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REVIEW

Cascade of care for children and adolescents with chronic hepatitis C

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Abstract

Chronic hepatitis C virus (HCV) infection presents a significant global public health burden. In 2015, over 400000 deaths worldwide were attributed to HCV infection. This led the World Health Organization (WHO) in 2016 to set the ambitious goal of eliminating HCV by 2030. Adult-centered guidelines have been established in order to provide direction for healthcare professionals, allowing integration of the newest screening policies and therapeutic strategies into their practices. However, for children and adolescents, HCV is a significant, unrecognized public health problem. HCV infection rates in the United States in women of childbearing age and those who are pregnant have increased in parallel with the rising opioid epidemic. An estimated 29000 women with HCV infection gave birth each year from 2011 to 2014 in the United States, with approximately 1700 of their infants being infected with HCV. Newer HCV-specific therapeutics, namely direct acting antivirals (DAA), has brought a new and highly successful approach to treatment of hepatitis C. Recent studies have confirmed similar levels of effectiveness and safety of DAA therapies in the pediatric population. Thus, an enhanced cascade of care, which should include the population under 18 years of age, can help achieve the WHO goal by focusing on elimination in the youngest populations. This review will present an overview of the natural history, clinical features, and management of HCV in children and adolescents.

Key Words: Hepatitis C virus; Hepatitis C education; Hepatitis C elimination

Core Tip: In 2020, the landmark series of accomplishments which started with the discovery of the hepatitis C virus (HCV) and led to the development of pharmaceutical

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agents capable of curing HCV infection was underscored by the awarding of the Nobel Prize in Medicine. These innovative cures are now being applied to the pediatric population. Furthermore, programs such as The Kentucky Hepatitis Academic Mentorship Program have been developed to train general pediatricians on HCV epidemiology, diagnosis, management, treatment and prevention. Thus this cascade of care will hopefully help achieve the World Health Organization goal of eliminating HCV by 2030.

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INTRODUCTION

The arc of discovery–from identification of the hepatitis C virus (HCV) as the causative agent of what was termed "Non-, Non-B Hepatitis" in 1989 to the development of pharmaceutical agent capable of efficiently curing HCV infection was remarkably short. In October 2020, this landmark series of accomplishments was underscored by the awarding of the Nobel Prize in Medicine to Drs. Alter, Houghton, and Rice. The Nobel committee recognized that their work transformed molecular virology/immunology and revolutionized the management of infected patients worldwide[1,2].

HCV infection presents a significant global public health burden. It is currently estimated that over 70 million individuals are chronically infected with HCV and that many are unaware of their infectious status[3]. In 2015, over 400000 deaths worldwide were attributed to HCV infection. This led the World Health Organization (WHO) in 2016 to set the ambitious goal of eliminating HCV by 2030. Despite the advances in HCV therapeutics, significant cost and access to care are the major barriers to the achievement of this goal[4].

Adult-centered guidelines have been established in order to provide direction for healthcare professionals, allowing integration of the newest screening policies and therapeutic strategies into their practices. For children and adolescents, HCV is a significant, unrecognized public health problem. Perinatal transmission accounts for the majority of recognized HCV infections in the pediatric population. HCV infection rates in the United States in women of childbearing age and those who are pregnant have increased in parallel with the rising opioid epidemic. An estimated 29000 women with HCV infection gave birth each year from 2011 to 2014 in the United States, with approximately 1700 of their infants being infected with HCV[5].

Newer HCV-specific therapeutics, namely direct acting antivirals (DAA), have brought a new and highly successful approach to treatment of hepatitis C. Recent studies have confirmed similar levels of effectiveness and safety of DAA therapies in the pediatric population. Thus, an enhanced cascade of care, which should include the population under 18 years of age, can help achieve the WHO goal by focusing on elimination in the youngest populations.

This review will present an overview of the natural history, clinical features, and management of HCV in children and adolescents.

EPIDEMIOLOGY OF HEPATITIS C

All 6 HCV genotypes have been diagnosed in the pediatric population; based on limited reporting, the genotypic distribution appears to mimic what is seen in the adult population, with genotype 1 predominating[6].

HCV infection is most often asymptomatic in the pediatric population; therefore, it is difficult to estimate the true global prevalence. Schmelzer *et al*[7] combined past modelling and epidemiological work in 104 countries and territories to estimate the prevalence in children in 2018. They reported the global estimated viremic prevalence in the population under 18 years of age to be 0.13%, corresponding to 3.26 million children with HCV in 2018, with wide variability. The prevalence increased with age in all countries and territories. The strongest predictor of HCV prevalence in children

aged 0-4 years was the HCV prevalence in women of childbearing age. The proportion of HCV infections in adults who inject drugs was significantly associated with HCV prevalence in children aged 15-19 years[8]. In view of the wide heterogeneity, reliable country- or territory-specific and age-specific HCV prevalence estimates will be required in order to allow countries and territories to improve national HCV elimination and treatment strategies.

The true prevalence of pediatric HCV infection in the United States is also unknown due to a lack of uniform screening strategies. In 2020, the United States Preventive Services Task Force (USPSTF) issued revised recommendations that encourages clinicians to screen all adults aged 18 to 79 years for HCV infection[9]. Previously, they had expressed a concern that HCV screening might be associated with negative psychological and social consequences. However, treatment with DAA therapy has been associated with improved quality of life in addition to high rates of curing HCV[10]. Thus, as screening tests for HCV are highly accurate, they now conclude that the combination of screening with DAA therapy indicates improved long-term outcomes.

The USPSTF recommendations specifically suggest HCV screening for all pregnant women during each pregnancy. This is important since the rate of HCV infection in pregnant women has continued to increase, with an associated increase in the number of infants exposed to HCV.

The risk of perinatal transmission is confined to HCV infected women who have detectable HCV RNA. The risk of transmission is increased with higher levels of HCV viremia, as well as co-infection with HIV[11-14]. The mode of delivery (vaginal *vs* cesarean-section) does not typically affect risk of transmission[13]. However, if the mother is co-infected with HIV, then there may be a protective affect by undergoing a cesarean-section for delivery[11]. HCV RNA may be detected in breast milk and colostrum; however, breast feeding does not appear to increase the rate of HCV transmission (with the exception of HIV co-infected mothers)[15].

While prenatal care settings are potential venues for expanding HCV testing, implementation is sporadic. Epstein *et al*[8] characterized the HCV diagnostic cascade for women attending an obstetric clinic serving individuals with substance use disorders. They reported successfully screening for HCV among pregnant women with opioid use. In retrospective cohort study of infants exposed to HCV who were enrolled in the Tennessee Medicaid program, testing was conducted in only 23% of infants and less frequently among African American infants[16]. These two observations indicate that infant HCV screening is currently imperfect, emphasizing the need for programmatic changes to improve both mother and infant follow-up to bridge gaps in the cascade to cure. Because current testing recommendations may not properly address the barriers to HCV testing among high-risk infants, contributing to missed HCV infections, new policies (such as universal pediatric testing) may address the gaps[6,17,18].

Screening in adolescents may also be improved. Epstein *et al*[19] reported that only 30% of adolescents with identified opioid, amphetamine, or cocaine use were tested for HCV; 7% were found to be positive. Barritt *et al*[20] reported that in the United States from 2006-2012, the hospitalization rates of children with HCV increased by 37%; the majority of these patients were adolescents. This further reflects that our attempts at identifying and treating HCV in early childhood and adolescents are inadequate.

NATURAL HISTORY

Children with chronic HCV infection are typically asymptomatic[6,17]. An estimated 20%-40% will undergo spontaneous clearance within the first 5 years of life[21,22]. A combination of perinatal transmission and genotype 1a is associated with decreased rates of clearance, persistent viremia, and higher likelihood of development of end-stage liver disease in children who are treatment naïve[23]. Albeit uncommon, progression to cirrhosis has been described and hepatocellular carcinoma (HCC) secondary to HCV and cirrhosis in a child has also been reported[24].

Younossi *et al*[25] reported that HCV infection in adolescents was associated with poor social functioning and health-related quality of life (HRQoL). Children chronically infected with HCV had a significant reduction in a wide range of intelligence and memory testing. Vocabulary, reading comprehension, abstract visual reasoning, and short-term memory were all statistically inferior in HCV infected children compared to healthy controls[26]. Treatment of HCV led to improved quality of life, using multiple validated patient reported outcome instruments[25]. Therefore,

while the liver disease in HCV infected children is often absent or mild, treatment may lead to improved HRQoL in addition to prevention of cirrhosis and end-stage liver disease.

CLINICAL FEATURES AND OUTCOMES

Jaundice, fatigue, dyspepsia, and abdominal pain are the most common signs and symptoms reported in adults[27]. Unfortunately, there is less robust prospective data regarding clinical symptoms in children and adolescents. When reported, minimal nonspecific and brief symptoms are found in approximately 15% of children. These symptoms can be in the form of fatigue, anorexia, nausea, vomiting, and abdominal colic[28].

Extrahepatic manifestations of HCV infection are well documented in the adult population. These include glomerulonephritis, polyarteritis nodosa and cryoglobulinemia. Other non-specific extrahepatic symptoms reported in adult studies include fatigue, renal impairment, lymphadenopathy, fever, and thyroid dysfunction[29-31]. Although there appears to be a low incidence of extrahepatic manifestations in children, careful monitoring is still recommended. Indolfi *et al*[32] noted that subclinical thyroiditis (not autoimmune thyroid disease) has been reported in children with HCV. Other extrahepatic manifestations such as myopathy and opsoclonus-myoclonus syndrome have also been reported.

It is rare for HCV-associated liver disease to advance to the point of requiring liver transplant in children or adolescents. Based on retrospective analysis of the United Network of Organ Sharing, Gupta *et al*[33] found that children transplanted for HCV had a one-year survival of 97% and a three-year survival of 89% in the post-pediatric end-stage liver disease era. These findings are consistent with best practice liver transplant outcomes in children.

DIAGNOSIS AND SCREENING CHILDREN AND ADOLESCENTS

In children older than 18 mo of age, diagnostic criteria are the same as those established for adults. An enzyme immunoassay is used to detect antibody (anti-HCV); however, the presence of anti-HCV alone is unable to distinguish if the patient has an active or resolved infection. Thus, in children with detectable anti-HCV antibodies, the next step is to verify viral infection by detecting HCV RNA. This is accomplished *via* polymerase chain reaction (PCR) testing. The diagnosis of chronic HCV infection is made based on presence of detectable HCV RNA for more than 6 mo[34,35].

The diagnosis of perinatal transmission in infants under 18 mo of age is confounded by the passive transfer of maternal antibodies, which can last for one year or more postnatally. Thus, anti-HCV testing is of limited value during the infantile period. Diagnosis in this age group can be reliably established by HCV RNA positivity on two or more occasions after two months of age[36-38]. Criteria for spontaneous clearance requires two negative HCV RNA tests spread at least 6 mo apart, followed by negative anti-HCV testing after 18 mo of age[39,40].

For the population < 18 years of age, the screening guidelines are unclear. Assoumou *et al*[41] completed a cost-effective analysis which revealed improved quality of life years (QALY) gained if universal screening for HCV was expanded to include adolescents (15 years and older). However, as the diagnosis of infants is more difficult to interpret, studies on the cost effectiveness of screening younger patients are needed. Recent efforts in the United States have focused on infants born to HCV infected mothers[42,43].

Jhaveri *et al*[44] endorsed a national strategy for HCV screening that integrates follow-up of infants with HCV exposure by using a model similar to HIV mother-to-child transmission prevention programs. This, and related calls to action to primary care providers, will lead to enhanced recognition and screening for children with HCV exposure, similar to the efforts to combat the HIV epidemic[45].

TREATMENT

The arrival of DAA therapies has led to a paradigm shift in the treatment and

eradication of HCV in all populations[3,4]. The spate of DAAs available have been shown to be as safe and effective in children and adolescents as in the adult populations. Pegylated-interferon (PEG-IFN) and ribavirin (RBV), the initial recommended combination for treatment of HCV in children and adolescents, ae no longer recommended[46]. DAA therapies are specific and more effective at achieving sustained virologic response (SVR) in the pediatric population with few side effects. DAA therapies can also achieve SVR in no more than 12 wk of treatment, as compared to the RBV and PEG-IFN combination which required 48 wk of treatment, close monitoring, and significant side effect profiles including pancytopenia. Furthermore, regimens of PEG-IFN have sustained efficacy of only just above 50%, whereas DAA regimens have been shown to be persistently more effective (SVR > 95%) in children[3,4,17,46,47].

DAAs target three HCV proteins: (1) The nonstructural protein 3/4A (NS3/4A) protease inhibitors (PIs) which work by inhibiting HCV polyprotein processing; (2) NS5A inhibitors, which inhibit viral replication and assembly; and (3) NS5B polymerase inhibitors that block HCV RNA replication[48,49]. By combining two or more of these classes of drugs with different mechanisms attacking the Hepatitis C virus, DAAs are able achieve high SVR rates.

Over the past few years, several phase 2 clinical trials have been completed revealing the safety and efficacy of DAA therapy in children as young as 3 years of age (Table 1)[50-77]. For example, the first pediatric trial showed the safety and efficacy of Harvoni, the combination of Ledipasvir (90 mg) and sofosbuvir (400 mg), for treatment of HCV genotype 1 over a 12 wk period in children ages 12-17 years[50]. Subsequent clinical trials have been completed which show the efficacy and safety of newer combinations of DAA therapy for a wider range of HCV genotypes and pediatric age groups. For example, Jonas *et al*[74] reported the utility of the pangenotypic combination of glecaprevir (300 mg) and pibrentasvir (120 mg) in children ages 12-17 years. They found 100% SVR at 12 wk post therapy (SVR12) in as few as 8 wk of treatment. The safety profile was also consistent with that in adults. Wirth *et al*[75], reported that the fixed-dose combination of elbasvir/grazoprevir in children ages 3-17 years for HCV genotypes 1 and 4 was safe and efficacious in all study participants. Furthermore, SVR12 was achieved by all 57 participants. Sokal *et al*[76] also recently completed a study on the safety and tolerability of sofosbuvir/velpatasvir in pediatric patients aged 3–17 years with chronic HCV infection through 24-wk post-treatment. They found a 92% SVR12 rate regardless of HCV genotype, prior treatment experience, or presence of compensated cirrhosis.

Rosenthal *et al*[72] revealed that sofosbuvir plus ribavirin (RBV) was well-tolerated and highly effective in children aged 3 to < 12 years with chronic HCV genotype 2 or 3 infection. However, over one-third of the participants experienced gastrointestinal symptoms (vomiting, diarrhea), common side effects to RBV treatment. This combination is an option for young children until we have more published evidence for RBV-free DAA regimens. The hope is that in 2021, we will have approval by the United States Food and Drug Administration (FDA) for the use of a wide variety of DAA combination therapies.

Higher risk groups, such as children who are survivors of cancer, have also had high success rates with DAA therapy. El-Shabrawi *et al*[66] prospectively followed 20 childhood cancer survivors ages 8-17 years with HCV genotype 4 in Egypt. They all received Sofosbuvir plus Daclatasvir over a 12 wk period. They achieved 100% SVR12 in their study group without any treatment related adverse events. Furthermore, no relapses were detected during treatment and throughout the follow up period (36 wk) for either the original malignant disease or the HCV infection.

Studies are also assessing the efficacy of smaller doses and shorter duration[60,67,77]. For example, Behairy *et al*[77] reported the effect of a shortened 8-wk regimen of ledipasvir/sofosbuvir at smaller dosing of 45 mg and 200 mg respectively. They found that this regimen is safe and effective with 100% SVR12 in treatment-naïve children aged 4-10 years with chronic HCV infection genotype 4.

WHERE DO WE STAND?

The American Association for the Study of Liver Diseases (AASLD) published updated guidelines for the evaluation and management of HCV infection to reflect the DAA era[78]. The AASLD supports the use of ribavirin-free DAA regimens as early as possible (all children >3 years of age) to avoid future complications. A policy paper from the North American Society of Pediatric Gastroenterology Hepatology and

Table 1 Completed studies of direct acting antivirals regimens in children and adolescents

Ref.	Year	Participant age in years (*n*)	HCV genotype	Therapy (duration)	SVR12 (%)
Balistreri *et al*[50]	2016	12-17 (100)	1	Ledipasvir 90 mg + sofosbuvir 400 mg (12 wk)	98
Wirth *et al*[51]	2017	12-17 (52)	2 or 3	Sofosbuvir 400 mg + ribavirin (variable)	98
Hashmi *et al*[52]	2017	5-18 (35)	1 or 3	Sofosbuvir 400 mg + ribavirin (variable)	97
El-Khayat *et al*[53]	2018	12-17 (144)	1, 4-6	Ledipasvir 45 mg + sofosbuvir 200 mg (12 wk)	99
Murray *et al*[54]	2018	6-11 (90)	1	Ledipasvir 45 mg + sofosbuvir 200 mg (12 wk)	98
El-Karaksy *et al*[55]	2018	12-18 (40)	4	Ledipasvir 90 mg + sofosbuvir 400 mg (12 wk)	100
Leung *et al*[56]	2018	12-17 (38)	1 or 4	Ombitasvir/paritaprevir/ritonavir + dasabuvir +/- ribavirin (variable)	100
Alkaaby *et al*[57]	2018	7-18 (22)		Ledipasvir + sofosbuvir +/- ribavirin (variable)	91
Tucci *et al*[58]	2018	0.5 (1)	4	Ledipasvir 22.5 mg + sofosbuvir 100 mg (12 wk)	100
El-Shabrawi *et al*[59]	2018	6-12 (20)	4	Ledipasvir 45 mg + sofosbuvir 200 mg (12 wk)	95
El-Shabrawi *et al*[60]	2018	12-17 (10)	1-6	Sofosbuvir 400 mg + daclatasvir 60 mg (8 wk)	100
Yakoot *et al*[61]	2018	12-17 (30)	4	Sofosbuvir + daclatasvir (12 wk)	97
Quintero *et al*[62]	2019	6-18 (9)	1 or 4	Ledipasvir + sofosbuvir (variable)	100
Ghaffar *et al*[63]	2019	8-18 (40)	4	Sofosbuvir + daclatasvir (variable)	97.5
Fouad *et al*[64]	2019	11-17.5 (51)	4	Ledipasvir 90 mg + sofosbuvir 400 mg (12 wk)	100
Ohya *et al*[65]	2019	10-13 (3)	1b	Ombitasvir + paritaprevir + ritonavir (12 wk)Or glecaprevir + pibrenastavir (8 wk)	100
El-Shabrawi *et al*[66]	2019	8-17 (20)	4	Sofosbuvir + Daclatasvir (12 wk)	100
Serranti *et al*[67]	2019	12-17 (14)	1	Ledipasvir 90 mg + sofosbuvir 400 mg (8 wk)	100
Marascio *et al*[68]	2019	13, 16 (2)	4	Ledipasvir 90 mg + sofosbuvir 400 mg (12 wk)	100
Fouad *et al*[69]	2020	12-18 (46)	Not performed	Ledipasvir 180 mg + sofosbuvir 400 mg (12 wk)	98
Kamal *et al*[70]	2020	3-6 (22)	4	Ledipasvir 45 mg + sofosbuvir 200 mg (8 or 12 wk)	100
El-Araby *et al*[71]	2020	9-12 (100)	4	Ledipasvir 90 mg + sofosbuvir 400 mg (12 wk)	100
Rosenthal *et al*[72]	2020	3-11 (54)	1 or 4	Sofosbuvir 400 mg + ribavirin (variable)	98
Schwarz *et al*[73]	2020	3-< 6 (34)	1 or 4	Ledipasvir + sofosbuvir (variable)	97
Jonas *et al*[74]	2020	12-17 (47)	1-4	Glecaprevir 300 mg + pibrentasvir 120 mg (8-16 wk)	100
Wirth *et al*[75]	2020	3-17 (57)	1 or 4	Elbasvir + grazoprevir (12 wk)	100
Sokal *et al*[76]	2020	3-17 (216)	1-4, 6	Sofosbuvir + velpatasvir (12 wk)	92
Behairy *et al*[77]	2020	4-10 (30)	4	Ledipasvir 45 mg + sofosbuvir 200 mg (8 wk)	100

Table adapted from Squires *et al*[17]. HCV: Hepatitis C virus.

Nutrition (NASPGHAN), included pediatric guidelines for treating children with DAA therapy[79]. They agreed with starting applicable DAA therapy as early as 3 years of age.

Outside of North America, guidelines are being updated to reflect the advent of DAA therapy. The European Association for the Study of the Liver (EASL) recently published a new guidance for management of HCV[80]. They recommend treating HCV positive children (with or without cirrhosis) as young as 3 years of age with DAA regimens of either combined sofosbuvir and velpatasvir, or glecaprevir and pibrentasvir. Indolfi *et al*[46] as part of the European Society for Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) also updated their position to

recommend initiation of DAA therapy for children as young as 3 years of age with HCV, regardless of the presence of fibrosis or active inflammation. We agree with AASLD, NASPGHAN, EASL, and ESPGHAN recommendations for an aggressive approach to treating children 3 years of age and older with a RBV-free DAA combination. Furthermore, we agree with these worldwide guidelines that if there is any signs or evidence of fibrosis, then patients should continue to be monitored even after completing DAA therapy and achieving SVR (please refer to monitoring below).

Anecdotally, one of the main hurdles is determining an age when a young child is capable of daily compliance with the medications for the recommended 8-12 wk period. The advent of DAA therapies in the form of granules/pellets is a promising strategy for younger children who cannot swallow whole tablets. For example, Schwarz *et al*[73] allowed for the granules to be sprinkled on a spoonful of nonacidic soft food, such as pudding or ice cream. SVR12 was achieved in 97% of patients, with only one patient discontinuing the trial after 5 d due to "abnormal drug taste".

Cost-effective analyses for treating children with DAA therapy are limited. Nguyen *et al*[81] found that early DAA treatment in adolescent patients with chronic HCV infection was cost-effective compared with deferred treatment, with approximately $27000 per QALY gained after 30 years. Greenaway *et al*[82] published data comparing treatment at age 6 years *vs* delaying treatment until age 18 years. In their model covering 20 years and treating 10000 children early, 330 cases of cirrhosis, 18 cases of hepatocellular carcinoma, and 48 liver-related deaths would be avoided. The incremental cost-effectiveness ratio of early treatment compared to delayed treatment was approximately $12690 per QALY gained and considered cost-effective. Thus delaying treatment until age 18 years results in an increased lifetime risk of late stage liver complications that can otherwise be avoided. Early treatment is associated with saving money and lives, as well as improving quality of life.

IMPROVING THE CASCADE OF CARE

In addition to inadequate screening, a major barrier to treatment and elimination is access to care and treatment. However, several programs have been conceived in order to provide DAA therapy to more individuals, with a focus in the primary care setting. In Australia, DAA treatments are available through the national Pharmaceutical Benefits Scheme (PBS) as of 2016. The PBS is a publicly funded scheme which provides highly subsidized prescription drugs *via* Australia's universal healthcare system[83]. Australia was also one of the first countries to allow DAA treatment to be initiated by general practitioners. Since the advent of these practices, they have seen marked improvements in the cascade of hepatitis C care among patients attending primary care clinics[84].

In the United States, certain regions have much higher rates of HCV infection. For example, the Appalachian region leads the nation in reported new cases. Thus, new developmental strategies have been created focusing on these communities. The Kentucky Hepatitis Academic Mentorship Program (KHAMP) was created with the goal to build a hepatitis C elimination model which would then be easily modified and used to improve the health of rural and underserved communities throughout the Appalachian region. KHAMP has trained primary care providers on HCV epidemiology, diagnosis, management, treatment and prevention. General practitioners in this region are thus equipped with the skills needed to increase the number of individuals treated, ensuring that they will no longer be required to travel and consult with a specialist in order to prescribe DAA therapy[17,85,86].

This blueprint is being applied to the rest of the United States, continuing the focus on the Appalachian region. For example, West Virginia has recently implemented the West Virginia Hepatitis Academic Mentoring Partnership which will use the same strategies as KHAMP to provide education for primary care providers on HCV[86,87]. Virginia and Ohio are also participating to improve their education and access at the primary care level.

The advent of telemedicine has also had a positive impact towards treating HCV. Arora *et al*[88] developed the Extension for Community Healthcare Outcomes (ECHO) model. In a prospective cohort study, the ECHO model through use of video-conferencing technology, trained primary care providers to care for underserved populations with HCV infection who live in New Mexico. Results showed that ECHO was an effective approach to treating HCV infection in underserved communities. Piao *et al*[89] have implemented ECHO to California with improvements in SVR, advocating for such programs to be an essential part of HCV care moving forward.

In Australia, hepatitis C treatment (DAA therapy) using a decentralized, nurse-led telemedicine model of care has been highly effective at reaching a treating large numbers of prisoners, many of which are IV drug abusers[90]. Canada has also implemented a telemedicine program in order to effectively increase the use of DAA therapy with a high success rate of SVR (approximately 95%)[91]. As more programs are being initiated, the possibility of reaching the WHO goal of eradication by 2030 is still possible.

IMPACT OF TREATMENT ON PROGRESSION

Progression of HCV from an inflammatory hepatitis, to fibrosis, and eventually cirrhosis can occur starting in early childhood. In the past, most HCV-infected children would develop chronic HCV with a lifetime risk of liver disease. Modin *et al*[92] quantified the development of long-term liver disease and the effect of treatment in patients infected with HCV in childhood. They reported that liver disease developed in 32% of patients, a median of 33 years after infection; patients with perinatal exposure developed cirrhosis at an earlier age than the rest of the risk groups. The incidence of HCC was 5%, liver transplant 4% and death occurred in 3%. Among those treated there was a higher mortality rate among patients that did not achieve an SVR, and treatment was more effective in patients without cirrhosis. Disease progression was less frequent than in patients with cirrhosis at the time of therapy. The authors make a strong case for early treatment, before development of cirrhosis.

MONITORING

Finding non-invasive methods to assess for progression to fibrosis is an important aspect of monitoring children with chronic HCV. Transient elastography (TE) *via* ultrasound (US) evaluation of the liver is gaining traction in the pediatric population[93]. TE as a measurement of liver fibrosis has been validated in a variety of chronic liver diseases, including HCV[94-97].

Pokorska-Śpiewak *et al*[98] reported their prospective analysis on the prevalence of fibrosis in adolescents (12-17 years) with chronic HCV. Using TE, they found that over 10% of their patients had evidence of significant fibrosis (fibrosis score > 2), and that 9% had evidence of cirrhosis (Fibrosis score of 4). Other markers of liver fibrosis, such as the aspartate transaminase-to-platelet ratio index score, correlated positively with liver stiffness from TE. Otherwise, serial monitoring with in-clinic visits, as well as laboratory testing of aminotransferases and gamma-glutamyl transferase are recommended to occur at least twice yearly. Monitoring for signs of HCC with serum alpha-fetoprotein and US imaging is also warranted[29,99,100].

Based on the previously completed studies on DAA therapy in pediatrics, our current practice involves obtaining HCV PCR at baseline (prior to initiation of DAA therapy), at 4 wk, at 12 wk, and at 24 wk post initiation of therapy[50,54,70]. As long as there was no evidence of long-term damage (fibrosis, cirrhosis, *etc*.), then patients can have a repeat HCV PCR one year after completion of therapy to affirm SVR[50,54,72-76,81]. No pediatric studies with the children completing the DAA therapy has revealed evidence of children being unresponsive to DAA therapies.

Children with evidence of liver fibrosis should continue to be closely monitored even after eradication of their underlying HCV. However, adult studies are emerging which reveal that fibrosis may be to-some-extent reversed by DAA treatment[101-104]. One study revealed a 32% reduction in liver stiffness measurements after DAA completion among 392 adults with chronic HCV and fibrosis[105]. However, for patients with evidence of high-grade fibrosis or cirrhosis, they are still at high risk of developing HCC even after achieving SVR[106]. More histological data is needed to further support the hypothesis of improved liver scarring post DAA treatment. At this time, children with evidence of fibrosis must be closely followed given the continued risk of complications such as HCC and portal hypertension.

IMPACT OF DAA THERAPY ON LIVER TRANSPLANT

The number of patients requiring HCV-related liver transplant has decreased, increasing organ availability for other liver disorders, such as NASH. In addition,

given the safety and effectiveness of DAA therapy, the idea of placing HCV-infected livers into uninfected recipients is gaining traction. With the rising demand of liver transplant, treating recipients with an appropriate course of DAA therapy immediately after transplant appears safe and efficacious[107,108]. In one small study, 8 veterans received HCV-infected livers and all 8 became viremic with HCV. However, after a 12 wk course of DAA treatment, all 8 patients achieved SVR12[109]. Bohorquez *et al*[110] increased this sample size and, after completing an appropriate DAA regimen, had 100% SVR12 in all 51 HCV-naïve patients who received HCV positive livers. Therefore, solid organ transplant from HCV infected recipients appears to be safe, is associated with excellent outcomes, and should be considered for recipients who would benefit from receiving an organ earlier than they would if they waited for an organ from an uninfected donor. Thus, reducing wait-list associated mortality. The same concept applies to other solid organ transplants. While no studies has been performed, based on the efficacy of DAA therapy in children, using HCV-infected donors should be an option.

CORONAVIRUS DISEASE AND HCV

The coronavirus disease 2019 (COVID-19) pandemic has significantly impacted access and healthcare practices for many patients and providers, including the pediatric population. As children will now have access to DAA therapies and potential HCV cure, it is imperative that diagnosis and treatment of this population is not overlooked. A related issue is for patients with chronic liver disease to avoid COVID-19 exposure and infection, by educating patients/parents on the risk and the recommended precautions[111]. This is especially true in rare cases of children with cirrhosis or end-stage liver disease secondary to HCV, as there appears to be a higher risk of a severe course of COVID-19[112].

The advent of telemedicine is playing an important role in the care of children with HCV. This allows patients to undergo lab testing locally, for DAA prescriptions to be sent to their home, and allow providers to safely communicate, educate, and closely monitor their patients during treatment[113]. Thus this pandemic should not be a hindrance to continuing the goal of eradication of HCV in the pediatric population.

CONCLUSION

The discovery of the HCV and the related advances in biomedical research-the establishment and implementation of diagnostic tests to ensure the safety of blood products, and antiviral drug development has, and will continue to have, a major impact on health care outcomes for patients of all ages…including the smallest victims. Enhanced screening and awareness efforts and continued education of healthcare providers will improve the outcomes of HCV infection in the pediatric population.

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MINIREVIEWS

Primary localized gastric amyloidosis: A scoping review of the literature from clinical presentations to prognosis

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Abstract

Localized gastric amyloidosis (LGA) is a rare disease characterized by abnormal extracellular deposition of amyloid protein restricted to the stomach and it is confirmed by positive results of Congo red staining. Over decades, only a few cases have been reported and studies or research focusing on it are few. Although LGA has a low incidence, patients may suffer a lot from it and require proper diagnosis and management. However, the pathology of LGA remains unknown and no overall review of LGA from its presentations to its prognosis has been published. Patients with LGA are often asymptomatic or manifest atypical symptoms, making it difficult to differentiate from other gastrointestinal diseases. Here, we report the case of a 70-year-old woman with LGA and provide an overview of case reports of LGA available to us. Based on that, we conclude current concepts of clinical manifestations, diagnosis, treatment, and prognosis of LGA, aiming at providing a detailed diagnostic procedure for clinicians and promoting the guidelines of LGA. In addition, a few advanced technologies applied in amyloidosis are also discussed in this review, aiming at providing clinicians with a reference of diagnostic process. With this review, we hope to raise awareness of LGA among the public and clinicians.

Key Words: Gastroscopy; Changes of gastric mucosa; Primary localized gastric amyloidosis; Clinical presentations; Prognosis

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Core Tip: Localized gastric amyloidosis (LGA) is rare. Few case reports are available to the public. It is often misdiagnosed as other gastrointestinal diseases due to its atypical manifestation. However, no systemic reviews or guidelines of LGA is published now. Therefore, we present a detailed overview from its clinical manifestations to prognosis for the first time. Based on that, a clinical diagnostic procedure is provided and may benefit clinicians who manage LGA.

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INTRODUCTION

Amyloidosis is a group of conformational diseases characterized by the accumulative extracellular deposition of insoluble fibrils in various tissues and organs as a result of protein folding disorders[1]. Large deposits may lead to the loss of the normal structure of tissues, subsequent organ dysfunction, and even death. At present, 36 different amyloid fibrils have been identified that are associated with amyloidosis[1]. Amyloid fibrils determine the properties of the amyloid diseases, and the subtypes of amyloidosis are also named after the corresponding fibrils. For example, light chain (AL) indicates that amyloid fibrils are derived from immunoglobulin light chains, and the resulting disease is referred to as AL amyloidosis[2]. According to the amyloid distribution, amyloidosis is divided into systemic and localized amyloidosis. Systemic amyloidosis is universal, while localized amyloidosis is a rare condition that only comprises 12% of newly identified amyloidosis cases[3].

Amyloidosis is a rare disease, among which AL amyloidosis is the most common type. An epidemiological study in Sweden reported an incidence of nonhereditary amyloidosis of 8.29 per million person-years, among which AL amyloidosis accounted for 3.2 per million person-years[4]. A nationwide study in the United States reported an increasing incidence of AL amyloidosis, from 9.7 per million person-years in 2007 to 14.0 per million person-years in 2015[5]. Although rare, amyloidosis can result in a severe disease burden, as reflected by patients' poor scores on assessments of the health status compared to the general population in a recent study of 341 patients[6]. Moreover, without proper intervention, it may ultimately develop into a fatal disease. From 2000 to 2008, 0.58 per thousand deaths were due to amyloidosis in England, and its proportion of deaths has doubled, indicating a tendency to increase[7].

Gastrointestinal involvement manifests as systemic amyloidosis (79%), while it is relatively rare in localized cases (21%), according to a retrospective study of 76 patients of biopsy-proven gastrointestinal amyloidosis evaluated in 1998-2011[8]. Localized gastric amyloidosis (LGA) is an extremely unusual condition. Generally, it refers to amyloidosis confined to the stomach without evidence of potential plasma cell dyscrasia or the involvement of other organs, particularly the heart, liver, kidney, or nerve[3,8]. More specifically, the precursor protein of amyloid is produced and deposited in the stomach without detection in a remote site[9]. According to a retrospective study of gastrointestinal biopsies from 542 patients, the most common amyloid subtype in the stomach is AL (λ), followed by transthyretin (ATTR), AL (κ), and serum amyloid A (AA)[10].

Given the rare reports and unsolved problems associated with LGA, we present a case of LGA (Figures 1 and 2) and collect several recent case reports of LGA available in the literature to present its clinical manifestations, diagnosis and differential diagnosis, and treatment. In addition, we will describe our understanding of its pathogenesis.

PATHOGENESIS

To date, the pathogenesis of amyloidosis is unclear, but current studies and

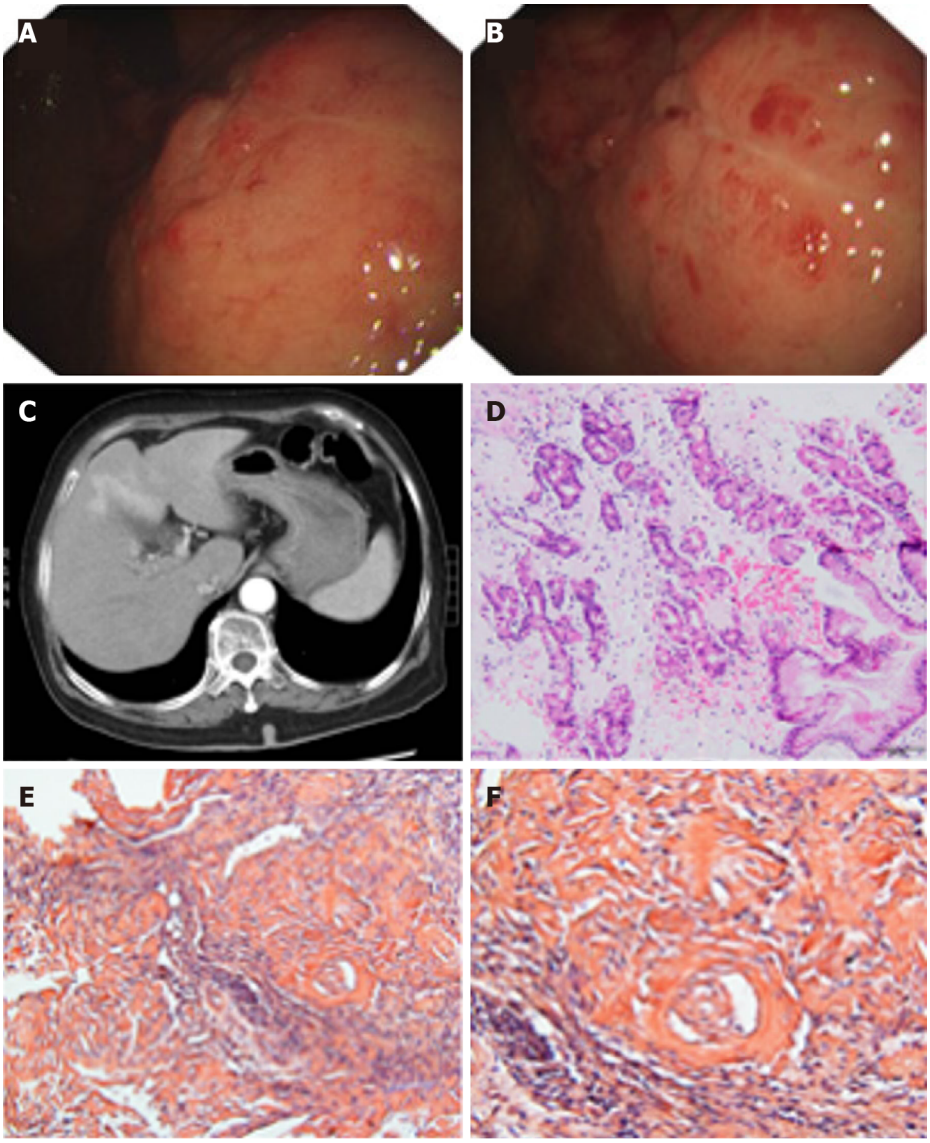

Figure 1 Pathological findings from a 70-year-old woman with localized gastric amyloidosis. The patient came to the hospital with a chief complaint of hematemesis for 2 wk. A and B: Endoscopic findings show multiple congested fragile ulcers scattered in the gastric body and fundus. A 4.0 cm × 4.0 cm area of the mucosa with edema, ulcers, and poorly delineated boundaries was observed in the anterior wall of the gastric body and fundus. The lesion appeared as a rough, congested area with edema, localized superficial fragile ulcers and active bleeding. Spot-like congested erosions exhibited a scattered distribution in the mucus of the sinus; C: CT reflected diffusely thickened gastric walls and shallow folds of the mucosa, while no abnormalities were observed in the enhanced images; D: H&E staining revealed massive amyloid fibrous connective tissues deposited in the interstitium with inflammatory cell infiltration; E and F: Congo red staining confirmed the existence of the amyloid protein (E: Congo red, × 200 magnification; F: Congo red, × 400 magnification).

hypotheses provide a few insights. All amyloid fibrils share the same antiparallel cross-β secondary structure, a structure with a high propensity for self-aggregation, as observed under an electron microscope[11,12]. Tightly bound β-sheets form the protofilament, and several protofilaments twist and eventually form amyloid fibrils[13]. Proteins may have a potential intrinsic propensity of misfolding that is influenced by multiple factors, such as aging and stably high concentrations in serum[11]. For example, wild-type TTR forms amyloid fibrils in older individuals, even at normal concentrations, while serum amyloid A and β_2M only become amyloidogenic at a persistently high concentration[14]. Different amyloid fibrils may be susceptible to different conditions that trigger misfolding. Mutations may induce the formation of amyloidogenic proteins[15,16]. Mutations in genes that encode amyloid fibrils, such as TTR, trigger familial amyloidosis[17]. Somatic mutations have been identified in AL amyloid fibrils. The N-terminal strand of the light chain variable domain prevents protein aggregation, and its mutation destabilizes the protein and accelerates light chain fibrillogenesis[18,19]. However, the exact relationship between mutations and amyloidosis remains unknown. In addition, further thermodynamic investigations reveal that many environmental factors, such as temperature and pH, are related to

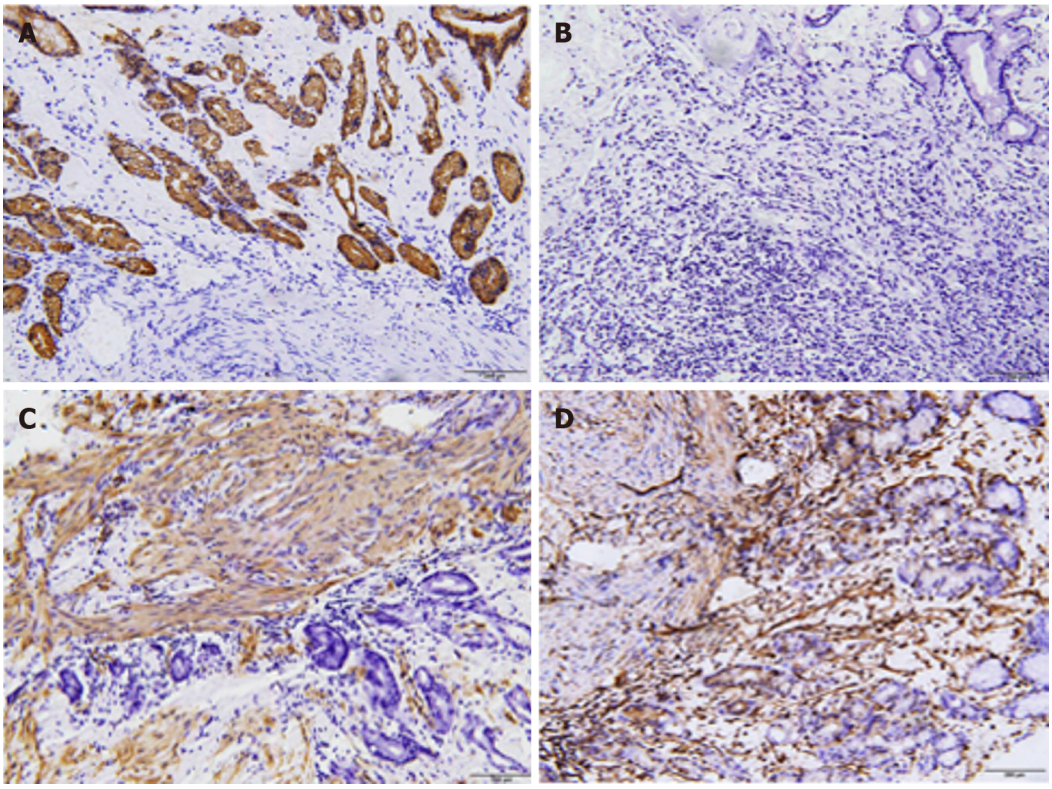

Figure 2 Results of immunochemical staining using several antibodies excluded the diagnosis of gastric cancer. A: CKPAN-positive staining in the glands; B: Periodic Acid-Schiff staining is negative; C: Smooth muscle actin-positive staining in the muscularis mucosa; D: Vimentin-positive staining.

the conformational stability of amyloid fibrils and may be critical factors contributing to the conversion of a normal protein into amyloid fibrils[13,20] (Figure 3).

In addition, several protein cofactors, nonfibrillar components, glycosaminoglycans (GAGs), and serum amyloid P (SAP) are present in all amyloid deposits and are believed to function in amyloidogenesis and persistence. GAGs, a main component of the extracellular matrix, are associated with amyloid fibrils in AL amyloidosis, and their size and charge may play an important role in the acceleration and stability of amyloid formation[19]. SAP is a type of plasma glycoprotein that binds to all types of amyloid fibrils in its calcium-bound state and protects them from proteolysis[21] Amyloid fibrils are digested *in vitro* by proteases and phagocytic cells, while amyloid fibrils likely exhibit relative stability *in vivo* with the assistance of SAP[22]. According to an *in vitro* study, SAP may accelerate and stabilize the formation of $A\beta_{42}$, the amyloid fibrils responsible for Alzheimer's disease[23]. The severity of amyloid deposition in SAP knockout mice is decreased considerably[24]. Therapies targeting SAP have been tested and confirmed to exert stable effects[25].

The mechanism by which amyloid deposits damage the organs and lead to dysfunction is unclear. The site of deposition may be related to multiple factors, such as the pH, protein concentration, proteolytic processing, and fibril seeds. Different amyloid fibrils exhibit a preference for specific organs; for example, β_2-microglobulin prefers joints[11]. In patients with LGA, amyloid is universally present in the walls of small vessels, and most of these amyloid deposits are classified as AL amyloidosis (12/22). In cardiac amyloidosis, amyloid deposits in small vessels lead to symptoms of cardiac ischemia[26]. In gastric amyloidosis, we are able to detect the same distribution patterns, but no connections have been reported to date. Furthermore, the toxicity of amyloidogenic light chain proteins (AL-LC) may be responsible for this condition. An investigation of the potential mechanism revealed that the injection of human AL-LC within a zebrafish model causes cell death. Human AL-LC induces intracellular oxidative stress and alters the cellular redox status, eventually leading to cardiac dysfunction, which is not attributed to the deposition of amyloid protein[27]. As shown in another study, AL-LC mediates cardiomyocyte apoptosis and dysfunction through the activation of p38 mitogen-activated protein kinases[28]. Lysosomal dysfunction has also been reported to provoke the proteotoxicity of AL-LC by contributing to impaired autophagy[29]. Investigations of the precise molecular mechanism of cardiac amyloidosis are ongoing and may explain how the amyloid protein contributes to

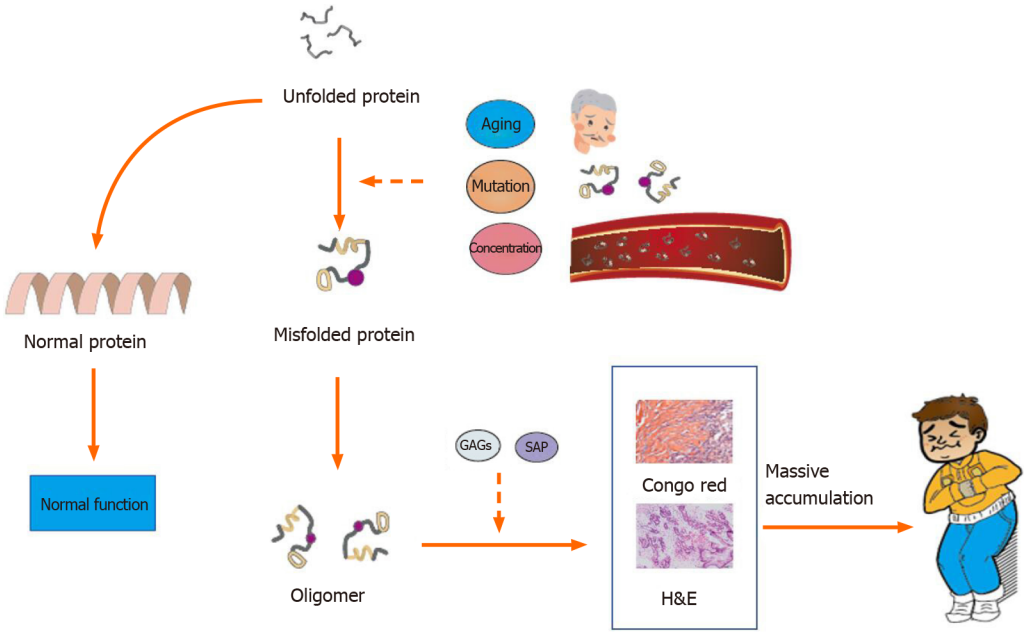

Figure 3 Potential molecular events leading to amyloidosis. Without intervention, the unfolded protein becomes the normal protein. Factors such as aging, mutation, and high blood concentrations may cause protein misfolding. The misfolded protein aggregates into oligomers and forms fibrils with the assistance of glycosaminoglycans and serum amyloid P. Massive deposition of amyloid fibrils leads to amyloidosis. GAGs: Glycosaminoglycans; SAP: Serum amyloid P.

organ damage. These studies may reveal the mechanism and inspire further studies of gastric amyloidosis.

CLINICAL MANIFESTATIONS

LGA mainly targets middle-aged and elderly people aged from 50 to 80 years. Equal numbers of male and female patients are affected, and no sex differences have been detected. The clinical manifestations of LGA often mimic other common gastric diseases and lack specificity, ranging from an asymptomatic disease to epigastric discomfort, pain, weight loss, anemia, heartburn, nausea, hematemesis, tarry stool, fatigue, and other symptoms (Figure 4). Generally, these manifestations depend on the sites and extent of amyloid involvement[30]. Patients in our reviewed case studies often presented without a chief complaint, and LGA was generally diagnosed based on the results of tests for other gastric diseases. Among the 22 cases that we reviewed, 13 cases of AL LGA and 1 case of AA LGA were identified. Most AL LGA cases manifest asymptomatically[31-35] or with epigastric discomfort[36,37], while AA LGA exclusively manifests as epigastric discomfort[38] (Table 1). To date, no association between symptoms and amyloid types has been confirmed[39]. Further discussion of whether a correlation exists between clinical presentations and amyloid fibrils in patients with LGA is worthwhile.

DIAGNOSIS

Imaging findings

Endoscopic results have identified variable features in patients. Amyloid deposits mainly invade the gastric body and antrum (Figure 5). Generally, the deposits manifest as single or multiple lesions in the form of a mass[40-43], ulcer[38,44], fold[36,45], elevation[37,46], or submucosal tumor-like feature[34,47]. The borders may be clear or unclear. These findings are consistent with those of previous studies. A remote study of 37 patients with gastrointestinal amyloidosis revealed that erosions were the most common presentation in the stomach, followed by granules, ulcers, and mucosal friability[48]. However, a recent study provided a different order of a normal appearance, followed by erythema, erosions, and nodularity[39]. The samples included both localized and systemic amyloidosis, and thus, the difficulty in distinguishing localized amyloidosis from systemic involvement simply based on endoscopic features

Table 1 Collection of recent case reports of localized gastric amyloidosis

Ref.	Age/sex	Symptom	Gross	Size	Location	Endoscopic ultrasound	biopsy	Amyloid type	Exclusion test	Suspected diagnosis	Treatment
Ikeda *et al*[59], 1978	68/F	Epigastric pain, nausea	One grayish-white mural elastic soft tumor with an irregular shape and poor margins; thickened and uneven mucosa, partly nodular; swollen mucosal folds	6 cm × 5 cm	Antrum	/	Congo red (+); amyloid deposits in vessel walls; H&E staining: Amyloid deposits with foreign-body reactions; small nodules of amyloid proteins with a scattered distribution	/	Biopsy of the skin rectum, gingiva and liver; urine Bence-Jones protein levels	Gastric carcinoma	Surgery
Dastur *et al*[40], 1980	50/M	Abdominal distension, worse after meals	One mass with central small ulceration and defective mucosa	/	Antrum	/	The mass extended from the mucosa to superficial muscularis and consisted of lymphocytes and germinal centers; normal plasma cells and amyloid proteins; Congo red (+)	/	Urine Bence-Jones protein (-)	/	Surgery
Björnsson *et al*[45], 1987	60/F	Hematemesis	A considerable amount of blood, bleeding and irregular, thickened mucosa folds	/	/	/	H&E staining: Amyloid deposits in the lamina propria and muscularis mucosae, infiltration of plasma cells, mucosa atrophy; Congo red staining: Amyloid deposits in the submucosa, muscularis propria and subserosa, mainly around vessels	AL (κ&λ)	Biopsy of rectum, gingiva, cervix and bone marrow; analysis of renal function; urine analysis	/	Surgery
Yanai *et al*[31], 1991	52/F	None	One irregular erosion	2.5 cm	The lower body of the stomach	Thickened mucosa and submucosa	Amyloid deposits in vessels of the mucosa and submucosa	AL (λ)	Examinations of blood, urine and skin; ultrasound and CT of the abdomen; simple X-ray examination of the chest and abdomen; biopsy of the rectum; ECG; ultrasound of the heart; barium enema; tests of antigens, urine Bence-Jones protein, rheumatoid factors and C-reactive protein levels	Gastric cancer	/
Wu *et al*[38], 2003	50/F	Epigastric discomfort, dull pain in the upper abdomen	One ulcer with heaped-up rough borders and erosive fragile mucosa	3 cm × 1 cm	Lesser curvature of the gastric body	Uneven hypoechoic thickened gastric wall with infiltrated submucosa	Amyloid deposits in the mucosa, submucosa and walls visualized using H&E and Congo red staining (-); atrophy of gastric glands and intestinal metaplasia	AA	Biopsy of the bone marrow and other gastrointestinal tissues (esophagus, duodenum, colon and stomach) (-)	Gastric cancer	Subtotal gastrectomy, clearance of perigastric lymph nodes
Shibukawa *et al*[44], 2004	51/F	Tarry stool	One irregular ulcer with swollen edges and dirty slough-like advanced cancer;	/	/	Structural loss of the first three layers of the gastric wall, a small amount of	H&E staining: Amyloid deposits infiltrated the submucosal connective tissues, lamina propria, and muscularis mucosae	AL or primary type	/	Carcinoma	Partial gastric resection

			bleeding			ascites	and were mainly observed around vascular walls in the submucosa; Congo red (+)				
Deniz *et al*[41], 2006	67/M	Fatigue, weight loss, poor appetite	One mass	5 mm × 5 mm × 5 mm	Paracardiac region	/	H&E staining: Amyloid deposits in the mucosa; Congo red (+)	/	Biopsy of other gastrointestinal tissues (-); urine Bence-Jones protein (-)	/	/
Rotondano *et al*[60], 2007	55/M	Epigastric pain, heartburn, weight loss	Two white-yellow granular-like circular areas	3 cm	Distal portion of the gastric body and angle of the stomach	Mucosal and submucosal layers exhibited slight thickening	H&E staining: Lymphocytes and polyclonal plasma cells infiltrated the lamina propria; Congo red (+)	/	Biopsy and endoscopy of the rectum, duodenum and esophagus (-)	/	None
Ebato *et al*[62], 2012	77/F	Anemia	One flat, depressed area	46 mm × 28 mm	Lower gastric body	/	H&E staining: Amyloid deposits in the mucosa and submucosa; DFS staining (+)	AL	/	/	Endoscopic removal
Sawada *et al*[46], 2012	72/F	/	Flat elevations, tumors; ulcers resemble advanced cancer, intramural hematomas	/	Scattered distribution in the antrum, proximal and middle stomach	Structural loss, thickened hypoechoic mucosa and submucosa	Congo red (+)	AL (κ&λ)	Biopsy and endoscopy of other gastrointestinal tissues (-)	/	/
Rivera *et al*[42], 2012	67/M	Melena, anemia	One round and erosive mass with errhysis	2.5-3 cm	Cardia	/	Confirmed amyloidosis	/	Biopsy of bone marrow (-)	Gastric adenocarcinoma	Surgery, hematology consultations
Kamata *et al*[36], 2012	76/F	Epigastric discomfort	Multiple swollen and reddish folds with a hemorrhagic and erosive mucosa	/	Greater curvature of the gastric body	Thickened submucosal layer	Amyloid deposits in the submucosa and mucosa, Congo red (+)	AL	Biopsy of the rectum and ileum (-); Bence-Jones protein (-); echocardiography (-)	Gastric carcinoma	None
Jin *et al*[61], 2014	33/F	Epigastric pain, dyspepsia, heartburn, acid reflux	One area with irregular borders and a hemorrhagic mucosa; another area with normal borders and smooth surfaces	1.2 cm × 1.2 cm; 10 mm × 20 mm	Lesser curvature of the gastric body; gastric fundus	Hypoechoic thickened stratum mucosum and lamina muscularis protruded in the lesser curvature; nonechoic lesions in the fundus	Amyloid deposits detected from the submucosa to muscularis propria and around small blood vessels using H&E staining; Congo red (+); van Gieson staining (-)	/	/	/	ESD; DMSO
Yamaguchi *et al*[47], 2015	49/M	/	One elevated lesion similar to a submucosal tumor	15 mm	Greater curvature of the lower body	A hypoechoic mass with hyperechoic spots in the submucosa and the muscular layer	Amyloid deposits in the submucosa; Congo red (+)	AL	Biopsy of other tissues in the gastrointestinal tract (-)	/	/
Kobara *et al*[37], 2015	80/M	Epigastric discomfort	One granular, elevated lesion	20 mm	Posterior wall of the prepyloric ring	A hypoechoic mass in the submucosa	Congo red (+)	AL	/	/	/

Kagawa *et al*[32], 2016	73/M	None	One pale, depressed area with clear borders	15 mm	The anterior wall of the lower gastric body	/	H&E staining: Amyloid deposits in the lamina propria and submucosa; Congo red (+)	AL	CT of the chest, abdomen and pelvis (-); urine Bence-Jones protein (-); electrocardiogram, echocardiography	/	None
Ahn *et al*[33], 2018	55/F	None	One pale, round, central-depressed area with irregular and heaped-up edges	20 mm	Lesser curvature of the mid-gastric body	/	Lymphoplasmacytes and Congo red (+) staining in the lamina propria	AL (κ&λ)	Biopsy of colon and duodenum (-); echocardiography (-); CT of chest abdomen and pelvis (-); antineutrophilic antibodies, rheumatoid factors, serum immunoglobulin and components, antinuclear antibodies and urine Bence-Jones protein	cancer	None
Ding *et al*[53], 2018	54/M	None	One well-defined lesion with irregularly distorted vessels	/	/	Thinned superficial mucosa, thickened deep mucosa	Congo red (+); H&E: Amyloid deposits in the mucosa	/	/	Early gastric cancer	Surgery
Kinugasa *et al*[34], 2018	64/M	None	One submucosal tumor with a hard elastic character	40 mm	Middle body of the greater curvature	In the second and third layers of the mucosa	Congo red (+) staining in the mucosal propria	AL (λ)	Bone marrow biopsy; biopsy of other gastrointestinal tissues; ultrasound and CT of the liver, kidney and heart	Myoma, malignant lymphoma, gastrointestinal submucosal tumor	/
Savant *et al*[43], 2018	64/M	/	One mass	3.6 cm	/	One hypoechoic mass in the muscularis propria	Congo red (+); H&E staining: Amyloid deposits with a foreign-body giant cell reaction	AL (λ)	CT, urine analysis and serology (-)	Gastrointestinal stromal tumor	/
Matsueda *et al*[35], 2019	59/M	None	Multiple pale and depressed lesions	/	Through the stomach	/	Congo red (+)	AL	Biopsy of other gastrointestinal tissues; Bence-Jones protein (-); ultrasound and CT	Healing gastric ulcer	/
Present case	70/F	Hematemesis	Multiple congestive erosions; one area of the mucosa with edema and ulcers exhibited unclear boundaries	4.0 cm × 4.0 cm	The anterior wall of the gastric body and fundus	/	Congo red (+); H&E staining: Massive amyloid fibrous connective tissues deposited in the interstitium with inflammatory cell infiltration	/	CT of the liver, colon, kidney; HRCT of the lung; ultrasound of the liver, heart, and kidney	/	None

ESD: Endoscopic submucosal dissection; DMSO: Dimethyl sulfoxide; CT: Computed tomography; ECG: Electrocardiograph; AL: Light chain; AA: Amyloid A; PFS: Progression-free survival.

is noted given the similarities in appearance. Furthermore, some patients appear normal in endoscopic evaluations, and endoscopy should therefore not be used for an independent diagnosis[39].

After reviewing our data, a reasonable hypothesis is that amyloid subtypes and endoscopic findings are correlated, based on the similar appearance and features noted among the patients with the same amyloid subtype. The endoscopic appearance corresponded to amyloid subtypes in the small intestine, which is the most frequent site of gastrointestinal amyloidosis. For instance, the endoscopic presentation of AL is mostly thickening (75%) and multiple polypoid protrusions (63%), whereas a fine

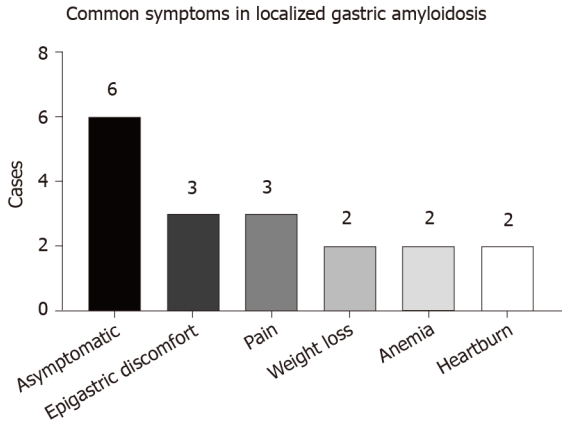

Figure 4 Common symptoms described in case reports of localized gastric amyloidosis and the present times.

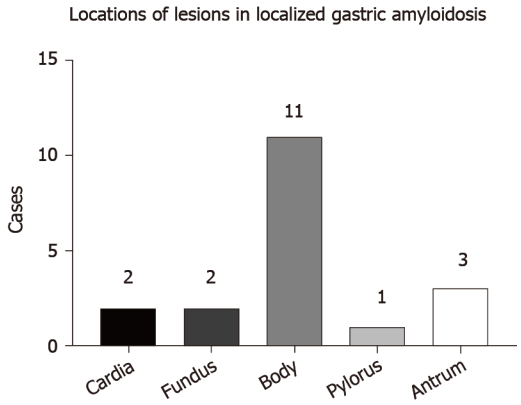

Figure 5 Common lesion locations of amyloid deposition in localized gastric amyloidosis and the present times.

granular appearance is predominant in patients with AA (85%)[49]. However, no association has been confirmed in the gastrointestinal tract according to a study with a small sample size, consistent with the results of the study by Samar M[39,50]. Considering the small sample sizes and limitations of published studies, further studies with large samples are required.

Narrow-band imaging with magnifying endoscopy (NBI-ME), a convenient technique that visualizes the mucosal morphology and microvascular architecture without a dye[51], is also highlighted in the amyloidosis diagnosis. Commonly, it is applied to detect malignancy in the colon and rectum through precise measurements of pit patterns with a promising accuracy (93.4%), sensitivity (100%), and specificity (75%)[52]. To date, the emerging use of NBI-ME has been reported not only in patients with LGA[32,46,47,53] but also in patients with amyloidosis of the rectum, trachea, and pleura[54-56]. Upon enhancement, special pathological patterns are detected in affected lesions, manifesting as abnormal distorted vascular networks on the mucosa with a grayish-green appearance and grooved surface, which may relate to amyloid protein deposits[32,46,56]. The application of NBI-ME provides a better method to reveal the amyloid deposits as a useful tool to assist with the diagnosis.

Endoscopic ultrasound (EUS) is also useful for determining the diagnosis by revealing the loss of the normal structure and thickened hypoechoic gastric walls, which mainly affects the mucosa and submucosa (Table 1). Its use in combination with other methods has certain clinical value, such as EUS-guided fine needle aspiration (EUS-FNA). In a retrospective study of 47 patients with amyloidosis presenting with lymphadenopathy (swollen lymph nodes), the involvement of the gastrointestinal tract was noted in 39% of cases[57]. In addition, EUS-FNA displayed a favorable sensitivity (83%), specificity (94%), and accuracy (86%) for distinguishing malignancy, although the size of the swollen lymph nodes may influence the accuracy[58]. The clearance of peripheral lymph nodes was once suggested as a preferred method to prevent malignancy[38]. However, with the assistance of EUS-FNA, the resection of swollen lymph nodes is not necessary in every case. Further use of EUS is expected.

Biopsy

Biopsy is an essential assessment for diagnosing amyloidosis. Direct biopsy verification in patients with symptoms is the main diagnostic criterion for gastrointestinal amyloidosis, according to the guideline of AL amyloidosis[30]. Specific staining methods, such as H&E staining and Congo red staining, are used to visualize the amyloid deposits. Under a light microscope, amyloid deposits typically manifest as amorphous eosinophilic hyaline materials after H&E staining[38]. Amyloids with foreign-body reactions[43,59] and plasma cell infiltration[40,45,60] are also noted. The gold standard for identifying amyloid protein is Congo red staining. When bound to the dye, amyloid deposits are orange under a light microscope, and exhibit green, orange, or yellow birefringence under a polarized microscope[1,12]. Congo red staining is commonly used to diagnose all types of amyloidosis and has been applied to various tissue samples. Most of the case reports collected in the present study use and present the results of both of H&E and Congo red staining to confirm the diagnosis. Biopsy findings also show that amyloids are mainly deposited in the lamina propria, submucosa, and mucosa. Amyloid involvement of blood vessels is also observed[31,38,59,61]. AA LGA is characterized by deposits in the mucosa, submucosa, and vascular walls[38]. AL LGA deposits are mainly located in the mucosal propria, submucosa, and mucosa[32,34,36,47,62]. In a study of 79 cases of gastric amyloidosis, depositions occurred in the muscularis mucosae, but our data do not reveal this characteristic, likely due to the limited number of cases and exclusion of patients with systemic gastric amyloidosis[39]. Interestingly, the amyloid subtypes may account for the difference in deposit locations, which has been verified in several studies. In patients with gastric amyloidosis, AA amyloid deposits are preferentially located in the lamina propria, while AL is mainly deposited in the muscularis mucosae[63].

Although useful, Congo red staining has limitations. For example, when inadequate amyloid labeling occurs or the procedure is performed by inexperienced examiners in poorly controlled conditions, it presents limitations and improved methods have been constantly introduced over the years[14,64]. The application of some hypersensitive techniques may improve the accuracy of amyloid detection. Luminescent dye-conjugated polymer (LCP) spectroscopy detects every deposit that Congo red does using an easy method and effectively reduces false positives[65]. Based on these findings, several probes have been used in experiments. In a study with a small sample of patients with systemic amyloidosis, heptameric formic thiophene acetic acid (h-FTAA) was reported to be more sensitive than Congo red staining when detecting amyloid in abdominal fat[66]. Furthermore, h-FTTA detects small amyloid-like structures that are negative for Congo red staining due to its high quantum field. Thus, this method might potentially achieve the early diagnosis of amyloidosis and allow considerable progress in early treatment. Moreover, h-FTTA possesses a high sensitivity and relatively low specificity, but its combination with Mayer's hematoxylin staining may compensate for its disadvantage in visual contrast[67]. Although the methods that we mentioned above are still in development, a reasonable expectation is the future development of a hypersensitive approach as a replacement for Congo red staining.

Assessment of amyloid typing

Different forms of amyloid lead to completely different prognoses, and amyloid typing is a routine test performed in patients with amyloidosis. Several assessments have been applied for amyloid typing, including immunohistochemistry (IHC), immunofluorescence (IF) staining, immunoelectron microscopy, and genetic testing[8,16,39]. However, only half (13/22) of the patients receive the test during the diagnosis of LGA, probably due to insufficient tissues and limited techniques[8]. IHC is currently used in the remaining patients and in patients with other forms of amyloidosis, as it is a convenient and rapid method. IHC was demonstrated to be a convincing approach with considerable specificity and sensitivity, according to a systematic study involving 117 patients[68]. However, some of its disadvantages include the presence of unclassifiable and misdiagnosed conditions due to the limited availability, specificity, and sensitivity of antibodies[16,65]. Laser microdissection/mass spectrometry (LMD/MS) requires a small amount of sample and exhibits a high specificity and sensitivity in typing clinical specimens[39,69]. LMD/MS has also been used to analyze samples that do not reach the standards for IF and exhibits a high detection rate of 92% compared to 45% using IHC[70,71]. Moreover, its use in combination with multiple reaction monitoring or liquid chromatography (LMD-LC-MS) results in the detection of amyloid protein that IHC fails to detect and achieves early amyloid detection, even when Congo red results are negative[72,73]. In addition,

the decellularization of an amyloid biopsy can facilitate the diagnosis of AL and ATTR amyloidosis, which are rich in plasma proteins. Here, decellularization provides a solution by removing the unnecessary proteins without altering the amyloid deposits and the basic structures of the biopsy[74,75]. Hopefully, these approaches may be used after further testing and reduce costs. Until then, we still call for the urgent increase in the use of IHC to diagnose LGA.

DIFFERENTIAL DIAGNOSIS

Systemic amyloidosis

The final diagnosis must be confirmed by the exclusion of the systemic involvement of other organs, which is generally performed using ultrasound of the heart and kidney and biopsy of the bone marrow and regions of the gastrointestinal tract, including the esophagus, duodenum, and colon. Occasionally, urine levels of the Bence-Jones protein and other antigens are tested. Under most conditions, the aforementioned tests are selected based on clinicians' experience with a screening procedure. Immunohistochemical examinations also provide a lead for the differentiation of systemic and localized amyloidosis, because the deposited protein exhibits specific distribution patterns. For example, AA and ATTR are often detected in the former type, while AL has been detected in both types[2,11].

Some radiopharmaceuticals present potential abilities to distinguish systemic and localized amyloidosis. SAP is one of the nonfibrillar components present in all types of amyloid deposits, and its abundant accumulation makes it an ideal radiotracer to visualize amyloid deposits in images of the body[2,22]. In a study of 189 patients with confirmed amyloidosis, ^{123}I SAP scintigraphy, a noninvasive qualitative method, presented a high sensitivity and specificity and was applied to diagnose most cases of AA and AL amyloidosis[76]. Through the use of whole-body scintigraphy, the injection of ^{123}I SAP obviously reveals the distribution and extent of amyloid deposits in images that a histopathological examination fails to detect, and the organ involvement identified using this technique exceed the results obtained in the clinic, which may be valuable in excluding systemic involvement. The only limitation is its failure in revealing the heart muscle[76]. It is gradually becoming a universal technique used in relevant studies for scanning systemic involvement in the whole body, except for the heart[3,77]. With the development of proper radiotracers and a considerable increase in use, we postulate that nuclear images will significantly contribute to the diagnosis of LGA.

^{18}F-fluorodeocyglucose positron emission tomography/computed tomography (^{18}F-FDG PET/CT) is also reported as a potential technique to distinguish systemic and localized amyloidosis. Under most conditions, it is introduced as a method for detecting lung malignancy in patients suspected of having the disease, and it is mainly used to scan amyloidosis in the lung[78,79]. Glaudemans *et al*[77] reported that ^{18}F-FDG PET/CT is able to visualize the difference between localized and systemic amyloidosis by imaging the inflammatory reaction of multinuclear giant cells, which are unique in localized amyloidosis, manifesting as positive FDG uptake at sites of amyloid deposits in patients with localized amyloidosis but negative uptake in patients with systemic amyloidosis[77]. However, Mekinian *et al*[80] also reported positive results in patients with systemic amyloidosis, which may be attributed to the limited number of samples or inappropriate designs[80]. To date, ^{18}F-FDG PET/CT is still an immature method to exclude systemic amyloidosis, and patients with gastric amyloidosis were not examined in either study. When used in conjunction with confirmed evidence obtained from other techniques, it may play a supporting role in determining the diagnosis.

Advanced cancer

Due to its rarity and nonspecific presentations, amyloidosis is typically not the first diagnosis suspected by clinicians, and differential diagnosis becomes an important step. Under most circumstances, patients are scheduled for a precise test screening for potential cancers and are confirmed to have amyloidosis. Most lesions were suspected to be advanced gastric cancers or some gastrointestinal tumors (9/22) since they share common appearances, such as ulcers, elevations, and tumor-like lesions. During gastroscopy or esophagogastroduodenoscopy, some of these lesions are easily suspected to be submucosal tumors[34,46]. The most reliable method for excluding the possibility of tumors is a tissue biopsy that does not detect tumor components and the confirmation of the presence of amyloid based on positive Congo red staining results.

Tumor biomarkers are occasionally involved in the examinations based on clinicians' experience. Although NBI-ME is a new technique, it may also facilitate differentiation, given its ability to exclude malignancies. Based on the clear visualization of the microvasculature and microstructure under NBI-ME, vascular and surface pattern classifications are proposed as a reference, and the typical hallmarks of advanced gastric cancer can be observed, thus providing a reliable evaluation of advanced gastric cancer[81]. A subsequent study reported an obviously increased sensitivity and accuracy of NBI-ME compared with routine approaches in scanning for advanced gastric cancer, and the use of NBI-ME was also beneficial to locate the most suspicious lesion for biopsy[82].

Compared to other case reports, we excluded advanced cancer using a more innovative screening method, namely, immunohistochemical staining, given our restricted conditions. Because gastric carcinoma is suspected in some cases[31,36,59], it may be a wise choice. The combination of several antibodies for immunohistochemical staining, including CKPAN, KL067, Periodic Acid-Schiff, spinal muscular atrophy and vimentin, with biopsy results did not reveal strong support for cancers and excluded the possibility of gastric cancer from various origins.

TREATMENT AND PROGNOSIS

Generally, the goal of therapy for amyloidosis is to eliminate harmful extracellular amyloid deposits and restore the normal function of affected organs as much as possible. The main treatments include surgery, observation, and radiotherapy[83]. Chemotherapy is recommended for patients with AL who present with myeloma.

Several novel techniques are also introduced here. As we mentioned above, SAP is a type of plasma protein present in all amyloid deposits, and its interaction with amyloid fibrils may prevent the digestion of amyloid, as evidenced by the results of *in vitro* experiments[21,22]. Because amyloidosis is delayed in SAP knockout mice, SAP clearance is introduced as a potential strategy for treating amyloidosis with the application of relevant antibodies and drugs[24]. Here, (R)-1-{6-[(R)-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl}pyrrolidine-2-carboxylic acid (CPHPC), a small-molecule drug with a high affinity for SAP, depletes most of the circulating SAP[84,85]. Its use in combination with the anti-SAP antibody dezamizumab results in a considerable reduction in the amount of residual amyloid protein visible in ^{123}I SAP scintigraphy, with no obvious side effects[85,86]. After treatment, patients tend to present an improved or stable state, suggesting its potential ability to ameliorate symptoms and reduce damage in the affected organs[84]. A subsequent study further confirmed its effects on the spleen, kidney, and liver[87]. Currently, studies targeting SAP are mainly conducted in mice and small samples of patients with systemic amyloidosis. Although complete removal of all amyloid deposits has not yet been achieved, its usage in patients with LGA as a rapid method for the early clearance of amyloid deposits is worthy of further exploration.

Currently, the first-line therapy for localized AL gastrointestinal amyloidosis is mainly observation/supportive care and the excision of amyloid deposits, and radiotherapy is rarely used, according to the experience of physicians from the Mayo Clinic[83]. Among 13 patients with LGA presented with clear therapeutic strategies, surgery was the main choice (8/13), and a few patients chose observation (4/13). The administration of dimethyl sulfoxide is also recommended to reduce the digestive symptoms and result in a visible improvement on endoscopy[61].

The prognosis of amyloidosis is generally related to the extent of organ damage. Unlike systemic amyloidosis, localized amyloidosis has an excellent prognosis and minimally affects patient survival[3]. After first-line treatment, most patients improved (53%) or achieved a stable state (31%), and only a few progressed (0.2%), according to a study enrolling 413 patients with localized AL amyloidosis. However, the study also mentioned an undeniable recurrence rate, as two or more recurrences occurred in 5% of the 413 patients, and the first 5 years after diagnosis is a crucial period for recurrence[83]. To date, no recurrence of LGA has been mentioned in the case reports published, and all patients presented a healthy state in follow-up visits, but close follow-up and regular examinations are necessary, particularly within the first 5 years (Figure 6).

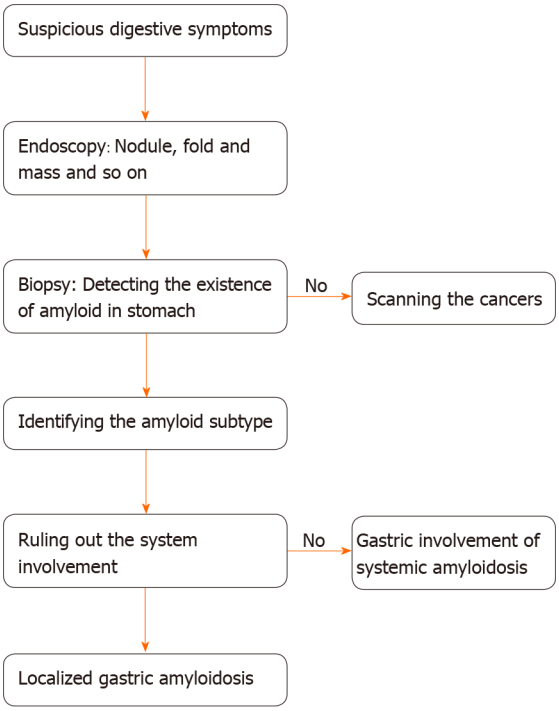

Figure 6 A diagnostic procedure from the perspective of clinicians. When a patient arrives at the hospital with suspected digestive symptoms, clinicians should initially perform endoscopy. Relevant endoscopic manifestations should lead to a biopsy examination to detect the existence of amyloid using Congo red staining. If a negative result is obtained, a screen for cancers is recommended, given the resemblance of their clinical manifestations. For a positive result, clinicians should identify the amyloid protein subtype. Then, a series of tests must be chosen by clinicians according to the patient' conditions to exclude the systemic involvement of amyloidosis. Finally, a diagnosis of localized gastric amyloidosis is determined.

CONCLUSION

Local gastric amyloidosis is such an extremely rare disease that only 22 cases have been reported in the past few decades and the disease is unknown to the public. It is commonly introduced as a human pathological state in which an abnormally misfolded protein accumulates in tissues, causing structure loss, organ dysfunction, and even death. Its pathogenesis remains a mystery, but a few influencing factors that may contribute to the formation of amyloid fibrils are studied and introduced here. Our review may inspire further investigations of the mechanism. Based on the 21 existing cases and our case, we present a detailed description of the main information available on LGA and conclusions regarding its clinical features, diagnostic tools, and treatment, with the goal of establishing future guidelines. Its clinical manifestations are complex and similar to those of other gastric diseases, such as advanced cancer, resulting in minimal awareness among clinicians. The diagnostic tools include biopsy, imaging, and amyloid typing. The final diagnosis mainly depends on the biopsy results, and Congo red staining remains the gold standard. Treatments for LGA mainly include supportive care and surgery. After treatment, most patients receive a good prognosis. Currently, due to its rare incidence, LGA lacks public awareness, and studies that explore its pathogenesis and its clinical features are often unspecific. For clinicians, LGA is a challenge to diagnose using regular tests. Building on that information, we describe the main clinical features and take the lead in proposing a process for diagnosing LGA from the clinicians' perspective, with the aims of promoting awareness of LGA and potentially contributing to the development of LGA guidelines.

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MINIREVIEWS

Emerging wearable technology applications in gastroenterology: A review of the literature

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Abstract

The field of gastroenterology has recently seen a surge in wearable technology to monitor physical activity, sleep quality, pain, and even gut activity. The past decade has seen the emergence of wearable devices including Fitbit, Apple Watch, AbStats, and ingestible sensors. In this review, we discuss current and future devices designed to measure sweat biomarkers, steps taken, sleep efficiency, gastric electrical activity, stomach pH, and intestinal contents. We also summarize several clinical studies to better understand wearable devices so that we may assess their potential benefit in improving healthcare while also weighing the challenges that must be addressed.

Key Words: Wearable technology; Wearables; Ingestibles; Smartphone; Remote patient monitoring; Gastroenterology

Core Tip: Wearable technology allows continuous health monitoring to provide a novel means of diagnosing and managing patients. Applications of wearable technology such as wrist wearables, abdominal wearables, smartphones and mobile apps, and ingestible sensors, are developing in gastroenterology. The aim of this review is to investigate current data from the literature that studies recent wearable technologies in several gastrointestinal diseases including inflammatory bowel disease, irritable bowel syndrome, and other functional gastrointestinal disorders.

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INTRODUCTION

Wearable devices are revolutionizing medicine and impacting healthcare by enabling continuous health monitoring outside of the clinic[1]. These wearables include devices that can be worn from head to toe and even swallowed. In patients with gastrointestinal diseases such as inflammatory bowel disease and irritable bowel syndrome, these devices collect physical activity, sleep quality, heart rate and rhythm, and more recently, gut activity and gas profiles. Despite the surge of consumer interest in these technologies, there is a lack of sufficient evidence to support their widespread use in clinical practice.

The field of gastroenterology has seen an emergence of wearable technology that has the potential to diagnose, manage, and even prevent disease. As technological advancements continue, classifying devices into categories will become essential. The purpose of this article is to offer focused insights into backgrounds for categorizing devices, the various uses of wearable technology, and future opportunities for clinical applications, with a focus on wrist wearables, abdominal wearables, smartphones, and ingestible sensors (Table 1).

In this review, we performed a PubMed search using the search terms "wearables," "wrist wearables," "abdominal wearables, "smartphones," and "ingestible sensors." We only selected manuscripts, which were original articles, and includes studies in several gastrointestinal diseases including inflammatory bowel disease, irritable bowel syndrome, and other functional gastrointestinal disorders. The objectives of this review were (1) to assess how wearable technology could assist physicians in investigating, diagnosing, or even treating our patients with gastrointestinal diseases; and (2) to recommend how wearable technologies could be applied in the future for several gastrointestinal diseases, including inflammatory bowel disease, irritable bowel syndrome, and other functional gastrointestinal disorders.

WEARABLE DATA TYPES AND USE

Wearable technology may be better understood by categorizing the types of data that can be collected. One type is data collection that requires active patient engagement with the device to obtain data that then can be transmitted in real time or uploaded to a stored source. This allows the user's data to be collected by a device such as a wrist wearable, which then can be uploaded to the electronic health record. For example, active patient engagement may be used to correlate certain symptoms of acute mesenteric ischemia with electrocardiographic assessment to detect the presence of a related arrhythmia. Another type is data collection that does not require active initiation other than the first step of wearing the device. Once the device is worn, it may passively collect data by continuously or intermittently obtaining data to be transmitted or stored and later uploaded. These passive data collections may include continuous measurements of heart rate, respiratory rate, tone of voice, caloric intake, and gastrointestinal activity in a patient with an underlying gastrointestinal condition.

Wearable data may be most useful in its ability to inform individuals and physicians of the effects of the patient's actions, management, or clinical status[2]. Ideally, these devices will provide data to offer decision support and even offer built-in therapies[3]. For example, we know that diet can be modified to modulate the microbiome[4] but to effectively design individualized diets, feedback is needed to close the loop between a prescription and its effects. This feedback can offer automated recommendations for instant modification of a patient's behavior and therapy. Even for devices that are unable to offer built-in therapy, the data collected can be used for diagnosis, prognosis, management, or prevention.

Table 1 Summary of wearable technology along with clinical applications

Ref.	Device name	Device type	Clinical applications
[5,12,13,20,21]	Fitbit; Apple Watch; Amazon Halo	Wrist Wearable	Daily activity monitoring (steps taken, energy expenditure, and sleep hygiene)
[11]	Sweatsenser	Wrist Wearable	Inflammatory bowel disease monitor and management
[27,28]	Electrogastrogram	Abdominal Wearable	Ambulatory monitoring, functional GI disorders screening, diagnosis, and management
[31,32,34]	AbStats; G-Tech Medical	Abdominal Wearable	Bowel sounds and movement monitoring, postoperative ileus and delayed gastric emptying
[27]	N/A	Smartphone App	Meal logs, exercise, bowel movement, and sleep synchronized to electrogastrogram recording
[39]	UCLA eIBD patient app	Smartphone App	Inflammatory bowel disease activity monitor
[40]	HealthPROMISE app	Smartphone App	Tracks symptoms, quality of life, follow up, and intervention integrated with electronic health record
[41,42]	StudentLife app	Smartphone App	Assess stress, sleep, activity, mood, mental well-being, and academic performance
[43]	PoopMD; Pooplog	Smartphone App	Records stool types, records bowel movements
[48]	N/A	Ingestible	Vital sign monitor, motility disorder diagnosis and management
[49]	IMBED	Ingestible	Gastrointestinal bleed diagnosis, management, and monitoring
[50]	N/A	Ingestible	Understand intestinal function, microbiota, and individual response to dietary change
[56]	Colon Capsule Endoscopy	Ingestible	Minimally invasive colonoscopy method
[57]	Digital Pills	Ingestible	Monitor medication adherence

IBD: Inflammatory bowel disease; UCLA: University of California Los Angeles; GI: Gastrointestinal; N/A: Not applicable.

WRIST WEARABLE DEVICES, INFLAMMATORY BOWEL DISEASE AND IRRITABLE BOWEL SYNDROME

Commercially available wrist wearable devices have grown rapidly in popularity during these recent years due to advancements in technology and the public's increased health consciousness. These wrist wearable devices such as Fitbit, Apple Watch, and the new Amazon Halo aim to provide the user with real-time feedback on various aspects of daily activities such as number of steps taken, energy expenditure, sleep hygiene, and time spent in different levels of activity[5]. They also provide personal goal setting options, data summary, and visualizations through synchronization with mobile- and computer-based apps such as health and fitness apps as well as options to connect to social media. Increasing consumer interest and improvement of data collecting capabilities of wearable technology has drawn attention to the devices as a potential avenue to improve the care of patients with inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS).

IBD, which includes Crohn's disease and ulcerative colitis, is characterized by chronic relapsing intestinal inflammation[6]. Although the etiology of IBD remains largely unknown, it is thought that IBD results from an abnormal and continuing immune response to the microbes in the gut, catalyzed by the genetic susceptibility of the individual[6]. Despite advances in therapeutic development, only 40%–60% of IBD patients can achieve remission at 1 year, and symptomatic relapse still occurs in at least 15% of patients per year[7,8]. Prediction of symptomatic relapse would be highly desirable in IBD patients as this would allow for early intervention or prevention. Studies have shown that quality of life for individuals with IBD was poorer than for healthy individuals, for both adults and children[9,10]. Effective and convenient strategies for prediction and prevention of relapse are needed.

IBD represents a chronic disease where the application of wearable technology may be able to improve management and predict or even prevent inflammatory disease flare. In a first study, Jagannath *et al*[11] used EnLiSense's Sweatsenser for noninvasive continuous monitoring of interleukin-1 (IL-1β) and C-reactive protein (CRP), two key biomarkers associated with IBD, in human eccrine sweat. The sensor device demonstrated capability to detect and real-time monitor IL-1β and CRP in sweat. This study signifies a promising non-invasive wearable microsensor device that has the

potential to empower patients to actively engage in monitoring and managing their IBD. This device may also give patients the chance to intervene earlier and help gastroenterologists understand whether treatment is effective.

Wiestler *et al*[12] investigated the association of quality of life with wearable-based physical activity in patients with IBD. A total of 91 patients with IBD were evaluated in terms of disease-specific quality of life, using the Inflammatory Bowel Disease Questionnaire (IBDQ), and physical activity, using an accelerometer. The IBDQ was significantly lower in patients with moderate-severe disease activity as compared to patients in remission, and the physical activity level was higher in remission than in active disease. This study found that parameters of physical activity were significantly correlated with the IBDQ, and steps per day, vigorous activity, and sleep efficiency were significantly associated with the IBDQ. Importantly, the data positively correlate with health-related quality of life and demonstrates the positive effect of physical activity for patients with IBD.

Hirten *et al*[13] surveyed 400 patients with self-reported IBD and found that 89% of them believed that wearable devices can provide important information about their health, and 93.8% reported that they would use a wearable device if it could help their physician manage their IBD. The patients specifically identified wrist wearables as the preferred device type and reported a willingness to wear them at least daily. Because of patients' willingness to participate, wearables allow them to actively engage in their health and further strengthen physician-patient collaboration, which will ultimately improve patient well-being and medicine as whole.

Irritable bowel syndrome, one of the most common disorders of gut-brain interaction worldwide, is a functional disorder of the gastrointestinal tract characterized by chronic abdominal pain or discomfort and bowel habit changes in the form of diarrhea, constipation, or alternating patterns between the two[14,15]. IBS is estimated to affect around 1 in 10 people globally[16] and is associated with reduced quality of life[17].

Many studies have shown that increased physical activity has positive long-term effects on IBS symptoms and psychological symptoms[18,19]. Hamaguchi *et al*[20] investigated the relationship between physical activity and gastrointestinal (GI) symptoms in 101 university students with IBS using the Gastrointestinal Symptoms Rating Scale and a pedometer, which measured gait steps for 1 wk. They found that the probability for daily locomotor activity to discriminate between 5 and 4 points on the Gastrointestinal Symptoms Rating Scale (*i.e.* likely to have reverse symptoms) decreased in accordance with increment of steps per day: 78% probability for 4000 steps, 70% probability for 6000 steps, 59% probability for 8000 steps, and 48% probability for 10000 steps. This study demonstrated that improvement in IBS symptoms increases with number of steps taken per day in IBS patients.

GI symptoms can also be triggered by several lifestyle factors including psychological distress, short sleep duration, and diet. Clevers *et al*[21] investigated the associations between selected lifestyle factors, measures of stress physiology, and GI symptoms. 1002 office employees were asked to report their GI symptoms, psychological distress, sleep times, and intake of caffeine, alcohol, and soft drinks for 5 d. They also recorded skin conductance, heart rate/variability, and acceleration using wearable sensors. Although the physiological variables such as skin conductance and heart rate variability were weakly associated with GI symptoms in this study, they found that short sleep duration was associated with next day GI symptoms and psychological distress mediated the association between short sleep duration and next day GI symptoms (61%).

Stress has been shown to play a major role in the onset and exacerbation of symptoms in IBS patients with stress related disorders such as anxiety and depression either preceding or following the development of IBS[22]. With wearables' capability of monitoring sleep, heart rate, physical activity, and tone of voice, these devices can alert patients of their well-being in real time and potentially recommend therapies to improve their well-being to serve as biofeedback to better control their stress and general health.

ABDOMINAL WEARABLE DEVICES, FUNCTIONAL GASTROINTESTINAL DISORDERS AND POSTOPERATIVE USE

The electrogastrogram (EGG) is a non-invasive device that is used for abdominal surface measurement of the gastric electrical activity of the human stomach[23]. However, it is rarely used due to inconsistent results and signal artifacts that make

interpretation and continuous monitoring difficult. Recent studies have shown the potential of EGG as an effective and non-stationary method to differentiate diabetic gastroparesis and functional dyspepsia patients[23].

Functional GI disorders can affect any part of the GI tract including the esophagus, stomach, and intestines. They are disorders of function, rather than structural or biochemical abnormalities. Examples of functional GI disorders include functional dyspepsia, gastroparesis, and irritable bowel syndrome (IBS). Functional dyspepsia[24], which is characterized by a sensation of pain or burning in the epigastrium, early satiety, fullness during or after a meal, or a combination of these symptoms, has a global prevalence between 5% and 11%[25]. Gastroparesis, which is characterized by delayed gastric emptying in the absence of mechanical obstruction, affects 4% of the United States population[26]. IBS, as stated above, is characterized by chronic abdominal pain and bowel habit changes, which deeply impairs and affects quality of life of many IBS patients. Functional GI disorders are typically diagnosed with subjective symptom-based assessment or objective but invasive procedures such as antroduodenal manometry, a procedure that measures motility with a catheter inserted through the mouth or nose with fluoroscopic or endoscopic guidance[27].

Gharibans *et al*[27] developed an innovative device that overcame the technical issues of the EGG with a wearable multi-channel system and artifact removal signal processing method, making it comparable to antroduodenal manometry, the gold standard diagnostic method. This non-invasive and easily administered approach potentially allows for patient monitoring outside of the clinic, helps better understand functional GI disorders, and leads to more effective screening, diagnosis, and management. Gharibans *et al*[27] also developed a smartphone app to enable the patients to document events or activities such as logging meals, exercise, bowel movement, and sleep, that are time-synchronized to the EGG recording for real-time feedback to the users.

The gut-brain axis consists of bidirectional communication between the central and the enteric nervous system, connecting emotional and cognitive centers of the brain with peripheral intestinal functions[28]. IBS is an example of the disruption of these complex relationships. Vujic *et al*[28] investigated the potential of using GI activity as an index of insula activity, which is the part of the brain associated with cognitive and affective functions. 33 participants with no known GI, neurological, or psychiatric disorders were connected to an EGG and EEG, presented emotionally salient film clips, and answered a self-assessment at the end of each clip. Although positive movie segments did not produce statistically significant changes (P = 0.4706), EGG signal analysis in the frequency domain demonstrated statistically significant changes from negative movie segments (P = 0.0209). Because EGG signals may be a sign of negative emotions, this gut-brain axis should be further studied in IBS patients in hopes of potential use of EGG in diagnosing and managing IBS.

Despite advances in surgical techniques, most patients develop temporary GI paralysis such as postoperative ileus (POI) and delayed gastric emptying (DGE) following abdominal surgery[29]. When prolonged or complicated, POI can worsen patient outcomes, increase resource utilization and cost, and extend hospital length of stay by 30%[30]. Data reveal that continuous audio recordings of bowel sounds strongly correlate with true intestinal motility as measured using antroduodenal manometry[31]. Spiegel *et al*[32] developed an acoustic gastro-intestinal surveillance (AGIS) biosensor – the Gastroinstestinal Logic AbStats system – a disposable plastic device embedded with a microphone that adheres to the abdominal wall and allows continuous and automated analysis of bowel sounds *via* noninvasive vibration and sound sensing. They compared intestinal rates using AGIS in 8 healthy controls, 7 patients tolerating feeding, and 25 with POI. Mean intestinal rates were 0.14, 0.03, and 0.016 events per second, respectively. AGIS separated patients from controls with 100% sensitivity and 97% specificity.

DGE following pancreaticoduodenectomy (PD) is a common complication, which occurs in up to 30% of cases[33]. In primary DGE, which is when not associated with other risk factors or intraabdominal complications, it is difficult to predict early on who will develop DGE after PD[34]. Dua *et al*[34] assessed whether the use of a novel, noninvasive wireless patch system (G-Tech Medical) that acquire gastric myoelectrical signals and transmit data by Bluetooth after PD is reproducible and can serve as an objective tool to identify patients who may be at risk of developing DGE. They found that tolerance of food was noted by 6 *vs* 9 d in the early versus late group by diet tolerance (P < 0.05) with higher cumulative gastric myoelectrical activity. Diminished gastric myoelectrical activity identified delayed tolerance to regular diet. This study introduces an abdominal wearable, wireless patch system capable of accurately monitoring gastric myoelectric activity after surgery, which can not only objectively

identify patients at risk for DGE but also potentially individualize feeding regimens to improve outcomes.

SMARTPHONES AND REMOTE PATIENT MONITORING OF GASTROINTESTINAL DISORDERS

The most common wearable device is the smartphone. The number of smartphone users has increased dramatically with smartphone ownership reported to be 43% globally and 72% in the United States[35]. Digital health refers to the use of digital, mobile, and wireless technologies to support achievement of health objectives, and the term is often interchangeably used with mobile health (mHealth) due to mobile devices' central role[36]. Due to its increasing popularity, smartphones provide one of the most promising platforms for mHealth interventions including activity trackers, telemedicine capabilities, and health-based apps. The integration of smartphones and mobile apps, remote sensor technologies like Fitbit, telemedicine, and electronic health records (EHR) allows for remote patient monitoring (RPM), which refers to digital tools capable of monitoring and reporting real-time data on patients' health activities outside of the usual healthcare settings[37,38].

Chronic GI disorders such as IBD and functional GI disorders are especially appropriate for RPM. Symptom flare risk and interventions required to control disease is heavily influenced by the patient's behaviors, which occurs outside of the healthcare setting and often are not adequately tracked or assessed such as stress levels, depression, smoking, or medication adherence. Because of these factors, patients with chronic GI disorders are ideal candidates for RPM to potentially improve self-management, quality of life, and collaboration.

Van Deen *et al*[39] developed an mHealth index that accurately monitors IBD activity using patient reported outcomes, which is currently implemented in the University of California at Los Angeles eIBD patient app and automated messages are sent to a nurse coordinator when the mHealth index indicates disease activity.

Atreja *et al*[40] created the HealthPROMISE app, a cloud-based patient reported outcome and decision support platform, which helps patients track their symptoms, quality of life, follow up, and interventions in real time and provides point of care intervention from physicians by integrating the app with EHR.

Wang *et al*[41,42] used the StudentLife app, a continuous sensing app that uses the smartphone's GPS, accelerometer, light sensor, and microphone integrated with call history, application usage, and texting patterns, to assess stress, sleep, activity, mood, sociability, mental well-being, and academic performance in college students. They found that the students' depression was significantly negatively correlated with sleep and conversation frequency and duration. These smartphone apps plus the new Amazon Halo, which captures mood using microphone, also have the potential to be integrated with EHR to monitor for depression and anxiety.

Franciscovich *et al*[43] used PoopMD, a mobile app that utilizes a smartphone's camera and color recognition software to analyze an infant's stool, and determined a sensitivity of 100% and specificity of 89%. They found that PoopMD accurately differentiates acholic from normal color stool and may be a valuable tool to help parents identify acholic stool and alert the infants' pediatricians. Apps like PoopMD and Pooplog, which allows patients to record bowel movements using the Bristol Stool Scale, can be further developed to be used in adult patients to identify various stools such as hematochezia and melena and even alert physicians of a possible GI bleed, infection, IBD flare, or constipation.

Studies have also shown that smartphones are widely used for social media and that a majority of social media is accessed through smartphones as compared to computers[44]. These social media such as Facebook, Instagram, and YouTube may have the potential to be used as platforms to broaden health education and outreach to a wider audience especially minority populations with cultural barriers to healthcare[45-47].

INGESTIBLES, GASTROINTESTINAL HEALTH AND BEYOND

Ingestible sensors, which are also known as swallowables, consist of a miniaturized detector and transmitter packed into a capsule that is swallowed and tracked through the intestine. Ingestibles are fast emerging with efforts continuously being made to

optimize these sensors for various clinical applications. These ingestible devices are noninvasive and provide information on pH, manometric pressure, temperature, medication adherence, vital signs, and intestinal lumen contents[48].

Dagdeviren *et al*[48] developed an ingestible sensor that settles on the stomach lining and allows for monitoring of vital signs and mechanical deformation of the gastric cavity. This flexible ingestible pieozoelectric device allows for possibilities in sensing mechanical variations and energy inside the GI tract, which may be applied in diagnosing and treating motility disorders and monitoring ingestion in obesity.

Ingestibles and microbiome

Mimee *et al*[49] created an ingestible micro-bio-electronic device that combines engineered probiotic sensor bacteria with microelectronics that communicates with an external device such as a smartphone. In this study, they engineered heme-sensitive probiotic biosensors and demonstrated accurate diagnosis of GI bleeds in swine (sensitivity and specificity of 83.3% at 60 min and 100% at 120 min). Thus, ingestible micro-bio-electronic device could transform diagnosis, management, and monitoring of GI health and disease.

The human gut is home to diverse microbes that play a fundamental role in the health and well-being of the host. The microbiota, which consists of bacteria, viruses, and eukaryotes, have been shown to interact with an individual's immune system to influence the development of diseases such as obesity, mental health issues, and atopic disease[50]. Gases of the gut, such as hydrogen carbon dioxide, nitrogen, and oxygen, have been significant in understanding the pathogenesis and diagnosis of gut disorders[51]. Gas production from bacterial fermentation is likely to produce symptoms in patients with diseases like IBS[52] and small intestine bacterial overgrowth[53]. Kalantar-Zadeh *et al*[54] developed an ingestible electronic capsule that can sense oxygen, hydrogen, and carbon dioxide. This study showed the potential of this gas-sensing capsule in understanding functional aspects of the intestine, the microbiota, and intestinal response to dietary changes. This allows for a novel diagnostic and monitoring tool that can be used for various clinical indications such as constipation and obesity and can aid in development of individualized diets and lead to more personalized medicine.

Colon cancer screening with ingestibles

Although conventional colonoscopy is currently the gold standard for bowel cancer screening, the colon capsule endoscopy (CCE) continues to be further developed and improved since its introduction in 2007[55]. The currently available second generation CCE has been developed to look at the inside of the gut wall using visible light and two video cameras that cover nearly 360 degrees and transmits images to an external monitor[56]. This is used primarily for incomplete colonoscopy, polyp detection, and IBD, but with further technological advancements and research, CCE has the potential to be a minimally invasive and reliable method for bowel cancer screening.

Ingestibles in medication monitoring

Medication nonadherence is a common issue in healthcare, which may lead to poor outcomes in many patients. Digital pills are an innovative drug-device technology that combines medications with a monitoring system that records in real-time medication adherence[57]. An ingestible event marker is embedded within tablets and activated in the stomach. Once activated, the ingestible event marker communicates to a patch, which is applied to the patient's torso, then the signal transmits *via* Bluetooth to an external device such as a smartphone or computer. These digital pills allow physicians to monitor adherence among patients in hopes of improving rates of adherence and can further remote patient monitoring.

DISCUSSION

Wearable technology could represent a vital method for gastroenterologists to diagnose, manage, and monitor patients with numerous GI conditions and may even prevent disease. Because of the many available technologies such as remote sensor wearables, smartphones and mobile apps, telemedicine, and electronic health records, remote patient monitoring is very promising in the near future. Wearable devices have the ability to connect wirelessly to other devices, allowing the transfer and exchange of information and placing these devices in a category of technology known as the Internet-of-Things[58]. The Internet-of-Things is one framework that will make such a

future possible by providing the framework for exchange and communication of data between sensors and health care providers[58]. This will benefit physicians and patients as wearable sensor systems can help reduce the costs associated with high-quality and continuous health care monitoring by reducing unnecessary hospital admission and length of stay[59], facilitate health behavior in the long run by monitoring and sending alerts to patients to give cues to modify behavior[60], and improve health in vulnerable populations[61].

Although wearable technology is a promising innovation in the field of gastroenterology, their use has also raised a number of concerns such as data accuracy and privacy issues (Table 2). Future studies could continue to investigate data accuracy of these various wearable technology as further developed and improved hardware and software algorithms are necessary before its use in daily clinical practice. Wearable devices store large amounts of information that is accessed by third parties, which creates a potential exposure of personal information to unauthorized users. Technological developments need to be carefully addressed to ensure that patients feel comfortable sharing a significant amount of data regarding their daily lives with health care providers, insurance companies, and data analytic companies[62]. Regulations will also need to evolve continuously to ensure the best interest of the general population. Nonetheless, wearable technology continues to expand and make great impacts in patients' lives from fitness to health and wellness monitoring to possible future diagnostic and management tools.

CONCLUSION

In general, remote patient monitoring in the field of gastroenterology are showing great promise for detection of GI conditions and managing and monitoring patients during their routine daily lives. They also show potential of reducing health care costs by encouraging better self-management and intervention approaches while allowing for a stronger physician patient collaboration and more personalized medicine. With rapidly advancing technological advancements, wearable technology has the potential to revolutionize how physicians provide high quality, reliable, and affordable health care to all.

Table 2 Benefits, challenges, and future advances of wearable technology

Benefits	Challenges	Future research
Method of diagnosis, management, monitoring, and prevention of various gastrointestinal conditions; Remote patient monitoring; Reduce healthcare costs; Encourage better patient self-management and intervention; Improve health in vulnerable populations; Reduce spread of disease and protective tool for healthcare workers; Facilitate physician patient collaboration towards personalized medicine	Data inaccuracy; Privacy issues	Investigate data accuracy with improved hardware and software algorithms; Technological developments to ensure patient privacy; Regulations to ensure patient comfort with sharing data

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ORIGINAL ARTICLE

Retrospective Cohort Study

Perioperative blood transfusion decreases long-term survival in pediatric living donor liver transplantation

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Abstract

BACKGROUND

The impact of perioperative blood transfusion on short- and long-term outcomes in pediatric living donor liver transplantation (PLDLT) must still be ascertained, mainly among young children. Clinical and surgical postoperative complications related to perioperative blood transfusion are well described up to three months after adult liver transplantation.

AIM

To determine whether transfusion is associated with early and late postoperative complications and mortality in small patients undergoing PLDLT.

Joaquim EHG, Seda-Neto J, da Fonseca EA, Pugliese RPS, Vintimilla AM, Carmona MJC, Auler Jr JOC, and D'Alburquerque LAC made critical revisions related to important intelectual content of manuscript; all authors have read and approved the final version of the manuscript.

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METHODS

We evaluated the effects of perioperative transfusion on postoperative complications in recipients up to 20 kg of body weight, submitted to PLDLT. A total of 240 patients were retrospectively allocated into two groups according to postoperative complications: Minor complications (n = 109) and major complications (n = 131). Multiple logistic regression analysis identified the volume of perioperative packed red blood cells (RBC) transfusion as the only independent risk factor for major postoperative complications. The receiver operating characteristic curve was drawn to identify the optimal volume of the perioperative RBC transfusion related to the presence of major postoperative complications, defining a cutoff point of 27.5 mL/kg. Subsequently, patients were reallocated to a low-volume transfusion group (LTr; n = 103, RBC ≤ 27.5 mL/kg) and a high-volume transfusion group (HTr; n = 137, RBC > 27.5 mL/kg) so that the outcome could be analyzed.

RESULTS

High-volume transfusion was associated with an increased number of major complications and mortality during hospitalization up to a 10-year follow-up period. During a short-term period, the HTr showed an increase in major infectious, cardiovascular, respiratory, and bleeding complications, with a decrease in rejection complications compared to the LTr. Over a long-term period, the HTr showed an increase in major infectious, cardiovascular, respiratory, and minor neoplastic complications, with a decrease in rejection complications. Additionally, Cox hazard regression found that high-volume RBC transfusion increased the mortality risk by 3.031-fold compared to low-volume transfusion. The Kaplan-Meier survival curves of the studied groups were compared using log-rank tests and the analysis showed significantly decreased graft survival, but with no impact in patient survival related to major complications. On the other hand, there was a significant decrease in both graft and patient survival, with high-volume RBC transfusion.

CONCLUSION

Transfusion of RBC volume higher than 27.5 mL/kg during the perioperative period is associated with a significant increase in short- and long-term postoperative morbidity and mortality after PLDLT.

Key Words: Liver transplantation; Child; Blood transfusion; Outcome; Liver cirrhosis; Mortality

Core Tip: This study aimed to assess whether perioperative transfusion is associated with early and late postoperative complications and mortality in small patients undergoing pediatric living donor liver transplantation (PLDLT). The volume of perioperative packed red blood cell (RBC) transfusion was the only independent risk factor for major postoperative complications. The perioperative volume of RBC > 27.5 mL/kg was an independent risk factor for mortality, increasing the risk by 3.031-fold, and was directly related to reduced patient and graft survival. In conclusion, not even massive transfusion, in the perioperative period, was associated with a significant increase in short- and long-term postoperative morbimortality after PLDLT.

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INTRODUCTION

Blood transfusion has been associated with increased morbimortality rates in major surgical procedures such as hepatic resection[1,2], cardiac[3] and non-cardiac major thoracoabdominal surgeries[4], and adult liver transplantation (LT)[5,6]. Regarding adult LT, Boyd *et al*[7] showed that intraoperative red blood cell (RBC) transfusion volume, a positive history of anti-RBC alloantibodies, and the immunosuppressive regimen used are associated with patient mortality. De Boer *et al*[5] reported that the indication for LT and the number of platelets or RBC units transfused during surgery are risk factors for 1-year graft survival; additionally, the number of RBC or platelet units transfused during surgery, cold ischemia time, and surgical team experience are risk factors for 5-year graft survival. Also, RBC transfusion has been associated with an increased rate of cancer recurrence. Indeed, the relative risk of digestive cancer recurrence has been reported to increase by 2.1-fold after the administration of ≥ 3 units[8]. Storage time and the timing of the transfusion related to surgery may also play a role[8]. In patients undergoing adult LT, all blood products (BP) are related to increased postoperative complications[9,10]; consequently, there is a trend towards reducing their use[11-13].

The harmful effects of perioperative transfusion in small pediatric patients are unclear pediatric LT. López Santamaría *et al*[11] suggested decreased graft survival associated with massive intraoperative blood transfusion, which was defined in their study as a loss greater than four volemias. They found four independent risk factors for mortality: Recipient's age < 3 years, retransplantation, severity of the underlying disease, and the transplant team's experience. Blood transfusion was not among them, however. To date, few reports have addressed the risks of transfusion in pediatric LT. In 2012, Nacoti *et al*[14] found that perioperative transfusion of fresh frozen plasma (FFP) RBC are independent risk factors for decreasing 1-year patient and graft survival. Nacoti *et al*[15] reported that intraoperative platelet and RBC transfusion are independent risk factors for developing major complications in the first year after pediatric LT.

Fanna *et al*[16] showed that high intraoperative bleeding is associated with pre-LT abdominal surgeries, factor V level ≤ 30%, and ex-situ parenchymal transection of the liver graft. Jin *et al*[17] identified high white blood cell count, low platelet count, and a deceased donor as independent risk factors for massive transfusion, which was defined as the administration of RBC volume ≥ 100% of the total blood volume (TBV). Although the graft failure incidence was higher in the massive group compared to the non-massive group, they found no difference in survival between the groups. Huang *et al*[18] evaluated pediatric living donor liver transplantation (LDLT) procedures and observed that younger patients with a lower weight, shorter stature, and preoperative prolonged international normalized ratio (INR) required larger blood transfusion volumes. Notwithstanding, preoperative INR was the only risk factor for massive blood transfusion. Kloesel *et al*[19] identified predictors of massive intraoperative bleeding (estimated blood loss of > TBV within a 24 h period): Preoperative hemoglobin (Hb) < 8.5 g/dL, INR > 1.5, platelet count < 100.000/mm^3, and surgery length > 10 h. Except for a longer intensive care unit (ICU) stay, there was no other correlation between massive transfusion and morbimortality.

Pediatric LT includes patients less than 18 years of age with either chronic or acute liver diseases involving deceased and living donors. Underlying pathologies may vary, and even the severity score is different under 12 years old [Pediatric end-stage liver disease (PELD) score]. Importantly, previous studies did not exclusively include young children. In addition, the grafts came from split and whole organs from deceased donors in most samples. Massive transfusion definitions vary across pediatric studies as BP transfusion from one[18] to four[11] volemias. There is still much controversy regarding the type, volume, and timing of BP transfusion and its association with postoperative morbimortality. Thus, this study assessed whether perioperative transfusion is associated with early and late postoperative complications and mortality in small patients undergoing pediatric LDLT (PLDLT).

MATERIALS AND METHODS

The Institutional Research Ethics Committees of ACCamargo Cancer Center and the University of São Paulo School of Medicine approved this observational, retrospective, and analytical cohort study according to the Helsinki Statement. All data were completely anonymized before they were accessed, and both committees waived the requirement for informed consent.

We investigated 254 pediatric patients weighing up to 20 kg with non-acute liver diseases who underwent first LDLT performed at the ACCamargo Cancer Center over 10 years. Fourteen patients were excluded: Five had fulminant hepatitis, three had their first transplant performed in other center, three were lost to follow-up, and three had missing data. All 240 enrolled patients underwent standardized procedures and techniques (total intravenous general anesthesia, piggyback inferior vena cava clamping, and exclusive use of hepatic left lateral segment grafts).

Study groups

Based on the severity of postoperative complications (graded according to the Clavien-Dindo classification during hospital stay), a total of 240 patients were initially allocated into two groups (Figure 1A): The minor complications (MiC) group (n = 109, either with no complications or with grade I-IIIa complications) and the major complications (MaC) group (n = 131, with at least one grade IIIb-V complication). Subsequently, all patients were reallocated into two further groups according to the RBC volume transfused during the perioperative period from 24 h before to 48 h after LT: The low-volume transfusion group (LTr n = 103, RBC ≤ 27.5 mL/kg) and the high-volume transfusion group (HTr n = 137, RBC > 27.5 mL/kg) (Figure 1B).

Classification of postoperative complications and postoperative follow-up

The Clavien-Dindo classification was applied to assess the relationship between blood transfusion and postoperative complications. In this pediatric population, general anesthesia was ostensibly required to ensure stillness and safety for diagnostic and treatment procedures. Thus, the original grade III was modified and subdivided into IIIa and IIIb according to the complexity of the procedures as low and high, respectively (Table 1). Data on mortality and complications were collected over a 10-year period post-LT. Patients who failed to attend outpatient follow-up after hospital discharge were considered lost to follow-up.

Complications were categorized by clinical presentation in 14 types: (1) Bleeding: Epistaxis, gastrointestinal, surgical wound, drains, and systemic hemorrhages due to portal hypertension or coagulopathy with or without need for surgery; (2) Cardiovascular: Systemic arterial hypertension, mismatches of cardiac rhythm, heart failure, hemodynamic instability, and cardiorespiratory arrest; (3) Dermatologic: Skin manifestations of drugs, food, and environmental factors; (4) Gastrointestinal: Malnutrition with weight-for-age z-score, height-for-age z-score[20], weight-for-height z-score, or body-mass-index z-score (BMIZ) ≤ -2 standard deviation, need for enteral or parenteral diet, persistent vomiting or diarrhea ≥ 3 wk, gastroesophageal reflux disease with or without brochoaspiration, visceromegaly, or ascites caused by maintained portal hypertension; (5) Infectious: Positive cultures with clinical or laboratory manifestations except from respiratory infections; (6) Malignancy: Post-transplant lymphoprolipherative disease (PTLD), lymphomas, skin tumors, and relapse of tumors; (7) Metabolic: Hydro-electrolytic serum changes such as hyponatremia (sodium < 133 mEq/L), hypernatremia (sodium > 147 mEq/L), hypokalemia (potassium < 3.0 mEq/L), hyperkalemia (potassium > 5.4 mEq/L), hypocalcemia (ionic calcium < 1.17 mmol/Lol/L), hypomagnesemia (magnesium < 1.8 mg/dL), hypophosphatemia (phosphorus < 2.5 mg/dL), arterial blood gases with pH < 7.2, acidosis and pH > 7.5, alkalosis, hyperlactatemia (lactate > 22 mg/dL), oliguria (diuresis < 0.5 mL/kg/h), adrenal insufficiency, diabetes mellitus, obesity (BMIZ > 2 standard deviations), or dyslipidemia (total cholesterol > 170 mg/dL, LDL fraction > 130 mg/dL, and triglycerides > 130 mg/dL); (8) Miscellaneous: Accidental injuries linked to LT procedure or postoperative follow-up; (9) Neuropsychiatric: Headache, vertigo, seizures, sedation withdrawal syndrome, delayed neuropsychomotor development, school learning difficulties, behavioral changes with psychomotor agitation, attention deficit, mood lability, anxiety, or depression; (10) Primary non-function (PNF) of the graft; (11) Rejection: Clinical and laboratory responsiveness to pulse therapy with methylprednisolone or anatomopathological documentation of acute or chronic rejection; (12) Renal: Renal failure was considered a decay of at least 50% of the estimated glomerular filtration rate (eGFR) applying the simplified revised Schwartz formula[21]; (13) Respiratory: Upper airway infections (rhinitis, sinusitis, otitis, tonsillitis, epiglottitis, pharyngolaryngitis), lower airway infections (tracheitis, bronchopneumonia, pneumonia), prolonged intubations (over 48 h), bronchospasm, atelectasis, effusions, pleural fistulas, hemothorax, pneumothorax, pneumomediastinum, non-cardiogenic edema, or acute respiratory failure; and (14) Surgical: LT specific complications (vascular thrombosis, biliary stenosis and fistulas, reoperation or retransplantation), hernias, dehiscence of anastomoses, or need for exploratory laparotomy except if caused by bleeding.

Table 1 Modified Clavien-Dindo classification for pediatric liver transplantation

Grade	Definition
I	Complication that requires the use of simple analgesics, antipyretics, anti-emetics, diuretics, electrolytes and physiotherapy
II	Complication requiring other drugs, different from grade I, blood transfusion or parenteral nutrition
III	Complication requiring surgical, endoscopic or radiologic intervention under any kind of anesthesia
IIIa	Low complexity procedures: Small and medium surgery, endoscopy and colonoscopy, US, CT, ERCP or PTCD, arteriography and angioplasty of portal vein or suprahepatic veins and hepatic artery, biopsies, simple teeth extractions, drainages, ostomies and central catheter passage for medication, dialysis or chemotherapy
IIIb	High complexity procedures: Large surgery, multiple teeth extractions, vascular and biliodigestive re-anastomosis, laparotomy and thoracotomy
IV	Life-threatening complication (including CNS[1] complications) requiring ICU admission
IVa	Dysfunction of one organ (including dialysis)
IVb	Dysfunction of two or more organs
V	Death of patient

[1]Cerebral hemorrhage, ischemic stroke, subarachnoid bleeding, excluding transient ischemic attack. Suffix "d" (for "disability") indicates that the patient still has complication, at time of discharge, requiring follow-up and must be entered to the degree of complication (*e.g.*, IVa-d degree). US: Ultrasound; CT: Computed tomography; ERCP: Endoscopic retrograde cholangiopancreatography; PTCD: Percutaneous transhepatic cholangiography or drainage; CNS: Central nervous system; ICU: Intensive care unit.

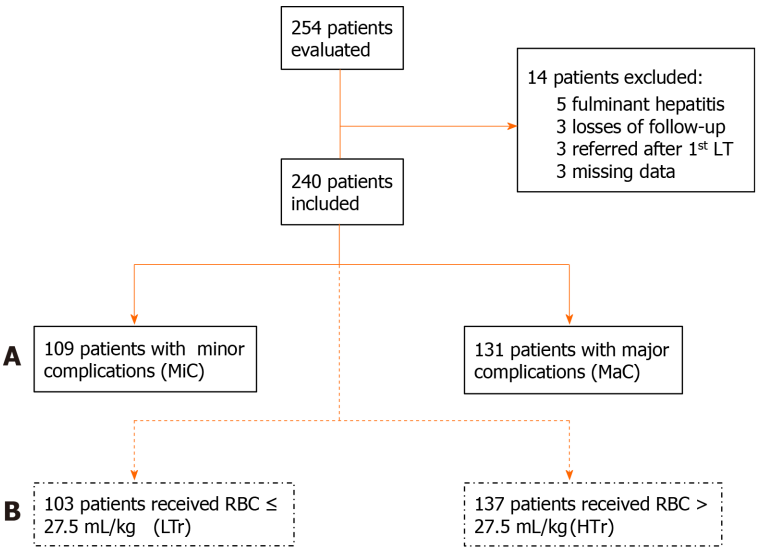

Figure 1 Patient flowchart. A: Minor complication group (grade I-IIIa) and major complication group (grade IIIb-V); B: Low red blood cell (RBC) volume transfusion group (RBC ≤ 27.5 mL/kg) and high RBC volume transfusion group (RBC > 27.5 mL/kg). LT: Liver transplantation; RBC: Red blood cell; MiC: Minor complication; MaC: Major complication; LTr: Low-volume transfusion; HTr: High-volume transfusion.

Statistical analysis

Multiple logistic regression analysis was performed to identify risk factors for major postoperative complications. The stepwise method was used for the selection of the variables. A perioperative RBC transfusion volume was identified as a single risk factor. A receiver operating characteristic curve was constructed using the perioperative RBC transfusion volume, and the occurrence of major complications were input parameters. A cutoff point of 27.5 mL/kg was identified using Youden's index (Figure 2).

Student's *t*-test or the Mann-Whitney test was used for quantitative variables while the chi-square test or Fisher's exact test was used for qualitative variables. Independent risk factors for mortality were identified using simple and multiple Cox regression analyses. Overall patient and graft survival analyses were performed using Kaplan–Meier survival curves, which were compared using the log-rank test. A P value level < 0.05 was used to define statistical significance. Statistical analyses were

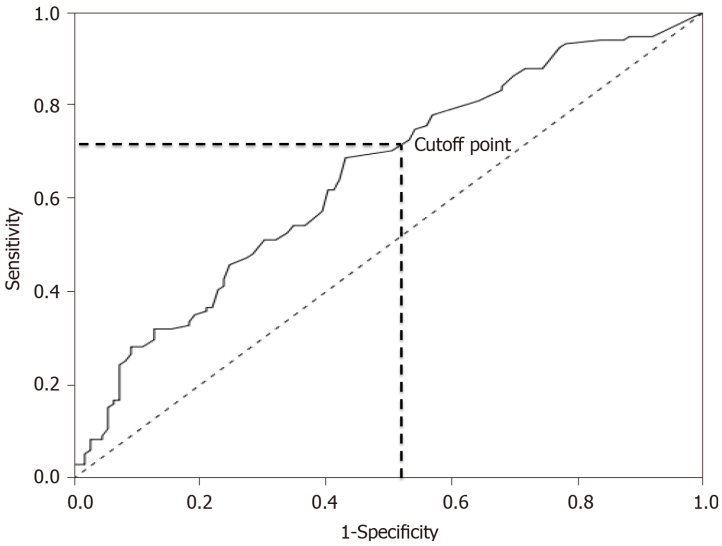

Figure 2 Receiver operating characteristic curve. A Receiver operation characteristic curve determined the optimal volume of perioperative red blood cells transfusion related to the presence of major postoperative complication. (Area under the curve = 0.648, $P < 0.0001$. Sensitivity = 68.7% and specificity = 56.9%. Cutoff point = 27.5 mL/kg; 95%CI: 0.578-0.717).

performed using SPSS version 23 (IBM Corp., Armonk, NY, United States) and R program version 3.2.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Patient characteristics

Of the 240 patients included in the study, 136 (56.7%) were females. The median patient age, weight, and stature were 12.4 mo, 8.07 kg, and 70 cm, respectively. Biliary atresia was found in 151 patients (62.9% of the underlying diseases) in our cohort. The Kasai procedure was previously performed in 111 cases (46.3%). The average pre-LT PELD score was 16 (± 7.7).

Perioperative risk factors for major postoperative complications

The overall incidence of major complications was 54.6%. In the MaC, all anthropometric measures, eGFR, Hb, sodium, and albumin levels were significantly lower. The INR, graft-to-body-weight ratio, and transfused BP volume were significantly higher than in the MiC. However, the only independent risk factor for major complications was perioperative RBC transfusion volume (Table 2).

Intraoperative and intensive care unit data

The HTr had lower Hb and sodium levels but significantly higher INR during the intraoperative period than the LTr. Additionally, the HTr had longer anesthetic and surgery time, a higher volume of crystalloids and colloids, higher diuresis rates, a lower incidence of extubation in the operating room, a longer intubation time, and a longer ICU and in-hospital stay than the LTr (Table 3).

Early postoperative complications

During hospitalization, the incidence of major complications *per* patient and the proportion of major complications were significantly higher in the HTr compared to the LTr (Table 4). Metabolic complications accounted for 28.2% of the complications during hospitalization. Additionally, complications such as gastrointestinal, malignancy, miscellany, neuropsychiatric, PNF, renal, and surgical were observed but with no significant difference between transfusion groups. The HTr had significantly more bleeding, respiratory, major cardiovascular, and major infectious complications but less dermatologic complications and rejections than the LTr (Table 5). Early LT-specific complications include PNF (2.1%), biliary fistula (6.2%), hepatic artery thrombosis (HAT) (3.3%), portal venous thrombosis (PVT) (9.2%), and retransplantation (1.2%); these were not related to a higher perioperative transfusion volume (Table 6). In terms of RBC transfusion volume, there was a significantly higher rate of

Table 2 Univariate and multiple logistic regression analyses of perioperative data stratified by the severity of complications

Variables	mean ± SD or No (%)		*P* value	OR	95%CI	*P* value
	MiC, *n* = 109	MaC, *n* = 131				
Male gender	54 (49.5)	52 (39.7)	0.081			
Age (d)	632 ± 440	512 ± 490	< 0.001			
Weight (kg)	9.67 ± 2.91	8.61 ± 3.09	< 0.001			
Height (cm)	76.6 ± 11.2	71.9 ± 11.6	< 0.001			
WAZ	-1.28 ± 1.24	-1.49 ± 1.31	0.105			
HAZ	-0.28 ± 1.15	-0.54 ± 1.24	0.012			
WHZ	-1.60 ± 1.34	-2.43 ± 1.48	< 0.001			
BMIZ	-0.20 ± 1.11	-0.36 ± 1.39	0.001			
Ascites	78 (71.6)	108 (82.4)	0.057			
PELD score	16.2 ± 7.0	17.8 ± 8.2	0.14			
Kasai surgery	50 (45.9)	61 (46.6)	0.698			
Portal hypertension	97 (88.9)	131 (100.0)	0.132			
Hepatopulmonary syndrome	16 (14.7)	15 (11.5)	0.414			
Infections 30 d pre-LT	0.4 ± 0.6	0.6 ± 0.7	0.081			
eGFR (mL/min/1.73 m^2)[1]	228 ± 121	187 ± 104	0.006			
Hemoglobin (g/dL)[2]	10.2 ± 1.5	9.3 ± 1.5	< 0.001			
Platelets (× 10^3/mm^3)[2]	196 ± 112	193 ± 118	0.789			
INR[2]	1.24 ± 0.25	1.49 ± 0.56	< 0.001			
Sodium (mEq/L)[2]	137 ± 2.7	135 ± 4.3	< 0.001			
Potassium (mEq/L)[2]	4.4 ± 0.5	4.3 ± 0.7	0.613			
Glucose (mEq/L)[2]	104 ± 59	92 ± 60	0.313			
Albumin (g/dL)[2]	2.9 ± 0.9	2.5 ± 0.8	0.002			
Total Bilirubin (g/dL)[2]	11.9 ± 7.3	13.4 ± 8.8	0.304			
GBWR (%)	3.3 ± 1.1	3.7 ± 1.2	0.035			
RBC (mL/kg)[3]	31.1 ± 25.4	46.7 ± 39.7	< 0.001	1.018	1.007-1.028	0.001
FFP (mL/kg)[3]	0.3 ± 2.6	3.0 ± 11.5	0.015			
Platelets (mL/kg)[3]	0.1 ± 0.8	1.2 ± 6.0	0.021			
Cryoprecipitate (mL/kg)[3]	0.0 ± 0.0	0.1 ± 1.2	0.196			

[1]Estimated glomerular filtration rate by simplified Schwartz's formula.
[2]Blood samples collected up to 72 h before liver transplantation (LT) anesthetic induction.
[3]Blood samples collected up to 2 h after the onset of LT anesthetic induction.
SD: Standard deviation; MiC: Minor complications group (I-IIIa); MaC: Major complications group (IIIb-V); WAZ: Weight-for-age z-score; HAZ: Height-for-age z-score; WHZ: Weight-for-height z-score; BMIZ: Body-mass-index-for-age z-score; PELD: Pediatric end-stage liver disease; LT: Liver transplantation; INR: International normalization ratio; GBWR: Graft-to-body-weight ratio; RBC: Red blood cells; FFP: Fresh frozen plasma; eGFR: Estimated glomerular filtration rate.

30 d reoperation (26.3% × 8.7%, $P < 0.001$) and 30 d mortality rate (6.6% × 0.0%, $P < 0.001$) in the HTr *vs* LTr, respectively (Table 6).

Late postoperative complications

Major cardiac complications were more frequent at 1, 5, and 10 years post-LT; major respiratory complications were more frequent at 5 and 10 years post-LT in the HTr than in the LTr. Dermatologic complications were less frequent up to 1 year in the HTr than in the LTr. Major infectious complications were more frequent, and rejections were less frequent in the HTr than in the LTr from 1 to 10 years post-LT. Minor

Table 3 Univariate analysis of preoperative, intraoperative and early postoperative data according to perioperative red blood cell volume transfused

Variables	mean ± SD or No. (%)		*P* value
	LTr, *n* = 103	HTr, *n* = 137	
Male gender	48 (46.6)	58 (42.3)	0.565
Age (d)	764 ± 544	419 ± 350	< 0.001
Weight (kg)	10.67 ± 3.20	7.88 ± 2.29	< 0.001
Height (cm)	80.0 ± 12.1	69.4 ± 8.7	< 0.001
WAZ	-0.90 ± 1.20	-1.75 ± 1.21	< 0.001
HAZ	-0.11 ± 1.41	-0.54 ± 1.24	0.012
WHZ	-1.60 ± 1.34	-2.43 ± 1.48	< 0.001
BMIZ	0.20 ± 1.20	-0.36 ± 1.39	0.001
Ascites	72 (69.9)	115 (83.9)	0.036
PELD score	14.1 ± 6.2	19.1 ± 7.9	< 0.001
Extra-hepatic cholestasis	67 (65.0)	105 (76.6)	0.029
Intrahepatic cholestasis	13 (12,6)	5 (3.6)	0.002
Cirrhosis	6 (5.8)	12 (8.8)	0.373
Metabolic diseases	7 (6.8)	10 (7.3)	0.852
Malignant diseases	7 (6.8)	3 (2.2)	0.159
Miscellany	3 (2.9)	2 (1.5)	0.751
Kasai surgery	40 (38.8)	74 (54.0)	0.016
Portal hypertension	96 (93.2)	132 (96.3)	0.132
Hepatopulmonary syndrome	19 (18.4)	12 (8.7)	0.043
Infections ≤ 30 d pre-LT	0 ± 1	1 ± 1	0.01
eGFR (mL/min/1.73 m^2)[1]	229 ± 121	188 ± 105	0.006
Hemoglobin (g/dL)[2]	10.2 ± 1.5	9.3 ± 1.5	< 0.001
Platelets (× 10^3/mm^3)[2]	196 ± 112	193 ± 118	0.789
INR[2]	1.24 ± 0.25	1.49 ± 0.56	< 0.001
Sodium (mEq/L)[2]	137 ± 2.6	135 ± 4.4	< 0.001
Potassium (mEq/L)[2]	4.3 ± 0.5	4.4 ± 0.6	0.613
Glucose (mEq/L)[2]	104 ± 59	92 ± 60	0.313
Albumin (g/dL)[2]	2.9 ± 0.9	2.5 ± 0.8	0.002
Total bilirubin (g/dL)[2]	11.9 ± 7.3	13.4 ± 8.8	0.304
Hemoglobin (g/dL)[3]	8.6 ± 1.5	7.6 ± 1.2	< 0.001
INR[3]	1.23 ± 0.42	1.67 ± 0.75	< 0.001
Sodium (mEq/L)[3]	135 ± 8	134 ± 5	0.002
Warm ischemia time (min)	42.2 ± 12.9	42.9 ± 11.4	0.515
Cold ischemia time (min)	53.3 ± 34.8	67.1 ± 56.3	0.118
Anesthetic time (h)	9.7 ± 1.6	10.8 ± 1.9	< 0.001
Surgical time (h)	7.8 ± 1.5	9.0 ± 1.9	0.001
Crystalloid (mL/kg)[4]	179 ± 67	251 ± 105	0.015
Colloid (mL/kg)[4]	33.6 ± 20.8	43.1 ± 19.7	< 0.001
Diuresis (mL/kg)[4]	47.5 ± 22.9	61.3 ± 36.6	0.013

GBWR (%)	2.9 ± 1.0	3.9 ± 1.1	< 0.001
Extubation in OR	24 (23.1)	16 (11.8)	0.014
Intubation period ≥ 48 h	7 (6.7)	35 (25.7)	< 0.001
ICU length of stay (d)	4 ± 5	8 ± 13	0.001
Hospital length of stay (d)	17 ± 13	23 ± 21	0.010

[1]Estimated glomerular filtration rate, preoperative calculated by Schwartz's formula.
[2]Blood samples collected up to 72 h before liver transplantation (LT) anesthetic induction.
[3]Blood samples collected up to 2 h after the onset of LT anesthetic induction.
[4]Intraoperative volume indexed by body's weight. Extra-hepatic cholestasis, extra-hepatic biliary atresia, coledocus cyst; intrahepatic cholestasis, Alagille's syndrome, non-syndromic biliary hypoplasia, primary sclerosing cholangitis, progressive intrahepatic familial cholestasis; cirrhosis, idiopathic, autoimune and cryptogenic; metabolic diseases, glycogenesis, Cligger-Najar's disease, tyrosinemia, cystic fibrosis, alpha-1 anti trypsin deficiency, urea cycle defects, type 1 oxaluria; malignant diseases, hepatoblastoma and hepatocarcinoma and hepatic miscellany diseases, Budd-Chiari syndrome, Caroli's disease and unclarified fibrosis. SD: Standard deviation; LTr: Low-volume transfusion group; HTr: High-volume transfusion group; WAZ: Weight-for-age z-score; HAZ: Height-for-age z-score; WHZ: Weight-for-height z-score; BMIZ: Body-mass-index-for-age z-score; PELD: Pediatric end-stage liver disease; LT: Liver transplantation; INR: International normalized ratio; GBWR: Graft-to-body-weight ratio; OR: Operating room; ICU: Intensive care unit; eGFR: Estimated glomerular filtration rate.

Table 4 Comparative analysis of postoperative complications during the hospitalization period using the modified Clavien–Dindo classification

Variables	All patients, *n* = 240		
	All complications during hospitalization, *n* = 783		
	mean ± SD or No. (%)	*P* value	
	LTr, *n* = 103	HTr, *n* = 137	
Patients with any complication	95 (92.2)	132 (96.4)	0.264
Patients with major complications (IIIb-V)	41 (39.8)	90 (65.7)	< 0.001
Complications *per* patient	2.5 ± 1.7	3.8 ± 3.6	0.007
Number of major complications (IIIb-V)	54/262 (20.6)	207/521 (39.7)	< 0.001
Median of the highest degrees of complication	IIIa (I-IVb)	IVa (I-V)	< 0.001
Median grade of complications *per* patient	II (I-IIIb)	II (I-V)	< 0.001

SD: Standard deviation; LTr: Low transfusion group; HTr: High transfusion group.

neoplastic complications were more frequent at later points in follow-up in the HTr than in the LTr (Table 5).

Late LT-specific complications were observed after 30 d over 10 years of follow-up, as biliary stenosis (12.1%), HAT (1.2%), PVT (3.7%), reoperation (7.1%) and retransplantation (2.5%), none of these were related to perioperative transfusion (Table 6). Overall, 10-years mortality rate, with respect to RBC transfusion volume, was significantly higher (25.5% *vs* 7.8%, $P < 0.001$), respectively, in the HTr compared to the LTr (Table 6).

Independent risk factors for death

Simple and multiple Cox regression analysis identified perioperative RBC volume > 27.5 mL/kg and preoperative eGFR as independent risk factors for mortality over 10 years of follow-up after LT (Table 7).

Patient and graft survival according to outcome and blood transfusion

The overall patient survival rates were 87.1%, 81.5%, and 80.3%, whereas the overall graft survival rates were 87.1%, 77.7%, and 75.6% at 1, 5, and 10 years post-LT, respectively. The graft survival rates were significantly lower in the MaC than in the MiC at 1, 5, and 10 years post-LT (81.5% *vs* 94.4%, 73.8% *vs* 84.6, and 72.2% *vs* 81%, respectively); however, no significant difference was seen in the patient survival rates between the MaC and the MiC. The patient survival rates were significantly lower in the HTr than in the LTr at 1, 5, and 10 years post-LT: 82.7% *vs* 97.7%, 73.9% *vs* 93.8%,

Table 5 Comparative analysis of type and grade of postoperative complications along the four periods of follow-up

Complication		Hospitalization			1 yr			5 yr			10 yr		
Type	Grade	LTr	HTr	*P* value	LTr	HTr	*P* value	LTr	HTr	*P* value	LTr	HTr	*P* value
Bleeding (%)	Minor	1(0.4)	6(1.1)	0.252	5(0.9)	11(1.3)	0.61	5(0.5)	13(0.9)	0.237	5(0.5)	13(0.9)	0.236
	Major	0(0)	9(1.8)	0.211	1(0.2)	9(1.0)	0.465	1(0.1)	9(0.7)	0.467	1(0.1)	9(0.6)	0.297
	Subtotal	1(0.4)	15(2.9)	0.016	6(1.1)	20(2.3)	0.178	6(0.6)	22(1.6)	0.052	6(0.6)	22(1.4)	0.068
Cardiovascular (%)	Minor	17(6.5)	29(5.6)	0.754	19(3.6)	32(3.6)	0.662	24(2.6)	39(2.8)	0.518	25(2.5)	42(2.7)	0.381
	Major	0(0)	17(3.3)	0.028	0(0)	18(2.0)	0.017	0(0)	18(1.3)	0.005	0(0)	18(1.2)	0.005
	Subtotal	17(6.5)	46(8.9)	0.319	19(3.6)	50(5.8)	0.098	24(2.6)	57(4.1)	0.075	25(2.5)	60(3.9)	0.282
Dermatologic (%)	Minor	9(3.4)	5(1.0)	0.093	25(4.7)	16(1.8)	0.009	52(5.6)	58(4.1)	0.322	59(5.8)	71(4.6)	0.583
	Major	0(0)	1(0.2)	1	1(0.2)	1(0.1)	0.406	7(0.8)	10(0.7)	0.168	8(0.8)	10(0.7)	0.114
	Subtotal	9(3.4)	6(1.2)	0.049	26(4.9)	17(1.9)	0.003	59(6.4)	68(4.8)	0.134	67(6.6)	81(5.3)	0.209
Gastrointestinal (%)	Minor	5(1.9)	13(2.5)	0.336	15(2.8)	30(3.4)	0.353	30(3.3)	44(3.1)	0.721	33(3.2)	49(3.2)	0.65
	Major	0(0)	1(0.2)	1	1(0.2)	4(0.5)	1	3(0.3)	6(0.5)	0.704	3(0.3)	6(0.4)	0.713
	Subtotal	5(1.9)	14(2.7)	0.627	16(3.0)	34(3.9)	0.488	33(3.6)	50(3.6)	1	36(3.5)	55(3.6)	1
Infectious (%)	Minor	21(8)	32(6.1)	1	86(16.2)	116(13.2)	0.577	188(20.3)	235(16.7)	0.434	207(20.3)	257(16.8)	0.394
	Major	32(12.2)	82(15.8)	0.013	43(8.1)	106(12.1)	0.028	56(6.0)	132(9.4)	0.02	60(5.9)	133(8.7)	0.022
	Subtotal	53(20.2)	114(21.9)	0.66	129(24.3)	222(25.3)	0.729	244(26.3)	367(26.1)	0.948	267(26.2)	390(25.5)	0.743
Malignancy (%)	Minor	0(0)	0(0)	1	4(0.8)	9(1.0)	0.576	13(1.4)	32(2.3)	0.067	13(1.3)	35(2.3)	0.027
	Major	0(0)	0(0)	1	4(0.8)	3(0.3)	0.058	9(1.0)	13(0.9)	0.137	11(1.1)	14(0.9)	0.07
	Subtotal	0(0)	0(0)	1	8(1.6)	12(1.4)	1	22(2.4)	45(3.2)	0.296	24(2.4)	49(3.2)	0.253
Metabolic (%)	Minor	86(32.8)	134(25.7)	0.786	89(16.8)	140(16.0)	0.494	96(10.4)	146(10.4)	0.329	102(10.0)	151(9.9)	0.416
	Major	1(0.4)	0(0)	0.207	2(0.4)	0(0)	0.052	2(0.2)	0(0)	0.069	2(0.2)	0(0)	0.077
	Subtotal	87(33.2)	134(25.7)	0.085	91(17.2)	140(16.0)	0.605	98(10.6)	146(10.4)	0.942	104(10.2)	151(9.9)	0.846
Miscellany (%)	Minor	8(3.1)	25(4.8)	0.067	30(5.7)	48(5.5)	0.093	66(7.2)	71(5.1)	0.179	73(7.2)	74(4.8)	0.1
	Major	1(0.4)	5(1.0)	1	3(0.6)	7(0.8)	0.702	4(0.4)	14(1.0)	0.792	5(0.5)	14(0.9)	1
	Subtotal	9(3.5)	30(5.8)	0.217	33(6.3)	55(6.3)	1	70(7.6)	85(6.1)	0.18	78(7.7)	88(5.8)	0.07
Neuropsyquiatric (%)	Minor	3(1.1)	2(0.4)	0.391	7(1.3)	4(0.5)	0.135	17(1.8)	14(1.0)	0.2	24(2.4)	23(1.5)	0.297

	Major	1(0.4)	1(0.2)	0.371	1(0.2)	1(0.1)	0.406	1(0.1)	1(0.1)	0.459	2(0.2)	1(0.1)	0.188
	Subtotal	4(1.5)	3(0.6)	0.169	8(1.5)	5(0.6)	0.134	18(1.9)	15(1.1)	0.116	26(2.6)	24(1.6)	0.109
PNF (%)	Minor	0(0)	0(0)	1	0(0)	0(0)	1	0(0)	0(0)	1	0(0)	0(0)	1
	Major	0(0)	5(1.0)	0.587	0(0)	5(0.6)	0.593	0(0)	5(0.4)	0.332	0(0)	5(0.3)	0.329
	Subtotal	0(0)	5(1.0)	0.175	0(0)	5(0.6)	0.164	0(0)	5(0.4)	0.164	0(0)	5(0.3)	0.164
Rejection (%)	Minor	40(15.3)	32(6.1)	0.004	73(13.8)	66(7.5)	0.003	111(12.0)	102(7.3)	0.106	117(11.5)	125(8.2)	0.03
	Major	0(0)	1(0.2)	1	1(0.2)	4(0.5)	1	4(0.4)	8(0.6)	0.526	5(0.5)	8(0.5)	0.363
	Subtotal	40(15.3)	33(6.3)	<0.001	74(14.0)	70(8.0)	<0.001	115(12.4)	110(7.9)	<0.001	122(12.0)	133(8.7)	<0.001
Renal (%)	Minor	2(0.8)	3(0.6)	1	3(0.6)	3(0.3)	0.693	4(0.4)	3(0.2)	0.469	4(0.4)	4(0.3)	0.731
	Major	2(0.8)	5(1.0)	0.637	2(0.4)	7(0.8)	1	5(0.5)	9(0.7)	0.535	5(0.5)	9(0.6)	0.547
	Subtotal	4(1.6)	8(1.6)	1	5(1.0)	10(1.1)	0.796	9(0.9)	12(0.9)	0.945	9(0.9)	13(0.9)	1
Respiratory (%)	Minor	8(3.1)	24(4.6)	0.093	75(14.2)	110(12.6)	0.934	161(17.4)	247(17.6)	0.141	177(17.4)	261(17.1)	0.106
	Major	7(2.7)	36(6.9)	0.539	9(1.7)	53(6.0)	0.096	14(1.5)	70(5.0)	0.029	15(1.5)	71(4.7)	0.017
	Subtotal	15(5.8)	60(11.5)	0.014	84(15.9)	163(18.6)	0.217	175(18.9)	317(22.6)	0.038	192(18.9)	332(21.8)	0.041
Surgical (%)	Minor	8(3.1)	9(1.8)	0.617	19(3.6)	23(2.6)	0.631	33(3.6)	49(3.5)	0.732	43(4.2)	66(4.3)	0.487
	Major	10(3.8)	44(8.4)	0.711	12(2.3)	51(5.8)	0.509	20(2.2)	56(4.0)	1	20(2.0)	58(3.8)	0.682
	Subtotal	18(6.9)	53(10.2)	0.166	31(5.9)	74(8.4)	0.092	53(5.8)	105(7.5)	0.118	63(6.2)	124(8.1)	0.079
Total minor complications (%)		208(79.4)	314(60.3)	<0.001	450(84.9)	608(69.3)	<0.001	800(86.4)	1053(75.0)	<0.001	882(86.6)	1171(76.7)	<0.001
Total major complications (%)		54(20.6)	207(39.7)		80(15.1)	269(30.7)		126(13.6)	351(25.0)		137(13.4)	356(23.3)	
Total complications per group (%)		262(33,5)	521(66,5)		530(37,7)	877(62,3)		926(39,7)	1404(60,3)		1019(40,0)	1527(60,0)	
Total complications per period (%)		783(30,8)			1407(55,3)			2330(91,5)			2546(100)		

HTr: High-volume transfusion group; LTr: Low-volume transfusion group; PNF: Primary non-function.

and 72.6% *vs* 90.9%, respectively. Likewise, the graft survival rates were significantly lower in the HTr than in the LTr at 1, 5, and 10 years post-LT: 79.5% *vs* 97.7%, 67.2% *vs* 92.3%, and 67.2% *vs* 87%, respectively (Figure 3).

Table 6 Patients with early and late liver transplantation specific complications and 30 d and 10 years mortality rate

Variables	Early complications			Late complications		
	No. (%)	No. (%)	*P* value	No. (%)	No. (%)	*P* value
	LTr, *n* = 103	HTr, *n* = 137		LTr, *n* = 103	HTr, *n* = 137	
PNF	0 (0.0)	5 (3.6)	0.072	0 (0.0)	0	NA
Biliary fistula	4 (3.9)	10 (7.3)	0.267	0 (0.0)	1 (0.7)	NA
Biliary stenosis	0	0	NA	15 (14.6)	14 (10.2)	0.307
Hepatic arthery thrombosis	2 (1.9)	6 (4.4)	0.478	0 (0.0)	3 (2.2)	0.261
Portal vein thrombosis	6 (5.1)	16 (11.7)	0.113	3 (2.9)	6 (4.4)	0.554
Reoperation	9 (8.7)	36 (26.3)	< 0.001	4 (3.9)	13 (9.5)	0.298
Retransplantation	0 (0.0)	3 (2.2)	0.262	2 (1.9)	4 (2.9)	0.306
30 d mortality	0 (0.0)	9 (3.6)	0.008	NA	NA	NA
10 yr mortality	NA	NA	NA	8 (7.8)	26 (19.0)	0.137

LTr: Low-volume transfusion group; HTr: High-volume transfusion group; PNF: Primary non-function.

DISCUSSION

Determining predictive factors for complications of pediatric LT may be hindered by patient heterogeneity and a lack of standardization in the definition of complications. This study is the first retrospective study to assess short- and long-term transfusion-associated postoperative complications in a large number of small pediatric patients with chronic liver diseases who received the same type of graft from living donors.

The Clavien-Dindo classification[22] was first used by Clavien *et al*[23] to assess postoperative complications in adult LT patients and has been increasingly used in most pediatric surgical areas with some adaptations[24]. Beck-Schimmer *et al*[25] classified post-LT complications as minor and major providing the basis for the use of this modified classification primarily because general anesthesia was induced for almost all procedures in this pediatric population (*e.g.*, imaging exams, biopsies, catheter insertions, and other minor invasive procedures).

We defined the interval from 24 h pre- to 48 h post-LT as the perioperative period. Over this period, patients had a greater need for transfusion. Our transfusion goals throughout this period were to keep Hb > 8 and < 10g/dL, INR < 3.5, platelet count > 45 × 10^3/mm^3, and fibrinogen level > 80 mg/dL. Viscoelastic methods were unavailable at our center at this time. Only 16 study participants were not transfused during the perioperative period. Of the 224 transfused patients, 7.1%, 98.8%, and 37.5% received RBC before, during, and after surgery, respectively. Some patients remained within a suitable range during the intraoperative period yet this level dropped during the early postoperative period requiring subsequent transfusion. In this study, the average volume of transfusion of other BP was minimal compared to RBC.

Massive bleeding is usually defined as the loss of 100% or more of circulating TBV within 24 h[26]. Massive transfusion can also be defined as the transfusion of over 10% of TBV *per* minute or 50% in 3 h[27]. Nevertheless, TBV in pediatrics depends on the child's age and weight ranging from 65 to 100 mL/kg. In our study, the child's average age was 567 d (1.5 years) corresponding to a TBV of 75 mL/kg. The mean perioperative RBC volume transfused in the HTr was 57.7 mL/kg. A cutoff point of 27.5 mL/kg was used when the perioperative RBC volume represented less than 37% of TBV transfused within 96 h. Therefore, most of our patients did not meet the definition for massive bleeding or transfusion. Though there was no massive transfusion in the perioperative period in most patients; this lower volume has already been associated with major postoperative complications in PLDLT.

This study identified a perioperative transfusion volume of RBC > 27.5 mL/kg and preoperative eGFR as independent risk factors for mortality in PLDLT patients. A perioperative RBC transfusion volume higher than 27.5 mL/kg was a strong independent risk factor for mortality and increased the risk by 3.031-fold *vs* lower or equal volumes. This volume of RBC is definitely below that reported in other studies, which analyzed risk factors for survival in LT.

Table 7 Independent risk factors for death identified by simple and multiple Cox regression

			All patients *n* = 240			
Variables	**Estimative**	**HR**	**95%CI**	***P* value**	**HR**	***P* value**
		Univariate			**Multivariate**	
Male gender	-0.062	0.94	0.514-1.718	0.841		
Age (d)	-0.024	0.977	0.950-1.004	0.095		
Weight (kg)	-0.17	0.844	0.738-0.964	0.012		
Height (cm)	-0.044	0.957	0.924-0.990	0.013		
WAZ	-0.243	0.784	0.623-0.986	0.037		
HAZ	-0.085	0.918	0.734-1.148	0.455		
WHZ	-0.235	0.791	0.649-0.963	0.019		
BMIZ	-0.084	0.919	0.723-1.168	0.49		
PELD score	0.022	1.022	0.982-1.064	0.282		
Biliary Atresia	-0.426	0.653	0.357-1.195	0.167		
Kasai surgery	-0.214	0.808	0.440-1.483	0.491		
Infections ≤ 30 d pre-LT	0.348	1.416	1.023-1.960	0.036		
eGFR (mL/min/1.73 m^2)[1]	-0.005	0.995	0.991-0.999	0.010	0.995	0.023
Surgical time (hours)	0.012	1.013	0.856-1.197	0.884		
Hemoglobin (g/dL)[2]	-0.206	0.814	0.657-1.008	0.059		
INR[2]	0.259	1.295	0.722-2.322	0.386		
Sodium (mEq/L)[2]	-0.014	0.986	0.916-1.061	0.704		
Albumin (g/dL)[2]	-0.116	0.89	0.615-1.290	0.539		
Lactate (mmoL/L)[3]	-0.021	0.979	0.914-1.048	0.541		
GBWR (%)	0.149	1.16	0.909-1.481	0.232		
RBC > 27.5 mL/kg	1.316	3.73	1.730-8.042	0.001	3.031	0.009
Presence of major complication	0.384	1.469	0.799-2.701	0.261		

[1]Estimated glomerular filtration rate, preoperative calculated by Schwartz's formula.
[2]Blood samples collected up to 72 h before liver transplantation (LT).
[3]Blood samples collected up to 2 h after the onset of LT anesthetic induction. WAZ: Weight-for-age z-score; HAZ: Height-for-age z-score; WHZ: Weight-for-height z-score; BMIZ: Body-mass-index-for-age z-score; PELD: Pediatric end-stage liver disease; LT: Liver transplantation; INR: International normalized ratio; GBWR: Graft-to-body-weight ratio; RBC: Red blood cells; eGFR: Estimated glomerular filtration rate; HR: Hazard ratio.

Matinlauri *et al*[28] identified transfusion of RBC > 20 units as a risk factor for graft survival post-LT in adults. Nacoti *et al*[14] evaluated the effect of transfusion on 1-year patient and graft survival in pediatric LT and estimated a mortality risk of 3.15 for ≥ 3 units of RBC. Preoperative eGFR was a weak risk factor for mortality in our study probably because malnourished children tend to have underestimated serum creatinine values, and the simplified revised formula [eGFR = 0.413 × Height (cm)/serum creatinine (mL/min/1.73 m^2)] increases the error of the results at higher GFR values[29]. In the meantime, serum creatinine is known to be an independent risk factor for mortality in adult LT[30] and in pediatric LT with deceased donor[15].

Though LDLT is currently a standard treatment with good outcomes for young children with end-stage liver diseases, the postoperative complication rate can lead to a high morbimortality[31]. Among the 240 children, early postoperative complications were observed in 94.6% of patients of whom 54.6% had major complications associated with higher rates of graft loss. Although, the 1-year survival rate of 87.1 was slightly lower than in other centers that reported overall survival higher than 90%, the 5 and 10-year survival rates of 81.5% and 80.3%, respectively, are comparable to rates higher than 80%, reported by others[32-34]. This could be attributed to the fact that we studied

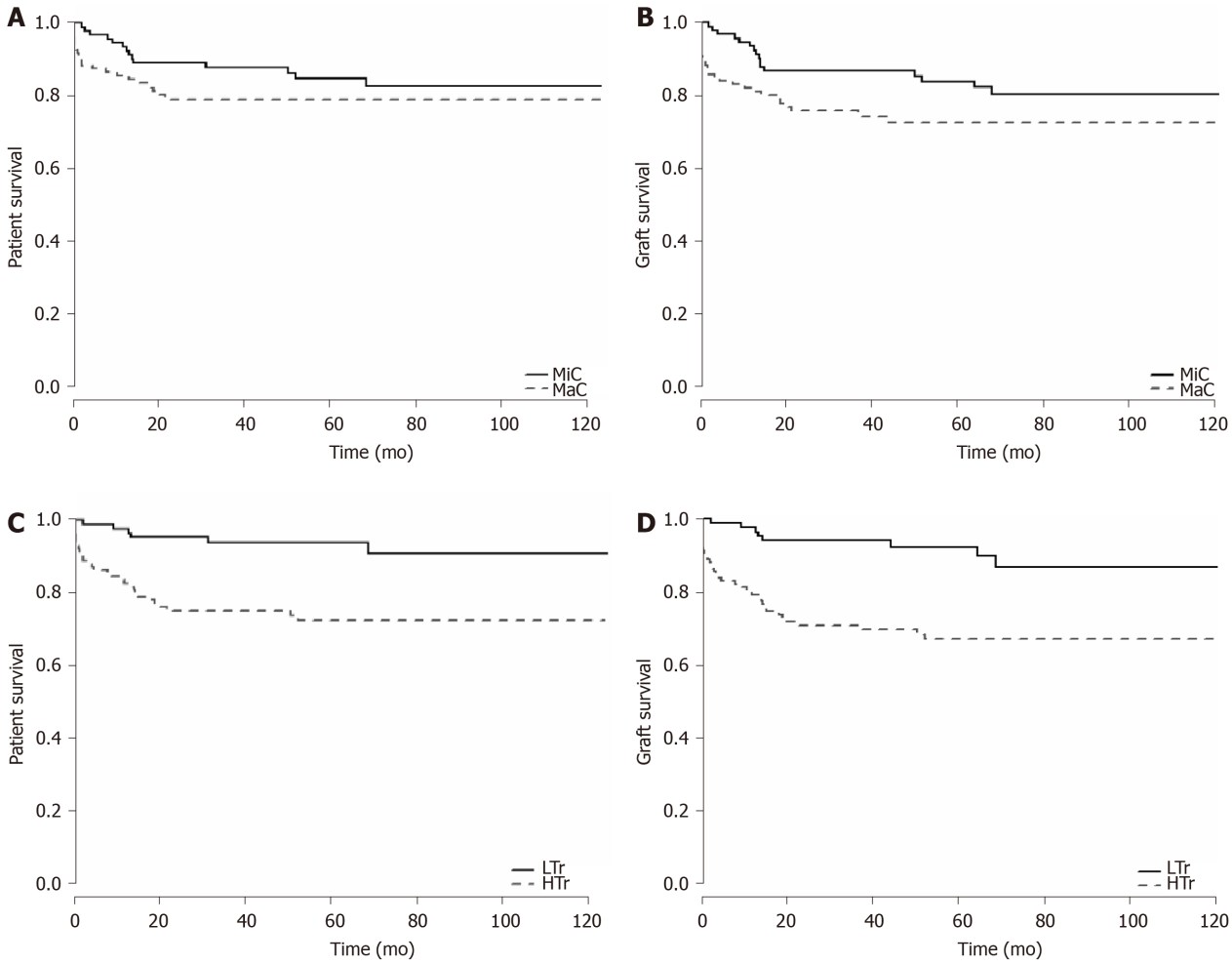

Figure 3 Patient and graft survival curves. A: Patient survival analysis comparing the minor complication (MiC) group (grade I-IIIa, *n* = 109) and the major complication (MaC) group (grade IIIb-V, *n* = 131) with number of patients at risk. Log-rank test: Chi-square = 12.6, degrees of freedom (*df*) = 1, *P* = 0.12; B: Graft survival analysis comparing the MiC and MaC groups. Log-rank test: Chi-square = 14.6, *df* = 1, *P* = 0.03; C: Patient survival analysis comparing the low-volume transfusion (LTr) group (*n* = 103) and the high-volume transfusion (HTr) group (*n* = 137). Log-rank test: Chi-square = 12.6, *df* = 1, *P* < 0.001; D: Graft survival analysis comparing the LTr (*n* = 103) and HTr (*n* = 137) groups. Log-rank test: Chi-square = 14.6, *df* = 1, *P* < 0.001. MiC: Minor complications group (I-IIIa); MaC: Major complications group (IIIb-V); LTr: Low-volume transfusion group; HTr: High-volume transfusion group.

exclusively small children, with inherent risks of early childhood, in comparison to other studies that include patients aged up to 18 years. In this study, the only risk factor for major postoperative complications after PLDLT was the volume of perioperative RBC transfused.

Transfusion was more frequently employed for younger and undernourished patients with extrahepatic cholestasis, previous Kasai procedure, increased rates of ascites, higher preoperative INR, lower Hb and sodium levels, and worse pulmonary and renal function. These findings are consistent with many reports on adult LT[35-37].

BP transfusion has often been reported to be associated with increased rates of both early and late LT complications[38,39]. Our sample consisted of 234 (97.5%) outpatients and 6 (2.5%) patients previously admitted to the ICU. During hospitalization, the total complication rates were similar between patients of the LTr and HTr groups (92.2% *vs* 96.4%). The HTr had more patients with severe complications (65.7% *vs* 39.8%), a higher average of complications *per* patient (3.8 ± 3.6 *vs* 2.5 ± 1.7), and a higher median number of major complications (IIIa *vs* IVa). Although the median grade of complications *per* patient was equal in the groups (II), there was a greater variability (I-V *vs* I-IIIb) in the HTr than in the LTr. Higher transfusion volumes were associated with longer anesthetic and surgery time, prolonged intubation, and longer ICU and in-hospital stays; these conclusions are corroborated by the findings of Massicotte *et al*[39] and Ramos *et al*[40] in adult LT patient studies.

Other specific complications might be related to perioperative BP transfusion in LT patients. Feltracco *et al*[41] reported that intraoperative BP transfusion was a risk factor for early postoperative pulmonary complications. Li *et al*[9] observed an increase in

infectious complications during in-hospital and ICU stays among LDLT adults in the early postoperative period. Furthermore, Pereboom *et al*[12] reported a higher incidence and mortality associated with intraoperative platelet transfusion and acute lung injury related to transfusion. Benson *et al*[42] also reported similar findings with plasma containing BP and a higher incidence of early postoperative infections with dose-dependent RBC transfusion in adult LT.

During hospitalization occurred 30.8% of the total complications observed within the 10 years of follow-up. Minor metabolic complications (*e.g.*, hydro electrolytic disorders, hypo- or hyper-glycemia, acidosis) were the most common. Nevertheless, no association between this type of complication and RBC transfusion volume was observed. Renal complications were not significantly different between groups, and dialysis was performed in seven patients during this period. HTr patients exhibited a higher frequency of major infectious complications (15.7% of complications, *e.g.*, severe sepsis, with hemodynamic instability), general respiratory complications (11.5% of complications, *e.g.*, pulmonary edema, pleural effusion, bronchospasm, pneumonia, and acute respiratory distress syndrome), general bleeding (2.8% of complications, *e.g.*, oozing and draining with a need for reoperation), and major cardiovascular complications (2.1% of complications, *e.g.*, hemodynamic instability requiring vasoactive drugs, severe arrhythmias, and cardiorespiratory arrest) than LTr patients. HTr patients had a lower frequency of rejections (6.3% of complications, *e.g.*, mild acute cellular reaction) and dermatologic complications (1.2% of complications, *e.g.*, oral ulceration and dermatitis.

Kloesel *et al*[19] did not observe significant differences in perioperative complications between the major and minor transfusion groups within the hospitalization period in pediatric LT patients despite an incidence rate of 43% for massive bleeding. In this last study, conflicting results might be attributed to the fact that all patients received an RBC transfusion and 88% of them received FFP transfusion thus creating a bias in the comparison between groups. Aside from increasing the risk of complications, RBC transfusion is a predictive factor for survival in adult[28] and pediatric LT[43]. In our study, patients with MaC presented reduced graft survival but patient survival was not affected relative to patients with no or MiC. Meanwhile, patients who underwent a HTr showed both worse graft and patient survival relative to patients of LTr.

The late period had 69.2% of the total complications with 55.3% up to the first year. Among the late postoperative complications of LT, infections are the leading cause of death[44]. In line with this, the frequency of major complications in HTr patients was higher at 1 up to 10 years post-LT. The most frequent major complications were infectious (15.8%-8.7% of complications, *e.g.*, severe sepsis), pulmonary (5.0%-4.7% of complications, *e.g.*, severe pneumonia requiring mechanical ventilation), and cardiovascular (2.0%-1.2% of complications, *e.g.*, cardiorespiratory arrest). In the HTr patients, the frequency of minor neoplastic complications was significantly higher at 10 years (2.3% of complications, *e.g.*, PTLD) than in LTr patients. The frequency of rejections was lower in the HTr at 1 to 10 years post-LT (8.0%-8.7% of complications, *e.g.*, mild acute cellular reaction) than in the LTr. Renal complications were still not different between groups, and dialysis was necessary in 14 patients up to 10 years. Dermatological complications are common among LT patients and are related to genetics, allergic factors, and the side effects of immunosuppressive drugs. Surprisingly, patients with HTr had fewer dermatological complications than patients with LTr up to 1 year after LT (1.9%). This could be attributed to a lower frequency of rejections and, consequently, less of a need for immunosuppressive drugs. However, this would fail to explain why the rate of dermatological complications does not differ significantly afterward while rejections remained lower in HTr throughout the study. A higher-volume transfusion of RBC might have had some influence. Notwithstanding the fact that such complications are usually related to LT[45], there is no strong evidence of the association of perioperative BP transfusion and long-term outcomes in pediatric LT. We found a significant difference in 30 d and 10-year postoperative mortality rates between the LTr and the HTr (0 *vs* 6.6% and 7.8 *vs* 25.5%), respectively, confirming that HTr patients generally experienced more severe complications.

Concerning LT-specific complications, the incidence of PNF corresponded to 2.1% and was not related to transfusion. This is consistent with others who reported an incidence of 0.9%-8.5%[46]. Hypercoagulability is a risk factor for vascular thrombosis in LT, mainly in children, as determined by rebalanced hemostasis in cirrhosis, technical vascular issues, inflammatory response to trauma, and massive transfusion[47,48]. Incidences of HAT have been reported to be 4%-8% and PVT of 5%-10%[49-51]. In our study, the overall incidence of HAT and PVT was 4.6% and 13.3%, respectively. Nevertheless, no relation between transfusion volume and an increase in arterial or venous thrombosis were observed, in both, short- and long-term periods. The lower

incidence of HAT is likely related to the microsurgical anastomosis technique whereas the higher incidence of PVT may be attributed to previous portal hypoplasia frequently observed in patients with biliary atresia-this was the most prevalent underlying disease in our cohort. Portal vein graft interposition was employed in 37 (15.4%) patients of the total cohort, and only three cases evolved with PVT. Biliary complications accounted for 18.3% of the total cases, and neither fistulas nor stenosis were related to RBC transfusion. The incidence rate of biliary complications in PLDLT has been reported to be 10%-20%[52], depending on the size of the graft and subsequent technical difficulties. Reoperations corresponded to 25.8%, which is consistent with others who reported an incidence of 8%-29%[33] in PLDLT. In the short-term period, reoperation (18.7%) was three-fold higher in the patients who received an RBC volume higher than 27.5 mL/kg (HTr), due mostly to bleeding and intestinal injuries. In the long-term period, reoperations were not related to perioperative transfusion. Retransplantation corresponded to 3.8% and was not related to RBC transfusion. The incidence was below the historical average of 9%-29%[53], probably because of the lower incidence of total hip arthroplasty and the good quality of the grafts. Although the HTr had fewer minor rejection episodes, such as a mild acute cellular reaction over 10 years, this fact did not impact the retransplantation rate in both, short- and long-term periods.

Immunosuppression associated with blood transfusion occurs *via* a decrease in the number and function of natural killer cells, a decrease in cytotoxic T-cell function, an increase in the number of suppressor T-cells, and a reduction in macrophage and monocyte function[54]. Blood transfusion may induce immunomodulatory effects (both proinflammatory and immunosuppressive) that are of variable intensity and long-term duration. These antagonistic effects are associated with a decrease in rejection episodes[55] as well as an increase in the frequency of infection[56], neoplasia[57], and tumor recurrence[8]. We essentially found that HTr patients displayed more major infections and fewer rejections during early and late postoperative periods and more minor neoplastic complications in the late postoperative period than LTr patients.

Detailing the type, severity, and chronology of postoperative complications is of paramount importance for a better understanding of the clinical evolution. It can assist in the implementation of preventive measures that may positively impact the outcome of PLDLT.

Several strategies have been adopted to decrease perioperative transfusion in adult LT patients. They can be classified into three groups of measures: (1) Prophylactic such as the recognition of patients at risk for bleeding and the previous suspension of drugs that interfere with coagulation; (2) Technical such as maintenance of low central venous pressure, controlled hypotension, use of vascular clamping, ultrasonic or argon scalpels, and capture and reuse of blood lost; guided fluid therapy by multiparametric data, reduction of transfusion trigger values, and viscoelastic tests; and (3) Pharmacological such as erythropoietin, desmopressin, vasopressin, antifibrinolytics, prothrombin complex, lyophilized fibrinogen, recombinant factor VIIa, fibrin sealants, and vasoactive drugs[58,59]. Certainly, not all of them apply to this population, that remain to be a challenge in conducting LT. Fluid management in small children undergoing LT cannot be guided by minimally invasive multiparametric monitors. These are of limited use, once their softwares are designed for adult patients. Nonetheless, the analysis of the trend curves can assist in decision-making, there is a lack of accuracy in the assessment of volemia, hemodynamic state and effect of vasoactive drugs during the perioperative period of pediatric LT. Besides, techniques as hemodilution and controlled hypotension are not validated in this group. Hemodilution increases hydrostatic pressure in the portal vein and inferior vena cava system and worsens the coagulopathy, exacerbating surgical bleeding. Controlled hypotension is a debatable issue and might be of potential risk for target organ damage. It is crucial to recognize that small children with chronic liver disease have a tenuous rebalance of the hemostatic system, not entirely understood, which might be easily disrupted by hasted interventions, pushing the patient towards hemorrhage and/or thrombosis. Prophylactic use of FFP is not advised, because it can increase intravascular pressure and increase RBC transfusion. Routine prophilatic use of antifibrinolytic drugs is no longer recommended, tranexamic acid and aminocaproic acid are possibly useful for patients in hyperfibrinolysis, demonstrated by microvascular oozing or viscoelastic tests. Prophylactic use of recombinant factor VIIa, should be avoided in all, except for highest-risk procedures[60]. Although, preoperative blood transfusion has been demonstraded to be independently associated to morbidity up to 30 d of postoperative period and harmful in neonates undergoing general pediatric surgery, neurosurgery, otolaringology, cardiothoracic, plastics and urology surgery[61], no strong evidence is found in pediatric LT in the long-term period.

Concurrent transfusion of "red" and "yellow" BP, in adult liver resection with compromised function, was associated with a significantly higher risk of postoperative morbidity compared to only RBC or only FFP transfusion, what might be attributed to synergistic effects[2]. Though, no similar study was conducted in pediatric LT. If there is an absence of universal definition of massive bleeding or massive transfusion and a scarcity of studies relating survival to specifc BP dosages, ratios, timing and guidance even in adult trauma victims[62], let alone pediatric LT in small children. Specific transfusion trigger thresholds in pediatric LT have not been validated and need to be determined by prospective controlled studies that seek to standardize patient samples, according to age or weight, underlying diseases, type of donor and type of graft.

This study does have some limitations. First, this was a retrospective study performed in a single center. Second, the collection of data was refined on an ongoing basis since the implementation of the LT program; the learning curve may have influenced the results. Third, and most importantly, complications due to increased transfusion volume may be an epiphenomenon related to a sicker patient and of higher technical difficulty, or, indeed, a risk factor for postoperative morbimortality. Nonetheless, this study has several strengths, such as the size and homogeneity of the sample as well as the standardization of the anesthetic/surgical approaches and the immunosuppression regimen. The follow-up was conducted in the same center, which included facilities for patients and their families to remain close during local treatment thus improving patient recruitment and reducing loss to follow-up throughout the study period.

CONCLUSION

In this study, blood transfusion volumes less than one total blood volume, though not considered massive transfusions, were already associated with a higher incidence of more serious complications and mortality as assessed by hospitalization up to 10 years after PLDLT. A perioperative RBC transfusion volume higher than 27.5 mL/kg is associated with not only increased rates of infectious, cardiovascular, respiratory, and neoplastic complications but also decreased frequency of rejection episodes. Furthermore, a perioperative volume of RBC transfusion higher than 27.5 mL/kg is an independent risk factor for mortality and is directly related to reduced patient and graft survival in PLDLT. These results underscore the need for more restrictive criteria to guide the use of blood transfusion in PLDLT patients to prevent potentially related postoperative complications.

Appropriate protocols should be tailored to each center according to the infrastructure, clinical staff experience, and patient's profile. Indeed, some strategies to reduce blood consumption should be implemented. An accurate nutritional assessment with specific dietary support and early supplementation is mandatory. Treatment with iron and vitamins should be considered. Prophylaxis of digestive bleeding and treatment of renal dysfunction and infection can decrease the incidence of preoperative anemia. The use of recombinant human erythropoietin therapy is controversial. Reduce blood tests and perform microsampling. During surgery, a more restrictive fluid management and a reduction of Hb trigger values to less than 8.0 g/dL, could reduce blood transfusion especially when combined with low doses of continuous infusion of norepinephrine. This could mitigate fluid overload, reduce portal hypertension, restore splanchnic and central circulatory imbalances and optimize tissue oxygenation. Assessment of coagulation with viscoelastic tests to improve blood management in pediatric surgery is feasible, but specific algorithms must be developed. The goals are to optimize the erythrocyte mass, minimize blood loss, increase tolerance to anemia and maintain hemostatic balance. As demonstrated, a small reduction in perioperative RBC transfusion volume may determine a better outcome in the short- and long-term postoperative periods. The evaluation of risk, effectiveness, and cost-benefit assessment of these strategies in young children with liver diseases is outside the scope of the present study and should be carried out in future research.

ARTICLE HIGHLIGHTS

Research background

Pediatric living donor liver transplantation (PLDLT) is a multidisciplinary procedure

of high complexity and potential risk of bleeding. The association between transfusion and short- and long-term postoperative complications is poorly established especially in small children. Blood transfusion is frequently indicated in the perioperative period of liver transplant, though there is little robust evidence of associated postoperative complications. Given the good survival results, in the past decade, it is now necessary to identify risk factors for complications in order to improve the long-term evolution.

Research motivation

To study in depth the short- and long-term evolution of this specific group of highly fragile pediatric patients, in order to improve the proficiency acquired in 20 years of working with PLDLT, and to be able to share knowledge.

Research objectives

This study assessed whether perioperative transfusion is associated with early and late postoperative complications and mortality in small patients undergoing PLDLT.

Research methods

Postoperative complications along 10 years of follow up were graduated with Clavien-Dindo modified classification in order to assess relationship between blood transfusion and postoperative complications. Multiple logistic regression analysis identified risk factors for major postoperative complications. Perioperative red blood cells volume was identified as a single risk factor and a receiver operating characteristic curve identified a cutoff point of 27.5 mL/kg. Cox regression analyses identified independent risk factors for mortality. Overall patient and graft survival analyses was performed using Kaplan–Meier survival curves, which were compared using the log-rank test and a $P < 0.05$ was considered statistically significant.

Research results

In terms of red blood cells (RBC) transfusion volume, there was a significantly higher rate of 30 d reoperation (26.3% × 8.7%, $P < 0.001$) and 30 d mortality rate (6.6% × 0.0%, $P < 0.001$) in the high-volume transfusion (HTr) *vs* low-volume transfusion (LTr), respectively. Early liver transplantation (LT)-specific complications include primary non-function, biliary complications, vascular thrombosis, and retransplantation that were not related to a higher perioperative transfusion volume. Over 10 years of follow-up, with respect to RBC transfusion volume, there was a significantly higher rate of reoperation (36.5% × 12.6%, $P < 0.001$) and mortality (25.5% × 7.8%, $P < 0.001$), respectively, in the HTr compared to the LTr. Perioperative RBC volume > 27.5 mL/kg and preoperative estimated glomerular filtration rate were identified as independent risk factors for mortality over 10 years of follow-up after LT. The patient survival rates were significantly lower in the HTr than in the LTr at 1, 5, and 10 years post-LT: 82.7% *vs* 97.7%, 73.9% *vs* 93.8%, and 72.6% *vs* 90.9%, respectively. Likewise, the graft survival rates were significantly lower in the HTr than in the LTr at 1, 5, and 10 years post-LT: 79.5% *vs* 97.7%, 67.2% *vs* 92.3%, and 67.2% *vs* 87%, respectively.

Research conclusions

A perioperative RBC transfusion volume > 27.5 mL/kg is associated with not only increased rates of infectious, cardiovascular, respiratory, and neoplastic complications but also decreased frequency of rejection episodes. Furthermore, a perioperative volume of RBC transfusion higher than 27.5 mL is an independent risk factor for mortality, and is directly related to reduced patient and graft survival in PLDLT.

Research perspectives

The detailed analysis of this study allows the construction of strategy protocols to reduce the need for transfusion of patients undergoing PLDLT improving short- and long-term outcome.

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ORIGINAL ARTICLE

Retrospective Study

R2* value derived from multi-echo Dixon technique can aid discrimination between benign and malignant focal liver lesions

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Abstract

BACKGROUND

R2* estimation reflects the paramagnetism of the tumor tissue, which may be used to differentiate between benign and malignant liver lesions when contrast agents are contraindicated.

AIM

To investigate whether R2* derived from multi-echo Dixon imaging can aid differentiating benign from malignant focal liver lesions (FLLs) and the impact of 2D region of interest (2D-ROI) and volume of interest (VOI) on the outcomes.

METHODS

We retrospectively enrolled 73 patients with 108 benign or malignant FLLs. All patients underwent conventional abdominal magnetic resonance imaging and multi-echo Dixon imaging. Two radiologists independently measured the mean R2* values of lesions using 2D-ROI and VOI approaches. The Bland–Altman plot was used to determine the interobserver agreement between R2* measurements. Intraclass correlation coefficient (ICC) was used to determine the reliability between the two readers. Mean R2* values were compared between benign and malignant FFLs using the nonparametric Mann–Whitney test. Receiver operating characteristic curve analysis was used to determine the diagnostic performance of R2* in differentiation between benign and malignant FFLs. We compared the diagnostic performance of R2* measured by 2D-ROI and VOI approaches.

RESULTS

This study included 30 benign and 78 malignant FLLs. The interobserver reproducibility of R2* measurements was excellent for the 2D-ROI (ICC = 0.994)

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and VOI (ICC = 0.998) methods. Bland–Altman analysis also demonstrated excellent agreement. Mean R2* was significantly higher for malignant than benign FFLs as measured by 2D-ROI ($P < 0.001$) and VOI ($P < 0.001$). The area under the curve (AUC) of R2* measured by 2D-ROI was 0.884 at a cut-off of 25.2/s, with a sensitivity of 84.6% and specificity of 80.0% for differentiating benign from malignant FFLs. R2* measured by VOI yielded an AUC of 0.875 at a cut-off of 26.7/s in distinguishing benign from malignant FFLs, with a sensitivity of 85.9% and specificity of 76.7%. The AUCs of R2* were not significantly different between the 2D-ROI and VOI methods.

CONCLUSION

R2* derived from multi-echo Dixon imaging whether by 2D-ROI or VOI can aid in differentiation between benign and malignant FLLs.

Key Words: R2*; Multi-echo Dixon imaging; Hypoxia; Malignant lesion; Benign lesion; Focal liver lesion

Core Tip: Our study showed that mean R2* value of malignant focal liver lesions (FLLs) was significantly higher than that of benign FLLs. R2* derived from multi-echo Dixon imaging is a potential biomarker to differentiate malignant from benign FFLs. The multi-echo Dixon sequence is easy to perform and requires only a single breath-hold of 16 s to image the entire liver, which holds a good potential for clinical application.

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INTRODUCTION

Liver cancer is the sixth most common cancer and the fourth leading cause of cancer deaths worldwide[1]. The liver is also the most frequent site for distant metastases[2]. Clinically, once a focal liver lesion (FLL) is identified, it is essential to distinguish between benign and malignant lesions, as this differentiation determines the individual's prognosis and subsequent treatment strategy[3]. Contrast-enhanced computed tomography (CT) and magnetic resonance imaging (MRI) are widely used to detect and characterize FLLs[4-7]. However, the use of iodine and gadolinium-based contrast agents is sometimes contraindicated; for example, in patients with severe kidney impairment due to the potential development of contrast-induced nephropathy[8] or nephrogenic systemic fibrosis[9]. Several imaging techniques without the need of contrast agents have been used to diagnose FFLs, including diffusion-weighted image (DWI), intravoxel incoherent motion, diffusion kurtosis imaging, and magnetic resonance elastography, although these techniques have shown mixed success with limited clinical application[10-13].

A hypoxic microenvironment is a hallmark in biology for solid tumors[14,15]. It is known that R2* estimation (R2* = 1/T2*) is inversely related to partial tissue pressure of oxygen, and reflects the paramagnetism of the tumor tissue, such as the presence of deoxygenated hemoglobin[15-17]. Previous studies have demonstrated that R2* can be used to assess oxygenation status in several malignancies[18,19] and offer additive value in identifying metastatic lymph nodes in breast cancer[20]. However, whether R2* can be used to differentiate between benign and malignant FLLs remains to be determined. Besides, 2D region of interest (2D-ROI) and volume of interest (VOI) analyses, which are better for R2* measurement in FFLs, remain elusive.

In this study, the diagnostic performances of R2* derived from multi-echo Dixon imaging in differentiating between benign and malignant FLLs based on 2D-ROI and

VOI analyses were investigated. The purpose of this study was to determine whether R2* derived from multi-echo Dixon imaging can aid in differentiating benign from malignant FLLs, and the impact of 2D-ROI and VOI on the outcomes.

MATERIALS AND METHODS

Patients

This retrospective study was approved by the Institutional Ethics Review Board of our hospital (approval No. SYSEC-KY-KS-2020-147), and the requirement for informed consent from the patients was waived. From January 2019 to December 2019, consecutive patients with FLLs were identified from the hospital database. Patients were included if they had: (1) A solid malignant or benign FLL confirmed by histology, and follow-up contrast-enhanced CT/MRI examination for at least 6 mo, or positron emission tomography (PET)-CT; and (2) Multi-echo Dixon imaging. The exclusion criteria were as follows: (1) Diffuse liver inflammation (n = 5); (2) Maximal lesion diameter < 10 mm (n = 5); (3) Lower signal-to-noise ratio on R2* images; and (4) Obvious breathing artifacts on R2* images (n = 5).

MRI acquisition

MRI was performed on a 3.0 T unit (MAGNETOM Skyra; Siemens Healthcare, Erlangen, Germany). The sequences consisted of conventional sequences and multi-echo Dixon imaging. Conventional MRI included axial BLADE T2-weighted imaging (T2WI) [repetition-time/echo-time (TR/TE) = 9672.9-12331.7/84 ms; flip angle = 130°; averages = 1; matrix = 320 × 320; field of view = 100 mm; slice thickness = 5 mm], axial and coronal T1-weighted imaging (T1WI) volume interpolated breath-hold examination (VIBE) (TR/TE = 3.97/1.29 ms; flip angle = 9°; averages = 1; matrix = 320 × 180; field of view = 75 mm; slice thickness = 3 mm), and axial DWI (TR/TE = 4900/66 ms; flip angle = 90°; averages = 12; matrix = 192 × 113; field of view = 78.125 mm; slice thickness = 5 mm; b values = 0 and 800 s/m^2). The multi-echo Dixon imaging was performed with T2* correction. The acquisition parameters were: TR = 9 ms; six-echo with TE = 1.05/2.46/3.69/4.92/6.15/7.38 ms; averages = 1; matrix = 160 × 136; field of view = 450 mm; slice thickness = 3.5 mm; number of slices = 64; a flip angle = 4° was used to minimize the effects of T1 weighting[21]. This sequence was acquired in a breath-hold of 16 s. After these sequences, multiphase contrast-enhanced imaging was performed after administration of gadolinium contrast medium (Magnevist; Bayer Schering Pharma, Berlin, Germany) using a fat-suppressed dynamic contrast enhancement sequence with the following acquisition parameters: TR/TE = 3.8/1.23 ms; averages = 1; slice thickness = 2.5; field of view = 80.56; matrix = 288 × 186; flip angle = 10°. Then, all patients underwent axial and coronal contrast-enhanced T1WI–VIBE (TR/TE = 3.97/1.26 ms; flip angle = 9°; averages = 1; slice thickness = 2.3 mm; matrix = 320 × 180; field of view = 75 mm).

Image analysis

All the images were assessed by using the ImageJ software (http://rsb.info.nih.gov/ij/). A low flip angle multi-echo Dixon sequence was used to derive R2* to minimize T1-related bias and improve the separation of water and fat. The improved fitting of the signals within fatty tissues allows more accurate R2* mapping and T2* correction of the water-fat separation[22]. Two experienced radiologists (Shi GZ and Gao M, with 6 and 12 years of experience in liver diagnostic imaging, respectively) who were blinded to the diagnosis of patients manually delineated the lesions on R2* maps. For 2D-ROI, a single freehand ROI was drawn to cover the whole tumor area on the section showing the maximal tumor dimension. For VOI, the freehand ROI was placed slice by slice to cover the entire tumor volume. The mean R2* values measured by 2D-ROI and VOI were used for analysis (Figure 1).

Laboratory and anthropometric evaluations

Hepatitis B virus infection, α-fetoprotein (AFP), carbohydrate antigen 19-9 (CA 19-9), and carcinoma embryonic antigen (CEA) were measured using standard reagents. Liver cirrhosis was determined by Masson trichrome staining. The normal ranges are: AFP ≤ 25 ng/mL, CA 19-9 ≤ 34 U/mL, and CEA ≤ 5 ng/mL. Laboratory examination was performed before clinical treatment. The time between laboratory examination and multi-echo MRI examination was within 1 wk.

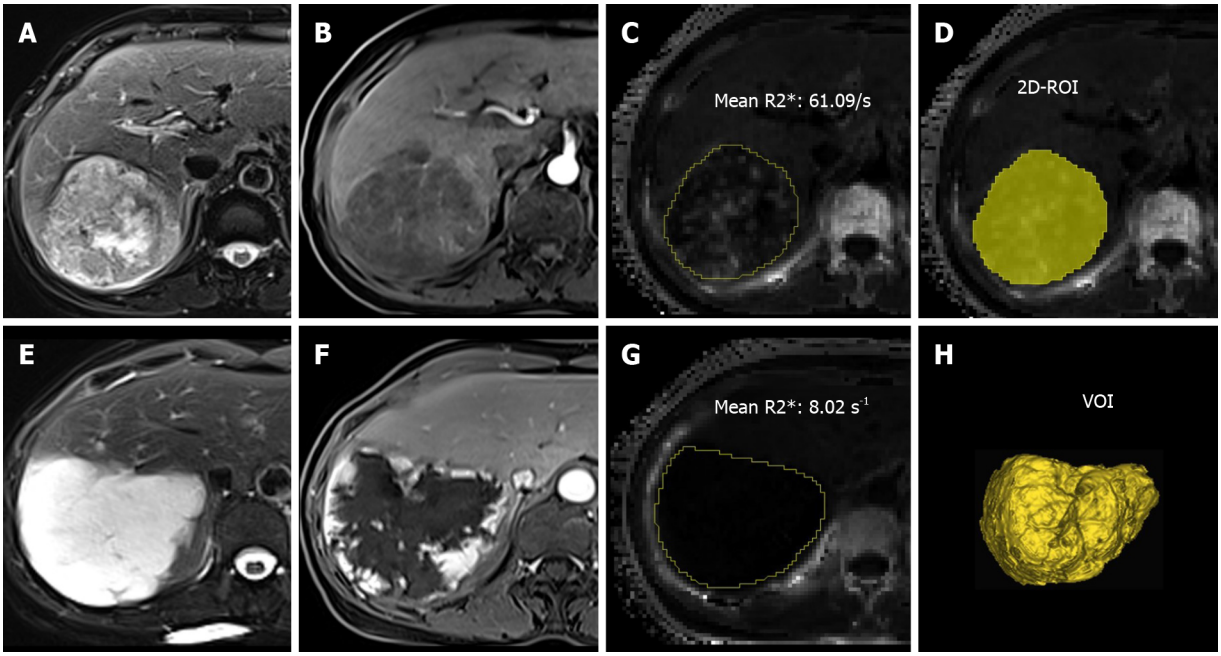

Figure 1 Two-dimensional region of interest and volume of interest. A-C: T2-weighted imaging (T2WI) (A), arterial phase contrast-enhanced T1-weighted imaging (T1WI) (B), and R2* map showed liver metastasis (yellow line) (C) confirmed by histology in a 59-year-old woman with lung cancer; D: Two-dimensional region of interest was drawn on the section showing the maximal tumor dimension; E-G: T2WI (E), arterial phase contrast-enhanced T1WI (F), and R2* map showed a live hemangioma (yellow line) (G) in a 59-year-old woman; H: Volume of interest was placed covering the entire tumor volume on R2* map. 2D-ROI: Two-dimensional region of interest; VOI: Volume of interest.

Diagnosis of FLLs

All analyzed lesions were diagnosed by contrast-enhanced MRI, follow-up contrast-enhanced CT/MRI examination within at least 6 mo, fluorine 18 (^{18}F) fluoro-deoxyglucose (FDG) PET-CT, or histopathological findings (hepatectomy or biopsy)[5,22-25]. Diagnostic reference standard was established based on histopathological confirmation in 29/32 hepatocellular carcinomas (HCCs), 6/9 intrahepatic cholangiocarcinomas (IHCCs), 7/37 metastases, 5/25 hemangiomas, and 2/3 focal nodular hyperplasias (FNHs). In the remaining 69 FLLs without histopathological results, diagnoses were established by well-accepted imaging findings in all acquired MRI sequences (*e.g.*, T1WI, T2WI, T2-SPAIR, DWI, and contrast-enhanced T1WI). Criteria were determined by consensus reading of two experienced radiologists (R1, Shi GZ; and R2, Gao M) by consideration of all acquired images. Further reference standards were required: (1) FFLs were diagnosed as primary malignant FFLs if they showed (a) characteristic imaging appearance during a 6-mo imaging follow-up combined with (b) clinical symptoms and serological results; (2) FFLs were diagnosed as liver metastasis in patients with primary malignancies (pathologically confirmed) when at least one of the following criteria was satisfied: (a) Newly developed lesion or an increase in size with typical imaging appearance during a 6-mo imaging follow-up; and (b) abnormal ^{18}F FDG uptake at PET-CT examination; and (3) FFLs were diagnosed as benign lesions if (a) they were stable at 6-mo imaging follow-up with characteristic imaging appearance in subjects at low risk; and (b) no malignant tumor was found in patients with benign FLLs during imaging examination.

Three HCCs, three IHCCs, and 19 metastases were diagnosed according to 6-mo imaging follow-up. Eleven metastases were confirmed by PET-CT. In liver metastasis patients, the primary tumors were bladder cancer (n = 9), lung cancer (n = 2), colorectal cancer (n = 7), cervical cancer (n = 4), gastric cancer (n = 3), gallbladder cancer (n = 1), breast cancer (n = 1), and HCC (n = 10). For benign FLLs, 20 hemangiomas and one FNH were confirmed by 6-mo imaging follow-up. Two liver abscesses had typical imaging findings in all the MRI sequences and typical imaging findings in a 6-mo follow-up MRI examination after clinical treatment.

Statistical analysis

Numerical data are expressed as the mean ± SD. The Bland–Altman plot was performed to determine the interobserver agreement on R2* measurements. Intraclass correlation coefficient (ICC) was used to determine the reliability between the two

radiologists in R2* measurements using 2D-ROI and VOI methods (0-0.20 poor; 0.21-0.40 fair; 0.41-0.60 moderate; 0.61-0.80 good; and 0.81-1.0 excellent correlation). Mean R2* values from the two readers were used for the final analysis. Nonparametric Mann–Whitney test was used to compare the difference in R2* values between the malignant and benign groups. The receiver operating characteristic (ROC) analysis was used to evaluate the diagnostic performances of R2*. The area under the ROC curve (AUC), optimal cut-off values, sensitivity, and specificity were determined as the maximum Youden index. Differences in the diagnostic performance of the two different ROI positioning methods were analyzed by comparing ROC curves according to the method developed by DeLong *et al*[26]. $P < 0.05$ (two-tail) indicated a statistically significant difference.

RESULTS

Clinicopathological characteristics

A total of 108 FLLs were found in 73 patients, including 78 malignant FLLs (mean maximum diameter, 48.2 ± 37.7 mm; range, 11-163 mm) and 30 benign FLLs (mean maximum diameter, 32.3 ± 22.5 mm; range, 14-94 mm). Forty-nine patients had malignant FFLs (30 men and 19 women; mean age, 56.3 ± 10.3 years; range, 40-81 years), and 24 patients (11 men and 13 women; mean age, 52.1 ± 12.9 years; range, 31-73 years) had benign FLLs. The malignant FFLs included 32 HCCs, nine IHCCs, and 37 liver metastases. Benign FFLs included 25 hemangiomas, three FNHs, and two liver abscesses. The mean maximum diameter of liver metastases, HCCs, and IHCCs was 29.1 ± 24.1 mm (range, 11-122 mm), 66.3 ± 43.0 mm (range, 15–163 mm), and 61.9 ± 25.9 mm (range, 32-111 mm), respectively. In benign FFLs, the mean maximum diameter of hemangiomas, FNHs, and liver abscesses was 29.4 ± 21.8 mm (range, 14-94 mm), 32.0 ± 8.5 mm (range, 23-40 mm), and 69.5 ± 12.0 mm (range, 61-78 mm), respectively. Clinicopathological characteristics and laboratory evaluations of FFLs are shown in Tables 1 and 2.

R2* analysis

Figure 2 shows the Bland–Altman plot measurement of R2* of FLLs for the two readers. For 2D-ROI analysis, the 95% limits of agreement of R2* for the two readers were from -5.68 to 5.04/s, and the mean difference for the two readers was -0.32/s. For VOI analysis, the 95% limits of agreement of R2* for the two readers were from -3.65 to 3.28/s, and the mean difference for the two readers was -0.18/s. The differences between the two readers using two different methods were relatively small. ICC for the 2D-ROI method was 0.994 and ICC for the VOI method was 0.998. The interobserver agreement was excellent.

The mean R2* values measured by 2D-ROI and VOI methods were significantly higher in the malignant group than in the benign group (2D-ROI: 37.99 ± 17.71 *vs* 18.6 ± 8.43/s, $P < 0.001$; VOI: 41.11 ± 19.01 *vs* 20.61 ± 9.01/s, $P < 0.001$). For 2D-ROI measurement, the mean R2* value of liver metastases was 44.17 ± 21.90/s, and the mean R2* values of HCCs and IHCCs were 33.45 ± 10.15 and 28.72 ± 10.21/s, respectively. The mean R2* values of hemangiomas, FNHs, and abscesses were 16.66 ± 8.18, 26.21 ± 5.61, and 23.29 ± 9.31/s, respectively. For VOI measurement, FFLs had a mean R2* value of 48.42 ± 23.61/s for liver metastases, 35.41 ± 10.04/s for HCCs, 31.34 ± 9.65/s for IHCCs, 19.36 ± 8.93/s for hemangiomas, 27.87 ± 7.46/s for FNHs, and 25.29 ± 10.46/s for abscesses. Malignant FFLs had higher R2* values than benign FLLs regardless of ROI placement methods (Table 3).

ROC analysis

The AUC of 2D-ROI was 0.884 (95%CI, 0.819 to 0.950) at a cut-off of 25.2/s, with a sensitivity of 84.6% and specificity of 80.0% for differentiating benign from malignant FFLs. The VOI method yielded an AUC of 0.875 (95%CI: 0.806 to 0.945) at a cut-off of 26.7/s in distinguishing benign from malignant FFLs, with a sensitivity of 85.9% and specificity of 76.7%. There was no significant difference between the AUCs for 2D-ROI and VOI positioning methods for discriminating benign from malignant FFLs ($Z = 1.069$, $P = 0.285$) (Figure 3).

Table 1 Baseline characteristics of malignant and benign focal liver lesions of 73 patients

Characteristic	Malignant	Benign	Total
Per-patient basis			
No. of patients (%)	49 (67.1)	24 (32.9)	73
Age (yr)			
mean ± SD	56.3 ± 10.3	52.1 ± 12.9	55.0 ± 11.2
Range	40-81	31-73	31-81
Sex, *n* (%)			
Male	30 (61.2)	11 (45.8)	41
Female	19 (38.8)	13 (54.2)	32
Per-lesion basis			
No. of lesions	78 (72.3)	30 (27.8)	108
Maximum diameter (mm)			
mean ± SD	48.2 ± 37.7	32.3 ± 22.5	43.8 ± 34.8
Range	11-163	14-94	11-163
Methods of diagnosis (%)			
Pathology	42 (38.9)	7 (6.5)	49 (45.4)
Imaging follow-up	25 (23.1)	23 (21.3)	48 (44.4)
PET-CT	11 (10.2)	–	11 (10.2)

FFL: Focal liver lesion; PET-CT: Positron emission tomography-computed tomography.

DISCUSSION

Our study showed that the mean R2* value of malignant FLLs was significantly higher than that of the benign FLLs. R2* derived from multi-echo Dixon imaging is a potential biomarker to differentiate malignant from benign FFLs.

The combined use of MRI, CT, and ultrasound has a high diagnostic performance for the identification of FLLs, but requires the administration of gadolinium or iodine contrast agents[7]. Gadolinium contrast is contraindicated in patients with severe renal impairment, because it may induce nephrogenic systemic fibrosis, and may even be a greater risk in patients with liver dysfunction[27,28]. Iodinated contrast administration for CT may aggravate renal failure[8]. Currently, no alternative imaging methods have been widely advocated for these patients. Hypoxia is an important factor in cancer progression, affecting the autonomous functions of tumor cells and nonautonomous processes such as angiogenesis, lymphangiogenesis, and inflammation[29]. Hypoxia causes an increase in the concentration of deoxygenated hemoglobin in the tumor. Deoxyhemoglobin can be used as an endogenous hypoxia tracer that may produce local magnetic field inhomogeneities to reduce T2* relaxation time[30]. Furthermore, higher local deoxyhemoglobin may result in a decrease in proton T2* relaxation time and a corresponding increase in R2*, which indicates a link between R2* and the oxygen concentration of local tissues[15]. Recently, susceptibility-weighted imaging, which was originally called blood-oxygen-level-dependent (BOLD) venographic imaging, has demonstrated advantages in the detection of hemorrhagic events due to its sensitivity to paramagnetic substances[31]. Also, BOLD MRI has shown ability in assessing tumor oxygenation and indirectly hypoxia, by detecting signal changes secondary to changes in blood flow and oxygenation[32]. These two sequences were commonly used in the central nervous system[33,34]. Currently, T2* has been used in assessing tissue oxygenation status *in vivo* based on the paramagnetic properties of deoxyhemoglobin[35]. Besides, this technique has been shown to be feasible and accurate in the detection of HCC[27,32]

Previously, R2* values have been used to distinguish cancerous from normal prostatic regions, with higher mean R2* values being related to a higher tumor Gleason score[36]. In addition, higher R2* values were found in high-grade bladder cancer[15] and clear cell renal cell carcinoma[37] than those of low-grade malignancies. In

Table 2 Clinicopathological characteristics of 108 focal liver lesions

Characteristic	Malignant		Benign			
	HCC	IHCC	Hemangioma	FNH	Abscess	
No. of lesions (%)	32 (29.6)	9 (8.3)	37 (34.3)	25 (23.1)	3 (2.8)	2 (1.9)
Maximum diameter (mm)						
mean ± SD	66.3 ± 43.0	61.9 ± 5.9	29.1 ± 24.1	29.4 ± 21.8	32.0 ± 8.5	69.5 ± 12.0
Range	15-163	32-111	11-122	14-94	23-40	61-78
Methods of diagnosis (%)						
Pathology	29 (26.9)	6 (5.6)	7 (6.5)	5 (4.6)	2 (1.9)	0 (0)
Imaging follow-up	3 (2.8)	3 (2.8)	19 (17.6)	20 (18.5)	1 (0.9)	2 (1.9)
PET-CT	–	–	11 (10.2)	–	–	–
Viral infection						
HBV	30	6	34	9	1	2
Non-HBV	2	3	3	9	1	0
NA	0	0	0	7	1	0
Cirrhosis on pathology (%)						
Yes	25	–	–	–	–	–
No	1	–	–	–	–	–
NA	6	–	–	–	–	–
AFP (ng/mL)						
≤ 25	12	9	29	–	–	–
> 25	20	0	7	–	–	–
NA	0	0	1	–	–	–
CA 19-9 (U/mL)						
≤ 34	21	4	15	–	–	–
> 34	9	5	20	–	–	–
NA	2	0	2	–	–	–
CEA (ng/mL)				–	–	–
≤ 5	27	7	15	–	–	–
> 5	5	2	22	–	–	–
NA	0	0	0	–	–	–

APF: -fetoprotein; CA 19-9: Carbohydrate antigen 19-9; CEA: Carcinoma embryonic antigen; FFL: Focal liver lesion; FNH: Focal nodular hyperplasia; HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma; IHCC: Intrahepatic cholangiocarcinoma; NA: Not available; PET-CT: Positron emission tomography-computed tomography. Data are shown as the mean SD.

our study, the mean R2* value of malignant FLLs was significantly higher than that of the benign FLLs. This may be attributed to the rapid growth of liver malignancies, resulting in a relatively hypoxic state and an increase in deoxyhemoglobin[15]. Consequently, the corresponding increase in R2* value may correlate with the degree of malignancy of FFL. R2* may be used as a quantitative imaging biomarker to provide additional information for tumor differential diagnosis.

In our study, mean R2* values, whether derived from 2D-ROI or VOI segmentation positioning methods, were highly reproducible. Moreover, the AUC of R2* measured by 2D-ROI was 0.884 with a sensitivity of 84.6% and specificity of 80.0%, while AUC of R2* measured by VOI yielded an AUC of 0.875 with a sensitivity of 85.9% and specificity of 76.7%, in distinguishing benign from malignant FFLs, respectively. Campo *et al*[38] demonstrated that a large ROI that refers to as large an area of the liver as possible can improve the reproducibility and repeatability of R2* measurements in

Table 3 Mean R2* values for different focal liver lesions

FFL	2D-ROI method	VOI method
Malignant		
Liver metastasis	44.17 ± 21.90	48.42 ± 23.61
HCC	33.45 ± 10.15	35.41 ± 10.04
IHCC	28.72 ± 10.21	31.34 ± 9.65
Benign		
Hemangioma	16.66 ± 8.18	19.36 ± 8.93
FNH	26.21 ± 5.61	27.87 ± 7.46
Abscess	23.29 ± 9.31	25.29 ± 10.46

Numerical data are expressed as the mean ± SD. 2D-ROI: Two-dimensional region of interest; FFL: Focal liver lesion; FNH: Focal nodular hyperplasia; HCC: Hepatocellular carcinoma; IHCC: Intrahepatic cholangiocarcinoma; VOI: Volume of interest.

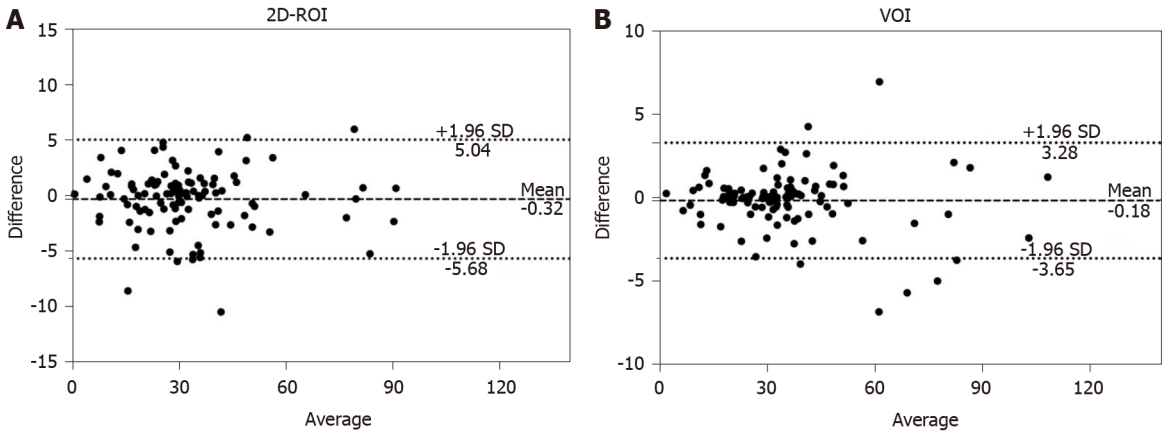

Figure 2 Bland–Altman plots showing interobserver variability in two-dimensional region of interest and volume of interest measurements. A: Two-dimensional region of interest (ROI); B: Volume of interest. The differences between the two readers using the two different ROI positioning methods were small. 2D-ROI: Two-dimensional region of interest; VOI: Volume of interest.

patients with low and high liver iron content. McCarville *et al*[39] reported excellent interobserver agreements in liver R2* for both small (≥ 1 cm diameter) and whole liver ROI methods for iron overloaded patients who underwent biopsy. Sofue *et al*[40] found that R2* measurements of whole liver volume and colocalized ROIs in three different hepatic segments were repeatable between examinations. However, these studies investigated ROI location of R2* measurements in diffusive liver lesions rather than FLLs. To the best of our knowledge, our study was the first to investigate R2* measurements in FFLs.

We found similar results in differentiating between benign and malignant FLLs by using 2D-ROI and VOI methods for R2* measurement. ROC curve analysis demonstrated no significant difference between the AUCs for 2D-ROI and VOI positioning methods for discriminating benign from malignant FFLs. R2* measured by VOI analysis showed an AUC of 0.875, while 2D-ROI analysis showed an AUC of 0.884 in differentiating between benign and malignant FLLs. These results indicate that the impact of the different ROI positioning methods could be ignored for the differential diagnosis of benign and malignant FFLs. Thust *et al*[41] obtained the same results in volumetric and 2D measurements of apparent diffusion coefficient in distinguishing glioma subtypes. Compared with VOI, 2D-ROI is easier to delineate and easily incorporated into clinical practice. The easy implementation of R2* measurements using 2D-ROI will facilitate its clinical application.

There were several limitations to this study. First, this was a single-center study, and the number of patients in the cohort was relatively small. A larger patient cohort in a multicenter setting is needed to validate our findings. Second, R2* is an indirect method for monitoring tumor PO_2[42]. In addition to the oxygenation state, R2* can also

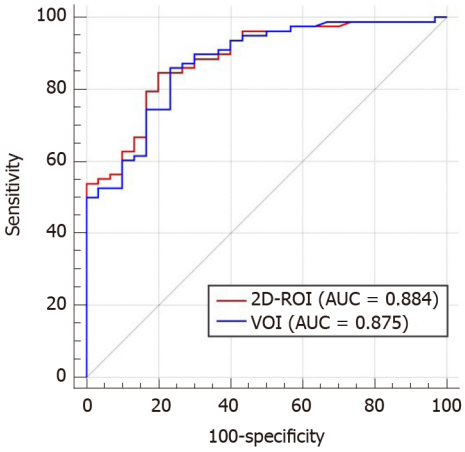

Figure 3 Receiver operating characteristic curve analysis of the two positioning methods in differentiating between malignant group and benign group. Two-dimensional region of interest and volume of interest methods yielded similar results. 2D-ROI: Two-dimensional region of interest; VOI: Volume of interest; AUC: Area under the curve.

be affected by other factors, such as hemoglobin levels, blood volume, and vasculature[15]. Nevertheless, various studies have found that T2WI is a highly sensitive technique for reliably assessing paramagnetic deoxyhemoglobin, methemoglobin, or hemosiderin in lesions and tissues in body imaging[30,35,37]. R2* quantification can yield hypoxia information about malignancies in a noninvasive manner[19,42]. In addition, the sequence used in our study is easy to perform and requires only a single breath-hold of 16 s to image the entire liver, and no image postprocessing is required.

CONCLUSION

In conclusion, R2* values derived from multi-echo Dixon imaging can aid in discrimination between benign and malignant FLLs. 2D-ROI and VOI methods do not affect the diagnostic performance of R2*. R2* measured by 2D-ROI can be adopted to improve diagnostic accuracy of FFLs, particularly in patients with a contraindication to contrast agents.

ARTICLE HIGHLIGHTS

Research background

It is essential to distinguish between benign and malignant focal liver lesions (FLLs), as this differentiation determines the individual's prognosis and subsequent treatment strategy. Since the use of iodine and gadolinium-based contrast agents is contraindicated, imaging techniques without the need of contrast agents have been used to diagnose FFLs, including diffusion-weighted imaging, intravoxel incoherent motion, diffusion kurtosis imaging, and magnetic resonance elastography.

Research motivation

Imaging techniques without the need of contrast agents have shown mixed success with limited clinical application. R2* estimation is inversely related to partial tissue pressure of oxygen, and reflects the paramagnetism of the tumor tissue, which may be helpful to differentiate between benign and malignant FLLs.

Research objectives

To investigate whether R2* derived from multi-echo Dixon imaging can aid differentiating benign from malignant FLLs. The findings obtained can provide information for differential diagnosis of FLLs using R2*.

Research methods

This study retrospectively enrolled 73 patients with 108 benign or malignant FLLs. All patients underwent conventional abdominal magnetic resonance imaging and multi-

echo Dixon imaging. The mean R2* values of lesions were measured using 2D region of interest (2D-ROI) and volume of interest (VOI) approaches. Mean R2* values were compared between benign and malignant FFLs using the nonparametric Mann–Whitney test. Receiver operating characteristic curve analysis was used to determine the diagnostic performance of R2* in differentiation between benign and malignant FFLs. The diagnostic performance of R2* measured by 2D-ROI and VOI approaches was compared.

Research results

The study included 30 benign and 78 malignant FLLs. Mean R2* was significantly higher for malignant than benign FFLs as measured by 2D-ROI ($P < 0.001$) and VOI ($P < 0.001$). The area under the curve (AUC) of R2* measured by 2D-ROI was 0.884 at a cut-off of 25.2/s, with a sensitivity of 84.6% and specificity of 80.0% for differentiating benign from malignant FFLs. R2* measured by VOI yielded a AUC of 0.875 at a cut-off of 26.7/s in distinguishing benign from malignant FFLs, with a sensitivity of 85.9% and specificity of 76.7%. The AUCs of R2* were not significantly different between the 2D-ROI and VOI methods. However, due to the relatively small sample size, a large population from multiple centers is needed for further validation of our findings.

Research conclusions

R2* derived from multi-echo Dixon imaging can aid in differentiation between benign and malignant FLLs. 2D-ROI and VOI methods do not affect the diagnostic performance of R2*.

Research perspectives

This study describes that R2* value derived from multi-echo Dixon imaging can aid in differentiation between benign and malignant FLLs. The multi-echo Dixon sequence is easy to perform and requires only a single breath-hold of 16 s to image the entire liver, which holds a good potential for clinical application.

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ORIGINAL ARTICLE

Retrospective Study

Cytapheresis re-induces high-rate steroid-free remission in patients with steroid-dependent and steroid-refractory ulcerative colitis

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Abstract

BACKGROUND

It is a crucial issue for patients with refractory ulcerative colitis (UC), including steroid-dependent and steroid-refractory patients, to achieve and maintain steroid-free remission. However, clinical studies focused on the achievement of steroid-free remission in refractory UC patients are insufficient. Cytapheresis (CAP) is a non-pharmacological extracorporeal therapy that is effective for active UC with fewer adverse effects. This study comprised UC patients treated with CAP and suggested the efficacy of CAP for refractory UC patients.

AIM

To clarify the efficacy of CAP in achieving steroid-free remission in refractory UC patients.

METHODS

We retrospectively reviewed the collected data from 55 patients with refractory UC treated with CAP. We analyzed the following points: (1) Efficacy of the first course of CAP; (2) Efficacy of the second, third, and fourth courses of CAP in patients who experienced relapses during the observation period; (3) Efficacy of CAP in colonic mucosa; and (4) Long-term efficacy of CAP. Clinical efficacy was evaluated using Lichtiger's clinical activity index or Sutherland index (disease activity index). Mucosal healing was evaluated using Mayo endoscopic subscore.

Written or oral informed consent was obtained from patients and/or parents of patients aged younger than 20 years.

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The primary and secondary endpoints were the rate of achievement of steroid-free remission and the rate of sustained steroid-free remission, respectively. Statistical analysis was performed using the paired t-test and chi-squared test.

RESULTS

The rates of clinical remission, steroid-free remission, and poor effectiveness after CAP were 69.1%, 45.5%, and 30.9%, respectively. There were no significant differences in rate of steroid-free remission between patients with steroid-dependent and steroid-refractory UC. The mean disease activity index and Lichtiger's clinical activity index scores were significantly decreased after CAP ($P < 0.0001$). The rates of steroid-free remission after the second, third, and fourth courses of CAP in patients who achieved steroid-free remission after the first course of CAP were 83.3%, 83.3%, and 60%, respectively. Mucosal healing was observed in all patients who achieved steroid-free remission after the first course of CAP. The rates of sustained steroid-free remission were 68.0%, 60.0%, and 56.0% at 12, 24, and 36 mo after the CAP. Nine patients (36%) had maintained steroid-free remission throughout the observation period.

CONCLUSION

Our results suggest that CAP effectively induces and maintains steroid-free remission in refractory UC and re-induces steroid-free remission in patients achieving steroid-free remission after the first course of CAP.

Key Words: Ulcerative colitis; Cytapheresis; Steroid-dependent; Steroid-refractory; Steroid-free remission; Inflammatory bowel disease

Core Tip: Management of steroid-dependent and steroid-refractory ulcerative colitis (UC) is a critical issue, and the goal of the therapy for such refractory UC should be steroid-free remission. However, clinical studies focused on the achievement of steroid-free remission in refractory UC patients are insufficient. In this study, we demonstrated that cytapheresis (CAP) was effective in inducing and maintaining steroid-free remission even in both steroid-dependent and steroid-refractory UC patients. Furthermore, it is notable that we also showed that CAP re-induced high-rate steroid-free remission repeatedly in such refractory UC patients who achieved steroid-free remission after the first course of CAP.

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INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD) of unknown etiology, which can affect the entire colon. Several treatments for UC are available to induce and maintain the clinical remission of the disease. Among these treatments, corticosteroids (CSs) were first introduced by Truelove and Witts[1] and currently remain the first-line treatment to induce remission in moderate to severe UC patients. Faubion *et al*[2] reported that 34% of UC patients were treated with CSs and that immediate outcomes were complete remission in 54%, partial remission in 30%, and no response in 16% of patients. They also showed that 1-year outcomes were prolonged response in 49%, CS dependence in 22%, and operation in 29% of patients[2]. Despite the effectiveness of CSs in inducing clinical remission in UC patients, it has been reported that 16%-18% of patients had no response to steroids (steroid-refractory), and the rate of steroid dependence was 17%-22% at 1 year following treatment with the initial CS therapy and increased to 38% mostly within 2

years[2-7].

Refractory UC generally includes both steroid-dependent and steroid-refractory UC. Along with the recent advancements of the treatment for UC, several breakthrough treatments, including biologics, have been developed for refractory UC[8-23]. A meta-analysis showed that anti-tumor necrosis factor-α (TNF-α) antibodies had more clinical benefits than placebo control as evidenced by the former's increased frequency of clinical remission, steroid-free remission, endoscopic remission, and decreased frequency of colectomy[24]. It was also reported that the rates of induction of steroid-free remission in refractory UC patients with anti-TNF-α antibodies ranged from 40.0% to 76.5%[6,9,11,15,16,18]. However, studies that analyzed the efficacy of biologics focused on the achievement of steroid-free remission in refractory UC patients are insufficient. On the contrary, despite the efficacy of anti-TNF-α antibody for UC, secondary loss of response (LOR) is a common clinical problem with its incidence rate ranging from 23% to 46% at 12 mo after anti-TNF-α initiation[25]. Moreover, it was reported that the incidence rates of LOR were 58.3% (adalimumab) and 59.1% (infliximab) during maintenance therapy (mean follow-up: 139 wk and 158.8 wk, respectively)[26]. Regarding vedolizumab, it was also reported that the cumulative rate for LOR in UC patients was 39% at 12 mo[27]. Concerning the adverse events of biologics, similar with other biological therapies, anti-TNF-α therapy may lead to serious infection, demyelinating disease, and associated mortality[28]. It was also reported that the use of anti-TNF-α antibody combined with thiopurines was associated with an increased risk of lymphoma in IBD[29].

Thiopurines have been conventionally used for the treatment of steroid-dependent UC[30-35]. Two randomized controlled trials have shown that the rates of the induction of CS-free remission with thiopurines in steroid-dependent UC patients were 44% and 53%, respectively[34,35]. However, Jharap *et al*[32] reported that thiopurine therapy has failed in approximately one-quarter of IBD patients within 3 mo after treatment initiation, which is mostly due to drug intolerance or toxicity. Moreover, thiopurines are associated with potential serious adverse events, such as an increased risk of lymphoma and nonmelanoma skin cancer[33].

Cytapheresis (CAP) is a non-pharmacological extracorporeal therapy and has been developed as a treatment for UC[36-42]. CAP is performed using two methods, namely, granulocyte and monocyte adsorptive apheresis (GMA), which uses cellulose acetate beads (Adacolumn, JIMRO Co., Ltd., Gunma, Japan), and leukocytapheresis (LCAP), which uses polyethylene phthalate fibers (Cellsorba., Asahi Kasei Medical Co., Ltd., Tokyo, Japan)[42,43]. GMA selectively depletes elevated granulocytes and monocytes from the patients' circulation, but spares most of the lymphocytes[42]. LCAP exerts anti-inflammatory effects by removing activated leukocytes or platelets from the peripheral blood through an extracorporeal circulation[43]. It has been shown that CAP is an effective therapeutic strategy for patients with active UC with fewer adverse effects[36-42]. However, to date, the number of studies focused on the efficacy of CAP in both steroid-dependent and steroid-refractory UC has been limited[43-54].

Despite the excellent therapeutic effects of CS for UC patients, prolonged CS therapy can result in multiple serious side effects such as diabetes mellitus, infection, osteonecrosis, and steroid-associated osteoporosis[55]. Furthermore, McCurdy *et al*[56] showed that IBD patients receiving CSs and immunomodulators were more likely to be diagnosed with cytomegalovirus diseases than IBD patients not receiving CSs and immunomodulators. Therefore, management of refractory UC patients is a crucial issue, and the goal of the treatment for such patients should be steroid-free remission. However, as described above, clinical studies focused on the achievement of steroid-free remission in refractory UC patients are insufficient. We had treated many UC patients with CAP and consequently suggested the efficacy of CAP for refractory UC patients. Considering these backgrounds, we retrospectively analyzed the efficacy of CAP specifically focused on the achievement of steroid-free remission in patients with steroid-dependent and steroid-refractory UC.

MATERIALS AND METHODS

Patients

We retrospectively reviewed the collected data from 55 (male 29, female 26) patients aged 16-82 years (mean ± SD, 38.7 ± 16.7 years) with active refractory UC (steroid-dependent type 33, steroid-refractory type 21, refractory but refused steroid therapy 1) treated with CAP (GMA 38, LCAP 17) between September 2002 and December 2019 (Table 1). The detailed clinical profiles of the patients enrolled in this study are shown

Table 1 Patients' characteristics in this study

Characteristics	
Age (yr, mean ± SD)	16-82 (38.7 ± 16.7)
Sex	Male 29, female 26
Disease duration from diagnosis (mo, mean ± SD)	1-384 (59.4 ± 78.8)
Disease extent	
Left-sided colitis	12
Pancolitis	43
Disease refractory type	
Steroid-dependent	33
Steroid-refractory	21
Refusal of steroids	1
Clinical type	
One-attack	4
Relapsing-remitting	50
Chronic continuous	1
Mean CAI (mean ± SE) (pre first course of CAP)	9.0 ± 0.62
Mean DAI (mean ± SE) (pre first course of CAP)	11.3 ± 0.55
Medication (pre first course of CAP)	
PSL (oral)	Yes 54, no 1
5-ASA	Yes 52, no 3
Thiopurines	Yes 12, no 43
TNF-α antibodies	Yes 1 (adalimumab), no 54
Vedolizumab, tofacitinib, tacrolimus, ustekinumab	Yes 0, no 55
Dose of PSL at the start of CAP (mean ± SD)	0-60 mg (33.4 ± 19.2)
Type of CAP	GMA 38, LCAP 17
Observation period after the first course of CAP (mo, mean ± SD)	18-193 (81.5 ± 47.3)

CAI: Lichtiger's clinical activity index; DAI: Sutherland index (disease activity index); PSL: Prednisolone; 5-ASA: 5-Aminosalicylic acid; CAP: Cytapheresis; GMA: Granulocyte and monocyte adsorptive apheresis; LCAP: Leukocytapheresis.

in Table 1. The dosage of prednisolone and the concomitant therapies at apheresis commencement are also shown in Table 1. The rates of concomitant use of prednisolone, 5-aminosalicylic acid, and immunomodulators were 98.2% (54/55), 94.5% (52/55), and 21.8% (12/55), respectively. Anti-TNF-α antibody (adalimumab) was administered to one patient. In most patients, concomitant medications except prednisolone were continued at the same dosage. The dosage of prednisolone was tapered or discontinued according to patients' clinical improvement during the CAP therapy.

This retrospective study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of Akita Red Cross Hospital (approval No: 195) and Akita University School of Medicine (approval No: 2419). Written or oral informed consent was obtained from patients and/or parents of patients aged younger than 20 years.

The primary endpoint of this study was the rate of achievement of steroid-free remission in refractory UC patients after the CAP therapy. The achievement of steroid-free remission included the induction of steroid-free remission in the first course of CAP and re-induction of steroid-free remission in the second, third, and fourth courses of CAP. The secondary endpoint was the rate of sustained steroid-free remission in refractory UC patients after the CAP therapy.

Definition of steroid-dependent and steroid-refractory UC

Steroid-dependent UC was defined as the disease that initially responds to steroids but could not maintain control of symptoms without steroids and requires low doses of steroids to remain symptom-free[6, 57]. Steroid-refractory UC was also defined as active UC characterized by the failure to respond to 0.75-1.5 mg/kg per day of prednisolone administered over at least 1 wk[43,57].

CAP

Each patient was treated with 5 to 20 GMA or LCAP sessions (mean ± SD, 8.8 ± 3.8 sessions). A total of 20 patients were treated with 5 sessions of CAP, 29 patients with 10 sessions, 1 patient with 9 sessions, 1 patient with 15 sessions, 1 patient with 18 sessions, and 3 patients with 20 sessions. Under the Japanese health insurance treatment system, the 11th CAP session was performed at 1 mo after the 10th CAP session in patients who received more than 10 CAP sessions. CAP was performed once weekly in principle. However, in some patients with severe UC, CAP was exceptionally performed twice a week for the first 2-3 wk (intensive CAP). CAP was also exceptionally performed once 2 wk for the last several weeks in some patients whose symptoms improved to mild after the treatment with several sessions of CAP.

Exclusion criteria

Patients with serious cardiac, kidney, or liver diseases; malignancy; coagulation disorders; infections; history of hypersensitivity to heparin; severe dehydration, granulocytopenia, anemia, thrombocytopenia; and patients taking angiotensin-converting enzyme inhibitor were excluded.

Evaluation of the efficacy of CAP

Efficacy of the first course of CAP: Clinical efficacy between April 2008 and December 2019 were evaluated using the Lichtiger's clinical activity index (CAI)[58] and that between September 2002 and March 2008 was evaluated using Sutherland index (disease activity index, DAI)[59]. Clinical remission was defined as decreased Lichtiger's CAI in 4 or less or decreased DAI in less than 2.5[60]. In this study, we assessed patients who did not achieve clinical remission after CAP, suggesting the "poor effectiveness of CAP". We evaluated the efficacy of CAP approximately 4 wk after the last apheresis session. We also examined the rate of steroid-free remission. We have defined "steroid-free" as the point when both oral steroids and enemas including steroids were discontinued. However, suppositories including small amounts of steroids were permitted, as an exception.

Laboratory data (C-reactive protein level, serum albumin concentration, neutrophil count, and monocyte count) before and after CAP were also examined in 28 patients treated between April 2008 and December 2019.

Efficacy of the second, third, and fourth courses of CAP: Efficacy of the second course of CAP in patients experiencing a relapse during the observation period was assessed. Furthermore, efficacy of the third and fourth courses of CAP was also assessed specifically in patients who achieved steroid-free remission after the first course of CAP and experienced relapses during the observation period.

Efficacy of CAP in colonic mucosal inflammation: Endoscopic findings after the first course of CAP in patients who achieved steroid-free remission were evaluated using the Mayo endoscopic subscore[61]. A score ≤ 1 suggested mucosal healing.

Long-term efficacy: Long-term efficacy of CAP in patients who achieved steroid-free remission after the first course of CAP was examined by assessing (1) the rate of sustained steroid-free remission at 12, 24, and 36 mo after the first course of CAP and (2) overall rate of maintaining sustained steroid-free remission throughout the observation period.

The surgical operation rate: The surgical operation rates of the patients within 6 mo, 3 years, and throughout the observation period after the first course of CAP were examined.

Statistical analysis: Statistical analysis was performed using the paired *t*-test, and chi-squared test, and a P value < 0.05 was considered statistically significant.

RESULTS

Efficacy of the first course of CAP

The rates of clinical remission, which includes steroid-free remission and clinical remission but not steroid-free remission, steroid-free remission, and poor effectiveness after CAP were 69.1%, 45.5%, and 30.9%, respectively (Figure 1). The rates of clinical remission, steroid-free remission, and poor effectiveness after GMA were 69.2%, 43.6%, and 30.8%, respectively. The rates of clinical remission, steroid-free remission, and poor effectiveness after LCAP were 68.8%, 50.0%, and 31.2%, respectively. There were no significant differences in the rates of both clinical remission and steroid-free remission after CAP between patients who received GMA therapy and patients who received LCAP.

In this study, thiopurines were concomitantly used in 12 patients. The rates of clinical remission, steroid-free remission, and poor effectiveness after CAP in patients who concomitantly received thiopurines were 66.7%, 41.7%, and 33.3% respectively. There were no significant differences in the rates of both clinical remission and steroid-free remission after CAP between patients who concomitantly received thiopurines and patients who did not receive thiopurines.

For patients with steroid-dependent UC, the rates of clinical remission, steroid-free remission, and poor effectiveness after CAP were 69.7%, 42.4%, and 30.3%, respectively (Figure 2). On the contrary, the rates of clinical remission, steroid-free remission, and poor effectiveness after CAP in patients with steroid-refractory UC were 66.7%, 47.6%, and 33.3%, respectively (Figure 3). There were no significant differences in both rates of clinical remission and steroid-free remission between patients with steroid-dependent UC and patients with steroid-refractory UC.

DAI and CAI scores (mean ± SE) before and after the first course of CAP are shown in Figures 4 and 5. The mean DAI score before CAP was 11.4, which decreased significantly to 3.36 after the CAP therapy ($P < 0.0001$) (Figure 4). The mean CAI score before CAP was 9.0, which decreased significantly to 3.63 after the CAP therapy ($P < 0.0001$) (Figure 5).

Laboratory data before and after CAP are shown in Table 2. As shown in Table 2, the inflammatory parameter (C-reactive protein) and the nutritional parameter (serum albumin concentration) significantly improved after CAP. Neutrophil count significantly decreased after CAP therapy. Monocyte count tended to decrease after CAP, but no significant difference was observed.

The rates of steroid-free remission after the second course of CAP

The second course of CAP was performed in 24 patients (12 patients who achieved steroid-free remission after the first course of CAP, 8 patients who achieved clinical remission but not steroid-free remission after the first course of CAP, 4 patients who had poor effectiveness in the first course of CAP) experiencing a relapse or worsening condition during the observation period. The rates of steroid-free remission after the second course of CAP in patients who achieved steroid-free remission after the first course of CAP, patients who achieved clinical remission but not steroid-free remission after the first course of CAP, and patients who had poor effectiveness in the first course of CAP were 83.3% (10/12), 12.5% (1/8), and 0% (0/4), respectively (Figure 6). The rate of steroid-free remission after the second course of CAP was significantly higher in patients who achieved steroid-free remission after the first course of CAP compared with that in patients who achieved clinical remission but not steroid-free remission after the first course of CAP ($P = 0.0018$) and that in patients who had poor effectiveness in the first course of CAP ($P = 0.0029$).

The rates of steroid-free remission after the second, third, and fourth courses of CAP in patients who achieved steroid-free remission after the first course of CAP

As shown above, the rate of steroid-free remission after the second course of CAP in patients who achieved steroid-free remission after the first course of CAP was 83.3%. In these patients, the rate of steroid-free remission after the second course of CAP in patients with steroid-dependent UC (83.3%) was the same as that of patients with steroid-refractory UC (83.3%).

The third and fourth courses of CAP were performed in 6 patients and 5 patients, respectively, who achieved steroid-free remission after the first course of CAP and experienced relapses during the observation period. The rates of steroid-free remission after the third and fourth courses of CAP in these patients were 83.3% (5/6) and 60% (3/5), respectively (Figure 7).

Table 2 Laboratory data obtained (mean ± SE) before and after cytapheresis

	Before CAP	After CAP	*P* value
CRP (mg/dL)	1.795 ± 0.721	0.312 ± 0.130	$P = 0.0396$
Albumin (g/dL)	3.579 ± 0.139	3.911 ± 0.117	$P = 0.0358$
Neutrophil count (μL)	6826 ± 561	5475 ± 456	$P = 0.0124$
Monocyte count (μL)	588 ± 73	425 ± 46	$P = 0.0626$

CRP: C-reactive protein; CAP: Cytapheresis.

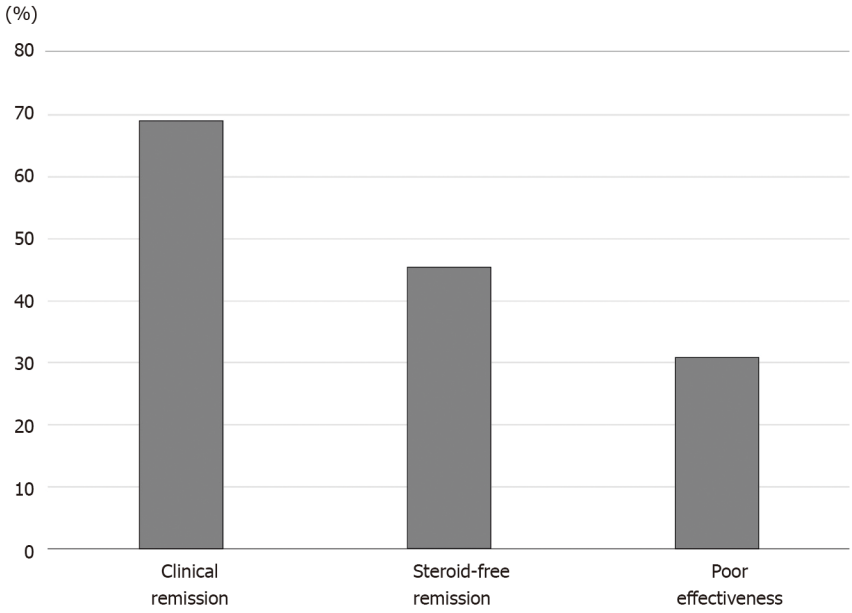

Figure 1 Efficacy of the first course of cytapheresis. The rates of clinical remission, which includes steroid-free remission and clinical remission without steroid-free remission, steroid-free remission, and poor effectiveness after cytapheresis were 69.1%, 45.5%, and 30.9%, respectively.

Endoscopic findings of patients who achieved steroid-free remission after the first course of CAP

Colonoscopic examination was performed in 21 out of the 25 patients (84%) who achieved steroid-free remission after the first course of CAP. Mucosal healing was observed in all 21 patients after the first course of CAP [Mayo endoscopic subscore 0 in 17 patients (81.0%), Mayo endoscopic subscore 1 in 4 patients (19.0%)]. None of the patients showed a Mayo endoscopic subscore ≥ 2 after the CAP. Endoscopic images before and after the CAP therapy of 5 patients are shown in Figure 8.

Long-term efficacy of CAP in patients achieving steroid-free remission in the first course of CAP

We could correctly follow the rate of sustained steroid-free remission for 3 years (36 mo) in all 25 patients who successfully achieved steroid-free remission after the first course of CAP. The rates of sustained steroid-free remission in these patients were 68.0% at 12 mo, 60.0% at 24 mo, and 56.0% at 36 mo after the first course of CAP (Figure 9). The rates of sustained steroid-free remission in patients with steroid-dependent UC were 69.2% at 12 mo, 53.8% at 24 mo, and 46.1% at 36 mo, respectively. On the other hand, the rates of sustained steroid-free remission in patients with steroid-refractory UC were 63.6% at 12 mo, 63.6% at 24 mo, and 63.6% at 36 mo, respectively.

The mean observation period of these 25 patients was 81.5 ± 9.7 mo (mean ± SE). Although the observation periods varied in these 25 patients, 9 patients (36.0%) had maintained sustained steroid-free remission throughout the observation periods. The mean period of maintained steroid-free remission of these 9 patients was 86.6 ± 14.3 mo (mean ± SE). Periods of sustained steroid-free remission and refractory type of the

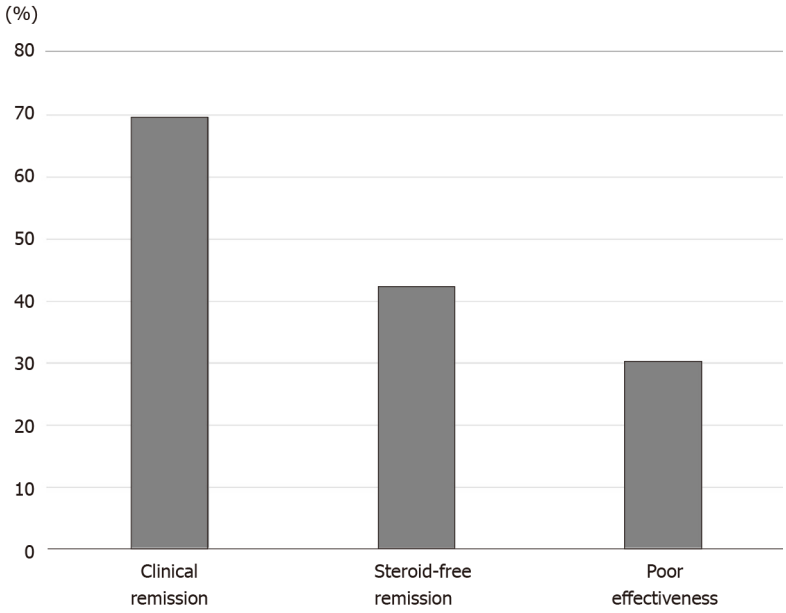

Figure 2 Efficacy of the first course of cytapheresis in the patients with steroid-dependent ulcerative colitis. The rates of clinical remission, steroid-free remission, and poor effectiveness after cytapheresis were 69.7%, 42.4%, and 30.3%, respectively.

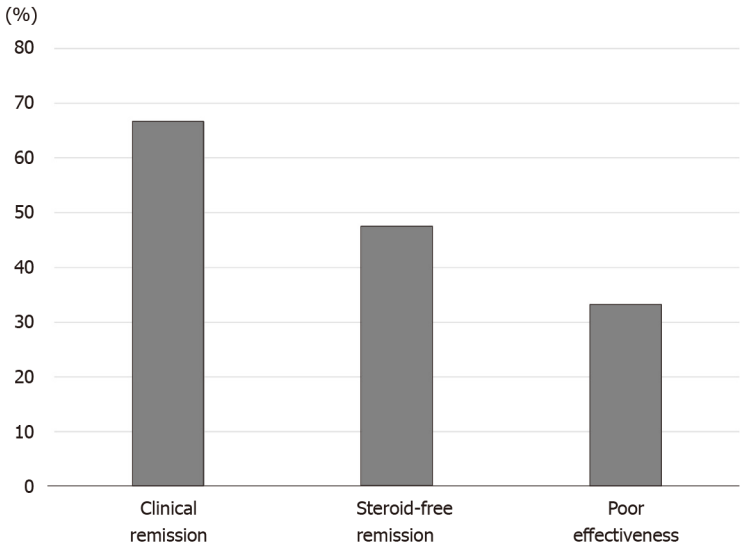

Figure 3 Efficacy of the first course of cytapheresis in the patients with steroid-refractory ulcerative colitis. The rates of clinical remission, steroid-free remission, and poor effectiveness after cytapheresis were 66.7%, 47.6%, and 33.3%, respectively.

9 patients are shown in Figure 10. Two patients had maintained sustained steroid-free remission over 10 years after the first course of CAP. The summary of the results of this study is shown in Figure 11.

The surgical operation rates

The surgical operation rate of the patients within 6 mo after the first course of CAP was 9.1% (5/55). The surgical operation rate within 6 mo after the CAP was significantly lower in patients who achieved steroid-free remission after the first course of CAP (0%) compared with that in patients who had poor effectiveness in the first course of CAP (29.4%) (P = 0.0039). The surgical operation rate within 3 years after the first course of CAP was 12.7% (7/55). The surgical operation rate within 3 years after the CAP was significantly lower in patients who achieved steroid-free remission after the first course of CAP (4%) compared with that in patients who had poor effectiveness (29.4%) (P = 0.0209). The surgical operation rate throughout the observation period [18-193 mo (81.5 ± 47.3 (mean ± SD)] after the first course of CAP was 20 % (11/55). The surgical operation rate throughout the observation period after

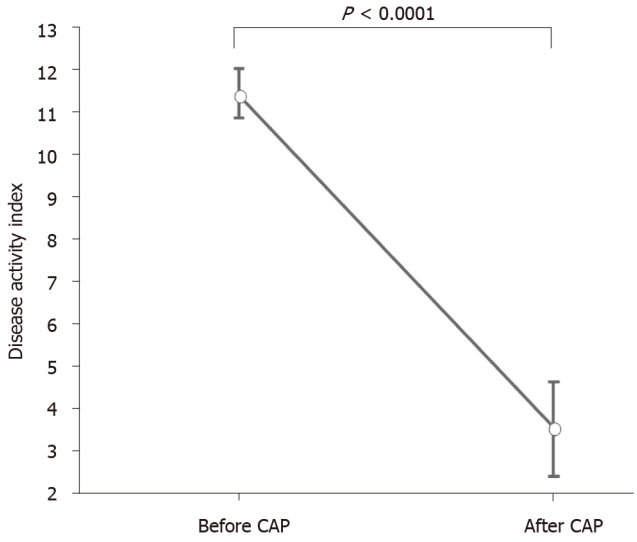

Figure 4 Mean disease activity index score before and after cytapheresis. Disease activity index score (mean ± SE) before and after cytapheresis is shown. The mean disease activity index score before cytapheresis was 11.4, which decreased significantly to 3.36 after treatment ($P < 0.0001$). CAP: Cytapheresis.

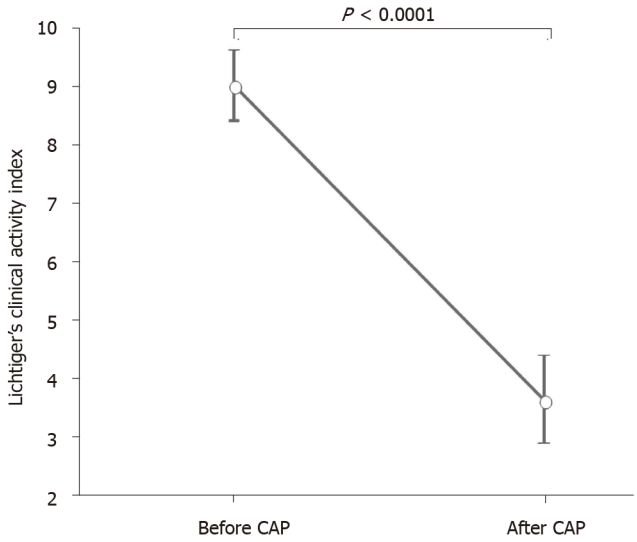

Figure 5 Mean Lichtiger's clinical activity index score before and after cytapheresis. Lichtiger's clinical activity index score (mean ± SE) before and after cytapheresis is shown. The mean Lichtiger's clinical activity index score before cytapheresis was 9.0, which decreased significantly to 3.63 after treatment ($P < 0.0001$). CAP: Cytapheresis.

the CAP was significantly lower in patients who achieved steroid-free remission after the first course of CAP (12%) compared with that in patients who had poor effectiveness in the first course of CAP (41.2%) ($P = 0.0293$).

Adverse events

Headache and slight fever were observed in one patient during the CAP therapy. No serious adverse events were observed in all patients in this study.

DISCUSSION

The primary endpoint of this study was the rate of achievement of steroid-free remission in refractory UC patients after the CAP therapy. In this context, we demonstrated that CAP effectively induced steroid-free remission not only in patients with steroid-dependent (42.4%) but also in patients with steroid-refractory (47.6%) UC. We also showed that mucosal healing was observed in all patients who achieved steroid-free remission after the first course of CAP. Previous studies examining the

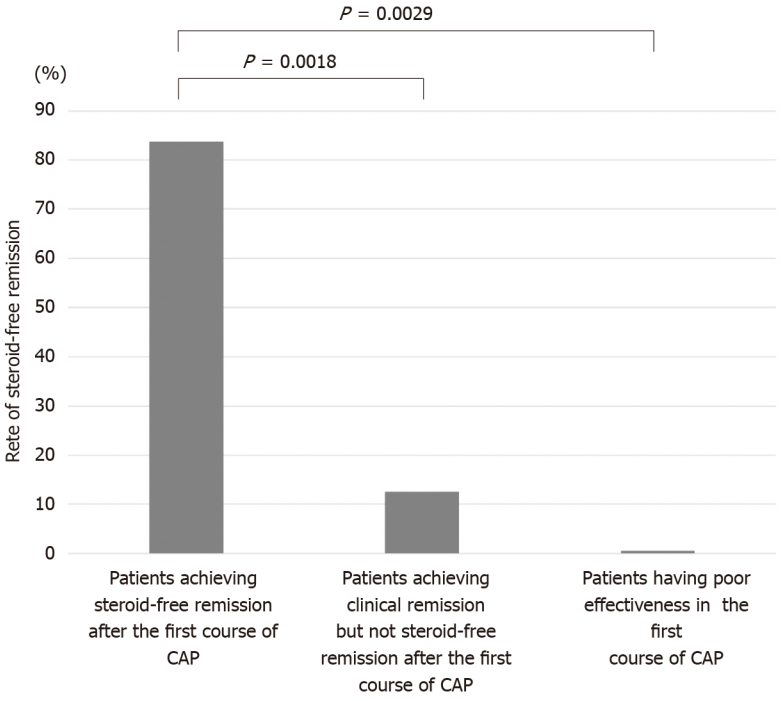

Figure 6 The rates of steroid-free remission after the second course of cytapheresis. The rate of steroid-free remission after the second course of cytapheresis (CAP) was significantly higher in patients who achieved steroid-free remission after the first course of CAP (83.3%) compared with that in patients who achieved clinical remission but not steroid-free remission after the first course of CAP (12.5%, $P = 0.0018$) and that in patients who had poor effectiveness after the first course of CAP (0%, $P = 0.0029$). CAP: Cytapheresis.

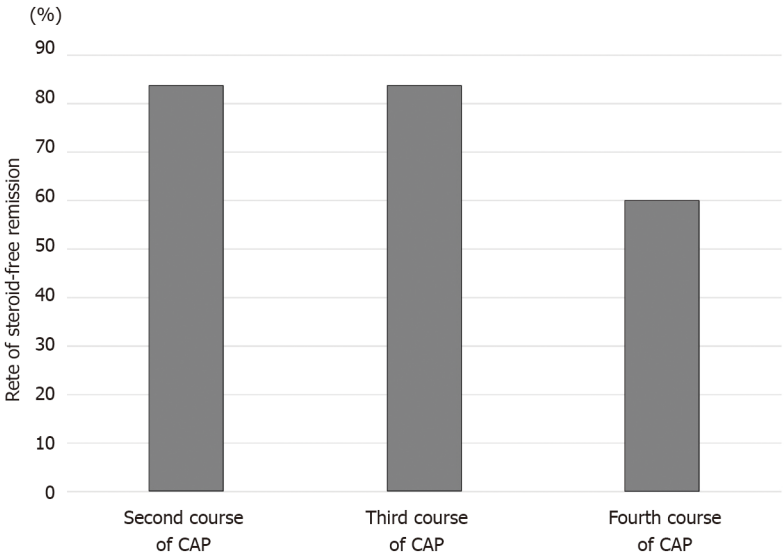

Figure 7 Rates of steroid-free remission after the second, third, and fourth courses of cytapheresis in patients who achieved steroid-free remission after the first course of cytapheresis. The rates of steroid-free remission after the second, third, and fourth courses of cytapheresis in patients who achieved steroid-free remission after the first course of cytapheresis and then experienced relapses were 83.3%, 83.3%, 60%, respectively. CAP: Cytapheresis.

efficacy of CAP in refractory UC and the results of this study are shown in Table 3[43-53]. In these studies, eight studies[45-48,50-53] examined the efficacy of CAP for induction of steroid-free remission and three studies[43,44,49] examined that for induction of only clinical remission. Here, we discuss the eight studies examining the efficacy of CAP for induction of steroid-free remission. In the eight studies, seven studies[46-48,50-53] examined the rate of the induction of steroid-free remission in patients with steroid-dependent UC and one study[45] examined that in patients with steroid-refractory UC. Regarding steroid-refractory UC, it is difficult to evaluate the results of the study because there is only one study, which only comprised eight steroid-refractory patients, that assessed

Table 3 Previous studies examining the efficacy of cytapheresis in refractory ulcerative colitis

Ref.	Refractory type	Number of patients	Evaluation items[1]	Rate of remission (evaluation time)	Rate of steroid- free remission (evaluation time)
Naganuma *et al*[44] (2004)	SR	10	Induction	Severe 20%, moderate 70%	
Giampaolo *et al*[45] (2006)	SR	8	Induction, sustained remission	100%	100%, 12.5% (12 mo)
Ricart *et al*[46] (2007)	SD	20	Induction, sustained remission	42.1% (17 wk)	36.8% (17 wk), 85.7% (12 mo)
Cabriada *et al*[47] (2010)	SD	18	Induction, sustained remission		55% (1 mo), 75% (12 mo)
Cabriada *et al*[48] (2012)	SD	142	Induction, sustained remission		37% (1 mo), 51%[2] (12 mo)
Sacco *et al*[49] (2013)	SD + SR	83 (SD 55, SR 28)	Induction, sustained remission	71%, 48% (12 mo)	
Yokoyama *et al*[43] (2014)	SD + SR	401 (SD 229, SR 172)	Induction	SD: 64.6% (2 wk), SR: 70.9 (2 wk)	
Dignass *et al*[50] (2016)	SD	86	Induction	39.3% (12 wk)	22.6% (12 wk)
Imperiali *et al*[51] (2017)	SD	33	Induction		36% (12 mo)
Dignass *et al*[52] (2018)	SD	95	Induction	34.0% (24 wk), 33.0% (48 wk)	19.2% (24 wk), 19.2% (48 wk)
Domènech *et al*[53] (2018)	SD	63	Induction		13% (24 wk)
Present study	SD + SR	55 (SD 33, SR 21)	Induction, sustained remission	SD: 69.7% (4 wk), SR: 66.7% (4 wk)	SD: 42.4% (4 wk) SR: 47.6% (4 wk); SD: 69.2% (12 mo), SR: 63.6% (12mo)

[1]Evaluation items, which include Induction (evaluation for induction of remission or steroid-free remission) and sustained remission (evaluation for maintenance of sustained remission or steroid-free remission).
[2]Rate of sustained steroid-free remission in the patients with no additional apheresis. SR: Steroid refractory; SD: Steroid dependent.

this type of UC[45]. With regard to steroid-dependent UC, according to the seven previous studies[46-48,50-53], the rates of the induction of steroid-free remission ranged from 13% to 55% (mean 31.4%). Although it is difficult to compare the results of these studies with that of our study because of the diversity of the patients' background enrolled in the studies, the rate of the induction of steroid-free remission of our study is higher than that of the six previous studies[46,48,50-53]. Based on the following reports[43,46,50], we suggest that the differences of the history of previous medication and the differences of the methods of CAP treatment of the studies might influence the rates of steroid-free remission. Dignass *et al*[50] showed that remission was achieved at week 12 after Adacolumn apheresis by 40.3% of patients who failed on immunosuppressants, but only 27.8% of patients who failed on anti-TNF-α treatment. On the other hand, Yokoyama *et al*[43] showed that a multivariate logistic regression analysis comparing the patients' backgrounds, concomitant medications, and therapeutic variables of LCAP between the remission and nonremission groups identified intensive LCAP (≥ 4 LCAP treatments within the first 2 wk) as the only factor that was significantly related to remission after LCAP. On the contrary, Ricart *et al*[46] showed that increasing the number of apheresis sessions affords a significant steroid-sparing effect in steroid-dependent UC. Looking back with reference to these reports, in our study, only one patient who had insufficient response to anti-TNF-α treatment was included, and intensive CAP was performed in some severe cases in contrast to the six previous studies[46,48,50-53] performing weekly apheresis in all patients. Additionally, it appears that patients in our study received more CAP sessions [5-20 sessions (mean 8.8)] compared with the previous studies. We suggest that a selection of an appropriate CAP treatment method for each patient is important to induce steroid-free remission effectively in refractory UC patients.

Regarding the achievement of steroid-free remission, assessing the rate of re-induction of steroid-free remission with CAP in patients who experience relapse after the first course of CAP is also required. In this regard, our study showed that the

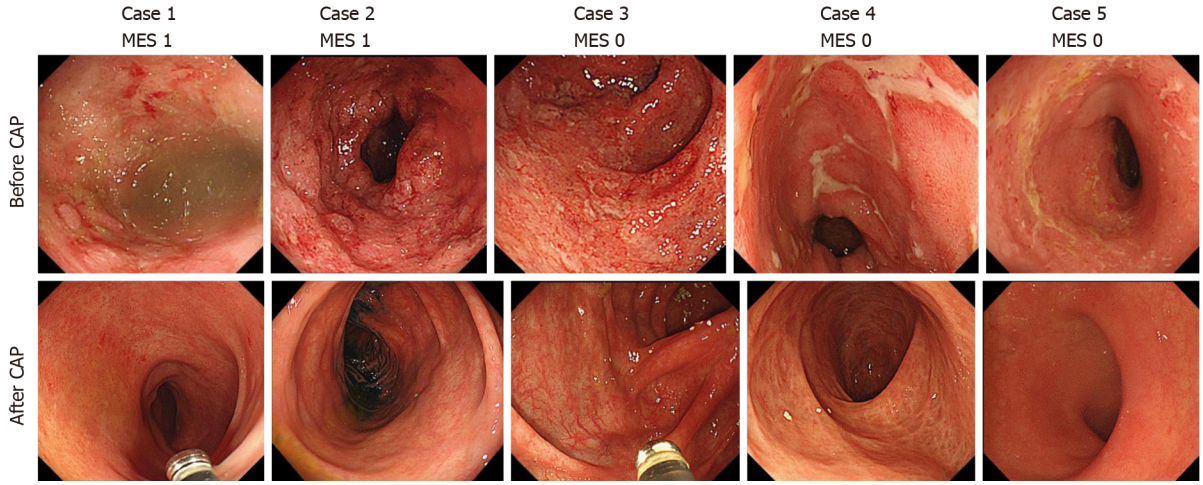

Figure 8 Endoscopic images of 5 patients who achieved steroid-free remission after the first course of cytapheresis. Endoscopic images before and after the cytapheresis (CAP) therapy of 5 patients who achieved steroid-free remission after the first course of CAP are shown. Active inflammation (Mayo endoscopic subscore ≥ 2) was observed in the colonic mucosa in all 5 patients before the CAP therapy. On the contrary, mucosal healing (Mayo endoscopic subscore ≤ 1) was observed in all 5 patients after the CAP therapy. MES: Mayo endoscopic subscore; MES 1/MES 0: Mayo endoscopic subscore after cytapheresis; CAP: Cytapheresis.

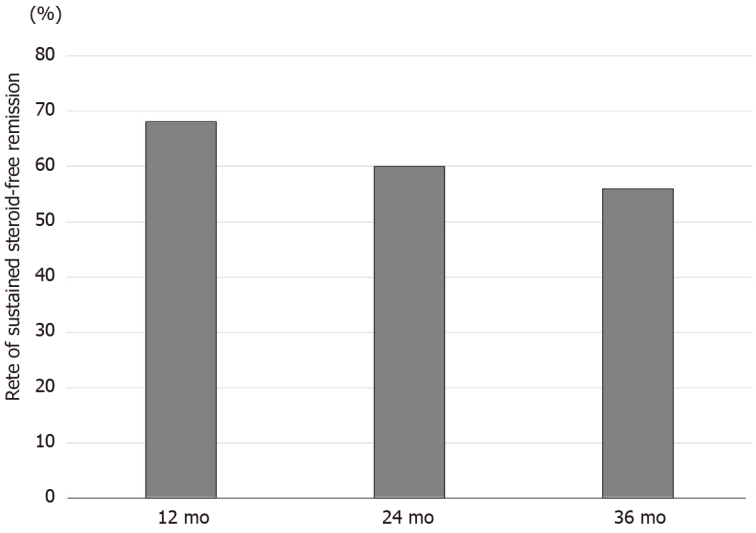

Figure 9 Rates of sustained steroid-free remission at 12, 24, and 36 mo after the first course of cytapheresis in patients who achieved steroid-free remission after the first course of cytapheresis. The rates of sustained steroid-free remission in patients who achieved steroid-free remission after the first course of cytapheresis were 68.0% at 12 mo, 60.0% at 24 mo, and 56.0% at 36 mo after the first course of cytapheresis.

second course of CAP effectively re-induced steroid-free remission (83.3%) in both steroid-dependent and steroid-refractory UC patients who had achieved steroid-free remission in the first course of CAP. The rate of re-induction of steroid-free remission was significantly higher in patients who achieved steroid-free remission in the first course of CAP (83.3%) compared with that of patients who had achieved clinical remission but not steroid-free remission (12.5%) and that of patients who had poor effectiveness in the first course of CAP (0%). Furthermore, our study also showed that the third and the fourth courses of CAP repeatedly induced steroid-free remission at a high rate in patients who achieved steroid-free remission in the first course of CAP. Based on these results, we suggest that patients achieving steroid-free remission in the first course of CAP are significantly likely to have a high sensitivity to CAP, namely, high responders to CAP. There have been no studies assessing the rate of re-induction of steroid-free remission of CAP in patients with steroid-dependent and steroid-refractory UC. However, there have been two studies that examined the re-efficacy of CAP in patients with active UC or Crohn's disease (CD)[37,41]. Takayama *et al*[41] examined the effects of the second course of CAP in UC patients with moderate to severe activity experiencing relapse during the disease course. They showed that the

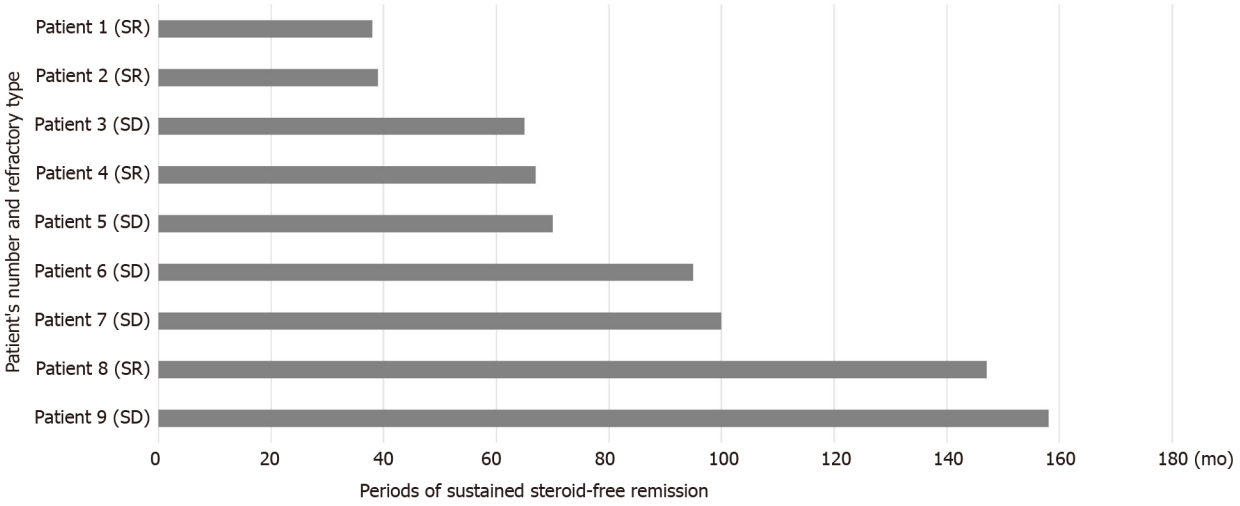

Figure 10 Periods of sustained steroid-free remission and refractory type of the 9 patients who had maintained steroid-free remission throughout the observation periods. Nine patients (36.0%) had maintained sustained steroid-free remission throughout the observation periods. Periods of sustained steroid-free remission of the 9 patients are shown in the figure. The mean period of maintained steroid-free remission of these 9 patients was 86.6 ± 14.3 mo (mean ± SE). Nine patients included 5 steroid-dependent patients and 4 steroid-refractory patients. Periods of sustained steroid-free remission of the 9 patients are shown in the figure. SR: Steroid-refractory patient; SD: Steroid-dependent patient.

percentage of remissive and effective responses of the second course of CAP was 79% in patients who had remissive and effective responses in the first course of CAP, whereas 40% in patients who had noneffective responses in the first course of CAP. Lindberg *et al*[37] presented 14 patients (UC 4, CD 10) who experienced relapse after showing initial remission and were re-treated with GMA. Although the remission rates of the re-treatments of GMA in UC patients were unclear, they showed that 13 of the 14 patients (93%) achieved a second remission. They also showed that following further relapses, all patients were successfully re-treated with GMA for the third, fourth, and fifth time. Thus, the previous two studies also showed that re-treatment of CAP seemed to be effective in UC patients who had remissive responses in the first course of CAP, supporting our results.

The secondary endpoint of this study was the rate of sustained steroid-free remission in refractory UC patients after the CAP therapy. In this regard, we showed that CAP had good long-term efficacy for the maintenance of sustained steroid-free remission (68% at 12 mo, 60% at 24 mo, 56% at 36 mo) in refractory UC patients who achieved steroid-free remission in the first course of CAP. Furthermore, interestingly, 36% of patients had maintained sustained steroid-free remission throughout the observation periods, and two patients had maintained it over 10 years. Previous studies examining the rate of sustained steroid-free remission after the CAP therapy in refractory UC patients are also shown in Table 3. Among them, three studies examined the rate of sustained steroid-free remission in patients with steroid-dependent UC[46-48]. The rates of sustained steroid-free remission at 12 mo after CAP of the three studies ranged from 51% to 85.7% (mean 70.6%). Thus, these studies and our study (69.2% in steroid-dependent patients) showed good long-term efficacy in the rates of sustained steroid-free remission. In this regard, in our study, mucosal healing was observed in all patients who achieved steroid-free remission after the first course of CAP. Ricart *et al*[46] also showed that all patients who experienced clinical remission also experienced endoscopic remission and good long-term efficacy. Cabriada *et al*[48] showed that among those patients in steroid-free remission, 96% also achieved endoscopic remission. They also showed that a tendency for sustained remission at 1 year was observed when initial endoscopic remission was achieved[47]. Based on these findings, we suggest that endoscopic mucosal healing was closely involved in the maintenance of sustained steroid-free remission and good long-term efficacy of CAP.

In this study, no serious adverse events were observed during the CAP therapy. It has been reported that other therapies, such as anti-TNF-α antibody administration, are associated with risk of serious infections, lymphoma, and associated mortality in IBD[28,29,50]. In this context, several studies reporting on the safety of CAP have been considered important[39,42,43,49,50]. Among these studies, Hibi *et al*[39] evaluated the safety and clinical efficacy of Adacolumn in 697 patients with UC in 53 medical institutions. They showed that no serious adverse events were observed, and mild to moderate

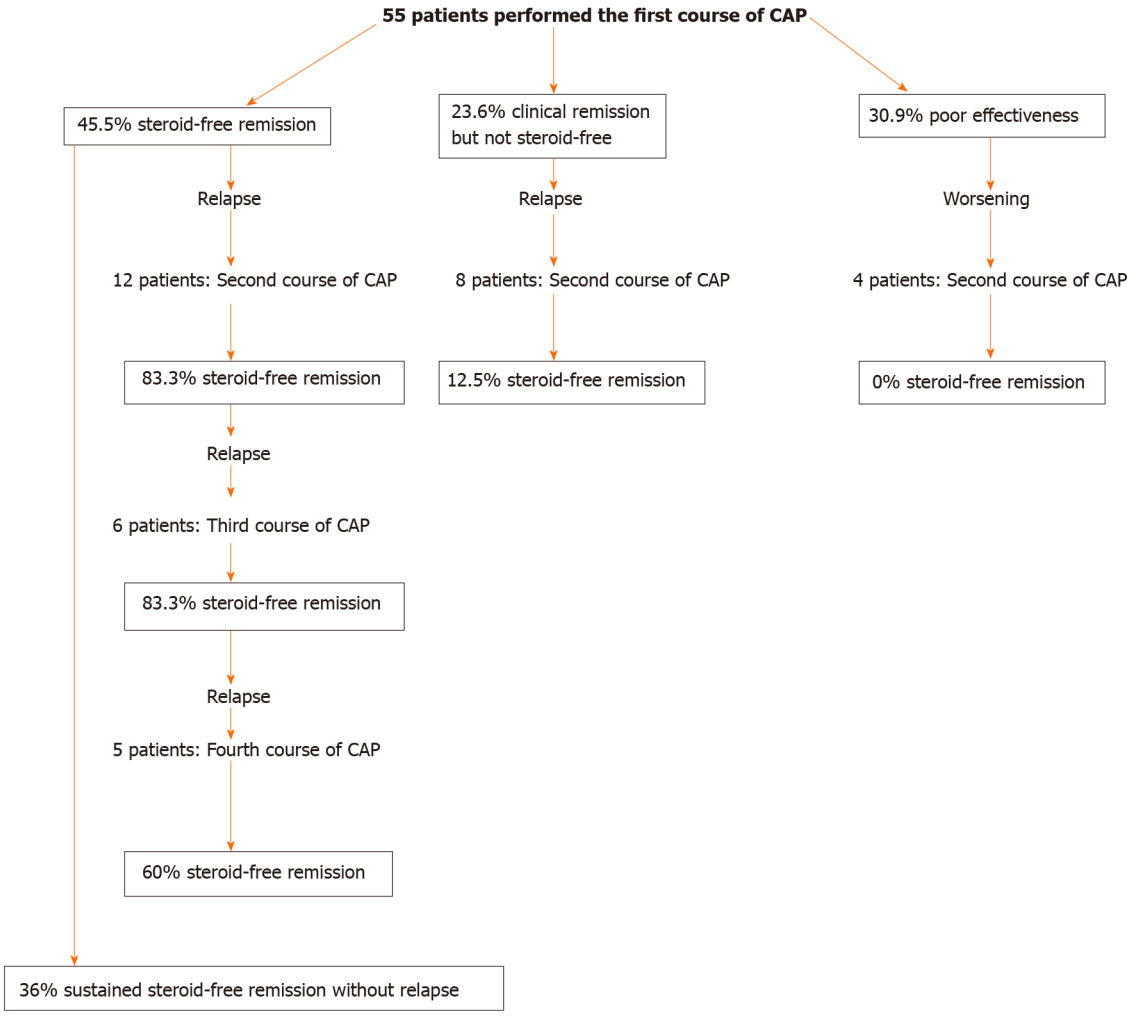

Figure 11 Summary of the results of the study. The results of this study are summarized in the figure. CAP: Cytapheresis.

adverse events were observed in 7.7% of patients. Motoya *et al*[42] conducted a retrospective multicenter cohort study that evaluated the safety and effectiveness of GMA in 437 IBD patients under special situations. They showed that the incidence of adverse events among elderly patients was similar in all patients.

There have been several studies comparing the impact of CAP in the clinical practice with the conventional pharmacotherapy for UC[53,62-64]. A meta-analysis showed that GMA is effective for inducing clinical remission in patients with UC compared with CS [odds ratio (OR), 2.23; 95% confidence interval (CI): 1.38-3.60] and that the rate of adverse events by apheresis was significantly lower than that by CS (OR, 0.24; 95%CI: 0.15-0.37)[62]. Another meta-analysis showed that comparing with conventional pharmacotherapy including CS, LCAP supplementation presented a significant benefit in promoting a response rate (OR, 2.88, 95%CI: 1.60-5.18) and remission rate (OR, 2.04, 95%CI: 1.36-3.07) together with significant higher steroid-sparing effects (OR, 10.49, 95%CI: 3.44-31.93) in patients with active moderate-to-severe UC[63]. In this regard, Domènech *et al*[53] showed that the addition of 7 weekly sessions of GMA to a conventional course of oral prednisolone did not increase the proportion of steroid-free remissions in patients with active steroid-dependent UC. On the other hand, Tominaga *et al*[64] showed that GMA produced efficacy equivalent to prednisolone and was without safety concern. Although they also showed that the average medical cost was 12739.4€/patient in the GMA group and 8751.3€ in the prednisolone group ($P < 0.05$), they concluded that the higher cost of GMA *vs* prednisolone should be compromised by good safety profile of GMA.

In summary, our study showed that CAP was effective in inducing steroid-free remission and maintained sustained steroid-free remission in both steroid-dependent and steroid-refractory UC patients. Additionally, our study also showed that CAP re-induced high-rate steroid-free remission repeatedly in patients who achieved steroid-free remission in the first course of CAP, namely, patients potentially having a high

sensitivity to CAP. Therefore, considering the high level of safety of CAP, we suggest that CAP should be one of the first-line therapies for steroid-dependent and steroid-refractory UC patients. We also suggest that CAP should be chosen as a first-line therapy for patients who achieve steroid-free remission in the first course of CAP and thereafter experience relapses during the disease course.

However, this study has some limitations; that is, this study is a retrospective study with small sample size that was conducted only in two medical institutions. Thus, a multicenter prospective study with large sample sizes is required to warrant our results.

CONCLUSION

In conclusion, our results suggest that CAP effectively induces and maintains steroid-free remission in refractory UC and re-induces high-rate steroid-free remission repeatedly in patients achieving steroid-free remission after the first course of CAP.

ARTICLE HIGHLIGHTS

Research background

Management of refractory ulcerative colitis (UC) patients is a crucial issue, and the goal of the treatment for such patients should be steroid-free remission. Although several breakthrough treatments, including biologics, have been developed for refractory UC, clinical studies focused on the achievement of steroid-free remission in refractory UC patients are insufficient.

Research motivation

Cytapheresis (CAP) is an effective therapeutic strategy for patients with active UC with fewer adverse effects. However, to date, the number of studies focused on the efficacy of CAP in both steroid-dependent and steroid-refractory UC has been limited. It is also important to assess the re-efficacy of CAP in patients who experience relapse after the first course of CAP.

Research objectives

The main objective of the study was to clarify the efficacy and re-efficacy of CAP in achieving steroid-free remission in refractory UC patients.

Research methods

We retrospectively reviewed the collected data from 55 patients with refractory UC treated with CAP. We analyzed the efficacy of the first course of CAP, efficacy of the second, third, and fourth courses of CAP, and long-term efficacy of CAP.

Research results

The rates of clinical remission, steroid-free remission after CAP were 69.1%, 45.5%, respectively, and the rates of steroid-free remission after the second, third, and fourth courses of CAP in patients who achieved steroid-free remission after the first course of CAP were 83.3%, 83.3%, and 60%, respectively. The rates of sustained steroid-free remission were 68.0%, 60.0%, and 56.0% at 12, 24, and 36 mo after the CAP. These results showed that CAP effectively induced steroid-free remission in refractory UC patients and that patients achieving steroid-free remission after the first course of CAP responded to CAP repeatedly after that and had good long-term efficacy.

Research conclusions

Our results suggest that CAP effectively induces and maintains steroid-free remission in both steroid-dependent and steroid-refractory UC patients and re-induces high-rate steroid-free remission repeatedly in patients achieving steroid-free remission after the first course of CAP. Considering the high level of safety of CAP, we suggest that CAP should be one of the first-line therapies for refractory UC patients and should be chosen as a first-line therapy for patients achieving steroid-free remission in the first course of CAP and thereafter experience relapses.

Research perspectives

A multicenter prospective study with large sample sizes is required to warrant our results.

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ORIGINAL ARTICLE

Observational Study

Risk perception and knowledge of COVID-19 in patients with celiac disease

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Abstract

BACKGROUND

We recently demonstrated that the odds of contracting coronavirus disease 2019 (COVID-19) in patients with celiac disease (CeD) is similar to that of the general population. However, how patients with CeD perceive their COVID-19 risk may differ from their actual risk.

AIM

To investigate risk perceptions of contracting COVID-19 in patients with CeD and determine the factors that may influence their perception.

METHODS

We distributed a survey throughout 10 countries between March and June 2020 and collected data on demographics, diet, COVID-19 testing, and risk perceptions of COVID-19 in patients with CeD. Participants were recruited through various celiac associations, clinic visits, and social media. Risk perception was assessed by asking individuals whether they believe patients with CeD are at an increased risk of contracting COVID-19 when compared to the general population. Logistic regression was used to determine the influencing factors associated with COVID-19 risk perception, such as age, sex, adherence to a gluten-free diet (GFD), and comorbidities such as cardiac conditions, respiratory conditions, and diabetes. Data was presented as adjusted odds ratios (aORs)

RESULTS

A total of 10737 participants with CeD completed the survey. From them, 6019 (56.1%) patients with CeD perceived they were at a higher risk or were unsure if they were at a higher risk of contracting COVID-19 compared to the non-CeD population. A greater proportion of patients with CeD perceived an increased risk of contracting COVID-19 when compared to infections in general due to their CeD (56.1% *vs* 26.7%, $P < 0.0001$). Consequently, 34.8% reported taking extra COVID-19 precautions as a result of their CeD. Members of celiac associations were less likely to perceive an increased risk of COVID-19 when compared to non-members (49.5% *vs* 57.4%, $P < 0.0001$). Older age (aOR: 0.99; 95%CI: 0.99 to 0.99, $P < 0.001$), male sex (aOR: 0.84; 95%CI: 0.76 to 0.93, $P = 0.001$), and strict adherence to a GFD (aOR: 0.89; 95%CI: 0.82 to 0.96, $P = 0.007$) were associated with a lower perception of COVID-19 risk and the presence of comorbidities was associated with a higher perception of COVID-19 risk (aOR: 1.38; 95%CI: 1.22 to 1.54, $P < 0.001$).

CONCLUSION

Overall, high levels of risk perceptions, such as those found in patients with CeD,

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may increase an individual's pandemic-related stress and contribute to negative mental health consequences. Therefore, it is encouraged that public health officials maintain consistent communication with the public and healthcare providers with the celiac community. Future studies specifically evaluating mental health in CeD could help determine the consequences of increased risk perceptions in this population.

Key Words: Celiac disease; Gluten; Risk; Infection; Knowledge; Perception; Coronavirus; COVID-19

Core Tip: Risk perceptions describe an individual's perceived susceptibility to a threat and directly influence their behavior. We conducted an international cross-sectional study to evaluate risk of contracting contracting coronavirus disease 2019 (COVID-19) in celiac disease and evaluated risk perception. Patients with celiac disease perceive they are at an increased risk of contracting COVID-19 due to their condition, which is opposite to current scientific evidence. A higher risk perception may have a negative impact in mental health, and therefore, we encourage healthcare providers, patient care groups, and public health officials to discuss the implications that COVID-19 may have on patients in relation to their specific conditions.

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INTRODUCTION

Coronaviruses, such as severe acute respiratory syndrome coronavirus and middle east respiratory syndrome coronavirus which arose in 2003 and 2012, respectively, represent a family of positive-stranded RNA viruses that infect the respiratory system[1]. Later, the Chinese city of Wuhan reported an outbreak of a novel infectious agent causing severe cases of pneumonia and alerted the World Health Organization (WHO) of its presence on December 31, 2019[2]. The disease caused by this infectious agent was later named coronavirus disease 2019 (COVID-19). Since the WHO declared COVID-19 a global pandemic in March 2020, there has been over 105 million confirmed cases of COVID-19 across 216 countries and territories and the disease has killed over 2300000 people worldwide[3].

Due to the rapid spread and detrimental health consequences of COVID-19, there is an urgent need to determine which groups of individuals may have an increased susceptibility to infection and understand the perceptions they have regarding their susceptibility. A particular group of interest are patients with celiac disease (CeD), a chronic immune-mediated gastrointestinal disease that is triggered by dietary gluten intake in genetically predisposed individuals. Numerous studies suggest that CeD is associated with an increased risk of respiratory infections, particularly pneumonia, tuberculosis, and influenza[4-6]. However, we have shown that the odds of contracting COVID-19 in patients with CeD is similar to that of the general population[7].

As a result of the discrepancies between the risks of contracting different infections in patients with CeD, patient-specific risk perceptions of COVID-19 are of particular interest. Risk perceptions describe an individual's perceived susceptibility to a threat and directly influence their health behaviors[8-10]. Further, risk perception is a complex, psychological construct that varies markedly between individuals and is influenced by their emotional, social, cultural, geographical, and cognitive state[11].

The concept of risk perception is especially important in the context of a pandemic because a group's perception of their susceptibility to infection influences their

willingness to cooperate with and adopt preventative safety measures such as travel restrictions, hand washing, social distancing, and personal protective equipment (PPE) use[11]. Although strict implementation of infection control measures, such as social distancing, can reduce infection rate, they may also increase the risk for mental health conditions[12]. These mental health risks are even more likely to occur in individuals who are or believe they are more vulnerable to COVID-19[13]. Patients with CeD, especially those with a higher risk perception, may be more vulnerable to the negative mental health consequences of COVID-19 due to the high rates of mood disorders commonly associated with CeD[14]. However, studies of risk perception in patients with CeD have been limited to an Italian study of 276 patients which found that 26.1% of their patients either felt neutral or felt they were at an increased risk of COVID-19 because of their CeD[15].

To validate and explore this further, we conducted an international, cross-sectional survey investigating the COVID-19 risk perceptions of patients with a self-reported diagnosis of CeD and examined the factors that may influence their perceptions.

MATERIALS AND METHODS

The study was approved by the Hamilton Integrated Research Ethics Board (Hamilton, Ontario), No. HIREB# 5414. The methods of this study were previously described[8].

Study design

This observational, cross-sectional study included participants of all ages with a self-reported diagnosis of CeD and non-celiac population residing in either Argentina, Australia, Canada, Italy, Mexico, New Zealand, Spain, Uruguay, or the United States. The survey was open to participants from other countries, but extensive distribution of the survey was limited to the above-mentioned countries.

We designed a web-based survey consisting of 41 items. Participants were offered a different link to the survey depending on whether they reported a diagnosis of CeD or not. The study questionnaire was divided into specific sections to capture information on their demographics, adherence to a gluten-free diet (GFD), symptomatology, comorbidities, medications, COVID-19 testing, and patient knowledge/perception of the relationship between COVID-19 and CeD[16]. Individuals who believed they were at an increased risk or were unsure if they are at an increased risk of contracting COVID-19 due to their CeD were considered to have high COVID-19 risk perceptions. Patient knowledge and perception was only assessed in the CeD population. After piloting and testing by the authors, the English survey was placed into the secure online electronic case report platform, Research Electronic Data capture[17], and later translated into Italian and Spanish by the authors. We further collected information on country-specific COVID-19 control and safety measures implemented during the study period.

Participants were recruited from March 2020 to June 2020. Recruitment of self-reported CeD patients was performed through national celiac associations (*via* electronic newsletter and social media) and at clinic visits.

Statistical analysis

Statistical analyses were carried out using IBM-SPSS (IBM-SPSS Inc, Version 25.0, Armonk, NY, United States) and STATA (Stata version 13.0 Corp, College Station, TX, United States). Graphics were created using Microsoft Excel and GraphPad Prism (GraphPad Software, Version 8.4 San Diego, CA, United States). Categorical variables were reported as frequencies and percentages, whereas continuous variables were reported as mean (SD) or median and interquartile range when applicable. Comparisons of categorical variables between groups were performed using χ^2 test. Haldane corrections were applied to χ^2 tests when necessary. A two-sided test was used and P values of < 0.05 were considered statistically significant. Logistic regression was used to assess the predictors of high COVID-19 risk perceptions. The model included COVID-19 risk perception as a dependent variable and factors including age, sex, adherence to a GFD, comorbidities, and use of corticosteroids, as independent variables.

RESULTS

Participant characteristics

Overall, out of the 18022 participants who completed the survey, 10737 participants self-reported a diagnosis of CeD. The demographics for the included population can be found in Table 1. Missing data constituted less than 3% for each variable and thus were not replaced.

The median age of the participants was 41 years, of which 1575 (14.8%) were male. The highest proportions of respondents were from Argentina and Canada followed by Australia, New Zealand, and the United States. The detailed geographical distribution of participants by country, states, provinces and departments can be found in Supplementary Table 1.

The majority of self-reported patients with CeD had been diagnosed *via* CeD-specific serology [anti-tissue transglutaminase immunoglobulin (Ig) A and/or anti-deaminated gliadin peptide IgG] and confirmed *via* duodenal biopsy (n = 7506; 69.9%). The median time since diagnosis was 7 years. Out of all the participants with CeD, 25.8% were affiliated with a regional/national celiac association.

The majority of patients with CeD reported following a strict GFD (65.7%) with 33.4% adopting a GFD with some transgressions and 0.9% following a diet without gluten restriction. Of the patients following a GFD, the median time of gluten restriction was 7 years. Further, 24.1% of patients with CeD reported having household members who were also following a GFD. The majority of participants with CeD reported having their symptoms well-controlled (68.3%), while 31.7% had persistent symptoms.

Risk perceptions for contracting COVID-19 in patients with CeD

Patients with CeD obtained information about the relationship between COVID-19 and CeD through the internet (n = 2942; 27.4%) or through celiac association websites (n = 2465; 23%) (Table 1). Only a small proportion of patients reported learning through their physicians or other healthcare team members (n = 604; 5.6%). When asked to comment on their understanding regarding the relationship between COVID-19 and CeD, the majority of participants with CeD (n = 8815; 63.6%) reported that they did not have a very good understanding of their risk of contracting COVID-19 in relation to their condition. Consequently, 63.6% of patients requested more information on how COVID-19 may affect them. Further, while only 26.7% of participants with CeD believed they either were or were unsure whether they were more susceptible to infections because of their CeD, this proportion increased significantly when asked about their susceptibility to contracting COVID-19 in particular (26.7% *vs* 56.1%, P < 0.0001) (Figure 1). Participants who were members of their local celiac associations had lower rates of perceiving an increased risk of contracting COVID-19 compared to non-members (49.5% *vs* 57.4%, P < 0.0001) (Table 2). There was a stepwise decline in the proportion of patients with high-risk perceptions of contracting COVID-19 as the pandemic progressed (Figure 2).

Country-specific COVID-19 risk perceptions were highest in the United States (73.1%), Australia (67.3%), New Zealand (65.0%), and Argentina (62.9%) and lowest in Spain (19.1%) and Uruguay (23.3%) (Table 3).

There were 3745 participants (34.8%) who reported that they were taking extra precautions for COVID-19 as a result of being diagnosed with CeD. The most common precautions included at least one of the following: Extensive isolation/social distancing (68.6%), extended PPE use (gloves, face masks) before widespread recommendations (32.7%), and consistent hand washing/sanitization (23.4%). Further infection control measures included paying extra attention to maintaining a GFD (6.1%), getting grocery/food delivery (2.9%), strict adherence to public health recommendations (2.4%), implementing vitamins, supplements, and healthy foods into their diet (2.0%), showering/washing clothes after returning home (1.4%), getting the influenza vaccine (0.8%), and one participant noted that they stopped their immunosuppressant medication use. Information on the country-specific infection control/safety measures implemented in the general population during the study period is shown in the Supplementary Material (Supplementary Table 2).

Factors influencing the risk perception for contracting COVID-19 in patients with CeD

Older age [odds ratios (aORs): 0.99; 95%CI: 0.99 to 0.99, P < 0.001], male sex (aOR: 0.84; 95%CI: 0.76 to 0.93, P = 0.001), and adherence to a strict GFD (aOR: 0.89; 95%CI 0.82 to 0.96, P = 0.007) were associated with a lower perception of COVID-19 risk. However,

Table 1 Demographic characteristics of study population

Demographic	CeD, *n* = 10737
Age (years), median (IQR)	41 (28-57)
Gender, *n* (%)	10646 (99.2)
Male	1575 (14.8)
Female	9017 (84.7)
CeD diagnosis, *n* (%)	10570 (98.4)
Bloodwork[1]	1304 (12.1)
Biopsy	1334 (12.4)
Bloodwork + biopsy	7506 (69.9)
Unsure	426 (4.0)
Years since diagnosis, median (IQR)	7 (3-13)
Member of celiac association, *n* (%)	2766 (25.8)
Diet, *n* (%)	
Unrestricted	61 (0.6)
Other restrictions-non gluten	29 (0.3)
GFD-sometimes	118 (1.1)
GFD-most of the time	283 (2.6)
GFD-rare intentional gluten	418 (3.9)
GFD-rare accidental gluten	1566 (14.6)
GFD-possible cross-contamination	1136 (10.6)
Strict GFD	7052 (65.7)
Years diet restriction, Median (IQR)	7 (3-12)
Any household member following GFD, *n* (%)	2688 (25.0)
Some members	1949 (18.2)
All members	409 (3.8)
Other	221 (2.1)
Management of CeD symptoms, *n* (%)	
Well controlled	7336 (68.3)
Symptoms < 2 mo	2362 (22.0)
Symptoms > 2 mo	938 (8.7)
Travel outside of the country[2], *n* (%)	202 (1.9)
Contact with COVID-19 positive, *n* (%)	175 (1.6)
Tested for COVID-19, *n* (%)	478 (4.5)
Fever[2], *n* (%)	252 (2.3)
Respiratory symptoms[2], *n* (%)	1124 (10.5)
Hospitalizations for respiratory infection[2], *n* (%)	21 (0.2)
Comorbidities, *n* (%)	
Chronic lung condition	708 (6.6)
Chronic heart condition	376 (3.5)
Diabetes	413 (3.8)
Receiving steroids	322 (3.0)
Receiving immune suppressive medications	393 (3.7)

Pregnancy, *n* (%)	96 (0.9)
Information about COVID-19 in celiac disease	
Internet	2942 (27.4)
My doctor	291 (2.7)
Other healthcare providers	313 (2.9)
Celiac association website	2465 (23.0)
Other	731 (6.8)

[1]Bloodwork includes celiac disease-specific serology anti-tissue transglutaminase immunoglobulin (Ig) A, and/or anti-deaminated gliadin IgA/IgG, and/or anti-endomysial antibodies IgA.
[2]In the last month. CeD: Celiac disease; IQR: Interquartile range; GFD: Gluten-free diet; COVID-19: Coronavirus disease 2019.

Table 2 Relationship between celiac association membership and coronavirus disease 2019 risk perceptions in patients with celiac disease

Member of a celiac association	CeD respondents, *n* = 7296	Respondents with high COVID-19 risk perceptions	*P* value
Yes	2766	1368 (49.5)	< 0.0001
No	4530	2600 (57.4)	

CeD: Celiac disease; COVID-19: Coronavirus disease 2019.

Table 3 Country-specific risk perceptions of contracting coronavirus disease 2019 in patients with celiac disease

Country	Infection rate[1]	ORs for contracting COVID-19 (CeD *vs* controls)	95%CI	CeD patients believing they are more susceptible to COVID-19, *n* (%)
Argentina	0.14	1.41	0.48-4.12	2637 (62.9)
Canada	0.04	0.80	0.31-2.01	1962 (52.1)
Australia	0	1.92	0.03-99.21	449 (67.3)
New Zealand	0	0.88	0.01-43.32	295 (65.0)
Spain	0.21	0.73	0.21-2.57	85 (19.1)
United States	0.16	3.28	0.61-17.44	304 (73.1)
Uruguay	0	0.24	0.01-6.68	78 (23.3)
Italy	0	0.27	0.01-6.37	85 (41.5)
Mexico	0.6	1.50	0.15-14.42	62 (42.8)
Other[2]	0.17	0.70	0.08-6.22	62 (68.1)

[1]Infection rate: Positive coronavirus disease 2019 test over total tested.
[2]Full list of other countries can be found in Supplementary Table 1 in the Supplementary material. ORs: Odds ratios; CeD: Celiac disease; COVID-19: Coronavirus disease 2019.

the presence of comorbidities such as chronic lung conditions, chronic heart conditions (including hypertension), and diabetes, was associated with an increased perception of COVID-19 risk (aOR: 1.38; 95%CI: 1.22 to 1.54, $P < 0.001$). The use of corticosteroids or immunosuppressants did not change risk perception levels for contracting COVID-19 (aOR: 0.86; 95%CI: 0.68 to 1.08, $P = 0.19$) (Table 4).

Table 4 Logistic regression analysis of coronavirus disease 2019 risk perception in patients with celiac disease

	Risk perception			
	Crude [OR (95%CI)]	*P* value	Adjusted[1] [OR (95%CI)]	*P* value
Older age	0.99 (0.99-0.99)	0.012	0.99 (0.99-0.99)	< 0.001
Sex (M)	0.84 (0.76-0.93)	0.001	0.84 (0.75-0.93)	0.001
Strict GFD	0.88 (0.81-0.95)	0.002	0.89 (0.82-0.96)	0.007
Comorbidities[2]	1.29 (1.17-1.43)	< 0.001	1.37 (1.22-1.54)	< 0.001
Use of corticosteroids or immunosuppressants	1.11 (0.91-1.37)	0.28	0.86 (0.68-1.08)	0.19

[1]By all variables listed.
[2]Comorbidities includes chronic lung conditions, chronic heart conditions, and diabetes. GFD: Gluten-free diet; OR: Odds ratio.

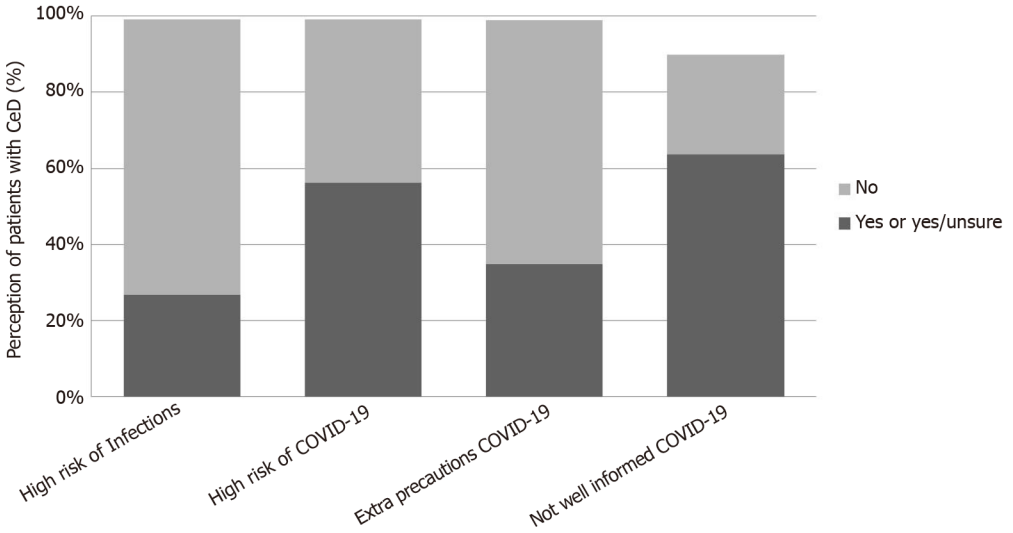

Figure 1 The risk perception of infections, contracting coronavirus disease 2019, need of taking extra precautions and obtaining information related to coronavirus disease 2019 in patients with celiac disease during the coronavirus disease 2019 pandemic. CeD: Celiac disease; COVID-19: Coronavirus disease 2019.

DISCUSSION

This study included over 10500 CeD patients and to our knowledge, is the first large-scale, international study to examine COVID-19 risk perceptions in patients with CeD. Despite demonstrating that the odds of contracting COVID-19 in patients with CeD is similar to that of the non-CeD population in our previous study[7], the majority of patients with CeD either believed they were at an increased risk or were uncertain of whether they were at an increased risk of contracting COVID-19 when compared to the general population.

A significant number of patients (44.0%) reported that their knowledge of the relationship between COVID-19 and CeD is poor or very poor with the majority of patients requesting more information. Further, while many patients learn about COVID-19 and CeD through the internet, very few learn about the relationship from their healthcare team. This is consistent with previous studies on CeD suggesting that patients are dissatisfied with the information offered by their physicians and feel like their general knowledge about CeD is inadequate[18]. Accordingly, previous reports suggest that many physicians have inadequate knowledge/awareness of the features associated with CeD which consequently has a direct impact on their patients' education regarding their condition[19,20]. As a result, while studies have demonstrated that CeD is associated with an increased risk of general infections, we found that only 26.7% of patients with CeD in our study believed they were at an increased risk. This supports the view that patients are generally uninformed about the potential consequences of CeD. This also represents a potential area for improvement as physicians and healthcare providers should be encouraged to thoroughly discuss the

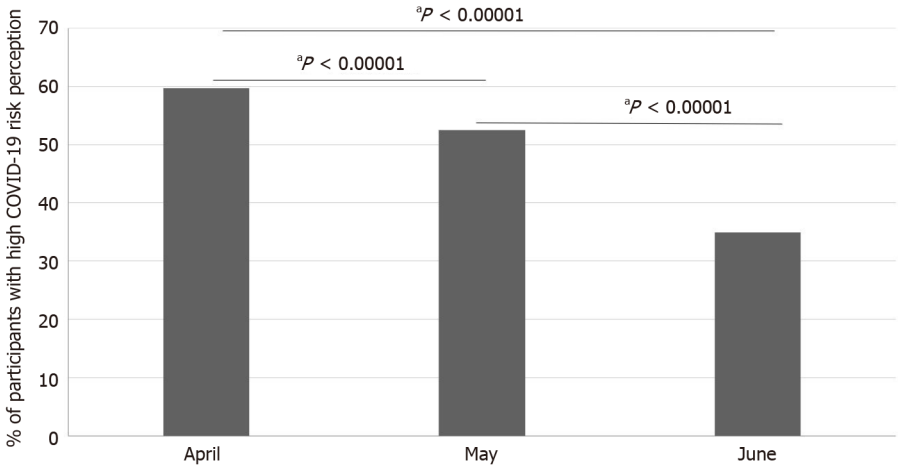

Figure 2 The risk perception of contracting coronavirus disease 2019 in patients with celiac disease decreased as the pandemic progressed. COVID-19: Coronavirus disease 2019.

implications of COVID-19 in relation to their patients' conditions based on the emerging evidence.

Patient perceptions are particularly relevant during the COVID-19 pandemic as high rates of depression and mood disorders have been associated with patients with higher risk perceptions of COVID-19[21]. Notably, in contrast to the generally low risk-perception that patients with CeD had regarding infections overall, more than half of our participants with CeD perceived they were at an increased risk or were unsure whether they were at a higher risk of contracting COVID-19 compared to the general population. As there are major uncertainties related to the novel coronavirus, this drastically affects the ability to properly and accurately inform patients of its potential implications. A recent study identified the lack of information regarding the virus as one of the major elements that contribute to fear and its associated high risk perception related to COVID-19[22]. Conversely, the study conducted by Siniscalchi *et al*[15] in March 2020, found that the majority of their patients with CeD (56.6%) did not feel more vulnerable to COVID-19 due to their condition[15]. One potential consideration contributing to the discrepancy between our results could be related to differences in study populations, as their study was limited to an Italian population. However, our results show the same trend when the analysis is sub-grouped by country and demonstrated similar results within our Italian participants (Table 3). These results suggest that risk perceptions vary markedly depending on the geographical area of the participants, as suggested by others[11]. In particular, country-specific differences in risk perception may be attributed to a variety of factors such as differences in culture, political climate, government communication, phase/timing of the pandemic, country-specific impacts of COVID-19, rates of infection, testing amounts and indications, and infection control measures (Supplementary Table 2). Notably, patients with CeD from Spain and Uruguay were found to have generally low risk perceptions for COVID-19. This aligns with previous studies that have found that the impact of COVID-19 has been relatively small in Uruguay as a result of swift lockdowns[23] and that individuals from Spain have been noted to have low personal concern about COVID-19[24]. Additionally, as the time of data collection was early in the pandemic for the study conducted by Siniscalchi *et al*[15], it is possible that patient perceptions may have changed as the pandemic progressed. Our analysis of risk perceptions by month found a stepwise decline in the proportion of participants with high COVID-19 risk perceptions as the pandemic progressed. This may be a result of timing because later in the study period, many countries have already passed the first wave of the pandemic and it may be due to the release of information regarding the link between COVID-19 and CeD from national celiac associations later on in the pandemic. It is also possible that this could be a result from "COVID-19 fatigue" as people become less concerned and more inclined to return to normal life as they recognize that the pandemic will persist for long periods of time.

We further investigated the different factors that we anticipated may modify the odds of having high risk perceptions for contracting COVID-19 in patients with CeD. Our results demonstrate a small, although significant, association between both younger age and female sex with higher risk perceptions of contracting COVID-19.

These results align with a study conducted by Rimal and Juon[25] which noted that younger, more educated individuals, have a higher risk perception of breast cancer. Further, our sex-specific findings are consistent with studies investigating both COVID-19 risk perceptions in the general population[11,26] and in patients with CeD[15].

Importantly, CeD has been associated with a large number of concomitant conditions such as cardiovascular conditions including hypertension, coronary artery disease, and arrhythmias, respiratory conditions such as asthma[27], and type 1 diabetes mellitus[28]. Further, it has been noted that the above-mentioned comorbidities may also predispose individuals to contracting COVID-19 and may contribute to a more severe disease course and mortality[29]. Accordingly, in our regression analysis, we noted that the presence of comorbidities such as chronic lung conditions, chronic heart conditions, and diabetes, increased the odds of patients believing they are at a higher risk of contracting COVID-19.

Notably, we found the use of corticosteroids or immunosuppressive therapies did not influence the odds of having high risk perceptions of COVID-19. This may potentially be attributed to nearly universal guidelines suggesting the continuation of immunosuppressive treatment during the pandemic and studies suggesting that the morbidity and mortality rates of patients who are immunosuppressed or have an autoimmune condition may be similar to that of the general population[30]. However, it is also possible that there was a selection bias if patients who perceived themselves to be at higher risk of contracting COVID-19 decided to stop the use of immunosuppressive therapies; as expressed in a comment by one participant. It also is possible that this action was taken by other participants; however, this was not systematically investigated in our study. Further, while it has been hypothesized that patients with active CeD (unmanaged or incompletely managed) may be at an increased risk of infection, our results demonstrate that individuals who follow a strict GFD have lower odds of perceiving themselves to be at an increased risk of contracting COVID-19.

Overestimation of risk can lead to being overly anxious, overly cautious, negative mental and physical health consequences[8], and not visiting a healthcare provider even when they believe they should[15,31]. Studies investigating healthcare use during the COVID-19 pandemic found significant reductions in emergency department visits, hospital admissions, and non-urgent healthcare visits[32,33]. Researchers have suggested that this decrease may be due to a perceived fear of contracting COVID-19 in high-risk areas such as hospitals. As a result, virtual patient care has been rapidly adopted to minimize this risk. However, patients in low-resource areas, such as those without technology or internet, are unable to access these alternate forms of healthcare and may be unequally affected by the pandemic[34]. Therefore, the role of governmental and non-governmental organizations in promoting awareness and knowledge in underserved/underdeveloped communities is especially important during the current health crisis.

In our participants with CeD, we found that 34.8% were taking extra COVID-19 precautions as a result of their condition. Importantly, we found that patients who were members of national celiac associations had overall lower risk perceptions for contracting COVID-19. Nearly a quarter of our patients reported learning about the relationship between COVID-19 and CeD through celiac association websites, which are often responsible for distributing patient-centred educational resources[35]. Previous studies have suggested that membership in a patient association has been correlated to increased physical/psychological well-being and social adjustment[36]. As over 60% of our participants reported that they would like more information on how COVID-19 may affect patients with CeD patient associations, such as national celiac associations, represent a promising avenue to help effectively disseminate health information, educate patients, and encourage healthy social relationships.

We acknowledge the presence of limitations associated with our study. First, this study did not assess risk perceptions in the general population and in the CeD group, there may have been potential selection/referral bias towards patients belonging to celiac associations as these associations acted as our primary mode of recruitment. Further, the cross-sectional nature of this study design only allowed us to evaluate COVID-19 risk perceptions during our study period which may change over time. Therefore, future prospective longitudinal studies may help assess changes over different time periods of the pandemic. In addition, although we investigated several dimensions of risk perception, some potential factors were not assessed. For example, we did not assess risk perceptions related to mortality or concerns of infecting family and friends. We also did not assess the mental health outcomes related to high levels of risk perception. As a result, future studies investigating additional factors and potential mediators of COVID-19 risk perception in patients with CeD will inform physicians and celiac associations on how to best design and communicate risk

mitigation strategies to support a potentially vulnerable patient population.

CONCLUSION

In conclusion, this international survey of patients with self-reported CeD demonstrates that a large proportion of patients with CeD perceive themselves to be at a high or unknown risk of contracting COVID-19. This association is more evident in females, those with comorbidities, and those who are not following a strict GFD. As a result of the uncertainty surrounding COVID-19, particularly at the start of the pandemic, and lack of information regarding the link between COVID-19 and CeD, patients typically have high levels of COVID-19 risk perceptions. Therefore, efforts should be made towards improving communication with patients with CeD and educating them based on emerging scientific evidence.

ARTICLE HIGHLIGHTS

Research background

We recently demonstrated that the odds of contracting coronavirus disease 2019 (COVID-19) in patients with celiac disease (CeD) is similar to that of the general population. However, how patients with CeD perceive their COVID-19 risk may differ from their actual risk.

Research motivation

Risk perceptions are important in the context of a pandemic because a group's perception of their susceptibility to infection influences their willingness to cooperate with preventative safety measures such as travel restrictions, hand washing, social distancing, and personal protective equipment use. However, overestimation of risk can contribute to negative mental and physical health consequences

Research objectives

The aim of this study was to investigate risk perceptions of contracting COVID-19 in patients with CeD and determine the factors that may influence their perception.

Research methods

We distributed an international survey throughout 10 countries and collected data on demographics, diet, COVID-19 testing, and risk perceptions of COVID-19 in patients with CeD. Risk perception was assessed by asking individuals whether they believe patients with CeD are at an increased risk of contracting COVID-19 when compared to the general population. Logistic regression was used to determine the influencing factors associated with COVID-19 risk perception.

Research results

A total of 10737 participants with CeD completed the survey. The majority of patients with CeD perceived they were at a higher risk or were unsure if they were at a higher risk of contracting COVID-19 compared to the non-CeD population. A greater proportion of patients with CeD perceived an increased risk of contracting COVID-19 when compared to infections in general due to their CeD. Consequently, 34.8% reported taking extra COVID-19 precautions as a result of their CeD. Members of celiac associations were less likely to perceive an increased risk of COVID-19 when compared to non-members. Older age, male sex, and strict adherence to a GFD were associated with a lower perception of COVID-19 risk and the presence of comorbidities was associated with a higher perception of COVID-19 risk.

Research conclusions

Overall, high levels of risk perceptions, such as those found in patients with CeD, may increase an individual's pandemic-related stress and contribute to negative mental health consequences. Therefore, it is encouraged that public health officials maintain consistent communication with the public and healthcare providers with the celiac community.

Research perspectives

Future studies specifically evaluating mental health in CeD could help determine the consequences of increased risk perceptions in this population.

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ORIGINAL ARTICLE

Observational Study

Risk stratification and geographical mapping of Brazilian inflammatory bowel disease patients during the COVID-19 outbreak: Results from a nationwide survey

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Author contributions: Kotze PG, Hino AAF and Queiroz NSF designed the study; Motta MP, Martins CA, Kotze PG, Queiroz NSF and Hino AAF participated in the acquisition, analysis and interpretation of the data and wrote the manuscript; Teixeira FV, Motta MP, Chebli LA, Martins CA, Quaresma AB, Silva AAP, Damião AOMC were involved in Grupo de Estudos da Doença Inflamatória Intestinal do Brasil COVID task force and critically reviewed the article for important intellectual content.

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Abstract

BACKGROUND

Coronavirus disease 2019 (COVID-19) pandemic is still evolving globally, and Brazil is currently one of the most affected countries. It is still debated whether

patients with inflammatory bowel disease (IBD) are at a higher risk for developing COVID-19 or its complications.

AIM

To assess geographical distribution of IBD patients at the highest risk and correlate these data with COVID-19 mortality rates in Brazil.

METHODS

The Brazilian IBD Study Group (Grupo de Estudos da Doença Inflamatória Intestinal do Brasil) developed a web-based survey adapted from the British Society of Gastroenterology guidelines. The included categories were demographic data and inquiries related to risk factors for complications from COVID-19. Patients were categorized as highest, moderate or lowest individual risk. The Spearman correlation test was used to identify any association between highest risk and mortality rates for each state of the country.

RESULTS

A total of 3568 patients (65.3% females) were included. Most participants were from the southeastern and southern regions of Brazil, and 84.1% were using immunomodulators and/or biologics. Most patients (55.1%) were at moderate risk, 23.4% were at highest risk and 21.5% were at lowest risk of COVID-19 complications. No association between the proportion of IBD patients at highest risk for COVID-19 complications and higher mortality rates was identified in different Brazilian states (r = 0.146, P = 0.467).

CONCLUSION

This study indicates a distinct geographical distribution of IBD patients at highest risk for COVID-19 complications in different states of the country, which may reflect contrasting socioeconomic, educational and healthcare aspects. No association between high risk of IBD and COVID-related mortality rates was identified.

Key Words: Crohn's disease; Colitis, Ulcerative; COVID-19; Inflammatory bowel disease; Brazil

Core Tip: The coronavirus disease 2019 pandemic is still evolving globally, and Brazil is currently one of the most affected countries. The Brazilian Inflammatory Bowel Diseases Study Group developed a web-based survey of 3568 patients that was adapted from the guidelines of the British Society of Gastroenterology. This study indicates a distinct geographical distribution of patients with inflammatory bowel disease at higher risk for coronavirus disease 2019 complications in different states of the country, which may reflect contrasting socioeconomic, educational and health aspects. No association between high risk and coronavirus disease 2019-related mortality rates has been identified.

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statement: The study was approved by the GEDIIB ethical review board under the protocol No. 002/2020 on October 28th, 2020. Informed consent was waived because the survey recruitment was self-selective. In addition, data were de-identified. Individual participant data were not published, which maintained confidentiality in all steps of study analysis. This study was conducted in compliance with regulations stated in the 1975 Declaration of Helsinki.

Informed consent statement: As this is a cross-sectional survey analyzing anonymous data, and information used derived from an unidentified database, informed consent from each individual was waived.

Conflict-of-interest statement: Kotze PG reports grants and personal fees from Takeda, grants and personal fees from Pfizer, personal fees from Janssen, personal fees from Abbvie, personal fees from Novartis, personal fees from Ferring, outside the submitted work.

Data sharing statement: Study data are de-identified. Data are provided only for the approved study. Data will not be shared with anyone outside of the named members of the investigator team.

STROBE statement: The authors have read the STROBE Statement-checklist of items, and the manuscript was prepared and revised according to the STROBE Statement-checklist of items.

INTRODUCTION

The World Health Organization declared coronavirus disease 2019 (COVID-19) a pandemic on March 11, 2020. Although many countries are already registering a reduction in the incidence of infections and starting vaccination programs, Brazil is

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currently one of the leaders in the world on a daily basis for both numbers of new cases and deaths. As of November 28, 2020, we have confirmed 6238250 infected patients and 171998 COVID-related deaths[1].

Available data suggests that patients with inflammatory bowel disease (IBD) are not at a higher risk for severe acute respiratory syndrome coronavirus-2 infection or the development of COVID-19 complications[2]. Moreover, the evolution of COVID-19 does not seem to be worse in patients with IBD, irrespective of their treatment. A recent analysis of 525 IBD patients from the Surveillance Epidemiology of Coronavirus Under Research Exclusion for Inflammatory Bowel Disease revealed that increasing age, comorbidities and corticosteroids are associated with worse outcomes of COVID-19 and that treatment with tumor necrosis factor inhibitors (TNFi) was not associated with severe COVID-19. The number of reported patients exposed to other medical options was insufficient to drive conclusions regarding risk for severe outcomes in this population[3]. Recently, another report from the Surveillance Epidemiology of Coronavirus Under Research Exclusion for Inflammatory Bowel Disease registry aimed to evaluate the association of IBD medications and their combinations on the risk of adverse COVID-19 outcomes. Based on data from over 1400 IBD patients, it was demonstrated that the combination of thiopurines with TNFi and thiopurine monotherapy are associated with a significantly increased risk of severe COVID-19 as compared with TNFi monotherapy. Anti-interleukins and anti-integrins were not associated with a significantly different risk than TNFi monotherapy[4]. Regardless of the risk of IBD medications, it is noteworthy that maintaining patients in remission with steroid-sparing treatments may be crucial during the pandemic period.

As the effect of immunosuppressive agents in IBD patients remains unclear during the pandemic, the British Society of Gastroenterology has issued guidance on risk stratification for IBD patients depending on medications in use, age, comorbidities and other risk factors[5]. Patients at highest risk for COVID-related complications are those who have a comorbidity and/or are over 70-years-old and are on any immunosuppressant therapy for IBD. Those of any age that are receiving ≥ 20 mg of prednisolone, undergoing new induction therapy with biologics and immunomodulators (combination therapy) within 6 wk with moderate to severe active disease with short gut syndrome or patients requiring parenteral nutrition are also considered at highest risk for severe COVID-19 outcomes. Patients receiving biological therapy, thiopurines, calcineurin inhibitors, Janus kinase inhibitors or combination therapy are stratified as moderate risk and the remaining IBD patients as lowest risk. Depending on the risk stratification, recommendations can be suggested. Patients at highest risk are advised to self-isolate, while patients at moderate risk or lowest risk should follow strict social distancing or social distancing as for the general population, respectively. Specific guidance on instructions for self-isolation (shielding education) and social distancing measures to reduce spread of the disease within the population and to protect vulnerable groups has been recently issued[6].

In order to identify Brazilian IBD patients who could be at high risk for COVID-19 complications, a taskforce group from the Brazilian IBD Study Group (Grupo de Estudos da Doença Inflamatória Intestinal do Brasil, GEDIIB) developed an anonymous web-based survey that allows self-identification risk assessment adapted from the British Society of Gastroenterology guidelines. Through a decision tree, patients were self-identified into different groups according to their risk of developing COVID-19 complications and received updated information according to their respective group (Table 1).

Currently, Brazil still has one of the fastest growing severe acute respiratory syndrome coronavirus-2 outbreaks in the world with one of the highest mortality rates, just behind the United States. Given that a higher absolute number of deaths in the context of an epidemic may reflect a strained healthcare system and economy, mapping IBD patients at highest risk for COVID-19 complications could help public authorities to delineate protection strategies to this possibly vulnerable population. Our study aimed to assess geographical distribution of IBD patients at highest risk and correlate these data with COVID-19 mortality rates in different states of Brazil.

MATERIALS AND METHODS

Data collection and participants

The GEDIIB COVID taskforce members in collaboration with experts in the field developed a questionnaire and established a decision tree to evaluate IBD patients pertaining to their risk of serious complications from COVID-19. The national survey

Table 1 Recommendations for inflammatory bowel disease patients according to their risk level

Highest risk
Stay home at all times and do not leave, even to buy food, medicine or to do exercises
Maintain attendance to infusions (only time to leave home) and the use of prescribed IBD medications
Stay at least 2 m (3 steps) from other people, including family members at home, whenever possible
Delivered products must be left outside the house by the courier
Anyone entering home must wash their hands thoroughly with soap for at least 20 s
Do not receive any visitors, unless help is needed
Moderate risk
Avoid contact with people with symptoms of COVID-19
Avoid using public transportation, crowds, public spaces and meetings with friends or family
Home office whenever possible
Use smartphones or virtual technology to contact physicians or other essential services
Lowest risk
All risk groups must follow the recommendations of the World Health Organization
Wash hands thoroughly with soap for at least 20 s, frequently
Use 70% alcohol gel if soap or water is not available
Avoid touching the face
Clean objects and surfaces that are frequently touched (such as door handles and phones)
Stay home to help prevent the spread of the virus
Leave home for very limited purposes: buying food and medication, exercise once a day (running, walking or cycling) alone or with a family member, help a vulnerable person or donate blood
Travel for professional purposes only if strictly necessary
When leaving home, minimize the time spent away and keep 2 m away from others

Adapted with permission from Queiroz *et al*[6]. COVID-19: Coronavirus disease 2019; IBD: Inflammatory bowel disease.

was available on the website (diicovid.com.br). Data was collected from April 14, 2020 to June 2, 2020. An informative article concerning this survey was posted at the official GEDIIB website, official mailing lists for patients and in national IBD patient association communication medias. All identified erroneous reports were removed for higher accuracy of the data.

The questionnaire consisted of fourteen questions (Supplementary Table 1). The included categories were demographic data (state and city of residence in Brazil, age, sex, smoking status) and questions related to the risk factors for complications from COVID-19 in IBD populations according to current previously published guidance[5,6]. The questions included IBD medications in use, self-reported comorbidities (hypertension, diabetes, cardiovascular disease and chronic pulmonary disease) and abdominal surgery for IBD performed in the past 30 d. Through a decision tree, patients were categorized as highest, moderate or lowest individual risk for the potential to develop serious complications from COVID-19 and received updated recommendations according to their risk level. Respondents were distributed according to their respective domiciliary states.

States of the national federation with a proportion of respondents at the highest risk for COVID-19 infection that were higher than the median rate of the overall country were considered for the analysis. Additionally, states with higher COVID-related cumulative mortality rates than the median of the country (as of June 2, 2020, the last available date of the survey) were also grouped. A possible correlation of these findings would suggest if there could be any coincidental relation between highest risk for COVID-19 complications and mortality in the same states.

Statistical analysis

Demographic and clinical characteristics of the study population were summarized by

descriptive statistics. Categorical variables were expressed as absolute counts and percentages and continuous variables as means and standard deviations. Data was presented initially for the total study population. Thereafter, it was organized according to the five Brazilian regions and respective states to evaluate their geographical distribution. The frequencies of patients using each therapeutic IBD regimen in combination with oral steroids were analyzed as subcategories.

Categorical variables were compared using a Chi-squared test, and continuous data was analyzed using Student's *t*-test. A two-tailed *P* value of 0.05 was used for statistical significance. The Spearman correlation test was performed to study a possible correlation between the proportion of highest risk patients and COVID-19 cumulative mortality in states with higher rates compared with the median national cutoffs for each variable. Data was exported and analyzed in SPSS Statistics 23 (IBM Corporation, Armonk, NY, United States).

Data regarding COVID-19 cumulative death rates from March 3 (first death registered in Brazil) to June 2 were obtained from the Brazilian Ministry of Health COVID-19 website, (https://covid.saude.gov.br/). We computed the COVID-19 mortality per 100000 people using the estimated populational data of 2019 available at the Statistical and Geographical Brazilian Institute for each of the Brazilian states and the federal district (https://datasus.saude.gov.br/populacao-residente/). In order to represent the mortality of COVID-19, we used classification into deciles. ArcMap 10.3® was used to generate the map representation.

Ethical considerations

The study was approved by the GEDIIB ethical review board under the protocol No. 002/2020 on October 28, 2020. Informed consent was waived because the survey recruitment was self-selective. In addition, data were de-identified. Individual participant data were not published, which maintained confidentiality in all steps of study analysis. This study was conducted in compliance with regulations stated in the 1975 Declaration of Helsinki.

RESULTS

A total of 3568 IBD patients participated in the national web-based survey and had data included. Six patients were excluded from the analysis due to inconsistent reported data. Overall demographic and baseline characteristics of respondents are illustrated in Table 2. Most respondents (55.6%) were 20-39-years-old, and 65.3% were females. Current smoking status was reported by 5.1% of the participants. The states with the highest response rates to the survey were São Paulo (29.6%), Rio de Janeiro (9.4%) Santa Catarina (7.7%), Paraná (7.7%), Bahia (6.0%) and the Federal District (5.3%). Details of the distribution of respondents per state are described in Supplementary Table 2.

The proportion of patients presenting with at least one self-reported comorbidity was 21.6%, and the most prevalent was hypertension (11.3%) followed by chronic pulmonary disease (4.6%) and recent (< 30 d) IBD-related abdominal surgery (3.8%). Most patients (84.1%) were on immune-mediated therapy (biologics 51.3% and/or immunomodulators 32.8%), 34.2% of respondents were using aminosalicylates and 13.3% had been recently treated with corticosteroids. Demographic, clinical and treatment characteristics by states is presented in Tables 3 and 4. The uptake of biological therapy was slightly higher among patients from the southeastern (52.9%) and southern (52.2%) regions compared with those from the northern region (33.3%). Demographic, clinical and treatment characteristics by regions are described in detail in Supplementary Table 3.

Overall, the majority (55.1%) of patients were at moderate risk, 23.4% were at highest risk and 21.5% were at lowest risk of developing COVID-19 complications. The proportion of IBD patients at highest, moderate and lowest risk for each state is represented in Figure 1A-C and for each county/city in Supplementary Figure 1. Thirteen states had higher proportional rates of patients at highest risk than the national median cutoff of 22.1% (Amapá, Rio Grande do Norte, Rio de Janeiro, São Paulo, Paraná, Amapá, Federal District, Santa Catarina, Ceará, Goiás, Espírito Santo, Acre and Paraíba). Paraíba was the state with the greatest proportion of IBD patients at highest risk (44.4%), followed by Acre (37.5%), Espírito Santo (29.9%) and Goiás (28.6%).

Geographical distribution of cumulative deaths from COVID-19 in Brazil as of June 2, 2020 by state (per 100000 people) is represented in Figure 1D. The national mortality

Table 2 Demographic, clinical and treatment characteristics from the whole sample of patients

Characteristics	*n* = 3568 (%)
Age (yr)	38.1 ± 12.3
0-9	10 (0.3)
10-19	120 (3.4)
20-29	769 (21.6)
30-39	1214 (34)
40-49	848 (23.8)
50-59	379 (10.6)
60-69	177 (5.0)
≥ 70	51 (1.4)
Sex	
Male	1238 (34.7)
Smoking	183 (5.1)
Clinical risk factors	
Hypertension	402 (11.3)
Diabetes	119 (3.3)
Cardiovascular disease	107 (3.0)
Chronic pulmonary disease	164 (4.6)
Recent abdominal surgery for IBD (< 30 d)	136 (3.8)
Overall IBD medications	
No medication	339 (9.5)
Oral steroids	473 (13.3)
5-ASA	1221 (34.2)
AZA/6-MP/MTX	1169 (32.8)
Biologics	1832 (51.3)
Therapeutic regimen	
Oral steroids monotherapy[1]	83 (2.3)
5-ASA monotherapy[1]	758 (21.2)
5-ASA + oral steroids[2]	115 (15.2)
AZA/6-MP/MTX monotherapy[1]	556 (15.6)
AZA/6-MP/MTX + oral steroids[2]	90 (16.2)
Biologic monotherapy[1]	1219 (34.2)
Biologic + oral steroids[2]	100 (8.2)
Combo therapy[3]	613 (17.2)
Combo therapy[3] + oral steroids[1]	85 (13.9)
COVID-related complications risk	
Highest	768 (23.4)
Moderate	1965 (55.1)
Lowest	836 (21.5)

[1]Monotherapy indicates no concomitant biologics or immunomodulator.
[2]These subcategories represent the frequency of patients in each monotherapy regimen requiring oral steroids.
[3]Combo therapy refers to biologics plus aminosalicylates/6-mercaptopurine/methotrexate.

5-ASA: Aminosalicylates; AZA: Azathioprine; 6-MP: 6-Mercaptopurine; MTX: Methotrexate; COVID: Coronavirus disease; IBD: Inflammatory bowel disease.

rate median cutoff in June 2 was 9.7/100000. The 13 states with mortality rates above the national median were Rio Grande do Norte, Maranhão, Alagoas, Espírito Santo, São Paulo, Acre, Roraima, Amapá, Pernambuco, Rio de Janeiro, Pará, Ceará and Amazonas. The states with higher mortality rates were Amazonas (50.7), Ceará (37.5), Pará (35.3), Rio de Janeiro (32.9) and Amapá (28).

When crossing overall proportion of highest risk patients with cumulative mortality rates, the Spearman rank correlation coefficient was not statistically significant (r = 0.146, P = 0.467). These data are illustrated in Figure 2.

DISCUSSION

This web-based survey analyzed important patient and treatment characteristics that could influence the IBD-related risk of having COVID-19 complications at a national level. Brazil is a continental country with different socioeconomic realities between its five different geographic regions (*i.e.* Northern, Northeastern, Southern, Southeastern and Midwestern). Most patients who participated in the survey were from the southeastern (n = 1886) and southern (n = 738) regions, which are more developed areas of the country. This may reflect patients who more often follow official mailing lists from the study group (as the call for participation in the survey) and might be treated in IBD tertiary referral centers. This could also mirror a higher prevalence of IBD in these regions of Brazil as compared to others as stated in a systematic review and some population-based studies[7-10]. Indeed, the findings of our study may not reflect a full national reality, as patients from the northern and northeastern regions may have a different IBD treatment profile. In the same line, it is noteworthy that the northern region had the highest proportion of patients with no current IBD medication (17.6%), and the southern and southeastern regions the highest proportion of patients under biological therapy (52.2% and 52.9%, respectively).

Our study suggests a different geographical distribution of IBD patients at highest risk for COVID-19 complications in different states of the country, which may reflect different socioeconomic, educational and healthcare issues that could potentially have affected our findings. Despite the fact that the states of Paraíba, Acre, Espírito Santo and Goiás had the greatest proportion of patients at highest risk, this was not reflected in higher COVID-related mortality according to official data from the Ministry of Health. Higher mortality for COVID-19 as of June 2, 2020 (last available date capturing responses in the survey) was observed in the states of Amazonas, Ceará and Rio de Janeiro.

Nevertheless, a clear correlation between the risk of COVID-19 complications in IBD patients and mortality was not demonstrated according to the Spearman test. This important finding is in tune with other previously published data from IBD cohorts, which did not identify worse COVID-19 disease courses in IBD patients[3,11]. This analysis underscores the important finding that the majority of respondents (55.1%) were classified at moderate risk, which means that they were currently having adequate IBD treatment with no increased rates of steroid therapy. Despite being treated with immunosuppressant agents, this population may not be as vulnerable as expected[12]. Although this still needs to be proven, speculation is undertaken if the reduction of the COVID-related "cytokine storm" can be achieved with adequate medical therapy for IBD[13]. More studies in this field are warranted. It is important to state that no longitudinal follow-up of these patients was evaluated in this study. The exact number of infected patients with IBD within our sample was out of the focus of the survey.

When data is analyzed by regions, the proportion of IBD patients at highest risk was 20%-25%, and the majority of patients comprised those using immunomodulators or biologics with no active disease, did not undergo recent IBD-related surgery and were not under steroid therapy. This may reinforce the fact that the survey reached more patients who were likely under regular follow-up with their specialists, a common practice in more developed areas. Another important point is that no significant difference between the regions in the low, moderate or high risk of COVID-related complications was demonstrated (P = 0.118; Supplementary Table 2). This may also be demonstrated by the fact that even in the same region, a particular state could have a

Table 3 Demographic, clinical and treatment characteristics by states in Brazil

Characteristics	São Paulo	Rio de Janeiro	Minas Gerais	Santa Catarina	Paraná	Bahia	Distrito Federal	Rio Grande do Sul	Espírito Santo	Ceará	Pernambuco	Maranhão	Goiás
	n = 1057 (%)	*n* = 336 (%)	*n* = 329 (%)	*n* = 278 (%)	*n* = 277 (%)	*n* = 217 (%)	*n* = 190 (%)	*n* = 183 (%)	*n* = 144 (%)	*n* = 107 (%)	*n* = 74 (%)	*n* = 57 (%)	*n* = 49 (%)
Clinical risk factors													
Age ≥ 70 yr	7 (0.7)	2 (0.6)	2 (0.6)	7 (2.5)	9 (3.2)	1 (0.5)	2 (1.1)	-	8 (5.6)	5 (4.7)	2 (2.7)	-	-
Hypertension	133 (12.6)	43 (12.8)	34 (10.3)	27 (9.7)	31 (11.2)	29 (13.4)	19 (10.0)	11 (6.0)	13 (9.0)	14 (13.1)	6 (8.1)	2 (3.5)	4 (8.2)
Diabetes	33 (3.1)	14 (4.2)	7 (2.1)	8 (2.9)	13 (4.7)	9 (4.1)	9 (4.7)	-	8 (5.6)	6 (5.6)	2 (2.7)	-	3 (6.1)
Cardiovascular diseases	23 (2.2)	10 (3.0)	4 (1.2)	10 (3.6)	12 (4.3)	9 (4.1)	9 (4.7)	3 (1.6)	8 (5.6)	6 (5.6)	2 (2.7)	3 (5.3)	1 (2.0)
Liver diseases	52 (4.9)	15 (4.5)	12 (3.6)	17 (6.1)	11 (4.0)	8 (3.7)	10 (5.3)	11 (6.0)	5 (3.5)	1 (0.9)	3 (4.1)	2 (3.5)	2 (4.1)
Abdominal surgery for IBD (< 30 d)	38 (3.6)	12 (3.6)	7 (2.1)	11 (4.0)	16 (5.8)	5 (2.3)	8 (4.2)	1 (0.5)	16 (11.1)	5 (4.7)	4 (5.4)	1 (1.8)	2 (4.1)
Overall IBD medications													
No medication	112 (10.6)	40 (11.9)	26 (7.9)	22 (7.9)	28 (10.1)	16 (7.4)	16 (8.4)	15 (8.2)	15 (10.4)	6 (5.6)	5 (6.8)	7 (12.3)	1 (2.0)
Oral steroids	137 (13.0)	50 (14.9)	52 (15.8)	32 (11.5)	30 (10.8)	34 (15.7)	27 (14.2)	23 (12.6)	10 (6.9)	16 (15.0)	11 (14.9)	6 (10.5)	7 (14.3)
5-ASA	357 (33.8)	90 (26.8)	124 (37.7)	110 (39.6)	90 (32.5)	88 (40.6)	68 (35.8)	73 (39.9)	20 (13.9)	21 (19.6)	37 (50.0)	18 (31.6)	19 (38.8)
AZA/6-MP/MTX	308 (29.1)	101 (30.1)	130 (39.5)	93 (33.5)	95 (34.3)	71 (32.7)	51 (26.8)	68 (37.2)	62 (43.1)	52 (48.6)	27 (36.5)	17 (29.8)	21 (42.9)
Biologics	575 (54.4)	164 (48.8)	156 (47.4)	145 (52.2)	153 (55.2)	95 (43.8)	93 (48.9)	87 (47.5)	92 (63.9)	64 (59.8)	28 (37.8)	29 (50.9)	29 (59.2)
Therapeutic regimen													
Oral steroid monotherapy[1]	17 (1.6)	14 (4.2)	8 (2.4)	5 (1.8)	2 (0.7)	8 (3.7)	12 (6.3)	4 (2.2)	1 (0.7)	1 (0.9)	3 (4.1)	-	-
5-ASA monotherapy[1]	212 (20.1)	57 (17.0)	67 (20.4)	67 (24.1)	49 (17.7)	68 (31.3)	45 (23.7)	46 (25.1)	11 (7.6)	12 (11.2)	23 (31.1)	12 (21.1)	14 (28.6)
5-ASA + oral steroids[2]	39 (18.4)	4 (7.0)	9 (13.4)	12 (17.9)	5 (10.2)	11 (16.2)	5 (11.1)	6 (13.0)	1 (9.1)	1 (8.3)	1 (4.3)	2 (16.7)	6 (42.9)
AZA/6-MP/MTX monotherapy[1]	141 (13.3)	61 (18.2)	72 (21.9)	39 (14.0)	45 (16.2)	30 (13.8)	24 (12.6)	31 (16.9)	25 (17.4)	24 (22.4)	15 (20.3)	9 (15.8)	5 (10.2)
AZA/6-MP/MTX + oral steroids[2]	28 (19.9)	12 (19.7)	11 (15.3)	2 (5.1)	10 (22.2)	6 (20.0)	3 (12.5)	2 (6.5)	2 (8.0)	6 (25.0)	2 (13.3)	2 (22.2)	-
Biologic monotherapy[1]	408 (38.6)	124 (36.9)	98 (29.8)	91 (32.7)	103 (37.2)	54 (24.9)	66 (34.7)	50 (27.3)	55 (38.2)	36 (33.6)	16 (21.6)	21 (36.8)	13 (26.5)
Biologic + oral steroids[2]	21 (5.1)	17 (13.7)	18 (18.4)	6 (6.6)	9 (8.7)	2 (3.7)	5 (7.6)	5 (10.0)	4 (7.3)	2 (5.6)	3 (18.8)	1 (4.8)	1 (7.7)
Combo therapy[3]	167 (15.8)	40 (11.9)	58 (17.6)	54 (19.4)	50 (18.1)	41 (18.9)	27 (14.2)	37 (20.2)	37 (25.7)	28 (26.2)	12 (16.2)	8 (14.0)	16 (32.7)

Combo therapy[3] + oral steroids[2]	32 (19.2)	3 (7.5)	6 (10.3)	7 (13.0)	4 (8.0)	7 (17.1)	2 (7.4)	6 (16.2)	2 (5.4)	6 (21.4)	2 (16.7)	1 (12.5)	-
Risk classification													
Low	221 (21.0)	71 (21.1)	67 (20.4)	65 (23.4)	53 (19.1)	51 (23.5)	50 (26.3)	46 (25.1)	15 (10.4)	16 (15.0)	20 (27.0)	11 (19.3)	11 (22.4)
Medium	579 (54.8)	184 (54.8)	193 (58.7)	139 (50.0)	156 (56.3)	118 (54.4)	91 (47.9)	108 (59.0)	86 (59.7)	62 (57.9)	40 (54.1)	40 (70.2)	24 (49.0)
High	256 (24.8)	81 (24.1)	69 (21.0)	74 (26.6)	68 (24.5)	48 (22.1)	49 (25.8)	29 (15.8)	43 (29.9)	29 (27.1)	14 (18.9)	6 (10.5)	14 (28.6)

[1]Monotherapy indicates no concomitant biologics or aminosalicylates/6-mercaptopurine/methotrexate.
[2]These subcategories represent the frequency of patients in each therapeutic regimen requiring oral steroids.
[3]Combo therapy refers to biologics plus aminosalicylates/6-mercaptopurine/methotrexate.
5-ASA: Aminosalicylates; AZA: Azathioprine; 6-MP: 6-Mercaptopurine; MTX: Methotrexate; IBD: Inflammatory bowel disease.

different frequency of high risk in comparison to its neighbors. As an example, the proportion of patients at highest risk was 28.6% in the state of Goiás and only 14.3% in Tocantins and 16.7% in Mato Grosso do Sul, all states from the midwestern region. This can also be justified by different types of IBD care and patient profiles in terms of comorbidities between the states, which demonstrates the complexity of analyzing data in a heterogeneous country such as Brazil.

Regarding COVID-related mortality rates in Brazil, it seems clear that the higher rates found in the states of Amazonas, Ceará, Pará, Rio de Janeiro and Amapá reflect difficulties in healthcare in these specific areas. Most of these states belong to less developed regions of the country (northern and northeastern). These are states with chronic difficulties in the public health system over the last decades, with limited resources, reduced numbers of hospitals and consequently disproportional intensive care unit beds per 100000 inhabitants[14]. Recent data revealed that Brazil has 15.6 intensive care unit beds per 100000 inhabitants. Considering only intensive care unit beds from the public health system, the average drops to 7.1 per 100000 inhabitants, and there are significant differences between the regions of the country. Among the population exclusively dependent on the public health system, 30.5% of the Northeast, 22.6% of the North and 21% of the Midwest regions live in places without intensive care unit beds[15].

Socioeconomical limitations may also be illustrated by the assessment of the Human Development Index (HDI) in these states because it is based on three aspects: Health, as measured by life expectancy at birth; Knowledge, as measured by the adult literacy rate; and A decent standard of living, as measured by gross domestic product per capita. Concerning the five geographic regions of Brazil, the first five states with the highest HDI are from the South, Southeast and Midwest regions and the last five states with the lowest HDI are from the North and Northeast regions[16]. Despite the northern and northeastern regions having a lower prevalence of IBD patients, the significant percentage of patients at highest risk for COVID-19 complications might reflect a similar healthcare system limitation, possibly with few available IBD specialists in the

Table 4 Demographic, clinical and treatment characteristics by states in Brazil (continuation of Table 3)

Characteristics	Piauí	Amazonas	Pará	Alagoas	Rio Grande do Norte	Mato Grosso	Paraíba	Acre	Sergipe	Mato Grosso do Sul	Tocantins	Rondônia	Roraima	Amapá
	n = 41 (%)	*n* = 38 (%)	*n* = 32 (%)	*n* = 31 (%)	*n* = 25 (%)	*n* = 22 (%)	*n* = 18 (%)	*n* = 16 (%)	*n* = 13 (%)	*n* = 12 (%)	*n* = 7 (%)	*n* = 6 (%)	*n* = 6 (%)	*n* = 3 (%)
Clinical risk factors														
Age ≥ 70 yr	-	-	-	-	-	-	3 (16.7)	2 (12.5)	-	1 (8.3)	-	-	-	-
Hypertension	5 (12.2)	4 (10.5)	1 (3.1)	4 (12.9)	1 (4.0)	2 (9.1)	5 (27.8)	3 (18.8)	1 (7.7)	3 (25.0)	-	3 (50.0)	3 (50.0)	1 (33.3)
Diabetes	-	2 (5.3)	-	1 (3.2)	-	-	1 (5.6)	2 (12.5)	-	1 (8.3)	-	-	-	-
Cardiovascular diseases	1 (2.4)	-	-	-	1 (4.0)	1 (4.5)	-	1 (6.3)	-	2 (16.7)	-	-	1 (16.7)	-
Liver diseases	2 (4.9)	2 (5.3)	5 (15.6)	1 (3.2)	1 (4.0)	2 (9.1)	2 (11.1)	-	-	-	-	-	-	-
Abdominal surgery for IBD (< 30 d)	2 (4.9)	4 (10.5)	2 (6.3)	1 (3.2)	-	-	-	1 (6.3)	-	-	-	-	-	-
Overall IBD medications														
No medication	5 (12.2)	5 (13.2)	5 (15.6)	1 (3.2)	-	2 (9.1)	-	2 (12.5)	1 (7.7)	3 (25.0)	2 (28.6)	1 (16.7)	3 (50.0)	-
Oral steroids	8 (19.5)	7 (18.4)	6 (18.8)	-	3 (12.0)	5 (22.7)	3 (16.7)	3 (18.8)	1 (7.7)	1 (8.3)	-	1 (16.7)	-	-
5-ASA	11 (26.8)	15 (39.5)	21 (65.6)	8 (25.8)	6 (24.0)	12 (54.5)	10 (55.6)	4 (25.0)	7 (53.8)	3 (25.0)	2 (28.6)	4 (66.7)	2 (33.3)	1 (33.3)
AZA/6-MP/MTX	9 (22.0)	11 (28.9)	7 (21.9)	13 (41.9)	5 (20.0)	5 (22.7)	7 (38.9)	4 (25.0)	4 (30.8)	2 (16.7)	3 (42.9)	1 (16.7)	-	2 (66.7)
Biologics	20 (48.8)	19 (50.0)	6 (18.8)	19 (61.3)	21 (84.0)	5 (22.7)	8 (44.4)	6 (37.5)	7 (53.8)	6 (50.0)	2 (28.6)	1 (16.7)	1 (16.7)	1 (33.3)
Therapeutic regimen														
Oral steroid monotherapy[1]	5 (12.2)	-	1 (3.1)	-	-	-	-	2 (12.5)	-	-	-	-	-	-
5-ASA monotherapy[1]	9 (22.0)	9 (23.7)	15 (46.9)	4 (12.9)	3 (12.0)	10 (45.5)	8 (44.4)	4 (25.0)	3 (23.1)	2 (16.7)	1 (14.3)	4 (66.7)	2 (33.3)	1 (33.3)
5-ASA + oral steroids[2]	-	3 (33.3)	4 (26.7)	-	-	4 (40.0)	1 (12.5)	-	-	-	-	1 (25.0)	-	-
AZA/6-MP/MTX monotherapy[1]	2 (4.9)	5 (13.2)	5 (15.6)	7 (22.6)	1 (4.0)	5 (22.7)	2 (11.1)	2 (12.5)	2 (15.4)	1 (8.3)	2 (28.6)	-	-	1 (33.3)
AZA/6-MP/MTX + oral steroids[2]	-	1 (20.0)	-	-	-	1 (20.0)	-	1 (50.0)	-	1 (100)	-	-	-	-
Biologic monotherapy[1]	13 (31.7)	13 (34.2)	4 (12.5)	13 (41.9)	17 (68.0)	5 (22.7)	3 (16.7)	4 (25.0)	5 (38.5)	5 (41.7)	1 (14.3)	-	1 (16.7)	-
Biologic + oral steroids[2]	1 (7.7)	3 (23.1)	-	-	1 (5.9)	-	-	-	1 (20.0)	-	-	-	-	-
Combo therapy[3]	7 (17.1)	6 (15.8)	2 (6.3)	6 (19.4)	4 (16.0)	-	5 (27.8)	2 (12.5)	2 (15.4)	1 (8.3)	1 (14.3)	1 (16.7)	-	1 (33.3)
Combo therapy[3] + oral steroids[2]	2 (28.6)	-	1 (50.0)	-	2 (50.0)	-	2 (40.0)	-	-	-	-	-	-	-

Risk classification														
Low	10 (24.4)	7 (18.4)	13 (40.6)	4 (12.9)	3 (12.0)	9 (40.9)	6 (33.3)	4 (25.0)	4 (30.8)	3 (25.0)	2 (28.6)	3 (50.0)	2 (33.3)	-
Medium	24 (58.5)	22 (57.9)	14 (43.8)	22 (71.0)	16 (64.0)	11 (50.0)	4 (22.2)	6 (37.5)	7 (53.8)	7 (58.3)	4 (57.1)	2 (33.3)	3 (50.0)	3 (100)
High	7 (17.1)	9 (23.7)	5 (15.6)	5 (16.1)	6 (24.0)	2 (9.1)	8 (44.4)	6 (37.5)	2 (15.4)	2 (16.7)	1 (14.3)	1 (16.7)	1 (16.7)	-

[1]Monotherapy indicates no concomitant biologics or aminosalicylates/6-mercaptopurine/methotrexate.
[2]These subcategories represent the frequency of patients in each therapeutic regimen requiring oral steroids.
[3]Combo therapy refers to biologics plus aminosalicylates/6-mercaptopurine/methotrexate.
5-ASA: Aminosalicylates; AZA: Azathioprine; 6-MP: 6-Mercaptopurine; MTX: Methotrexate; IBD: Inflammatory bowel disease.

respective regions.

It is not being an easy task for Brazilian health authorities to deal with the COVID-19 pandemic. The country is facing important economic and political challenges that likely contribute to the significant increase of the number of cases and deaths throughout the country. The Brazilian government has been a recurrent target for scientific and regular media worldwide[17]. We truly hope this manuscript can raise awareness and call the attention from national health authorities with respect to vulnerability of specific populations during this critical period our country is facing.

The present study is associated with some limitations, which must be considered for adequate interpretation of the results. First, as previously mentioned, the higher proportion of respondents coming from the southeastern and southern regions may not reflect the reality in other states, mostly from the northern and northeastern regions, which have more rural areas and lower HDI. Another important point is that the survey was simple, objective and analyzed a limited number of variables. As an example, even the simple diagnostic difference between ulcerative colitis and Crohn's disease was not evaluated. Disease activity at the time of the survey was not captured, which could have influenced the results. Data is also derived from self-reported personal and treatment-related characteristics, which may be biased by individual intellectual issues. Important additional limitations of our study include the absence of follow-up of the patients who replied to the survey. By not having this information, we could not describe in detail if patients who had COVID-19 infection continued their medications, possible differences between ulcerative colitis and Crohn's disease or common manifestations of severe acute respiratory syndrome coronavirus-2 among included patients due to methodological issues. Despite these limitations, the study's strength is based in the large number of patients who responded to the survey from all states of Brazil, and this represents one of the largest databases regarding COVID-19 risk for complications in IBD patients globally.

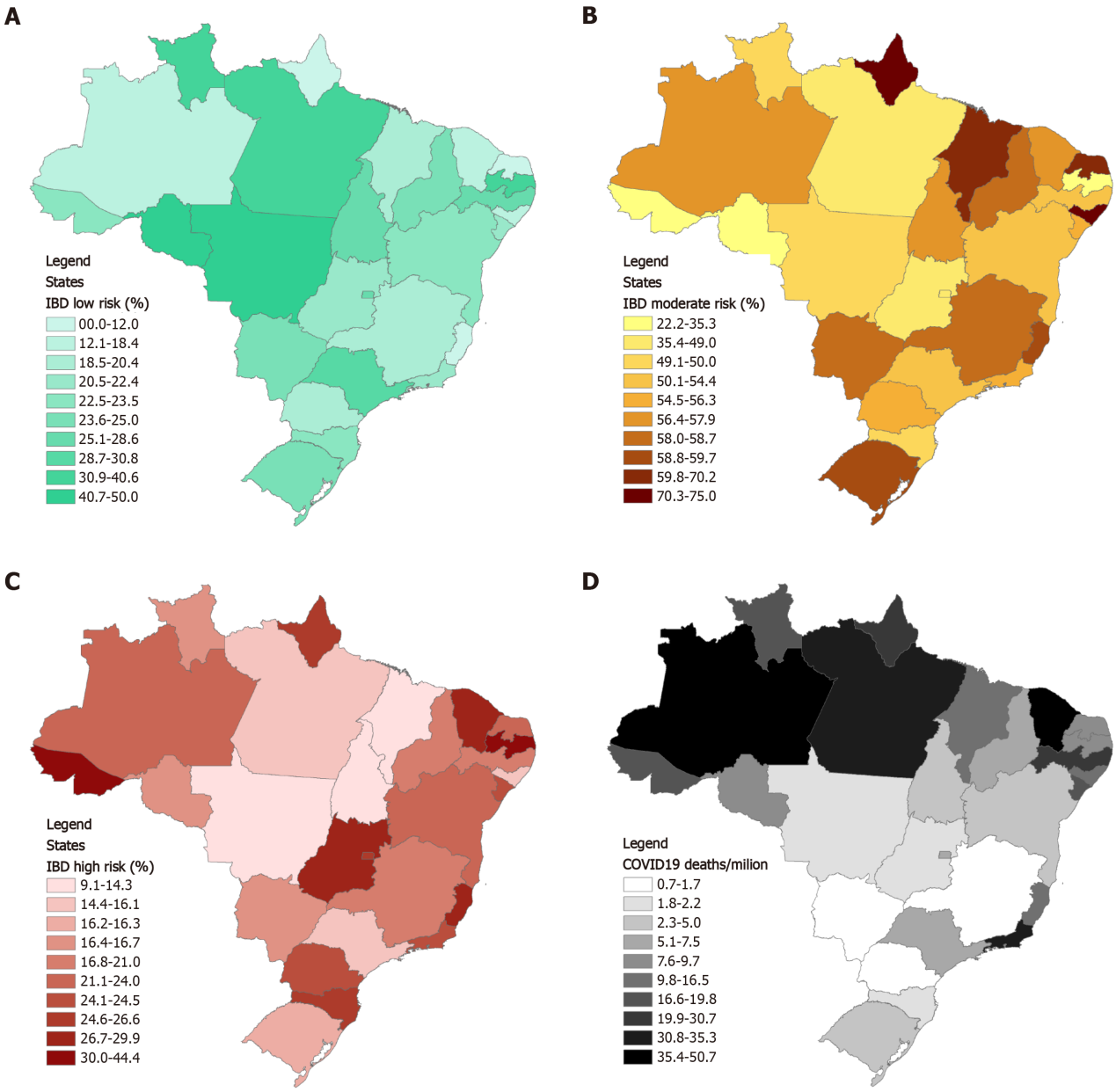

Figure 1 Inflammatory bowel disease patients at low, moderate and high risk for complications of coronavirus disease 2019 and coronavirus disease-related deaths in Brazil by state (per 100000 people). A: Low risk; B: Moderate risk; C: High risk; D: Coronavirus disease 2019 deaths. COVID: Coronavirus disease; IBD: Inflammatory bowel disease.

CONCLUSION

In summary, no correlation between the proportion of IBD patients at highest risk for COVID-19 complications and higher mortality rates was identified among Brazilian states. This can be related to the fact that the majority of the IBD patients are at moderate risk, which could possibly reflect adequate treatment and controlled disease. More epidemiological data comparing IBD and COVID-19 outcomes are suggested in large countries such as Brazil to properly position which IBD patients are more vulnerable in this pandemic period.

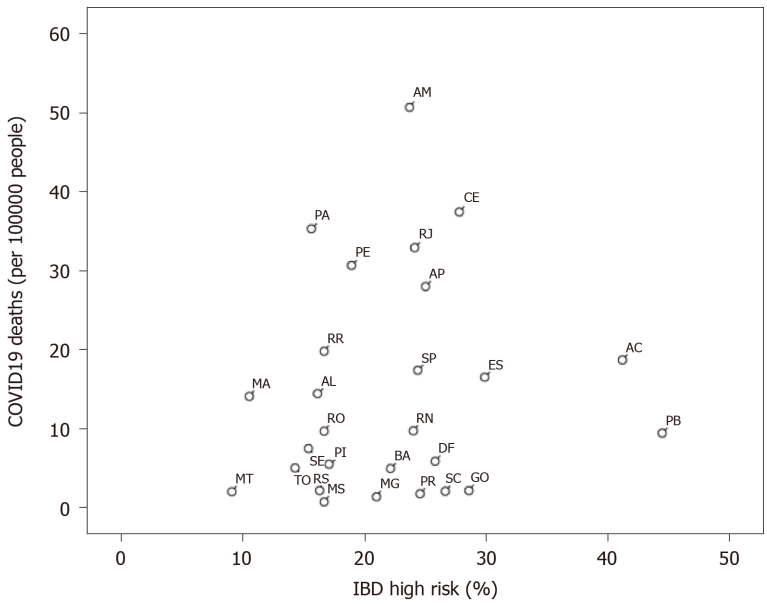

Figure 2 Spearman correlation test between the 27 states and cumulative coronavirus disease 2019 mortality rates. No significant correlation was identified (r = 0.146, P = 0.467). COVID-19: Coronavirus disease 2019; IBD: Inflammatory bowel disease.

ARTICLE HIGHLIGHTS

Research background

The coronavirus disease 2019 (COVID-19) pandemic is a public health emergency of international concern, and Brazil is currently one of the most affected countries.

Research motivation

It is uncertain whether patients with inflammatory bowel disease (IBD) are at a greater risk for developing COVID-19 or its complications. There are scarce data in large countries correlating IBD patients, the risk of COVID-19 complications and mortality.

Research objectives

This study aimed to evaluate geographical distribution of IBD patients at highest risk and correlate these data with COVID-19 mortality rates in different states of Brazil.

Research methods

It was a web-based survey adapted from the British Society of Gastroenterology guidelines. We included demographic data and risk factors for complications from COVID-19. Patients were categorized as highest, moderate or lowest individual risk.

Research results

The proportion of IBD patients at highest risk for COVID-19 complications depends on individual aspects and can vary in specific regions. No correlation between patients with IBD at highest risk and COVID-related mortality rates was demonstrated in different regions of the country.

Research conclusions

This is one of the largest studies analyzing the risk of patients with IBD during COVID-19 pandemic globally.

Research perspectives

These data can be important to large countries such as Brazil, United States, Russia and India, which are currently facing significant problems in terms of controlling the pandemic. Even European countries, facing a second wave of COVID-19 infection, can base future research or decisions using these data as an example.

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META-ANALYSIS

Hepatitis E in solid organ transplant recipients: A systematic review and meta-analysis

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Abstract

BACKGROUND

Hepatitis E virus (HEV) infection is underdiagnosed due to the use of serological assays with low sensitivity. Although most patients with HEV recover completely, HEV infection among patients with pre-existing chronic liver disease and organ-transplant recipients on immunosuppressive therapy can result in decompensated liver disease and death.

AIM

To demonstrate the prevalence of HEV infection in solid organ transplant (SOT) recipients.

METHODS

We searched Ovid MEDLINE, EMBASE, and the Cochrane Library for eligible articles through October 2020. The inclusion criteria consisted of adult patients with history of SOT. HEV infection is confirmed by either HEV-immunoglobulin G, HEV-immunoglobulin M, or HEV RNA assay.

RESULTS

Of 563 citations, a total of 22 studies (n = 4557) were included in this meta-analysis. The pooled estimated prevalence of HEV infection in SOT patients was 20.2% [95% confidence interval (CI): 14.9-26.8]. The pooled estimated prevalence of HEV infection for each organ transplant was as follows: liver (27.2%; 95%CI: 20.0-35.8), kidney (12.8%; 95%CI: 9.3-17.3), heart (12.8%; 95%CI: 9.3-17.3), and lung (5.6%; 95%CI: 1.6-17.9). Comparison across organ transplants demonstrated statistical significance (Q = 16.721, P = 0.002). The subgroup analyses showed that the prevalence of HEV infection among SOT recipients was significantly higher in middle-income countries compared to high-income countries. The pooled estimated prevalence of de novo HEV infection was 5.1% (95%CI: 2.6-9.6) and the pooled estimated prevalence of acute HEV infection was 4.3% (95%CI: 1.9-9.4).

CONCLUSION

HEV infection is common in SOT recipients, particularly in middle-income countries. The prevalence of HEV infection in lung transplant recipients is considerably less common than other organ transplants. More studies examining the clinical impacts of HEV infection in SOT recipients, such as graft failure, rejection, and mortality are warranted.

Key Words: Hepatitis E virus; Hepatitis E virus infection; Solid organ transplant; Prevalence

Core Tip: Hepatitis E virus (HEV) infection among patients with pre-existing chronic liver disease and organ-transplant recipients on immunosuppressive therapy can result in decompensated liver disease and death. The prevalence of HEV infection in solid organ transplant (SOT) recipients varies by countries and transplanted organs. This meta-analysis, demonstrates the prevalence of HEV infection in SOT recipients is 20.3% (highest in liver transplant recipients and lowest in lung transplant recipients). The prevalence of HEV infection is two-fold more common in middle-income countries compared to high-income countries. Our findings encourage future studies to describe the clinical impacts of HEV infection on patient and allograft outcomes.

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INTRODUCTION

Hepatitis E virus (HEV) results in approximately 20 million infections each year[1]. This virus is endemic to mostly developing countries in Asia, Africa, and Central America. There are additional cases of the disease manifesting in developed countries without patients having traveled to endemic areas[1,2]. As HEV is transmitted by the fecal-oral route, infection is more prevalent in areas with poor water quality and food contamination[1]. Patients typically demonstrate symptoms of fevers, gastrointestinal complaints, and jaundice within weeks of infection that self-resolve with supportive care[1]. Although most patients with HEV recover completely, HEV infection among patients with pre-existing chronic liver disease, pregnant women and organ-transplant recipients on immunosuppressive therapy can result in fulminant hepatitis with a 10%-30% mortality rate[3].

HEV has been noted to impact solid organ transplant (SOT) recipient outcomes. HEV infection has been cited to cause graft cirrhosis and subsequent failure in liver graft recipients secondary to chronic infection[4]. Furthermore, heart transplant recipients have been noted to have secondary liver infection and subsequent fibrosis[5]. In contrast, renal transplant allografts were found to have similar rejection and two-year graft survival between HEV seropositive and negative recipients, thus demonstrating HEV does not always impact non-liver allografts[6]. HEV reactivation from infected SOT allografts remains a risk as well, with case reports describing this occurrence in liver transplant recipients who receive an allograft with latent disease[7]. However, little evidence has demonstrated cases of HEV reactivation in renal transplant patients[7]. HEV viremia has also been found in non-SOTs such as hematopoietic stem cell transplant[8]. This suggests possible transmission of the virus through bone marrow products as well as SOT. Once infected with the virus, transplant recipients are at risk for developing chronic liver disease, especially with HEV genotype 3[9].

With the majority of this evidence being limited to case reports and some retrospective studies, there is very limited conclusive evidence illustrating the true HEV association, its related risk profile, and the clinical outcomes in transplant patients. Pooling the aggregate data of current studies will help elucidate the extent of risk and help stratify the clinical outcomes. We conducted this systematic review and meta-analysis to describe the prevalence of HEV infection in SOT patients. Our study is the first meta-analysis to emphasize the burden of HEV infection in SOT recipients.

MATERIALS AND METHODS

Search strategy

This manuscript follows the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA)[10] statement as well as Meta-analysis of Observational Studies in Epidemiology (MOOSE)[11] guidelines. A systematic search was conducted through the Ovid MEDLINE, EMBASE, and Cochrane Library from database inception to October 2020 using the following search terms: ("hepatitis E" OR "HEV") AND ("transplant" OR "transplantation") AND ("outcome*" OR "mortality" OR "graft loss" OR "graft function" OR "incidence" OR "death"). The detailed search strategy for each database is summarized in Supplementary search strategy. No language restrictions were applied.

Inclusion criteria

The eligibility of each study was determined by the following inclusion criteria: (1) The nature of study is observational or conference abstract; (2) Study population consisted of SOT recipients; and (3) The prevalence of HEV infection was reported as one of the outcomes of interest. Exclusion criteria consisted of pediatric patients, hematopoietic stem cell transplant recipients, and studies with a total sample size of less than 50 patients. The latter was to avoid inter-study variance. Study eligibility was independently evaluated by two investigators (PH and AT). Any disagreements were resolved by mutual consensus. The quality of each study was appraised using the Newcastle–Ottawa quality scale[12], which assesses six components including (1) Representativeness of the subjects; (2) Ascertainment of the exposure; (3) Demonstration of outcome of interest was not present at start of study; (4) Assessment of outcome; (5) Follow-up duration period was long enough for outcome to occur; and (6) Adequate follow-up duration.

Review process and data extraction

The titles and abstracts of all discovered studies were screened (PH and AT) prior to a full-text review. The full-text of the screened articles was reviewed to determine their eligibility for inclusion into the systematic review and meta-analysis. We created a standardized data collection form to extract the following information from the included studies: First author's name, year of publication, country of origin, study design, subject(s), sample size, transplanted organ (heart, lung, liver, kidney, and undifferentiated), age, male sex, ethnicity, prevalence of HEV, confirmatory test used to diagnose HEV infection, prevalence of acute HEV infection, death, other reported outcomes and follow-up duration. Country of research origin was classified into high-income and middle-income based on the definition by the World Bank[12]. De novo HEV infection is defined by post-transplant HEV infection in patients with negative pre-transplant HEV-immunoglobulin G (IgG), HEV-immunoglobulin M (IgM) or HEV-RNA. Acute HEV infection is determined by positive post-transplant HEV-IgM with or without positive HEV-RNA.

Measurements

The prevalence of HEV infection, prevalence of de novo HEV infection, and prevalence of acute HEV infection underwent meta-analysis and the results were reported in percentage along with 95% confidence interval (CI). Forest plot of each analysis was created. Results were presented in percentage for categorical data and in mean ± SD or median (interquartile range) for continuous data.

Network association

The prevalence of HEV infection in each organ transplant (heart, lung, liver, kidney, and undifferentiated) were individually compared using mixed-effects model. The association of each couple comparison was assessed with Cochrane's Q-test and its P value. P values less than 0.05 were considered statistically significant.

Subgroup analysis, meta-regression analysis, and publication bias

Subgroup analyses based on the following variables were performed: study year, country of origin, study design, sample size, mean age, male proportion, number of confirmatory tests used in the study, antibody assay, and follow-up duration. Mixed-effects model of analysis was used in subgroup analyses. Publication bias was evaluated by (1) Funnel plot (if the total number of studies was greater than ten[13]); and (2) Egger's regression intercept. An intercept P value of less than 0.05 was considered significant for potential publication bias. The quality of each study was appraised using the Newcastle-Ottawa quality scale[14].

Statistical analysis

All statistical analyses were performed by the Comprehensive Meta-analysis version 3 software (Eaglewood, NJ, United States) and SPSS version 23.0 (IBM Corp., Armonk, NY, United States). Statistical heterogeneity of the included studies was assessed using the Cochran's Q-test and I^2 statistics. An I^2 value of ≤ 25% represents insignificant heterogeneity, 25%-50% represents low heterogeneity, 50%-75% represents moderate heterogeneity, and > 75% represents high heterogeneity[15]. For analyses with I^2 > 50%, the results were analyzed by random-effects model to minimize the heterogeneity and external variance[16]. A P value of less than 0.05 represents statistical significance.

RESULTS

Study characteristics

Of 563 citations, a total of 20 studies consisting of 5842 subjects were included in this meta-analysis and systematic review. Figure 1 provides a flowchart of the literature search and study selection for this meta-analysis. Included studies were published from 2011 to 2020. The study design was retrospective (66.7%) and prospective (33.3%). The median age was 52.0 (11.9) years, 68.5% were male, and 27.7% were Caucasian. The median duration of follow-up was 13.7 (14.0) mo.

Prevalence of HEV infection

For the prevalence of HEV infection, a total of 18 studies (n = 4557) were included in the meta-analysis. Erken *et al*[17] was excluded as the authors only reported the prevalence of de novo HEV infection while Reekie *et al*[18] was excluded because of the

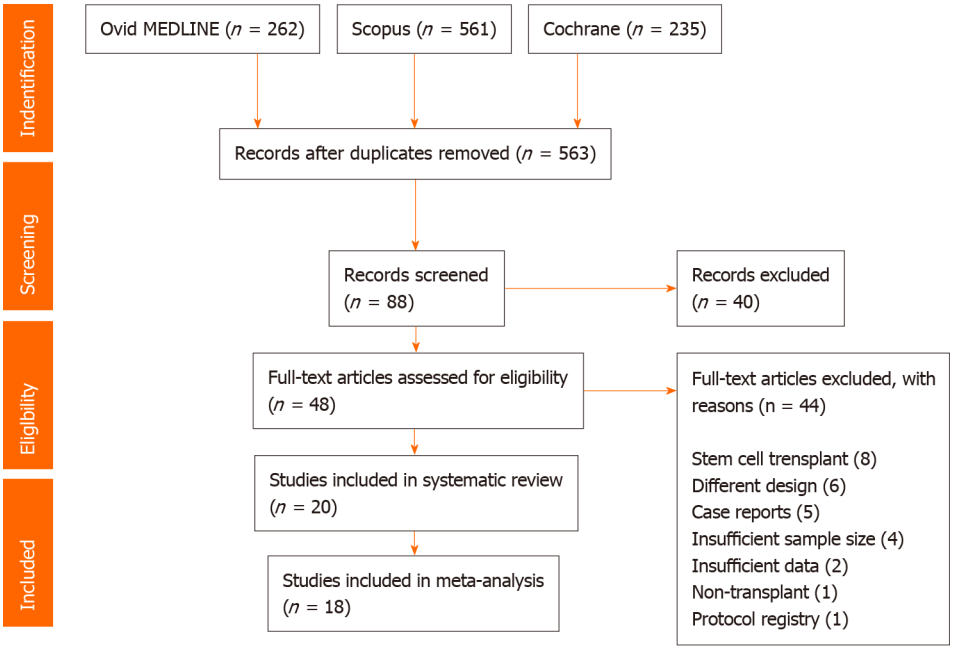

Figure 1 PRISMA flowchart of article search and selection.

potential risk of bias. All other articles had acceptable NOS scores (low risk of bias) for inclusion into meta-analysis for prevalence of HEV infection.

The pooled estimated prevalence of HEV infection in SOT recipients was 20.2% (95%CI: 14.9-26.8; I^2 = 95.3%; Figure 2A). The pooled estimated prevalence of HEV infection in each transplanted organ was: Liver (27.2%; 95%CI: 20.0-35.8; *n* = 11; *n* = 1887), kidney (15.3%; 95%CI: 6.6-31.5; *n* = 4; *n* = 1137), heart (12.8 %; 95%CI: 9.3-17.3; *n* = 1; *n* = 274), lung (5.6%; 95%CI: 1.6-17.9; *n* = 3; *n* = 625), and undifferentiated (29.6%; 95%CI: 10.1-61.1; *n* = 3; *n* = 634).

De novo HEV infection

A total of seven studies (*n* = 2004) were included in the meta-analysis of de novo HEV infection. The pooled estimated prevalence of de novo HEV infection in SOT recipients was 5.1% (95%CI: 2.6-9.6; I^2 = 90.8%). The forest plot is illustrated in Figure 2B.

Acute HEV infection

A total of nine studies (*n* = 1925) were included in the meta-analysis of acute HEV infection. The pooled estimated prevalence of acute HEV infection in SOT recipients was 4.3% (95%CI: 1.9-9.4; I^2 = 90.7%). The forest plot is illustrated in Figure 2C.

Network association analysis

We used subgroup analysis to compare the pooled estimated prevalence of HEV infection from two solid organs of interest at a time. Figure 3 depicts a diagram of the network association analysis. In brief, the prevalence of HEV infection in lung transplant was significantly lower than liver transplant patients (Q = 7.033, *P* = 0.008) and undifferentiated patients (Q = 4.322, *P* = 0.038). There were no statistically significant associations across all other comparisons.

Subgroup analyses

Subgroup analysis results are depicted in Table 1. Here, we analyzed the pooled estimated prevalence of HEV infection based on study characteristics. We applied mixed-effects model to minimize inter-study variance in the subgroup analyses. In brief, we found that the pooled prevalence of HEV infection was similar after adjustment for study year (< 2015 *vs* ³2015), study design (prospective *vs* retrospective), sample size (< 400 *vs* ³400), age (£ 50 years *vs* > 50 years), male proportion (£ 65% *vs* > 65%), number of confirmatory tests (> 1 marker *vs* single marker), and follow-up duration (£ 12 mo *vs* > 12 mo). Interestingly, we found that the prevalence of HEV infection was significantly higher in middle-income countries compared to high-income countries (41.8% *vs* 18.9%; Q = 22.375, *P* < 0.001). The seroprevalence of positive anti-HEV antibodies was significantly higher in studies that

Table 1 Subgroup analyses of all variables

Subgroup	*n* (%)	Incidence (%)	95%CI	
Year				
< 2015	10	17.1	9.9-27.9	
≥ 2015	8	26.8	19.5-35.7	Q = 2.248, *P* = 0.134
Country				
High-income	16	18.9	13.1-26.4	
Middle-income	2	41.8	37.6-46.1	Q = 22.375, *P* < 0.001[c]
Study type				
Prospective	6	22.3	13.4-34.7	
Retrospective	12	20.3	12.8-30.7	Q = 0.077, *P* = 0.782
Sample size				
n < 400	14	25.4	18.9-33.1	
n ≥ 400	4	10.4	3.2-28.8	Q = 2.613, *P* = 0.106
Mean age				
≤ 50 yr	3	17.7	5.4-44.8	
> 50 yr	10	20.2	13.4-29.3	Q = 0.051, *P* = 0.821
Male proportion				
≤ 65%	7	16.0	7.7-30.4	
> 65%	5	23.0	12.5-38.5	Q = 0.631, *P* = 0.427
Diagnostic test				
More than one marker	11	25.9	19-34.4	
Single marker	7	14.1	5.5-31.5	Q = 1.806, *P* = 0.179
Antibody assay				
Wantai assay	8	28.4	21.4-36.6	
Other assays	6	12.3	7.7-19.1	Q = 10.134, *P* = 0.001[b]
Follow-up				
≤ 12 mo	7	23.8	23.8	
> 12 mo	4	26.8	26.8	Q = 0.072, *P* = 0.789

[b]*P* < 0.01.
[c]*P* < 0.001. CI: Confidence interval; *n*: Number of studies.

utilized Wantai assay compared to studies with other assays (28.4% *vs* 12.3%; Q = 10.134, *P* = 0.001).

Evaluation for publication bias

The p-value of Egger's regression intercept for the analysis of pooled total prevalence of HEV infection, de novo HEV infection and acute HEV infection was 0.060, 0.054, and 0.136, respectively. These values indicated no potential publication bias. The funnel plot for the HEV pooled prevalence infection analysis in undifferentiated SOT recipients is illustrated in Supplementary Figure 1.

Systemic review

Table 2 illustrates study characteristics and outcomes included in this systematic review. Kamar *et al*[19] showed that the use of tacrolimus (OR 1.89; 95%CI: 1.49-1.97) and low platelet count (OR 1.02; 95%CI: 1.00-1.10) were associated with chronic HEV infection in SOT patients. Additionally, cirrhosis (OR 7.6; 95%CI: 4.4-13.1), liver transplantation (OR 3.1; 95%CI: 1.8-5.4) and Human immunodeficiency virus (HIV)

Table 2 Characteristics of studies included in the systematic review

Ref.	Country	Type	*n* (%)	Organ	Age	Male	White	HEV	Confirmed test	Acute HEV	Death	Outcome	Follow-up
Kamar *et al*[19], 2011	Monaco	R	85	UD	-	-	-	65.9% (total; 56/85). 32.1% (18/85) had clearance. 0% (0/18) reactivation	Persistently elevated LFTs and positive HEV-RNA	0%	-	Factors associated with chronic HEV infection: Tacrolimus (OR 1.89; 1.49-1.97). Low platelet (OR 1.02; 1.00-1.10)	6 mo
Legrand-Abravanel *et al*[35], 2011	France	R	700	Liver (*n* = 171), kidney (*n* = 529)	52	65.6%	-	15.1% (total; 106/700). 14.6% (25/171) for LTx. 15.3% (81/529 for KTx. 5.6% (de novo; 34/601). 0% (0/17) reactivation	Positive HEV IgG, IgM (Adaltis assay) or HEV-RNA	2.8% (17/611). 2.7% (4/150) for LTx. 2.8% (13/461) for KTx	-	-	22 mo
Pischke *et al* [22], 2012	Germany	P	274	Heart	57	80%	-	12.8% (total; 35/274). 7.3% (de novo; 20/274)	Positive HEV IgG (MP assay) or HEV-RNA	-	-	Heart transplant recipients had significantly higher seroprevalence of HEV-IgG than healthy individuals	8 mo
Crossan *et al*[21], 2014	Scotland	P	317	UD	56.4	93.4%	-	14.2% (total; 45/317)	Positive HEV IgG or IgM (Wantai assay)	0.9% (3/317)	-	Factors associated with chronic HEV infection: HBV coinfection (OR 7.4; 1.4-37). IgG positive is associated with HCC (OR 2.3; 1.1-4.8)	-
Mallet *et al* [36], 2013	France	R	267	Liver	-	-	-	31% (total; 83/267)	Positive HEV IgG (Wantai assay)	-	-	-	-
Pischke *et al*[37], 2014	Germany	R	95	Lung	-	-	-	5.3% (total; 5/95)	Positive HEV IgG (MP assay)	-	-	-	-
Riezebos-Brilman *et al*[38], 2013	Netherlands	R	468	Lung	40	60%	-	2.1% (total; 10/468)	Positive HEV-RNA	-	0.4%	-	6.5 mo
Abravanel *et al*[39], 2014	France	P	263	Liver (*n* = 52), kidney (*n* = 211)	53	64.3%	-	38.4% (total; 101/263). 42.3% for LTx (22/52). 37.4% for KTx (79/211). 1.9% (de novo; 3/162)	Positive HEV IgG, IgM (Wantai assay) or HEV-RNA	-	-	-	12 mo
Buffaz *et al* [40], 2014	France	R	206	Liver	41.1	63.0%	-	36.4% (total; 75/206). 5.3% (de novo; 11/206)	Positive post-transplant HEV IgG, IgM (Wantai assay) or HEV-RNA	-	-	-	32.8 mo
Riveiro-Barciela *et al*[20], 2014	Spain	R	625	Liver (*n* = 332), kidney (*n* = 296), dual (*n* =	54.5	60.8%	-	5.8% (total; 36/625). 3.7% (11/296) for KTx. 7.4% (25/332) for	Positive HEV IgG (MP assay)	-	-	Risk factors associated with HEV infection: Cirrhosis (OR 7.6; 4.4-13.1).	-

				6)				LTx				Liver transplantation (OR 3.1; 1.8-5.4). HIV infection (OR 2.4; 1.3-4.4)	
De Nicola *et al*[41], 2015	Italy	R	79	Liver	55	-	-	33% (total; 26/79). 5.7% (de novo; 3/53)	Positive HEV IgG, IgM (Wantai assay), HEV-RNA	0%	-	-	12 mo
Magnusson *et al*[42], 2015	Sweden	P	62	Lung	55	37.5%	100%	14.8% (total; 8/54). De novo N/A	Positive HEV IgG (recomWell assay)	25% (2/8)	17.8%	-	12 mo
Sherman *et al*[43], 2017	United States	P	171	Liver (*n* = 70), kidney (*n* = 101)	-	-	-	19.9% (total; 34/171). 21.4% (15/70) for LTx. 18.8% (19/101) for KTx	Positive HEV IgG (Wantai assay)	5.5% (3/55) for LTx. 0% for KTx	-	HIV-infected transplant recipients	24 mo
Erken *et al* [17], 2018	Netherlands	R	677	Kidney	-	-	-	0.7% (de novo; 2/300)	Positive HEV-RNA	-	-	Subjects are patients with ALT elevations	-
Reekie *et al* [18], 2018	United Kingdom	R	611	Liver (*n* = 262), kidney (*n* = 349)	-	-	-	0.5% (total; 3/611)	Positive HEV-RNA	-	-	-	36 mo
Samala *et al*[44], 2018	United States	R	232	Liver (*n* = 208), kidney (*n* = 10), both (*n* = 10), intestine (*n* = 4)	58	65%	70%	19.4% (total; 45/232)	Positive IgG, IgM (Wantai assay) or HEV-RNA	-	-	HEV seroprevalence was associated with older age and patients with the diagnosis of alcohol- or NAFLD-associated liver failure	-
Darstein *et al*[45], 2020	Germany	R	74	Liver	55	62.2%	-	28.8% (total; 21/73)	Positive HEV IgG (recomWell assay) or HEV-RNA	-	-	-	-
Komolmit *et al*[46], 2020	Thailand	P	108	Liver	58	69%	0%	44% (total; 48/108)	Positive IgG, IgM (Wantai assay) or HEV-RNA	2% (1/48)	0.9%	-	12 mo
Wang *et al* [23], 2020	China	R	408	Liver	50	81.1%	0%	41.2% (total; 168/408). 16.9% (de novo; 69/408)	Positive HEV-RNA more than 6 months	13.7% (56/408)	-	Alcoholic cirrhosis (OR 5.324; 1.36-20.98). Liver failure (OR 23.76; 2.78-203.08). Graft rejection (OR 0.217; 0.06-0.74)	13.7 mo
Zanotto *et al*[47], 2020	Italy	R	120	Liver	-	-	-	19.2% (total; 23/120)	Positive HEV IgG, IgM (N/A assay) or HEV-RNA	-	-	-	-

UD: Undifferentiated; HEV: Hepatitis E virus; LFTs: Liver function test; OR: Odds ratio; LTx: Liver transplant; IgG: Immunoglobulin G; MC: Multicenter; IgM: Immunoglobulin M; KTx: Kidney transplant; HCC: Hepatocellular carcinoma; HIV: Human immunodeficiency virus; ALT: Alanine aminotransferase; R: Retrospective; NAFLD: Non-alcoholic fatty liver disease; MP: Methylparaben.

infection (OR 2.4; 95%CI: 1.3-4.4) were significant risk factors for HEV infection in the Spanish cohort[20]. Another study[21] demonstrated that HBV coinfection was associated with chronic HEV infection in SOT recipients (OR 7.4; 95%CI: 1.3-37.0), and patients with positive HEV-IgG had higher odds of developing hepatocellular carcinoma (OR 2.3; 95%CI: 1.1-4.8). Pischke *et al*[22] emphasized the prevalence of HEV infection in

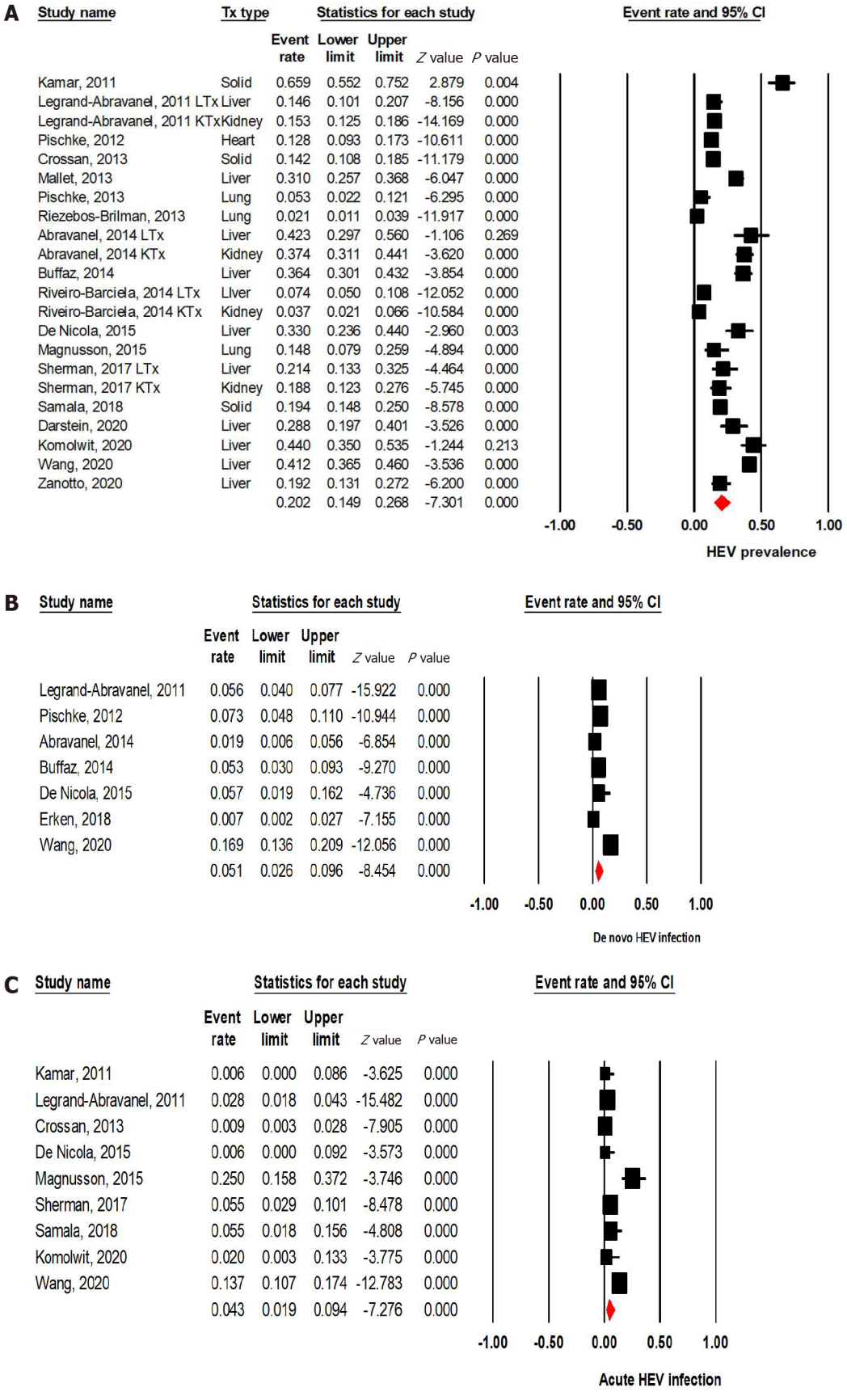

Figure 2 Forest plots of meta-analysis. A: The pooled prevalence of hepatitis E virus (HEV) infection (I^2 95.3%; Egger's intercept 0.060); B: The pooled prevalence of de novo HEV infection (I^2 90.8%; Egger's intercept 0.054); C: The pooled prevalence of acute HEV infection (I^2 90.7%; Egger's intercept 0.136). CI: Confidence interval; HEV: Hepatitis E virus.

heart transplant patients by demonstrating that these patients had a significantly higher seroprevalence of HEV-IgG than healthy individuals. Interestingly, in a Chinese cohort of 408 Liver transplant recipients[23], alcoholic cirrhosis (OR 5.3; 95%CI: 1.4-21.0) and liver failure (OR 23.8; 95%CI: 2.8-203.1) were associated with increased de novo HEV infection during a follow-up of 3 years while graft rejection (OR 0.22; 95%CI: 0.06-0.74) was surprisingly a protective factor.

DISCUSSION

The meta-analysis revealed prevelance of HEV in SOT recipients is 20%. De novo HEV infection and acute HEV infection accounted for less than 5% of infections. A recent meta-analysis of 419 studies comprised of 519,872 individuals showed an estimated global seroprevalence of anti-HEV IgG of 12.5% and a pooled estimated anti-HEV IgM seroprevalence of 1.5%[24]. Although our study did not provide a direct comparison to non-transplant patients, it can be extrapolated that the prevalence of HEV infection is higher in SOT patients (20.1% *vs* 12.5%). The prevalence of acute HEV infection was also higher in SOT patients compared to non-transplant patients (4.3% *vs* 1.5%). These findings emphasize the burden of HEV infection in SOT patients. To date, the United States has not issued national guidelines for the management of hepatitis E in SOT. However, recent guidelines from the British Transplantation Society have recommended screening for HEV infection in individuals with elevated liver enzymes (evidence 1D)[25]. Unfortunately, the evidence for this recommendation is relatively weak due to a lack of studies supporting the association between HEV infection and adverse post-transplant clinical outcomes. More studies on this particular topic are needed. Furthermore, our study indicated a high burden of de novo HEV infection and acute HEV infection in SOT patients. Whether these infections affect the post-transplant clinical outcomes different from chronic HEV infection is yet to be investigated.

It is possible that the seroprevalence of anti-HEV IgG could be affected by the assays used for antibody testing. Rossi-Tamisier *et al*[26] compared the positive rates of two different commercial microplate enzyme-immunoassays and found that the prevalence of seropositive IgG against HEV was higher in the Wantai assay compared to Adaltis assay[26]. Similarly, Li *et al*[24] conducted a meta-analysis and described that the seroprevalence of anti-HEV IgG was highest with the Wantai assay in comparison with other commercial assays[24]. In our subgroup analysis, we also observed that the seroprevalence of anti-HEV antibodies from studies that utilized the Wantai assay was significantly higher than other assays. Thus, the type of assay test should be taken into consideration when interpreting positive anti-HEV IgG or IgM results.

We also found that the prevalence of HEV infection was significantly higher in middle-income countries *vs* high-income countries. This finding is consistent with previously published. Li *et al*[24] suggested that the seroprevalence of anti-HEV IgG was at least two-fold higher in Africa and Asia in comparison to Europe and North America[24]. As HEV route of transmission *via* the fecal-oral route is similar to hepatitis A virus, patients with poor hygiene are predisposed to both hepatitis A and hepatitis E infection. Consumption of raw meat, exposure to soil, contact with dogs, residing in rural areas, and an education level attained less than elementary school is known risk factors for HEV infection[24]. However, our study did not include any articles that originated from low-income countries where the prevalence of HEV infection is anticipated to be high. This may be due to the lower rate of SOTs within this demographic. More studies from low- and middle-income countries are encouraged to reliably determine the global burden of HEV infection in SOT recipients.

We found that the prevalence of HEV infection was lowest in lung transplant recipients. It is unclear why lung transplant recipients had less HEV infection compared with liver transplant recipients. It is possible that the prevalence of HEV infection in lung transplant recipients is under-reported in the literature, given the smaller number of lung transplants annually, at least in the United States. The total number of lung transplants is three times fewer than the total number of liver transplants from the United States Organ Procurement and Transplantation Network[27]. However, it is also possible that lung transplant recipients may be predisposed to receiving ribavirin therapy for other indications, such as respiratory syncytial virus or hepatitis C virus infection. Ribavirin and interferon-a are two main antivirals that have been used to treat cases of HEV infection. There are several reports of successful use of ribavirin in chronic HEV infection to achieve overall sustained virologic response of up to 80%[28-30]. The underlying mechanism by which lung

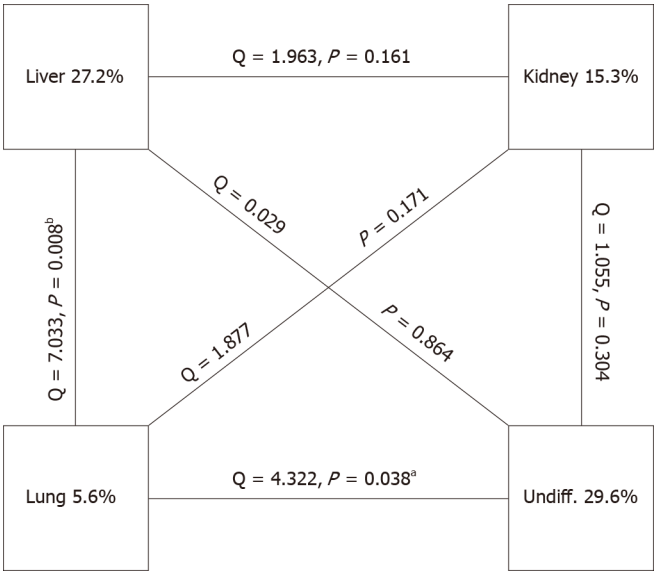

Figure 3 Network association analysis. [a]*P* < 0.05; [b]*P* < 0.01.

transplant patients had lower HEV infection should be investigated in future clinical studies.

Several risk factors for HEV infection in SOT patients have been identified from our systematic review. The use of tacrolimus (versus cyclosporine), low platelet count, cirrhosis, liver failure, human immunodeficiency virus (HIV) coinfection, and hepatitis B virus (HBV) coinfection are all significant risk factors for HEV infection. Hypothetically, tacrolimus generally delivers more immunosuppressive property than cyclosporine, which could predispose patients to contract HEV. This statement, however needs more supporting clinical evidence. Liver disease and associated manifestations including cirrhosis, liver failure, and low platelet count, are not specific to HEV infection; they may be attributed to HEV infection or one of many other etiologies of chronic liver failure. HIV and HBV coinfection raises concern for transfusion-associated HEV transmission, which has been reported in several studies worldwide[31-34].

Our study is subjected to certain limitations. First, all studies were observational in design, making them susceptible to selection bias. We attempted to minimize this bias by performing risk of bias assessment prior to inclusion of studies into our meta-analysis and systematic review. Second, the clinical impact of HEV infection was not meta-analyzed due to limited information from the original articles. More studies investigating the association between HEV infection and clinical outcomes are needed. Third, the genotype of HEV was not reported. Although it is well perceived that HEV genotype 3 and 4 are more common in immunocompromised patients[2], the prevalence of HEV genotype 3 and 4 infection in SOT patients remains inconclusive from our study. Fourth, only the status of recipients was evaluated in our study. HEV infection profile in donors was not taken into consideration due to the limited data in the original articles. HEV transmission *via* transplanted liver has been reported and would potentially impact the prevalence of HEV infection in the recipients. Fifth, generalization of our findings to heart transplant patients is limited because only one study included heart transplant patients. Finally, the majority of included studies were from high-income countries. Additional cohorts from low-income and middle-income countries are highly encouraged.

The future prospects include evaluation of the impact of HEV on SOT patients and graft analysis by meta-analysis or meta-regression analysis. Once the association between HEV infection and adverse clinical outcomes is conclusive, the role of ribavirin therapy for HEV eradication should be investigated in future clinical trials. The ultimate objective of this study is to help contribute to the core knowledge of improving the clinical outcomes of SOT recipients.

CONCLUSION

In conclusion, HEV infection is common in SOT recipients and accounts for 20.2%. It is at least two-fold higher in middle-income countries compared to high-income countries. The prevalence of HEV infection in lung transplant recipients is considerably less common than other organ transplants. More studies demonstrating the clinical impacts of HEV infection in SOT recipients, such as graft failure, rejection, and mortality, are warranted.

ARTICLE HIGHLIGHTS

Research background

Hepatitis E virus (HEV) infection among patients with pre-existing chronic liver disease and organ-transplant recipients on immunosuppressive therapy can result in decompensated liver disease and death.

Research motivation

The prevalence of HEV infection in solid organ transplant (SOT) recipients varies from one organ to another. The disease burden and clinical outcomes of HEV infection in such patients are under-investigated.

Research objectives

To demonstrate the prevalence of HEV infection in SOT recipients.

Research methods

Eligible articles were searched through Ovid MEDLINE, EMBASE, and the Cochrane Library. The inclusion criteria are adult patients with history of SOT. HEV infection is confirmed by either HEV-immunoglobulin G, HEV-immunoglobulin M, or HEV RNA assay.

Research results

Of 563 citations, a total of 22 studies (n = 4557) were included in the meta-analysis. The pooled estimated prevalence of HEV infection in SOT patients was 20.2% (95%CI: 14.9-26.8). The pooled estimated prevalence of HEV infection in each organ transplant was as followed: liver (27.2%; 95%CI: 20.0-35.8), kidney (12.8%; 95%CI: 9.3-17.3), heart (12.8%; 95%CI: 9.3-17.3), and lung (5.6%; 95%CI: 1.6-17.9). The comparison across all organ transplant was statistically significant (Q = 16.721, P = 0.002). The subgroup analyses showed that the prevalence of HEV infection among SOT recipients was significantly higher in middle-income countries compared to high-income countries. The pooled estimated prevalence of de novo HEV infection was 5.1% (95%CI: 2.6-9.6) and the pooled estimated prevalence of acute HEV infection was 4.3% (95%CI: 1.9-9.4).

Research conclusions

HEV infection is common in SOT recipients, especially in middle-income countries. The prevalence of HEV infection in lung transplant recipients is considerably less common than other organ transplants.

Research perspectives

The results of this study offer a preliminary perspective on the magnitude of disease burden from HEV infection, especially in middle-income countries. In the future, large-scale observational studies investigating these associations between HEV infection, patient outcomes, and allograft outcomes are needed to help guide the management of HEV infection in SOT recipients. We also highlight the need for studies from low-income and middle-income countries, as the prevalence of HEV infection from these countries is under-reported.

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