscope for examination in the absence of speculum, tenaculum, Pozzi forceps and cervical dilation. Vaginoscopic technique was adopted to reduce the pain generated by use of the above instruments. No anesthesia or analgesia was administered. The distention medium used was normal saline at room temperature. Diagnostic hysteroscopy revealed thickened and decidualized endometrium, but no obvious gestational sac was seen in the uterine *cavity* (Figure 1). Left tubal ostium was found (Figure 2), while the right tubal ostium could not be observed. Since pregnancy was suspected at that moment, we ceased the examination soon after.

Urine pregnancy test after hysteroscopy was positive. Since the pregnancy was so desired by the patient and her husband, serum human chorionic gonadotropin and progesterone were tested, and the results were 4917.67 mIU/mL and 54.1 nmol/L, respectively. A transvaginal ultrasound showed a 3-mm gestational sac in the uterine cavity (Figure 3). Minimal vaginal bleeding occurred in the following week. On a subsequent visit half a month later, the fetal heart was successfully detected by transvaginal ultrasound (Figure 4). Her pregnancy continued uneventfully until full term. An elective cesarean section was performed, and she gave birth to a normal boy weighing 3150 g. The woman has been followed up. The baby is now 11 mo old, and is as normal as other babies of the same age.

DISCUSSION

Termination of pregnancy such as induced abortion is regarded as one of the most important reasons for IUA[3]. Although clinical history, along with patient's manifestations and auxiliary examination like TVS, are helpful in the diagnosis of IUA, hysteroscopy is considered the gold standard for diagnosis and treatment of IUA. In general, hysterosalpingography (HSG), sonohysterography, TVS, and hysteroscopy are the most commonly used methods for detecting intrauterine lesions associated with infertility. However, many articles published have made the comparison among these techniques and have concluded that hysteroscopy is of higher value on the diagnosis of intrauterine lesions in infertile women[4-6]. With the progress of technology, hysteroscopy today is a simple, safe, and cost-effective outpatient procedure for diagnosis and treatment of intrauterine lesions.

Viable intrauterine pregnancy is a contraindication for hysteroscopy[1]. The procedure may cause infections or lead to abortion. A pregnancy test is regarded as a selective test but not a routine one before the procedure. It often depends on the complaints and clinical history of the patient[7]. In our case, the pregnancy test was not performed prior to hysteroscopy because the patient presented with "normal menstrual cycles", a history of abstinence during the preceding month and a long duration of infertility. We inadvertently performed hysteroscopy on the woman during her early pregnancy. However, the procedure, which was carried out with normal saline as the distention medium, did not disrupt the gestation. The woman eventually had a successful delivery.

Live birth outcomes have been reported in some

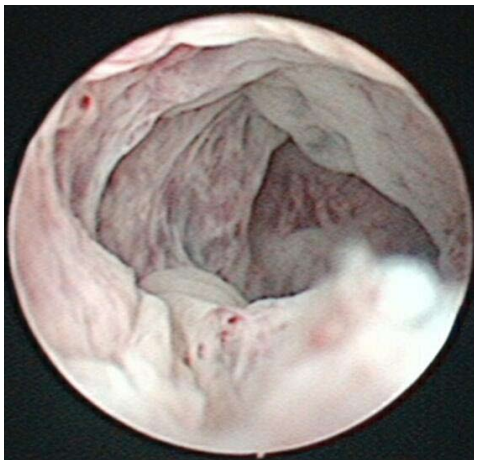

Figure 1 Image of intrauterine cavity under hysteroscopy. Thick and decidualized endometrium indicates the possibility of early pregnancy.

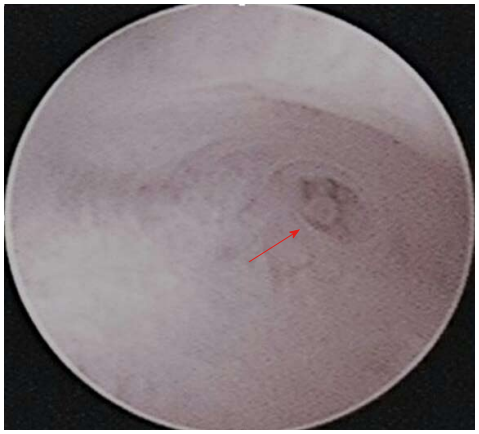

Figure 2 Left tubal ostium is seen, indicated by the red arrow.

cases when using invasive measures such as hysteroscopy, HSG, laparoscopy and chromotubation during pregnancy.

In 1992, Assaf *et al*[2] reported successful pregnancy outcomes after removal of intrauterine devices by CO_2 hysteroscopy during early pregnancy. As was reported, 31 of 50 patients achieved full-term pregnancy after the procedure[2]. Last year, Cohen *et al*[8] reported that seven pregnant patients who had undergone removal of intrauterine devices by hysteroscopy delivered at term without obstetric complications. Along with these two articles, some other similar articles have confirmed the effectiveness and safety of hysteroscopy in removing the intrauterine devices during pregnancy. Besides, McCarthy *et al*[9] reported a case that a levonorgestrel intrauterine system was successfully removed by ultrasound-guided hysteroscopy during early pregnancy. Moreover, despite exposure to the levonorgestrel intrauterine system during first-trimester, the female baby was normal[9]. AL-Mizyen *et al*[10] reported a live birth after bilateral ovarian diathermy and hysteroscopy performed during early pregnancy. Erenus and Sezen[11] described a case of ongoing pregnancy in a woman who underwent hysteroscopy during the implantation phase. Justesen *et al*[12] reported four cases of HSG performed inadvertently during early pregnancy. One of them had a live birth[12]. Kuo *et al*[13] reported a case of live birth after inadvertently performing HSG during early pregnancy. After seven years of follow-up by the authors, the growth and development of the child was found to be normal[13]. Opsahl[14] reported three cases of full-term deliveries after laparoscopy and chromotubation performed during the implantation phase. Dwivedee and Banfield reported a live birth after inadvertent hysteroscopy and laparoscopy in a patient of uterus didelphys in early pregnancy[15]. Last year, Pontré and McElhinney reported a live birth occurring post-radical laparoscopic excision of endometriosis, hysteroscopy, curettage and test of tubal patency during the early implantation phase[16]. All of these cases indicate that early pregnancy is invulnerable most of the time. However, images under hysteroscopy during early viable pregnancy are rare.

Although early pregnancy is invulnerable, it may become very fragile due to an inappropriate decision made during invasive procedures. Sometimes it is difficult for a doctor to make an accurate diagnosis when such uncommon images are visualized under hysteroscopy. It needs to be differentiated from endometrial lesions. Instead of immediate biopsy or even dilation and curettage, performing further confirming tests is the optimal choice. In our case, we ceased the procedure at once and prescribed a urine pregnancy test to confirm the tentative diagnosis of early pregnancy. However, instead of discontinuing the hysteroscopic procedure, the best option would be to pause the procedure, collect a urine sample and test it for urinary beta human chorionic gonadotropin at the time of surgery.

Hysteroscopy is often scheduled in the follicular phase of the menstrual cycle. However, performing hysteroscopy inadvertently during an unexpected early pregnancy seems inevitable. Menstrual-like bleeding may be regarded as normal, especially in oligomenorrhea. To avoid making mistakes, many units now rely on a history of abstinence or the use of contraception in the preceding month. As in our case, a preoperative discussion between doctor and the patient regarding contraception and unprotected sexual intercourse was done 2 mo prior to the procedure. However, the patient denied any history of sexual intercourse during the previous month. She provided a false history in order to not wait another month. This case also emphasizes the importance of effective and successful communication. Pregnancy test before hysteroscopy is regarded to not cost-effective and is not recommended for all patients[7]. However, according to Herr *et al*[17], one of 410 women presenting for HSG was found to have an unsuspected early pregnancy, which was detected with a point-of-care urine pregnancy test. The authors suggested a routine pregnancy test before HSG[17]. In the same way, consideration should be given to routine pregnancy testing of women with infertility before hysteroscopy

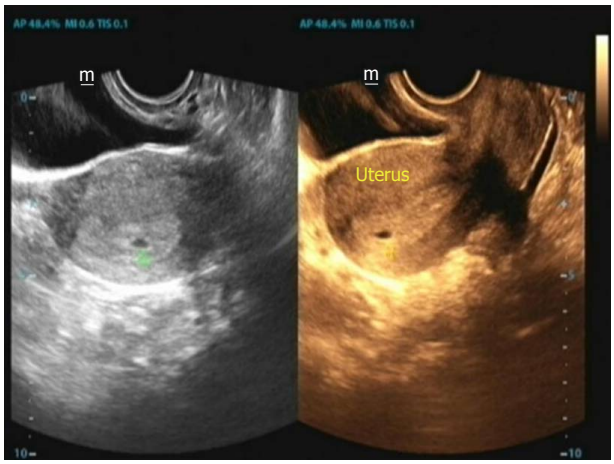

Figure 3 Transvaginal ultrasound after hysteroscopy. The image shows a 3-mm gestational sac in the uterine cavity.

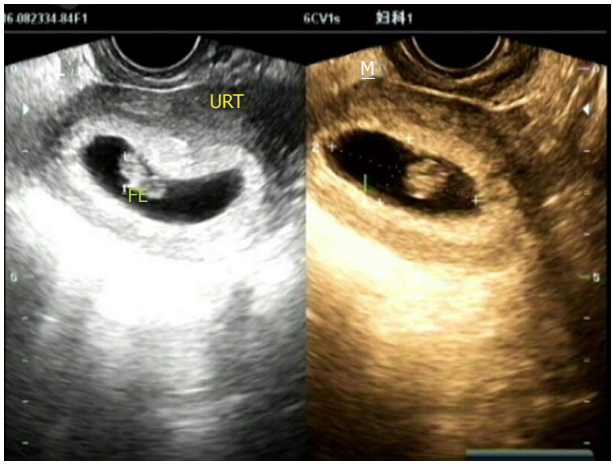

Figure 4 Transvaginal ultrasound 16 d after hysteroscopy. Fetal heart was detected.

because scheduling on the basis of menstrual cycle can be unreliable.

The case highlights the importance that hysteroscopy should be performed with caution at any time. An unexpected situation as in this case should always be kept in mind in order to avoid disturbance of a potential normal pregnancy during invasive procedures. Even a patient with a long history of infertility may acquire pregnancy at any time.

In addition, vaginoscopic technique is very important in such a case. It can reduce the pain caused by use of a speculum and cervix dilation, which may induce a miscarriage. Vaginoscopy is now recommended as a standard technique for outpatient hysteroscopy[1].

In conclusion, we report a case of live birth after hysteroscopy was inadvertently performed during early pregnancy. Although hysteroscopy is often scheduled in the follicular phase, potential pregnancy is unavoidable. Gentle and careful operation, timely identification of images under hysteroscopy and taking appropriate measures can benefit the patients to the largest extent.

ARTICLE HIGHLIGHTS

Case characteristics

It is inevitable to encounter a potential pregnancy under hysteroscopy during the follicular phase. Gentle and careful operation, timely identification of images under hysteroscopy and taking appropriate measures can benefit the patients to the largest extent.

Clinical diagnosis

Early pregnancy.

Differential diagnosis

Endometrial lesions.

Laboratory diagnosis

Urine pregnancy test confirmed the diagnosis of pregnancy.

Imaging diagnosis

Thickened and decidualized endometrium suggested a suspected diagnosis of early pregnancy.

Treatment

A follow-up of the patient until full-term delivery, without treatment or drug used.

Related reports

A few cases have been published on this issue. However, most of the patients in those cases underwent hysteroscopy during implantation phase and the images under hysteroscopy were normal. However, no hysteroscopic images were provided in these cases.

Term explanation

The levonorgestrel intrauterine system (LNG IUS) is a T-shaped, plastic, contraceptive IUS that releases the progestin hormone levonorgestrel into the uterus at a dose of 20 μg/d for up to five years. LNG IUS prevents pregnancy by thickening cervical mucus, inhibiting sperm motility, and suppressing the growth of the uterine wall.

Experiences and lessons

Consideration should be given to routine pregnancy testing of women with infertility before hysteroscopy, because scheduling on the basis of menstrual cycle dates can be unreliable. It is very important to perform hysteroscopy gently and carefully.

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CASE REPORT

Mesh migration into the sigmoid colon after inguinal hernia repair presenting as a colonic polyp: A case report and review of literature

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Author contributions: Ji F designed the study; Liu S collected the patient's clinical data and studied the relevant literature; Zhou XX, Zhong WX and Li L reviewed the data; Liu S analyzed the data and wrote the manuscript; Yu MS and Zhang H edited the manuscript and figures.

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Abstract

Mesh migration and penetration into abdominal viscera rarely occur after laparoscopic inguinal hernia repair. We present the first case of mesh migration into the sigmoid colon identified as a colonic polyp at initial colonoscopic examination. The patient complained of mild abdominal distention in the lower abdomen over the previous year without changes in bowel habits or stool appearance and without weight loss. By complementary endoscopic ultrasonography, a cavity-like structure beneath the suspected polyp was further confirmed. Enhanced abdominal computed tomography merely revealed local bowel wall thickening and inflammation of the colosigmoid junction. The migrating mesh, which was lodged in the sigmoid colon and caused intra-abdominal adhesion in the lower abdominal cavity, was finally identified via exploratory surgery. The components of inflammatory granulation tissue around the mesh material were diagnosed based on histological examination

of the surgical specimen after sigmoidectomy. In this patient, nonspecific endoscopic and imaging outcomes during clinical work-up led to the diagnostic dilemma of mesh migration. Therefore, the clinical, radiological and endoscopic challenges specific to this case as well as the underlying reasons for mesh migration are discussed in detail.

Key words: Colonoscopy; Surgical mesh; Hernia repair; Sigmoid colon; Colonic polyps; Computed tomography; Foreign bodies

Core tip: Mesh migration and penetration into abdominal viscera are rarely reported as a long-term complication after inguinal hernia repair. In this case, a migrating prosthetic mesh penetrated the sigmoid colon in a 59-year-old male patient after bilateral inguinal hernioplasty. The migrating mesh mimicked a "colonic polyp" under endoscopy, while it was almost absent on radiological imaging and caused no obvious symptoms. This has never been reported in the previous literature, and it enhanced preoperative diagnostic difficulty. Therefore, clinical, radiological and endoscopic aspects of the case and, more importantly, the possible factors accounting for mesh migration and erosion are analyzed and summarized.

Liu S, Zhou XX, Li L, Yu MS, Zhang H, Zhong WX, Ji F. Mesh migration into the sigmoid colon after inguinal hernia repair presenting as a colonic polyp: A case report and review of literature. *World J Clin Cases* 2018; 6(12): 564-569 Available from: URL: http://www.wjgnet.com/2307-8960/full/v6/i12/564.htm DOI: http://dx.doi.org/10.12998/wjcc.v6.i12.564

INTRODUCTION

The tension-free method with mesh as a muscle reinforcement technique is regarded as an important part of inguinal hernia repair since it reduces the hernia recurrence rate and recovery period. Superficial wound infection and chronic pain associated with prosthetic mesh are well-known complications[1], which mainly occur in the early postoperative period. However, serious complications, such as mesh migration and perforation of adjacent organs, are rarely reported and may present symptoms at different time intervals after inguinal hernia repair. Colon penetration by inguinal hernia repair mesh can possibly cause formation of inflammatory granulation tissue within the injured bowel wall leading to misdiagnosis or missed diagnosis. We report the first case of chronic mesh penetration into the sigmoid colon, where the migrating mesh appeared to be a colon polyp under endoscopy, while it was almost asymptomatic and invisible in enhanced computed tomography (CT).

CASE REPORT

The patient was a 59-year-old man with a history of Stoppa-repair for bilateral inguinal hernia 7 years previously. Shortly thereafter, he developed a mild superficial wound infection in the right groin region. However, the infection improved without any medical treatments and did not recur. One year ago, he underwent general examinations in a local hospital due to mild lower abdominal distention. Colonoscopy revealed a "polyp" (2.5 cm × 2.0 cm in size), which was hyperemic and erosive on mucosa and was situated on the upper segment of the sigmoid colon. The colonic lesion was suspected to be an inflammatory protuberance or malignant neoplasm, hence it was biopsied but left untreated. The "polyp" was defined as mucosal inflammation based on histological characteristics. Therefore, the patient was referred to our hospital for further examinations and treatment.

On admission to our gastroenterology department, the patient did not appear to be sick. Neither tenderness on palpation nor rebound tenderness was positive on physical examination. The stool test showed occult blood of 1+. The abdominal CT revealed local bowel wall thickening and inflammatory stranding involving the colosigmoid junction, accompanied by bowel gas accumulation and extension of the proximal sigmoid colon segment (Figure 1). Hyperemic polypoid mucosal changes in the sigmoid colon (approximately 28 cm from the anal verge) were observed *via* colonoscopic re-examination, which occupied 1/4 of the bowel wall circumference (Figure 2A). The lesion was oozing a pus-like substance and was covered with mucus. Complimentary endoscopic ultrasonography confirmed the mucosal lesion to be heterogeneously isoechoic or hypoechoic, with a cavity-like structure below (Figure 2B). It bled easily when biopsied. Pathological examination showed chronic mucosal inflammation with components of necrotizing inflammation. We presumed the colonic lesion to be atypical colonic diverticulitis or a localized abscess. However, there were no positive laboratory test results (white blood cell, erythrocyte sedimentation rate, peripheral blood culture) supportive of our suspicion; hence, we determined that the patient would not benefit greatly from antibiotic therapy, and therefore, no antibiotics were used. An exploratory surgery was undertaken, and endoscopic carbon nanoparticle tattooing was utilized to position the colonic lesion prior to surgery. Screening for enlarged intra-abdominal lymph nodes was negative during laparoscopic intraperitoneal observation. However, the sigmoid colon was found firmly adhered to the abdominal wall. The mesh material was not exposed in the abdominal cavity until adhesiolysis was performed. We identified the presence of left-sided hernia repair mesh penetrating the sigmoid colon from the preperitoneal space (Figure 3). No obvious sinus or fistulas were found between the mesh and colon lumen. Sigmoidectomy with remo-

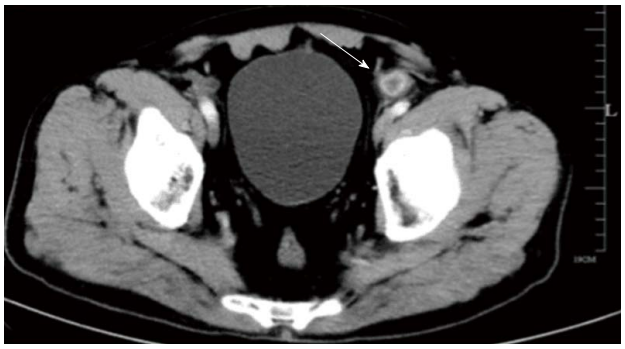

Figure 1 Abdominal computed tomography findings. Abdominal computed tomography showed bowel wall thickening and inflammatory stranding involving the colosigmoid junction (white arrow).

val of the mesh was performed. Simultaneously, mild adhesion between the right-sided abdominal wall and colon was also observed and separated. The patient received flurbiprofen for postoperative analgesia and prophylactic antibiotics (ornidazole combined with latamoxef intravenously for 3 d) perioperatively. Pathological analysis of surgical specimen confirmed the substance of the foreign body within the bowel wall along with adjacent inflammatory granulation tissue formation (Figure 4). He was discharged on postoperative day 6 without major complications and recovered uneventfully.

DISCUSSION

Over the past four decades, increasingly wide utilization of hernia-repair mesh during laparoscopic inguinal hernioplasty has significantly reduced the recurrence rate of inguinal hernia. With the introduction of trocars, mesh implantation is carried out distally from the trocar incision[2], and the superficial infection rate has decreased dramatically (less than 2%)[3]**.** In comparison, other complications induced by mesh, such as foreign body reaction, deep-seated infection, consequent mesh migration and perforation into viscera, have been reported sporadically. Incidence rates for such complications remain unknown[1]. The intestine and urinary bladder were involved in most cases of mesh migration reported from 2003 to 2017[1,4-10]. A relatively rare case of a migrated mesh in the retroperitoneal region mimicking a cystic adnexal mass was also documented previously[11].

Depending on the different positional relationship of migrating mesh with visceral organs, clinical manifestations vary significantly and may present from 1 to 20 years after inguinal hernia repair[12]. Lower abdominal pain and mild tenderness were described in the majority of cases[4,13], while weight loss, anorexia, symptoms of bowel obstruction, palpable abdominal mass were merely referred to by a few reports[9,14]. In our case, the male patient complained of mild abdominal discomfort, without other symptoms or positive physical signs, which led to diagnosis delay. Furthermore, the colon-embedded mesh was chronically mildly infected and surrounded by inflammatory granulation tissue, which limited the diagnostic value of colonoscopy and enhanced abdominal CT. Typical signs caused by gastrointestinal perforation or peritonitis were almost absent on the radiograph. In previously reported cases, migrating mesh plugs were neglected or misdiagnosed as a poorly defined mass[4], an intra-abdominal neoplasm[9] or sigmoid diverticulosis[13] based on radiological investigations.

Incomplete peritoneal repair, inadequate fixation or inappropriate amount of implantation space are possible reasons accounting for mesh migrating into intra-abdominal viscera, occasionally followed by fistulas formation or mechanical bowel obstruction[15]. In addition, the sharp edges of prosthetic mesh or tackers could injure the viscera serosal layer[16,17], initiating the intra-abdominal inflammatory process and subsequent mesh erosion. The bowel injury incidence rate ranged between 0.4% and 5.6% in previous studies[18]. Considering that the patient in our case was almost asymptomatic, the factors responsible for painless mesh migration are as follows: (1) The foreign body reaction to mesh enables gradual movement of the mesh through the anatomic planes in the abdominal cavity, particularly along the paths of low resistance[17]; (2) In some occasions, the mesh can be encapsulated by the omentum during its migration and create a channel into hollow organs along with inflammatory reaction and peristaltic bowel movement[5]; (3) Gram-positive cocci are generally responsible for superficial wound infection and can further trigger the deeper infection. Bacterial biofilm can develop over the mesh due to chronic contamination by staphylococcus species, which results in painless mesh migration through the tissue[19] and (4) Prosthetic mesh material decreases the formation of the mesothelial cell layer in peritoneal repaired defects, predisposing the irregular surface of mesh to be surrounded by scar tissue, thus the inflammation is localized.

To prevent further erosion of migrating mesh and preserve the function of affected viscera, total removal of the mesh via laparoscopy or laparotomy is advised in clinical practice, along with either partial or entire resection of the organ[1]. Meanwhile, the possible wound sinus or enteric fistulas linked to the mesh should be completely eradicated by excision in combination with medication therapy (antibiotics, somatostatin and parenteral nutrition). Tailoring the mesh, appropriate suture placement and adherence to principles of antisepsis during hernia repair surgery are crucial in avoiding long-term mesh-related complications.

ARTICLE HIGHLIGHTS

Case characteristics

A 59-year-old male patient developed mild lower abdominal distention 7 years after repair surgery of a bilateral inguinal hernia. A colonic lesion was found under his endoscopic examination and was suspected to be a polyp. However, the complementary radiological imaging and subsequent endoscopic ultrasonography (EUS) failed to provide enough clues for exact diagnosis. The patient was referred for explorative surgery, during which a prosthetic mesh was confirmed as migrating into the sigmoid colon from its original

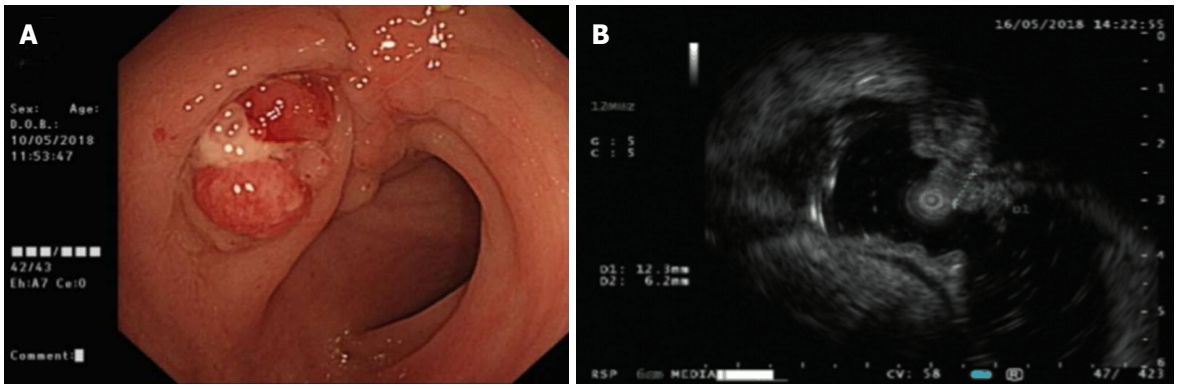

Figure 2 Endoscopic findings. A: Colonoscopy revealed a polypoid lesion in the sigmoid colon, which was hyperemic and oozed a pus-like substance; B: Endoscopic ultrasonography showed a mucosal lesion (1.23 cm × 0.62 cm) with a cavity-like structure below in sectional dimension.

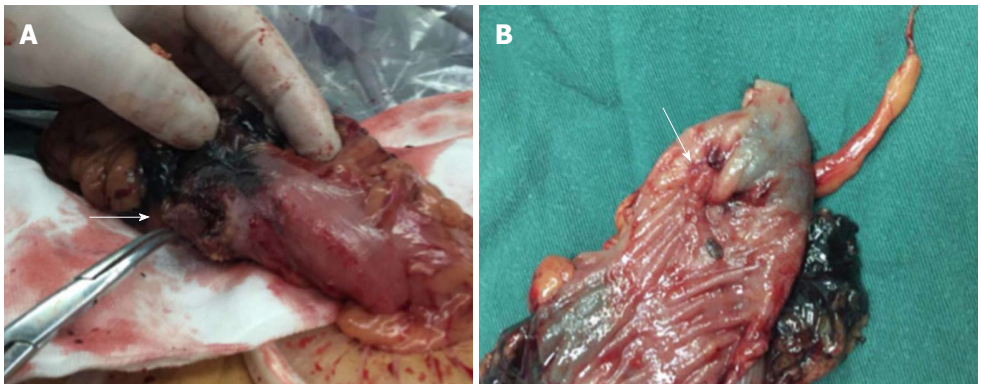

Figure 3 Intraoperative findings. A: Mesh (arrow) penetrated the sigmoid colon and was intimately involved in the bowel wall; B: The "polyp" (slanted arrow) was observed on the luminal side of the bowel wall.

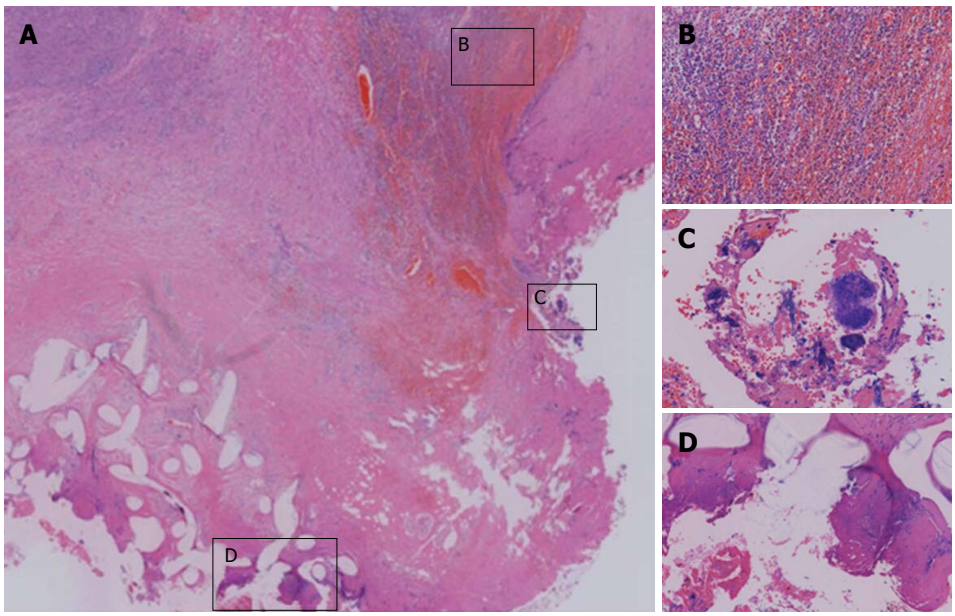

Figure 4 Histological findings revealed by Hematoxylin and Eosin staining of paraffin-embedded sections from the surgical specimen. A: The presence of a foreign body in the bowel wall, which caused inflammatory infiltrate and granulation tissue formation in the surrounding tissue (magnification × 10); B: Infiltration of massive inflammatory cells and formation of granulation tissue (magnification × 100); C: Foreign-body giant cells were observed (magnification × 200); D: Prosthetic mesh material (magnification × 100).

position. Sigmoidectomy with removal of the mesh was performed. Histological investigations also demonstrated the existence of a foreign body within the

affected bowel wall.

Clinical diagnosis
Mesh migration into the sigmoid colon after inguinal hernia repair.

Differential diagnosis
Based on the endoscopic and radiological investigations from this case, a colonic polyp caused by migrating mesh should be differentiated from an inflammatory protuberance, malignant neoplasm, atypical colonic diverticulitis or a localized abscess involved in the sigmoid colon. Abdominal distention occurring in the lower abdomen could indicate functional disorders of the intestine, intestinal infection, enteritis, bowel obstruction, habitual constipation, hernia, diseased urinary bladder or gynecological diseases (for women) and so on.

Laboratory diagnosis
A stool test showed occult blood of 1+. However, there were no positive results from other laboratory tests (*e.g.*, white blood cell, erythrocyte sedimentation rate, peripheral blood culture).

Imaging diagnosis
Abdominal computed tomography (CT) showed bowel wall thickening and inflammatory stranding of the colosigmoid junction with bowel gas accumulation and extension of the proximal colon segment. Hyperemic polypoid mucosal lesion was observed in the sigmoid colon via colonoscopy, which was located at approximately 28 cm from the anal verge. EUS identified a cavity-like structure beneath the heterogeneously isoechoic or hypoechoic mucosal lesion.

Pathological diagnosis
Chronic mucosal inflammation with components of necrotizing inflammation was confirmed by histological investigation of the endoscopic tissue biopsy from the "colonic polyp". Pathological features of the surgical specimen showed the substance of the foreign body within the defected bowel wall of the sigmoid colon, along with adjacent inflammatory granulation tissue formation.

Treatment
Sigmoidectomy with removal of mesh and lysis of the adhesions between lower abdominal wall and colon were performed. The patient received flurbiprofen for postoperative analgesia and prophylactic antibiotics (ornidazole combined with latamoxef) perioperatively. He recovered uneventfully, and abdominal distention was relieved thereafter.

Related reports
Penetration and erosion of migrating hernia repair mesh into the small bowel, cecum, transverse colon, sigmoid colon, urinary bladder or retroperitoneal region were previously reported in the literature. Most patients complained of abdominal pain and mild tenderness, and pain occasionally increased with food intake. An abdominal mass could be palpable when migrating mesh initiates severe adhesions between viscera. Meanwhile, symptoms including weight loss, anorexia, and fatigue could develop. Bowel obstruction could occur due to intraluminal penetration of migrating mesh.

Term explanation
Mesh migration and penetration into viscera are rare complications after laparoscopic inguinal hernia repair, which could present at different time intervals postoperatively. For the most part, the variable and nonspecific clinical manifestations caused by migrating mesh lead to diagnosis delay. Total removal of the deep-seated prosthetic mesh with organ resection via laparoscopy or laparotomy is first considered and advised in clinical practice. Possible wound sinus or enteric fistulas linked to mesh should be cautiously explored and completely eradicated by excision in combination with drug therapy (antibiotics, somatostatin and parenteral nutrition). Colonoscopic retrieval of intraluminal migrating mesh can be attempted in absence of enteric fistulas.

Experiences and lessons
Mesh migration after inguinal hernia repair is difficult to detect or distinguish *via* imaging modalities due to the nonradiopaque property of mesh prosthesis. Metal clips or tackers used to fasten mesh are radiopaque but still occasionally missed by internists. In addition, inflammatory tissue formation caused by foreign body can prevent an accurate preoperative diagnosis. Therefore, the case-based learning as well as detailed collection of patients' medical history provides clinicians with more clues to analyze CT scan with orientation. Overreliance on ultrasonic or radiological investigations occasionally leads to misdiagnosis and missed diagnosis of specific foreign bodies.

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CASE REPORT

CNKSR2 mutation causes the X-linked epilepsy-aphasia syndrome: A case report and review of literature

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Author contributions: Kong QX and Wang YL designed the study and wrote the manuscript; Sun Y, Liu YD and Xu ZF collected the clinical data and edited the manuscript.

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Abstract

The mutation in *CNKSR2* leads to a broad spectrum of phenotypic variability and manifests as an X-linked intellectual disability. However, we reported that the male patient in this study not only had intellectual disability but also epileptic seizures. In addition, there were progressive language impairment, attention deficit hyperactivity disorder and autism. Electroencephalograms showed continuous spike-and-wave during sleep. Genetic testing revealed a *de novo* mutation of the *CNKSR2* gene (*c.2185C>T*, *p.Arg729Ter*) in the child that was not detected in the parents. Therefore, the child was diagnosed with X-linked epilepsy aphasia syndrome. Deletion of the *CNKSR2* gene has been rarely reported in epilepsy aphasia syndrome, but no *de novo* mutation has been found in this gene. This report not only adds to the spectrum of epilepsy aphasia syndrome but also helps clinicians in diagnosis and genetic counseling.

Key words: Epilepsy; Language impairment; Mental retardation; *De novo* mutation of *CNKSR2*; X-linked epilepsy-aphasia syndrome

Core tip: Patient with epileptic seizures and progressive language impairment. Genetic testing revealed a *de novo* mutation of the *CNKSR2* gene in the child and was not detected in the parents. Therefore, the gene may lead to X-linked epilepsy aphasia syndrome.

Sun Y, Liu YD, Xu ZF, Kong QX, Wang YL. *CNKSR2* mutation causes the X-linked epilepsy-aphasia syndrome: A case report and review of literature. *World J Clin Cases* 2018; 6(12): 570-576 Available from: URL: http://www.wjgnet.com/2307-8960/full/v6/i12/570.htm DOI: http://dx.doi.org/10.12998/wjcc.v6.i12.570

INTRODUCTION

Atypical epilepsy-aphasia syndrome is caused by Landau-Kleffner syndrome (LKS) and epileptic encephalopathy, with a continuous spike-and-wave pattern during sleep[1]. The synapse is the core component of brain operations and executive functions, and its function plays an important role in brain neuron function[2]. *CNKSR2* is located on the X chromosome, and as a synaptic protein, it is involved in RAS/MAPK signal transduction[3]. It is highly expressed in the brain[4], and its mutation or deletion causes a wide range of neurodevelopmental defects[5]. Currently, *CNKSR2* deletion or mutation has been shown to induce symptoms that are part of the EAS spectrum[6-9]. In this paper, we review clinical data and genetic test results of a child with epilepsy and aphasia and have identified a *de novo* mutation: *CNKSR2* (*c.2185C>T*, *p.Arg729Ter*). We reviewed the literature and analyzed the clinical features of X-linked epilepsy-aphasia syndrome in order to assist clinicians in their diagnosis of this condition and to help provide genetic counseling.

CASE REPORT

An 8-year and 8-mo-old boy from China was admitted to the hospital due to paroxysmal unconsciousness for more than 6 years. The performance of paroxysmal loss of consciousness was associated with brief jerks of the limbs, eye staring, lip bruising, spitting foam from the mouth, and sometimes with urinary incontinence; however, fever was absent. Each episode lasted 2-3 min and resolved on its own. The episodes of epileptic seizures occurred in varying lengths of times; sometimes, the episodes occurred once every six months, and sometimes only once a month. The form of each seizure was similar.

During the clinical examination, the child did not have any abnormal physical signs. There was no abnormal language expression before epileptic episodes. After the onset of a seizure, he gradually showed signs of poor language expression, repeated speech, unanswerable questions, uncooperative actions, and attention deficit hyperactivity disorder (ADHD). The child had intellectual disability, childish behavior, poor communication skills with the outside world, and autism performance. Parents were healthy and involved in a nonconsanguineous marriage, and family history was not significantly abnormal. Maternal pregnancy was normal, and perinatal history of hypoxia and asphyxia was denied. The Apgar score was unknown.

Auxiliary check: There were no abnormalities in routine laboratory examinations, ultrasonography or craniocerebral MRI. His video-electroencephalogram (EEG) (Figure 1) results showed an abnormal EEG and supported the diagnosis of epilepsy.

Gene detection

Two milliliter of peripheral venous blood was extracted from the patient and her parents. Genomic DNA from the patient was extracted from blood using standard methods for whole exome sequencing. Mutation of *CNKSR2* gene was found in the children. Primers were designed based on the gene tested (chrX:21627228). The parents used Sanger sequencing after PCR to analyze the coding exons and flanking introns of the *CNKSR2* gene (NM_014927). The established variant was sequenced in both forward (AGTCCCCAAGCCCAAGCTAC) and reverse directions (ACTGGCTGTCTTGCGAATGG). A nucleotide variation of c.2185C>T (code no. 2185 nucleotides changed from C to T) was identified in the patient's *CNKSR2* gene. The mutation altered the codon sequence of the amino acid Arg into a termination codon (p.Arg729Ter). No abnormalities were identified at this site in the parents. The mutation was *de novo* (Figure 2). The child was eventually diagnosed with X-Linked EAS, and was treated with hormone and anti-epileptic drugs (Sodium valproate, Levetiracetan). After these treatments, his seizures had eased.

To highlight changes in the secondary structure of the *CNKSR2* gene, we used the more popular PSIPRED (http://bioinf.cs.ucl.ac.uk/psipred/)[10] for structural prediction. The codon of no. 729 amino acid Arg was altered into a termination codon (p.Arg729Ter), resulting in the inability to express the 729-1034 sequence of amino acids (Figure 3). RaptorX (http://raptorx.uchicago.edu)[11-13] can predict protein tertiary structures. After inputting the sequence, the 3D structure of the protein sequence can be predicted from the protein database (PDB) (Figure 4). Compared with the wild type, the patient's *CNKSR2* gene did not fold completely in its spatial structure, thus affecting protein function.

DISCUSSION

CNKSR2 (also known as *CNK2, KSR2, MAGUIN*)[8] interacts with synaptic scaffold molecules (S-SCAM) and the postsynaptic density (PSD)-95/synaptic-associated protein (SAP) 90 to form a complex[14]. The complex is involved in RAS/MAPK signaling and mediates neuronal proliferation, migration, differentiation and death, as well as RAS-mediated synapse formation[5-9]. It also connec-

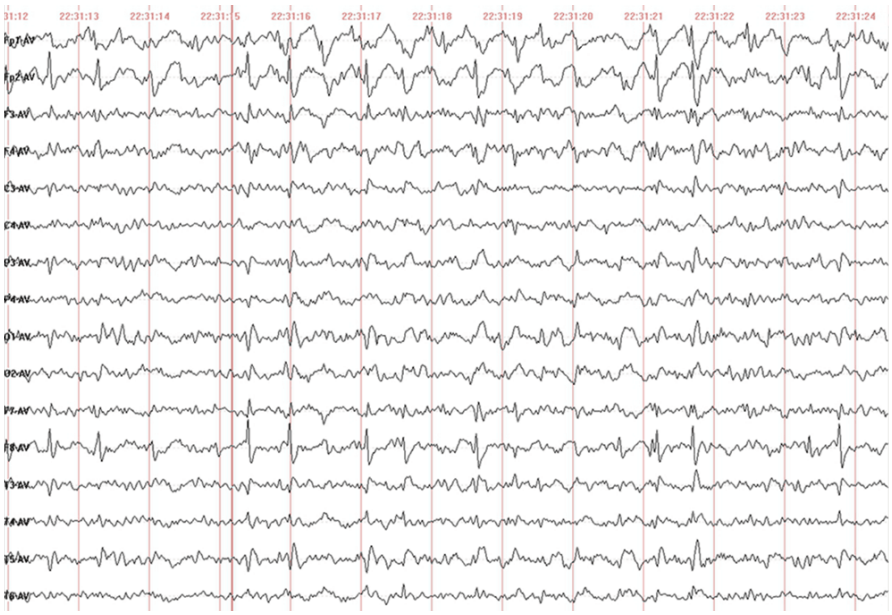


Figure 1 Electroencephalogram of the patient. It showed generalized continuous spike-and-wave patterns in the bitemporal and frontal lobes, noticeable on the left side. Abnormal discharge was more pronounced during the sleep-electroencephalogram. Slower on background activity.

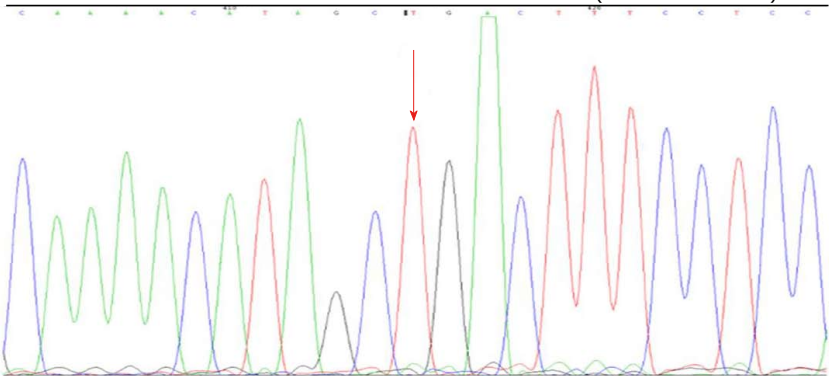
ts N-methyl-D-aspartate (NMDA) receptors to neuronal cell adhesion molecules[14]. The NMDA subunit encoded by *GRIN2A* is the first gene associated with EAS[2]. *GRIN2A* mutations reduce NMDA receptor trafficking and agonist potency–molecular profiling as well as functional rescue[15]. *GRIN2A* gene is a rare causative gene in Chinese patients with EAS, suggesting the possibility of other genes being involved in the pathogenesis[16]. Hence, we speculate that a mutation or deletion of *CNKSR2* may result in changes to the NMDA receptor activity and might affect downstream signaling cascades. Abnormal NMDA receptor will potentially damage the cortical thalamus network during sleep[17]. *CNKSR2* is highly expressed in the brain (especially in the hippocampus, amygdala, and cerebellum), and mutations result in loss of specificity and might also affect brain function[7], leading to seizures and neurodevelopmental disorders that especially affect the patient's speech expression[2]. *CNKSR2* is a gene located on the X chromosome, and its mutations or deletions lead to X linkage intelligence disorder (XLID)[8]. The main features of XLID are: (1) intellectual disability; (2) highly restrictive speech (especially expression of language); (3) ADHD; (4) transient childhood epilepsy; and (5) epilepsy with continuous spike waves of slow-wave sleep (CSWS) in early childhood[5].

Before experiencing seizures, our patient suffered from developmental delays and ADHD, which is consistent with the performance of X-linked intellectual disability. After seizure occurrence, the patients' speech expression gradually decreased, the EEG continued to show abnormal wave patterns during sleep, and a *de novo* mutation of the *CNKSR2* gene was identified. Therefore, we diagnosed this patient as X-linked epilepsy-aphasia syndrome. After definite diagnosis, patients were given immunoglobulin (400-500 mg/kg per day, 3-5 d for 1 course) and oral prednisone (from 1-3 mg/kg per day, and after one month, changed to 1 mg/kg per day), with a total course of 6 to 12 mo. Meanwhile, lamotrigine (75 mg/qd) and sodium valproate oral solution (6 mL/bid) were continued for antiepileptic treatment. At telephone follow-up one year later, the child had fewer epileptic seizures than before as well as partial improvement in verbal ability and an ability to repeat speech; however, the patient had no improvement in intelligence. The disease duration was more than 6 years. If diagnosed early and actively treated, the patient's intelligence, seizures, and language may have been better mitigated.

The underlying mechanism for EAS disorders occ urrence remains unknown, although environmental factors such as thalamic injury[18] and immunity disorders[19], with evidence of onconeural antibodies that can cause the EEG phenotype, have been reported. Studies have shown that the antibodies of brain endothelial cells and nuclei in children were elevated[20]. Additionally, inflammatory markers of children with electrical status epilepticus in sleep (ESES) may be increased[21]. Some researchers have proposed a potential autoimmune reaction secondary to blood-brain-barrier disruption from a thalamocortical uncoupling secondary to the spike-wave activation seen in slow-wave sleep[22]. Furthermore, few genetic causes of ESESS/CSWSS/epilepsy aphasia spectrum have been reported, where the common underlying pathway is channelopathy[23]. The different forms of seizures in EAS include partial seizure, generalized tonic-clonic seizure, atypical absence seizure, myoclonic seizure, atonic seizure, *etc*. Aphasia can occur before or after epilepsy. Moreover, 70% of patients with epilepsy-aphasia syndrome have epileptic seizures with EEG features that reveal spike-and-wave patterns in the unilateral or bitemporal lobes during the waking period.

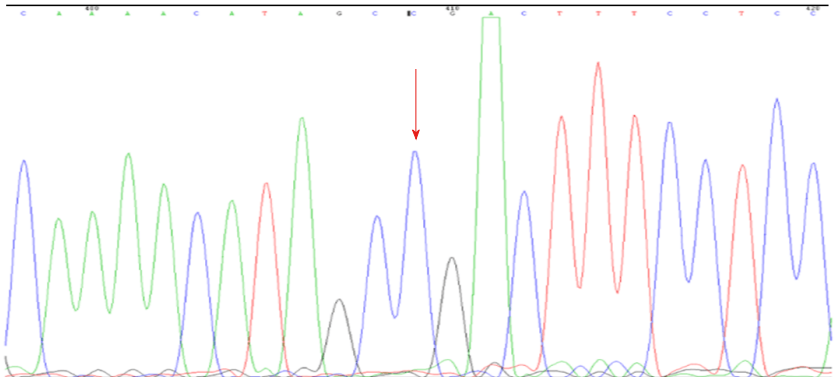
A

Sample	Gene	Site	Variation	Variant type
Patient	*CNKSR2*	chrX:21627228	*c.2185C>T*	Hemizygous (male X chromosome)



B

Sample	Gene	Site	Variation	Variant type
Father	*CNKSR2*	chrX:21627228	*c.2185C>T*	No variation



C

Sample	Gene	Site	Variation	Variant type
Mother	*CNKSR2*	chrX:21627228	*c.2185C>T*	No variation

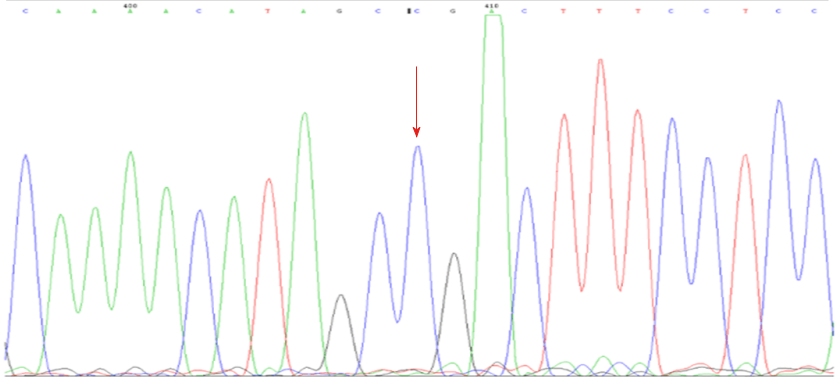


Figure 2 Gene sequences of three members in the family. A: *De novo* mutation of the *CNKSR2* gene (*c. 2185C>T*, *p.Arg729Ter*) in the patient; B, C: No mutation was observed at the same locus in the parents (arrows).

Generalized continuous spike-and-wave is seen in all leads during sleep, and bilateral synchronous discharge accounts for more than 85% of stage in abnormal discharge[1]. The other 30% of children do not seizures, but show EEG abnormalities (which does not meet the EEG standards of CSWS). Their EEG's showed the following during sleep: Induced focal epileptiform discharges were identified mainly at the center (but may also might be involved in other areas); or there was no bilateral synchronous activity; or synchronous activity accounted for less than 85% of NREM (non-rapid eye movement) sleep. These cases are all called intermediate epilepsy aphasia (IEADs). The speech recovery ability of IEADs patients is better than that of EAS.

Patients with epilepsy and speech disorders should be advised to undergo EEG monitoring and genetic testing to confirm the diagnosis. Currently, there are no specific medications for the treatment of X-linked epile-

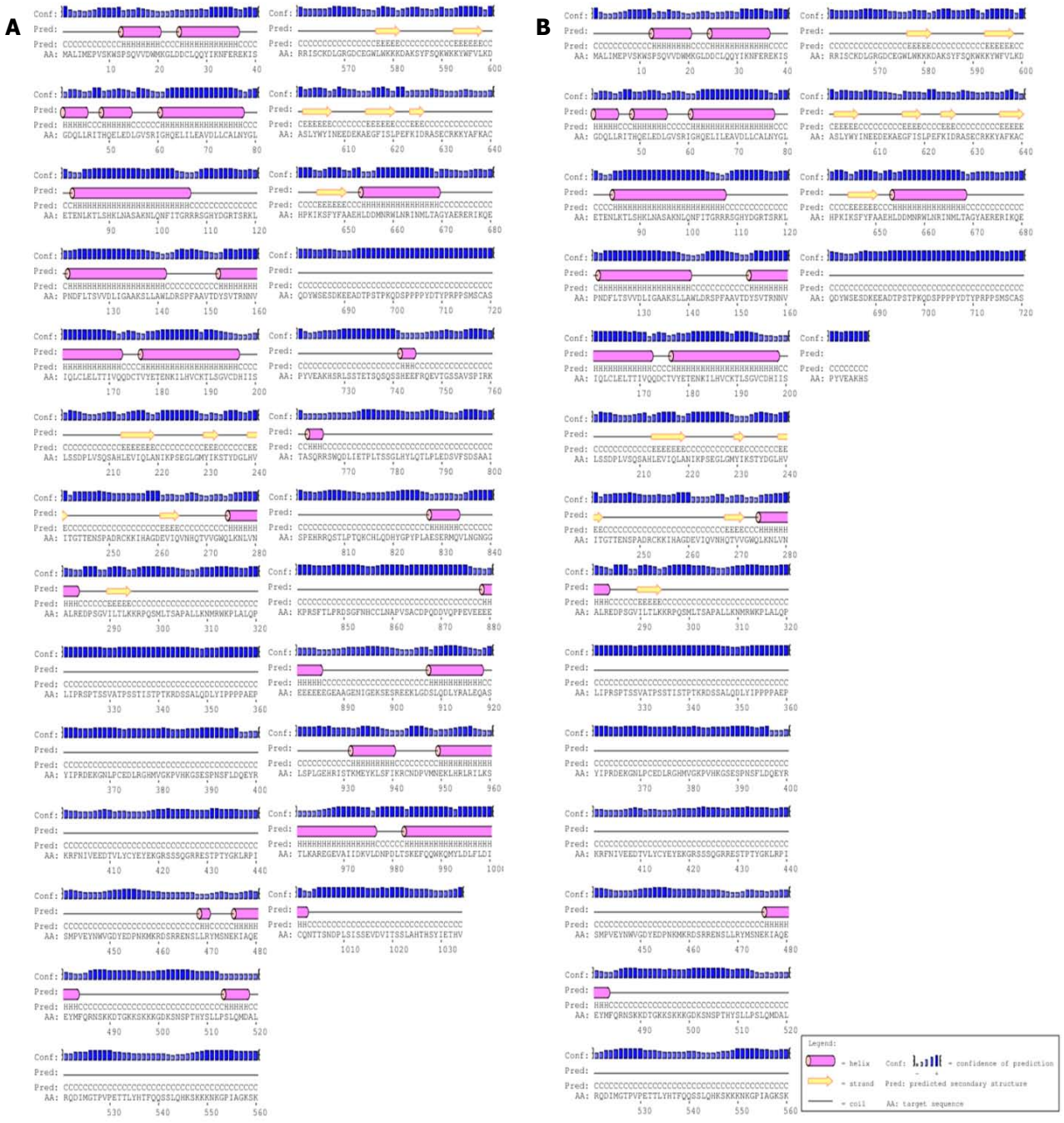


Figure 3 Secondary structures of wild-type and mutated *CNKSR2* proteins predicted by PSIPRED. A: The wild-type *CNKSR2* gene encodes an intact peptide chain of 1034 amino acids; B: The mutated *CNKSR2* gene leads to an early termination of the synthesis of the peptide chain and only No.1-728 amino acids are expressed.

psy-aphasia syndrome. The early diagnosis and early use of antiepileptic drugs as well as hormone therapy can recover speech comprehension to different degrees and improve abnormal discharge. Therefore, the overall prognosis of patients is good. Clinical seizures should be treated with antiseizure drugs, and barbiturates, carbamazepine, and phenytoin should be avoided as they can potentiate spike wave discharges during sleep[24,25]. Although there is evidence that mutations or deletions of *CNKSR2* lead to neurological development defects, such as epilepsy and intellectual disability, the pathogenesis remains unclear. Therefore, the next step is to screen a large number of epileptic encephalopathy individuals to delineate the phenotypic spectrum of the *CNKSR2* mutation. Second-generation gene sequencing can assist in the identification of hereditary etiology and discovery of new mutations while expanding on the early epilepsy encephalopathy clinical phenotype and genetic spectrum. Simultaneously, the pathogenesis of X-linked epilepsy-aphasia syndrome should be studied to assist clinicians in diagnosis and genetic counseling.

ARTICLE HIGHLIGHTS

Case characteristics

Before experiencing seizures, our patient suffered from developmental de-

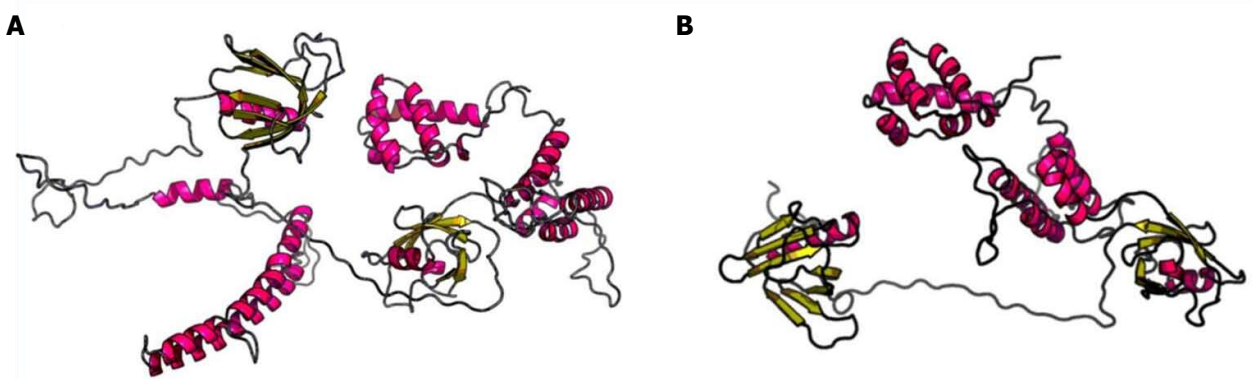


Figure 4 Tertiary structures of wild-type and mutated *CNKSR2* proteins predicted by RaptorX. The spatial structures of *CNKSR2* proteins are significantly different between the wild-type (A) and the patient (B).

lays and attention deficit hyperactivity disorder, which is consistent with the performance of X-linked intellectual disability. After seizure occurrence, the patients' speech expression gradually decreased, the electroencephalogram (EEG) continued to show abnormal wave patterns during sleep, and a *de novo* mutation of the *CNKSR2* gene was identified.

Clinical diagnosis
X-linked epilepsy-aphasia syndrome.

Differential diagnosis
Hysteria and childhood autism.

Laboratory diagnosis
A *de novo* mutation of the *CNKSR2* gene.

Imaging diagnosis
EEG continued to show abnormal wave patterns during sleep.

Treatment
Immunoglobulin, oral prednisone, lamotrigine and sodium valproate oral solution.

Related reports
Frequency of *CNKSR2* mutation in the X-linked epilepsy-aphasia spectrum has been reported in the journal of Epilepsia.

Term explanation
Epileptic encephalopathy with continuous spike-and-wave during sleep.

Experiences and lessons
This case will contribute to improvements in our understanding of X-linked epilepsy-aphasia syndrome. Patients with epilepsy and speech disorders should be advised to undergo EEG monitoring and genetic testing to confirm the diagnosis. The early diagnosis and early use of antiepileptic drugs as well as hormone therapy can recover speech comprehension to different degrees and improve abnormal discharge.

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