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Multidisciplinary approaches in pediatric gastrointestinal and liver disease

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Multidisciplinary approaches in pediatric gastrointestinal and liver disease

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Editorial: Multidisciplinary approaches in pediatric gastrointestinal and liver diseases

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KEYWORDS

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Editorial on the Research Topic
Multidisciplinary approaches in pediatric gastrointestinal and liver disease

1 Introduction

Pediatric gastrointestinal diseases are complex and can frequently include extra-digestive manifestations, which can sometimes be markers of initial signs and symptoms. Hence, their diagnosis is often challenging, as the clinical picture might be misleading and could potentially point to a completely different condition. The research topic "Multidisciplinary approaches in pediatric gastrointestinal and liver diseases" aimed to attract publications focusing on the involvement of different disciplines in the diagnosis and management of pediatric gastrointestinal and hepatic disorders. A total of 10 articles were published under the umbrella of this research topic: six original research articles, two case reports, and two review articles.

2 Artificial intelligence application in pediatric gastrointestinal disorders

In recent years, artificial intelligence has boomed spectacularly, and ongoing studies suggest that it could be useful in the early diagnosis and prediction of complications of various pediatric pathologies. These include necrotizing enterocolitis in newborns, for which Cui et al. have proposed a multimodal, artificial intelligence-based model, based on standard laboratory investigations and abdominal x-ray. The authors' unique combination of the multimodal model with the gradient-weighted class activation mapping (GradCAM) helps identify intestinal wall edema and gas, portal vein gas, and the dilation of the intestinal loops, improving the early recognition of necrotizing enterocolitis.

3 Gastrointestinal dysmotility conditions and pediatric bowel obstruction

Idiopathic constipation represents a problematic occurrence in children with neurological disorders, secondary to reduced intestinal motility. Within their study, Wang et al. analyzed the efficacy of Tui Na, a special massage therapy derived from Traditional Chinese Medicine, compared to that of oral lactulose in children with cerebral palsy, as provided in addition to basic treatment. The study proved that Tui Na performs similarly to traditional therapies such as lactulose in combating cerebral palsy-related constipation, proposing it is a viable alternative therapeutic approach. Addressing gastrointestinal dysmotility issues in children with psycho-motor delay can also involve therapies such as acetylcholinesterase inhibitors. Comisi et al. present a case report of a patient known with ATR-X syndrome, as well as gastro-esophageal reflux, dysphagia, and chronic constipation, in whom the barium enema highlighted a sub-stenosis of the recto-sigmoidian region. Pyridostigmine alleviated the abdominal pain and distention and improved the intestinal transit; thus, the need for surgery was avoided.

Foreign body ingestion is a concerning event in pediatrics, as toys made of components such as superabsorbent polymer (SAP) beads can lead to bowel obstruction. In an *in vitro* study, Hachem et al. assess the expansion of SAP in different liquid media, showing that gastric acid inhibits their expansion to a greater extent than water or alkaline solution. After administering solutions with high concentrations of polyethylene glycol (PEG), the size of the beads was effectively reduced, which makes PEG a prospective option in managing the ingestion of SAP beads in children.

Congenital or acquired bowel obstructions represent surgical emergencies that need to be recognized promptly and treated accordingly. Within a multicenter, retrospective study, Yang et al. point out that non-bilious vomiting is a frequent initial presentation in infants with intestinal malrotation. Still, abdominal ultrasound presents good sensitivity and specificity for the detection of intestinal malrotation complicated by intestinal obstruction, which makes further contrast examinations unnecessary. Acquired intestinal obstructions are uncommon complications of otherwise conservatively manageable conditions, such as eosinophilic gastrointestinal disease. Di Mari et al. report an unusual case of a male teenager with progressive duodenal stenosis, secondary to eosinophilic esophagitis and enteritis.

4 Hepatic and autoimmune diseases of the gastrointestinal tract

Hepatic congenital and autoimmune disorders have been thoroughly studied, but their diagnosis and outcome prediction still require further research, mainly based on the discovery of novel biomarkers. For example, activin A immunostaining of hepatic biopsy specimens, during hepatic portoenterostomy (HPE) performed for biliary atresia, has been proposed as a long-term outcome predictor of the surgical intervention. As Džepina et al. show in their research conducted on a group of infants with biliary atresia, the patients with the weakest positive reaction for activin A presented the highest survival rates, of over 90% 2 years after HPE, following native liver transplantation. The distinction of autoimmune hepatitis (AIH) from other AIH-like conditions, such as drug-induced hepatitis and infectious hepatitis, requires a thorough assessment of hepatic infectious antigens and antibodies, a hepatic antibody panel, as well as liver biopsy in some cases. Within a retrospective study, Ma et al. demonstrate that the identification of positive autoantibodies can aid in the early diagnosis of AIH. Furthermore, liver histology in AIH differs from other hepatic conditions through peculiar inflammatory modifications of the hepatic lobule, with lymphoplasmacytic infiltration.

Celiac disease is another condition that mainly leads to gastrointestinal symptoms and a malabsorption syndrome, but it behaves as a systemic disease, which can be associated with other immune-mediated diseases. Starcea et al. bring to attention the potential association between celiac disease and IgA nephropathy, as well as diabetic nephropathy, which both can lead to the development of chronic kidney disease, even at pediatric ages. Furthermore, a strong association exists between celiac disease and hyperoxaluria, a major risk factor for urolithiasis. These reported co-occurrences of renal disease in children call for periodic screening of renal function in pediatric patients with celiac disease.

5 Acute gastroenteritis in children

Acute gastroenteritis still remains a major problem in underdeveloped countries, accounting for one of the leading causes of mortality in children under the age of 5 years. Rotavirus is a major etiological factor of acute gastroenteritis outbreaks in both rural areas and hospital settings. Tian et al. have systematically reviewed the rotavirus outbreaks so far reported within the medical literature and concluded that group B rotaviruses lead to more severe outbreaks, characterized by a longer duration and a larger number of cases when compared to group A rotaviruses.

6 Conclusions

The research topic presented research articles on various topics; together, this outlines the importance of the implications of multiple domains and medical fields for the appropriate management and early recognition of pediatric gastrointestinal disorders. These included *in vitro* studies, artificial intelligence-based models, and studies/case reports focusing on surgical management or on the involvement of histology and immunostaining for the differential diagnosis and outcome prediction of hepatic conditions. Literature reviews of celiac

disease-related renal conditions and rotavirus outbreaks in children complete the content of this research topic, showcasing the systemic and potentially fatal involvement of these conditions.

Author contributions

MS: Conceptualization, Formal analysis, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. AL: Conceptualization, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. VL: Conceptualization, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Conflict of interest

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Duodenal stenosis, an unusual presentation of eosinophilic gastroenteritis: a case report

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Eosinophilic gastrointestinal diseases (EGIDs) are rare, chronic inflammatory disorders characterized by eosinophilic infiltration of the gastrointestinal tract. Symptoms and clinical presentations vary depending on the site and layer of the gastrointestinal wall infiltrated by eosinophils. Gastrointestinal obstruction is a serious, though uncommon, presentation. Management can be extremely challenging because of the rarity of the condition and the lack of robust scientific evidence. Current treatment approaches for EGIDs mainly focus on elimination diets, proton pump inhibitors and corticosteroids, which present high refractoriness rates. Novel targeted therapies are being investigated but not routinely used. Surgery should be avoided as far as possible; however, it may be the only option in gastrointestinal obstruction when long-term remission cannot be attained by any medical strategy. Herein we report the case of an adolescent boy affected by an eosinophilic gastrointestinal disease with progressive duodenal stenosis, refractory to medical therapy, who successfully benefitted from surgical management. He presented with a one-year history of gastrointestinal obstructive symptoms with feeding intolerance. After the diagnostic workup, he was diagnosed with an eosinophilic gastrointestinal disease (esophagitis and enteritis) with a duodenal involvement causing a progressive duodenal stenosis. Due to refractoriness to the conventional medical therapies and the consequent high impact on his quality of life, related both to the need for enteral nutrition and repeated hospitalizations, we decided to perform a gastro-jejunum anastomosis, which allowed us to obtain a clinical and endoscopic long-term remission. The early discussion of the case and the involvement of all experienced specialists, pediatricians and pediatric surgeons is essential.

KEYWORDS
eosinophilic gastroenteritis, stenosis, surgical treatment, quality of life, case report

Introduction

Eosinophilic gastrointestinal diseases (EGIDs) are a group of rare, chronic inflammatory disorders characterized by primary eosinophilic inflammation of specific segments in the gastrointestinal (GI) tract (1, 2). Multiple GI segments may be simultaneously or sequentially involved (1).

EGIDs' diagnosis is based on symptoms associated with histological findings of intestinal eosinophilic infiltration after excluding a secondary cause of tissue eosinophilia. Symptoms

differ according to the patient's age and the localization, extension, and depth of the eosinophilic infiltration through the intestinal wall. Mucosal disease is the most common form and presents with non-specific symptoms such as nausea, vomiting, diarrhea, failure to thrive, dysphagia, dyspepsia, abdominal pain, and gastrointestinal bleeding. The serosal form occurs in a minority of patients and is characterized by exudative eosinophil-rich ascites, bloating and abdominal pain (1). Patients with muscular involvement, often affecting the stomach and duodenum, may develop intestinal obstruction or sub-obstruction because of the eosinophilic inflammation and fibrosis of the muscular layer of the bowel (3).

Histological confirmation of EGIDs diagnosis may be challenging since uniformly accepted histological criteria for EGIDs, beyond Eosinophilic Esophagitis (EoE, ≥15 eosinophils per high power field, HPF), are still debated (1, 4). Collins and colleagues in 2018 proposed the following criteria for the histological diagnosis of non-EoE EGIDs: diagnosis of Eosinophilic Gastritis (EoG) includes an eosinophilic count ≥30/HPF in more than 5 HPF and ≥70/HPF in more than 3 HPF; diagnosis of Eosinophilic Enteritis (EoN) and Eosinophilic Colitis (EoC) includes an eosinophilic count at least double the peak number found in normal biopsies (EoN: >52/HPF in duodenal mucosa and >56/HPF in ileum; EoC: >100/HPF in cecum and right colon, >84/HPF in transverse and left colon, >64/HPF in rectum and sigma) (5).

Eosinophilic Esophagitis (EoE) represents the most known and common form, with a prevalence of 1/2,000 subjects (6). Eosinophilic Gastritis (EoG), EoN and EoC are rarer forms (2, 7) whose epidemiology and pathophysiology are still unclear (4). According to different cross-sectional studies, the prevalence of the latter three forms ranges, respectively, from 1.5 to 6.4/100.000, from 2.7 to 8.3/100.000 and from 1.7 to 3.5/100.000 subjects (8). Nevertheless, accurate data on incidence and prevalence of non-EoE EGIDs are difficult to establish because most of them derive from case reports and small retrospective series; moreover, in many case series, the diagnosis of EGID was based on non-standardized eosinophils cut-off values. Recently, an Italian multicenter study retrospectively described a cohort of 40 adult patients affected by EoC, which currently represents the largest reported cohort of EoC by adopting the stringent histological cut-off values proposed by Collins et al. (9).

First-line treatment approaches focus on elimination diets, proton pump inhibitors (for upper EGIDs), and topical and systemic corticosteroids. However, due to standard therapy refractoriness and high recurrence rate, effective maintenance treatments are needed both in adult and pediatric patients (10–13). Immunomodulators (e.g., azathioprine), sodium cromoglycate, or montelukast have been tried as maintenance therapy, but with poor efficacy (14–16). Novel targeted therapies, including monoclonal antibodies (targeting IL-13, IL-4, IL-5, integrins, Siglec-8) and non-biological treatments (targeting JAK-STAT and CHTR2 signaling pathways) seem to be promising and are currently being investigated, although not routinely used (14, 16–18).

Little has been published about the surgical management of EGIDs. Surgery may sometimes be required when complications like perforation or obstruction occur (19–21). It has been reported that about 40% of EGID patients may need surgery and about half of them may experience recurrence after surgery (22).

Gastrointestinal obstruction represents an unusual presentation of EGID and is generally associated with predominantly muscular disease. In infants, gastric outlet and duodenal strictures may mimic pyloric stenosis (3, 23, 24). More distal intestinal obstructions, although rare, have been described and may occur in the ileum (3) and jejunum (25). Obstructive symptoms are mostly reversible under corticosteroids. Surgery is reserved for recurrent forms when long-term remission cannot be obtained by any medical strategy (26, 27).

Diagnosis and treatment of EGIDs remain very challenging in the case of muscular and serosal involvement. Moreover, the chronic nature of the disease, long-term therapies, and strict follow-up may impair the quality of life of patients and their families (28–30).

Herein, we describe the case of an adolescent boy presenting with esophageal and small bowel EGID, complicated by a progressive duodenal stenosis, who failed to respond to long-term medical treatment and benefitted from surgical management. We want to highlight the diagnostic and therapeutic challenges, the importance of a strict, early, and continuous collaboration between pediatricians and pediatric surgeons, and the delicate and complex aspects of follow-up.

Case presentation

A 14-year-old boy was referred to our Pediatric Gastroenterology Unit at Vittore Buzzi Children's Hospital in Milan in June 2018, with a one-year history of upper abdominal pain, repeated nonbilious vomiting, and occasional dysphagia. Five kilograms were lost yearly; no diarrhea or gastrointestinal bleeding were reported. He had suffered from recurrent aphtous stomatitis and allergic oculorhinitis from age 8. Neither allergic asthma nor chronic rhino-sinusitis with polyposis were associated. His family history included allergic asthma and psoriasis.

At referral, his weight was 56 kg (0 SD); his height was 165 cm (0 SD). His clinical examination was unremarkable, except for upper abdominal tenderness.

Blood tests revealed mild eosinophilia (Eo 1,000/mm^3) and increased total IgE (341 kU/L). Erythrocyte sedimentation rate (ESR), C reactive protein (CRP), liver and pancreatic tests were normal; serum albumin was normal. Fecal calprotectin and parasitological examination resulted negative. Skin prick tests were positive for some inhalants, and negative for food allergens.

Abdominal ultrasound showed dilation of common hepatic, common bile, cystic and pancreatic ducts. The magnetic resonance cholangiopancreatography confirmed the dilation of the biliary tract, while the pancreas resulted normal. A congenital malformation of the biliary tree (choledochal cyst) was hypothesized. Moreover, a thickening of the wall of the descending duodenum was observed and confirmed by a magnetic resonance enterography. No other involvement of small bowel was detected.

Esophagus-gastro-duodenoscopy (EGD) revealed a pale, mild trachealized esophagus with longitudinal furrows (Figure 1), normal gastric mucosa, and the presence of an edematous

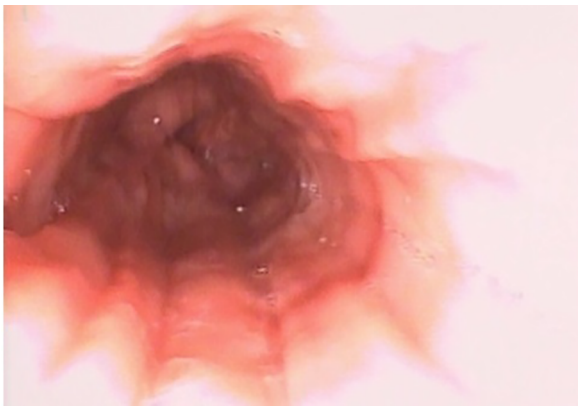

FIGURE 1
EGD documenting esophagitis in the 3rd esophageal tract characterized by reduced wall distensibility, trachealization and furrows.

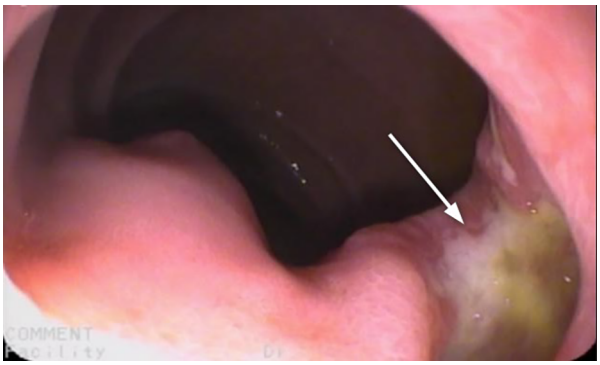

FIGURE 3
Post-stenotic juxta papillary ulceration (arrow) was identified with no active bleedings.

duodenal bulb associated with a tight stenosis of the descending duodenum (Figure 2); a neonatal endoscope was effective in overcoming the narrowing, and a post-stenotic juxta papillary ulceration was identified (Figure 3). Ileocolonoscopy was normal.

Eosinophilic inflammation was found in all the biopsies, but the gastric ones: >60 eosinophils/high power field (HPF) in the terminal ileum and colon, >30/HPF in the duodenum, >15/HPF in all the esophageal tracts; clusters of eosinophils were detected with focal epithelial infiltration. Neither parasites nor tumoral cells, morphological elements attributable to inflammatory bowel diseases or other microscopic colitis were identified.

The final diagnosis was EGID: eosinophilic esophagitis and enteritis; we could not confirm a colonic involvement due to the lack of assessment of the precise eosinophilic count in biopsies, as compared to the criteria proposed by Collins and colleagues (5), and the absence of symptoms suggestive of colitis.

A course of systemic steroids (prednisone 40 mg/day for 2 weeks, then tapered in 10 weeks) was prescribed, associated with proton pump inhibitors (PPI) (esomeprazole 40 mg/day), with a prompt clinical response. The follow-up EGD, performed after 4 weeks of treatment, showed a global improvement: the esophagus was easily distensible without trachealization and only mild exudate; the descending duodenum stenosis, though still present, was passable by a 9 mm endoscope; the duodenal mucosa in the post-stenotic tract appeared repaired. Histological examination documented the absence of eosinophilic inflammation in all the examinated fragments.

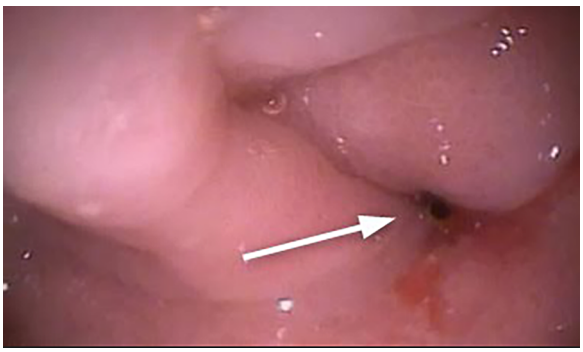

FIGURE 2
Duodenal stenosis (arrow): edema in the duodenum prevented the progression of the 9 mm endoscope. A 5 mm endoscope was used to overcome the stenotic tract.

As a maintenance treatment, a 3-food (milk, egg, and wheat) elimination diet was then prescribed; however diet therapy success was limited by poor patient adherence.

The patient experienced a clinical relapse 1 month after the discontinuation of steroids; the upper GI series confirmed the recurrence of duodenal stenosis (Figure 4).

Therapy with systemic steroids and PPI was successfully restarted and associated with azathioprine (2 mg/kg/day) as a maintenance treatment. Oral budesonide was started at prednisone tapering.

A 3-month-follow-up EGD revealed a passable duodenal stenosis. Histological examination of gastric and duodenal biopsies revealed the absence of eosinophilic inflammation.

A third relapse occurred six months later, requiring a course of intravenous methylprednisolone; a maintenance therapy of montelukast 10 mg/day was associated with azathioprine.

Due to oral feeding intolerance and weight loss, a total pre-pyloric enteral nutrition with hydrolyzed formula was started and administered by nasogastric tube.

The magnetic resonance enterography showed a worsening in duodenal wall thickening; moreover, gastric wall thickening was encountered, suggesting an eosinophilic muscular infiltration of the stomach. A non-passable stenosis was confirmed by EGD.

Given the ineffectiveness of medical therapy in preventing the recurrence of duodenal stenosis, with consequent persistent oral feeding intolerance and the high psychological impact related to enteral nutrition and repeated hospitalizations, we decided to perform, in September 2020, a video-laparoscopic side-to-side gastro-jejunum anastomosis by a stapler device without any resection, to obtain a surgical bypass of the obstructed duodenum. Endoscopic dilatation of the duodenal stenosis was not technically

FIGURE 4
Duodenal stenosis (arrow) confirmed by upper GI series.

possible because of the proximity to the biliary papilla. Unfortunately, it was impossible to take full-thickness biopsies of the thickened duodenal bulb and stomach because of the extreme stiffness of the wall.

Surgery effectively relieved the patient's symptoms, allowing a gradual withdrawal of enteral nutrition. Medical therapy with montelukast and oral budesonide was continued while azathioprine was suspended because of hematological side effects. Follow-up endoscopies, performed 6 and 18 months after the surgical treatment, showed an improvement in the duodenal stenosis, highlighting a possible beneficial effect of partial bowel diversion on EGID remission. After the 18-months-follow-up EGD, budesonide was suspended while therapy with montelukast is still ongoing, associated with omeprazole. Follow-up magnetic resonance enterography, performed 36 months later, showed stable duodenal involvement without pre-stenotic dilatation. Follow-up EGD is scheduled.

No clinical relapses occurred 36 months after surgery with a great amelioration and normalization in the patient's quality of life.

Discussion

EGIDs' long-term outcome has yet to be fully characterized (31). In a retrospective study conducted on 43 EGIDs patients over a follow-up period of 13 years, 42% of patients experimented no clinical relapse after the initial flare. In 37% of patients, multiple flares alternated with periods of full remission while 21% presented a chronic disease course (32). Another study, conducted on 44 pediatric and adult EGIDs patients, reported that only one-third remained in remission after a mean follow-up of 26.2 months, while most patients presented a persistent or progressive disease course. Better response rates were showed for oral corticosteroids than for elimination diets, leukotriene antagonists, H2 blockers and mast-cell inhibitors (33).

Oral systemic steroids have been shown to induce both clinical remission and a reduction in mucosal eosinophilia (34). Topical steroids (fluticasone, budesonide), commonly used for EoE, may be considered in EGIDs. Their efficacy in reducing symptoms and tissue eosinophilia has been reported in some retrospective studies (35–37).

However, relapses may occur at discontinuation of steroids. The chronic relapsing course of EGIDs, the need for restrictive diets, multiple medical treatment changes, and the frequent endoscopies needed during follow-up may hugely impact the quality of life of EGIDs patients (29, 30, 38), indicating the need for long-term maintenance therapies.

Sodium cromoglycate, montelukast and immunomodulators such us azathioprine demonstrated poor efficacy as maintenance therapies (14–16).

Novel biological drugs, targeting T helper 2 (TH2) (IL-25, IL-33, TSLP, IL-4, IL-5, IL-9, IL-13) and non-TH2 pathways, are being investigated to treat EGIDs. Dupilumab, an anti-IL-4 receptor alpha (IL-4Rα) monoclonal antibody, blocking IL-4 and IL-13 signaling, is already approved for atopic dermatitis in children older than 6 months, for asthma in children older than 6 years, and for nasal polyposis in adults. In May 2022, Dupilumab has been approved for EoE in patients older than 12 years (39). Efficacy of Dupilumab is currently being investigated in a phase 2 multi-center trial in adults and children older than 12 years affected by EoG (ClinicalTrials.gov Identifier: NCT03678545). Anti-IL-5 monoclonal antibodies (Reslizumab, Mepolizumab) have been demonstrated to significantly reduce the esophageal eosinophilic inflammation in children affected by EoE, but no difference emerged in reducing symptoms. Their efficacy in treating non-EoE EGIDs in adults and in adolescents is currently under investigation (40, 41). Lirentelimab is an antibody targeting the sialic acid-binding immunoglobulin-like lectin 8 (Siglec-8), which induces the apoptosis of activated eosinophils; in a phase 2 clinical trial conducted on adult patients affected by EoG and eosinophilic duodenitis, it was demonstrated to reduce gastrointestinal eosinophilia and symptoms (42). Vedolizumab, an α4β7 integrin inhibitor used in the treatment of inflammatory bowel diseases, may improve eosinophilic infiltration and reduce steroid dependency in refractory EoG and EoN (43).

A recent retrospective multicenter study, conducted both in children and adults affected by EGIDs, reported that only 2/142 (1%) patients with EoG, 4/123 (3%) patients with EoN, and 1/108 (1%) patients with EoC, were treated with a

monoclonal antibody, with clinical, endoscopic, and histologic improvements in short term follow-up (34).

The European and North American Societies for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN and NASPGHAN), in their recent joint guidelines, recommend considering oral systemic steroids to induce remission in children with non-EoE EGIDs; they confirm the lack of sufficient evidence to recommend for or against the use of leukotriene inhibitors, mast cell blockers, immunomodulators and biologics in this group of patients; they consider the possibility to evaluate the use of proton pump inhibitors in children affected by EoG or eosinophilic duodenitis with ulcerations and the use of topical steroids and empiric elimination diets in selected patients (44).

Little has been published about the surgical management of EGIDs. Sheick and colleagues described five cases of EGID-related gastrointestinal obstruction, with predominant involvement of the stomach and duodenum. Four out of 5 patients benefitted from medical treatment. A 71-year-old woman in their series was affected by eosinophilic gastritis, with thickened mucosal folds and multiple antral polypoid lesions causing recurrent gastric outlet obstruction. She failed to respond to steroids and sodium cromoglicate and successfully underwent antrectomy and gastrojejunostomy with consequent remission on low-dose corticosteroids and sodium cromoglicate (26). Shetty and Shetty reported two cases of recurrent subacute intestinal obstruction whose diagnosis of EGID was made after surgery; they both recovered well (27).

Given the rarity of complicated EGIDs, treatment strategies are adjusted each time, relying on empiric considerations and personal experience rather than evidence. There is no consensus or guidelines for treating patients with complex EGIDs forms, considering that the disease's pathogenesis is largely unclear.

The main concerns about surgery of EGIDs are the intervention timing, the execution of minimally invasive and reversible procedures, and the adequacy of the follow-up. Treatment decisions should be made on the extension of the gastrointestinal involvement and clinical severity.

Laparoscopy plays an important role in the diagnosis of EGID when a muscular or serosal involvement is suspected, since it allows us to perform full-thickness biopsies to prove the eosinophilic infiltration, which cannot be detected by endoscopic mucosal biopsies (45). The approach is minimally invasive and well tolerated even in pediatric ages.

Surgery may be required in complicated and life-threatening conditions, such as intestinal perforation or intussusceptions (19–21). It could also be needed in recurrent EGID-related obstructions, which are not reversible under corticosteroids; furthermore, in some uncertain cases, the histopathology of the surgically removed segments can confirm the diagnosis definitively (3, 45, 46).

Our patient presented with a duodenal stenosis, an unusual manifestation of the disease. The histopathologic evaluation of biopsies, negative for morphological elements attributable to inflammatory bowel diseases, other microscopic colitis or for tumoral cells, allowed us to rule out Crohn's disease and neoplasms. Patient medical history, parasitic tests on both stools and biopsies and an allergologic evaluation allowed for the exclusion of a secondary cause of tissue eosinophilia.

Our patient benefitted from a combined medical and surgical management of his condition, with a good outcome, similarly to other cases already reported in the literature (26, 27). As limitations, we could not accede to novel targeted treatments because of a lack of authorization in our pediatric center at the time of patient evaluation. Our case report highlights the role of surgery in progressive intestinal stenosis in EGID. Given the high risk of recurrence, it raises the need for a proper follow-up, notably with surveillance of the bind-ending intestinal loop and maintenance treatment.

Conclusion

EGIDs are rare, chronic relapsing conditions for which a high degree of clinical suspicion is necessary for diagnosis. Therapeutic approaches are still inadequate and poorly standardized, especially in maintaining remission (1). Surgery should be avoided as far as possible (4). Still, it can be useful for managing recurrent intestinal obstructions if medical treatments cannot attain long-term remission. A "more intense" treatment, including immunomodulators, targeted therapies, and endoscopic and/or surgical procedures, may be required in complicated disease. Each case should always be discussed between all the pediatric experts, starting from the initial stages of care, to ensure the best-tailored management and establish the proper intervention timing.

Data availability statement

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Ethics statement

Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

CD: Conceptualization, Data curation, Methodology, Supervision, Writing – original draft, Writing – review & editing. EP: Conceptualization, Data curation, Methodology, Supervision, Writing – original draft, Writing – review & editing. CM: Writing – original draft, Writing – review & editing. FD: Writing – original draft, Writing – review & editing. MM: Writing – original draft, Writing – review & editing. MC: Supervision, Writing – original draft, Writing – review & editing. AS: Writing – original draft,

Writing – review & editing. GP: Writing – original draft, Writing – review & editing, Validation. GZ: Writing – original draft, Writing – review & editing, Validation.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Unraveling chronic kidney disease in children: a surprising manifestation of celiac disease

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Celiac disease, firstly described in children, is a type of T-cell enteropathy that occurs in individuals genetically predisposed to gluten exposure. The estimated global prevalence of celiac disease is continuously increasing. Although, traditionally, celiac disease was diagnosed in children with failure to thrive and digestive issues, it is now recognized that may present with a wide range of symptoms beyond gastrointestinal ones. Celiac disease continues to pose significant challenges due to the continuous advancement of knowledge in understanding its pathophysiology, diagnosing the condition, managing its effects, and exploring potential therapeutic approaches. The prevalence of celiac disease is increased among individuals with chronic kidney disease, also. The most frequent associations are with diabetic nephropathy, IgA nephropathy and urolithiasis. A gut-kidney axis has been recognized to play a significant role in chronic kidney diseases. This literature review aims to review the chronic renal pathology associated with celiac disease, with emphasis on childhood.

KEYWORDS
celiac disease, chronic kidney disease, children, IgA nephropathy, diabetic nephropathy

1 Introduction

Celiac disease is an autoimmune condition that affects the entire body, causing disruptions in the internal balance and resulting in specific changes in the tissue structure of the small intestine. These changes include significant villi damage with increased crypt formation. Clinically, the disease presents a diverse range of symptoms, spanning from gastrointestinal issues like diarrhea, bloating, weight loss, and abdominal discomfort to non-digestive symptoms such as iron deficiency anemia, delayed puberty, and oral ulcers. These symptoms collectively stem from varying levels of malabsorption (1, 2).

Celiac disease is correlated with a number of autoimmune disorders, including type 1 diabetes and thyroid disease, which are classified as "associated conditions" or conditions that exhibit an elevated incidence but are not directly attributable to gluten consumption (2).

CD is a type of T-cell enteropathy characterized by an abnormal immune response to gluten, a protein found in wheat, barley, and rye. The estimated global prevalence of CD ranges from 0.7%–1.4% (3). CD can affect individuals of all ages and may present with a wide range of symptoms beyond just gastrointestinal problems (4).

CD continues to pose significant challenges due to the continuous advancement of knowledge in understanding its pathophysiology, diagnosing the condition, managing its effects, and exploring potential therapeutic approaches. As awareness of CD has increased, healthcare professionals have become better at identifying atypical and asymptomatic cases. Thus, more adults are now being diagnosed with CD, indicating that the disease's clinical spectrum is broader than previously understood (5).

The identification of tissue transglutaminase as an immunological target has offered a reliable first-line diagnostic test for CD (6). The introduction of screening for CD has enabled the diagnosis of certain cases even in the absence of typical digestive symptoms (7). According to recent studies (8, 9), the occurrence of CD is twice as common in children compared to adults, and 1.5 times higher to women than men. Although small intestinal biopsy remains the "gold standard" in the diagnosis of CD, current serological tests, such as tissue transglutaminase, endomysial gliadin and de-amidated peptide antibodies, have become more important in the diagnosis of the disease (5). The long-term effects of using this new no-biopsy approach on adherence to a gluten-free diet have yet to be evaluated (1, 10).

Currently, the only treatment for CD remains the strict gluten-free diet throughout life, which leads to improved quality of life, relief of symptoms and prevention of reducible complications, such as intestinal lymphoma and intestinal adenocarcinoma (11, 12). CD also presents a variety of extraintestinal manifestations, which affect the skin, liver or joints, nervous system or kidney (13, 14).

The prevalence of CD is increased among individuals with chronic kidney disease (CKD), as evidenced by current studies (15). Also, an increased risk of end-stage renal disease (ESRD) has been associated with CD (16). The most frequent associations are with diabetic nephropathy, IgA nephropathy and reno-urinary lithiasis (17, 18).

A gut-kidney axis has been recognized to play a significant role in chronic kidney diseases. Disruption of the gut barrier can lead to the abnormal entry of bacterial lipo-polysaccharide (LPS) endotoxin into the circulation. This abnormal entry contributes to uremic toxicity and systemic inflammation, which are factors implicated in the development and progression of chronic kidney diseases (19, 10).

This literature review aims to review the chronic renal involvement associated with celiac disease, with emphasis on childhood.

2 Material and methods

The scientific literature was browsed using Scopus, PubMed, and Embase da-ta-bases by searching the following keywords: "celiac disease," "chronic kidney disease," "T-cell enteropathy," and "children," in various combinations. The search was limited to open-access articles published in English within the last 10 years. Studies that mostly focused on creating a full definition of celiac disease in children were included. These studies were especially interested in the extradigestive symptoms of the disease, with a focus on kidney involvement. Articles that were vague and did not focus on specific aspects of the subject were excluded from the review process, but they were still used for general informational purposes.

3 Diabetic kidney disease and celiac disease in children

Diabetic kidney disease (DKD) has been observed concurrently with the increased incidence of diabetes mellitus (DM) in children. The kidney disease associated with DM in children and adolescents is represented by persistent albuminuria, arterial hypertension, the progressive decrease in the glomerular filtration rate, with the possibility of evolution towards the final stage (20). The association of type 1 diabetes (T1DM) with CD is known, and its prevalence in children varies from 3%–12% (20). A significant study was developed in Sweden, where patients with T1DM are routinely screened for CD. The risk of kidney disease in patients with type 1 diabetes mellitus and coexisting CD was evaluated in a cohort of 954 patients with both T1DM and CD, comparing the prevalence of CKD with that of patients diagnosed with T1DM alone (21). The authors found similar percentages of patients who developed chronic kidney disease in both groups.

Previous studies discussing the incidence of CKD in patients with T1DM and CD are limited to the adults and report conflicting results. Gopee et al., showed that the gluten-free regimen in children with CD + T1DM has a renoprotective effect, by decreasing albuminuria, compared to those who only have T1DM (22). The same HLA predisposition to CD influences the development and progression of albuminuria (23).

The Genetics of Kidneys in Diabetes (GoKinD) study included 1,879 individuals with long-term evolution of T1DM, half of them with diabetic nephropathy. Involvement of DRB1 is consistent with the known immunological processes in the pathogenesis of diabetic nephropathy, especially in the Late Autoimmune Diabetes of Adults, less in children. There is an inverse and separate relationship between the risk of CD and the age at which diabetes begins, with a greater risk observed in children under 4 years old compared to those over 9 years old. The mechanism of association of these two diseases involves a shared genetic background: HLA genotype DR3-DQ2 and DR4-DQ8 (23, 24).

Another study investigating the risk of ESRD in CD patient showed that the effect of type 1 diabetes on the risk was only marginal (16). The review by Boonpheng et al. (18) demonstrated that CD can be independently related to kidney dysfunction, regardless of the development of type 1 diabetes.

CD is also an independent risk factor for microvascular complications related to diabetes, but also for macrovascular complications (25) especially an increased risk the intima-medial thickness of the carotid arteries (26). The established association between CD and microvascular complications in diabetes is another independent argument in favor of screening for CD in the patient with T1DM (27). However, the mechanism behind how CD increases the risk of microvascular disease in patients with T1DM remains unclear and is likely to involve multiple

factors (28). One such factor is the association of unrecognized CD with elevated homocysteine levels, which may be attributed to deficiencies in folic acid and other B vitamins, contributing to endothelial dysfunction (29). A recent study indicated that supplementing vitamin B in individuals with CD was linked to significantly lower homocysteine levels, potentially reducing the risk of vascular disease (30).

4 IgA nephropathy and celiac disease

IgA nephropathy (IgAN), identified almost 60 years ago by Berger (31), is one of the most common glomerulonephritis reported worldwide (32). IgA antibodies are produced by B cells in the mucosal tissues, such as the lining of the gut and airways. When these antibodies are activated, they can form immune complexes that can deposit in the kidneys and damage the glomeruli (33). Immunoglobulin A, the hallmark of mesangial deposits of IgAN, is the main immunoglobulin in mucous secretions (34). Recent data suggest that the dysregulated gut mucosal immunity, genetic conditioning, gut dysbiosis, and diet play a combined role in development and progression of IgAN (35). People who have a genetic predisposition to IgA nephropathy in high-risk groups may be able to be prophylactically screened for the CD and closely monitored by immunohistochemical methods or identified by genetic testing. The prognosis for IgA nephropathy varies depending on the severity of the disease. However, with early diagnosis and treatment, most people with IgA nephropathy can live a normal life (36). The link between a dysregulated gut-associated lymphoid tissue (GALT) and IgAN was hypothesized following the observation of a higher association between IgA nephropathy and celiac disease (37). However, in people with IgAN, GALT can become dysregulated, leading to the production of abnormal IgA antibodies. These antibodies can then deposit in the kidneys, causing inflammation and damage. The link between GALT and IgAN has been supported by a number of studies. A genome-wide association study (GWAS) found that most loci associated with the risk of IgAN are also associated with immune-mediated inflammatory bowel diseases, maintenance of the intestinal barrier and regulation of response to gut pathogens. This suggests that exposure to intestinal microbes may play a role in the development of IgAN, particularly in people who are genetically predisposed to the disease (38).

The production of mucosal IgA is induced both by mechanisms dependent on T lymphocytes and by mechanisms independent of them (39). After priming, B cells migrate to the effector zone of the mucosa-associated lymphoid tissue (MALT), located in the lamina propria of the mucosa, where they release dimeric IgA, composed of two IgA molecules and a connecting chain (35). Secretory IgA has enhanced bacteriostatic effects due to antimicrobial peptides, such as defensins, which are secreted into the intestinal lumen.

In the last decade the role of gut microbiota in patients with IgAN has been intensive investigated (40). Toll-like receptors (TLRs), such as LPS (TLR4) and lipoteichoic acid (TLR2), are significantly expressed on mucosal epithelial cells and recognize the molecular patterns of microbes (41). Gut microbiota signals the gut injury response through TLRs (42). An altered intestinal barrier facilitates increased absorption and circulation of LPS (43). Studies have indicated the presence of increased intestinal permeability in patients with IgAN (35, 40).

The causal association between IgAN and CD was analyzed in prospective study, by screening patients with IgAN for CD and explored the utility of analysis of IgA anti-TG2 antibody deposits. IgA anti tTG2 Ab colocalization study in kidney biopsies of patients with suspected celiac associated IgA nephropathy were found, but a small proportion of patients with IgAN have associated CD (44).

The gluten-free diet has been shown to be effective in treating IgA nephropathy in people with celiac disease. The gluten-free diet helps to reduce the production of IgA antibodies, which can lead to the disappearance of IgA deposits in the glomeruli and improvement of the condition of the glomeruli (45).

Children with IgA Vasculitis or just IgAN have increased expression of TRL4 mRNA in the periphery lymphomononuclear cells, which was correlated with signs of activation of innate immunity and proteinuria (35, 46). These observations lead to the hypothesis that increased intestinal permeability in intestinal microbes it triggers, by activating TLR4, immune system activation detected in IgAN. This mechanism correlates with systemic inflammation and oxidative stress (35).

In celiac disease, increased intestinal permeability is a distinctive feature (47). Many studies suggest the existence of analogies between intestinal barrier disruption in patients with CD and those with IgAN. Gluten aggravates IgAN through the interaction of gliadin with CD89, favoring the formation of the IgA1–sCD89 complex (35, 45). In both IgAN and celiac disease, the transferrin receptor (TFR) is associated with transglutaminase 2 (TG2), both of which are present in enterocytes and mesangial cells.

Tight junctions represent the foremost junctional complex located at the apex of cellular structures, serving to establish a polarized arrangement that segregates the apical and basolateral poles of the cells (48). In celiac disease, increased intestinal permeability results from disruption of intestinal epithelial cell tight junctions (TJ), leading to increased absorption of dietary antigens (48, 49). Gliadin, the lectin component of gluten, is deamidated by TG2 and presented to T cells in the context of HLA-DQ2 or HLA-DQ8 molecules on dendritic cells or macrophages. Interaction with CD4+ T cells produces a TH1 response, generating proinflammatory cytokines, resulting in mucosal atrophy and a further increase in intestinal permeability. This process also generates a TH2 response, with the production of antibodies by B lymphocytes in the GALT (the lymphoid tissue associated with the intestinal mucosa). Thus, the production of IgA and IgG against gliadin and TG2 is stimulated. Most patients with CD associate the HLA-DQ2/DQ8 haplotype and show anti-gliadin and anti-TG2 antibodies (35) (Figure 1).

In patients with IgAN, it is hypothesized that a similar intestinal permeability mechanism might be active, with the release of anti-gliadin IgA into the circulation to form macromolecular aggregates with IgA1/sCD89, which ultimately

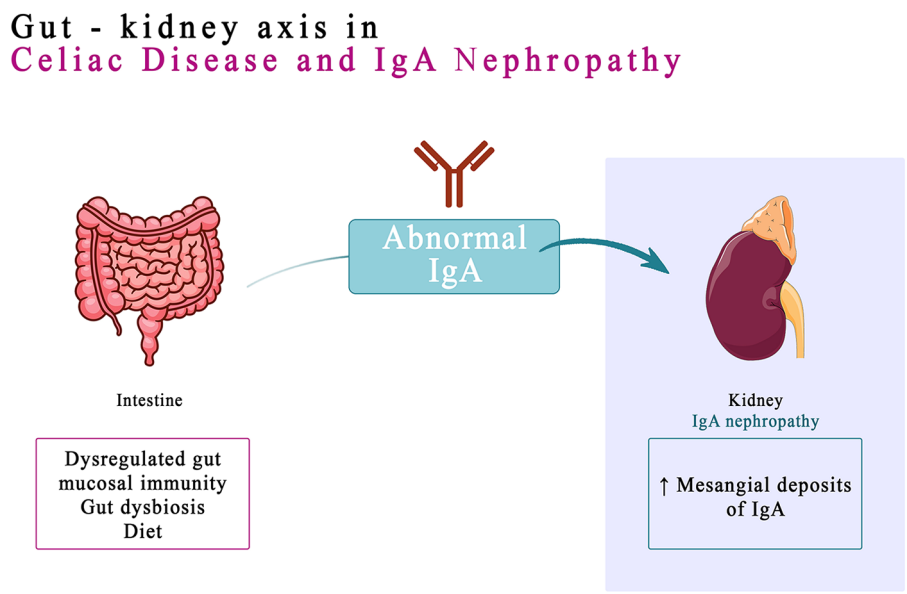

FIGURE 1
Pathogeny of IgA nephropathy in celiac disease.

deposit in the mesangial tissue (35). The detection of IgA anti-TG2 deposits in the mesangial tissue is a reliable technique for establishing an association with CD in these patients (43). The correlation is also supported by studies that show the remission of IgAN associated with CD in patients subjected to a gluten free diet (GFD) for at least 5 years (49). The link between IgA nephropathy and CD should be kept in mind. CD may be associated with a progressive evolution of CKD towards the end stage (50).

5 Urolithiasis and celiac disease

The first report of the association between CD and urolithiasis in children was made by Ogilvie et al. in the 1970s. The authors found that over half of CD patients had hyperoxaluria (51). Recent studies show increased risks of recurrent lithiasis, especially oxalic, in patients with CD (52, 53).

Urine supersaturation with calcium, phosphates, oxalates or cystine is necessary but not sufficient to explain the formation of calculations. The absorption defect of one or more compounds in the gut, or a defect in the renal handling of these compounds, causes supersaturation, primum movens in initiating the formation of kidney stones. Practically, the imbalance between the factors promoting and inhibiting the formation of stones (malabsorption, increased absorption of oxalates, cystine, calcium, vs. decreased absorption of citrate, magnesium, pyrophosphate at the intestinal level) leads to urinary supersaturation with the possibility of initiating crystallization on a support of cellular detritus. Alternatively, it can be speculated that autoantibodies present in CD may accompany urolithiasis. Relevant for this hypothesis, was a study in which anti-SS-A anti-bodies were detected in patients with urolithiasis, who developed Sjögren's syndrome. In another study, the antinuclear antibodies were positive in patients with hypocitraturia known favoring factor for urolithiasis (53–55).

Disturbances in the gut microbiome and metabolome may thus be determinants of early-onset disease and may explain the association between antibiotics and nephrolithiasis. In addition, decreased butyrate production and decreased oxalate degradation among people with early-onset kidney stones has been proven in studies. Roseburia species, (1% of all bacteria present in the gut microbiome) produce butyrate, a short-chain fatty acid that is a mediator of inflammation, helping to maintain the gut mucosal barrier and regulating the expression of oxalate transporters in the gut (56). Dysbiosis contributes to the loss of this species of beneficial bacteria, leading to the mediation of local inflammation, with increased oxalate absorption, which explains hyperoxalic lithiasis (57).

6 Other nephropathies and celiac disease

A few studies have reported the occurrence of other glomerulopathies (except IgAN) in the context of celiac disease, including membranous nephropathy and membranoproliferative nephropathy (4). Interestingly, in four case reports, membranoproliferative nephropathy showed improvement following a gluten-free diet (18).

The coexistence of CD and nephrotic syndrome is an exceptionally uncommon occurrence (58). In celiac disease, exposure to gliadin triggers the release of zonulin from enterocytes. High serum levels of zonulin activates protease-activated receptor 2 (PAR2) in a paracrine manner, and determine the disruption of the actin cyto-skeleton and cell-cell junctions in the intestinal epithelium (59, 60). PAR2 is also expressed on podocytes. The increased levels of serum zonulin induced by gluten ingestion may result in enhanced ligand binding to the PAR2 receptor on podocytes. Consequently, alterations in the podocyte cytoskeleton can impact cell motility and attachment to the glomerular basement membrane, potentially leading to proteinuria (61). Focal segmental glomerulosclerosis (FSGS) is a cause of nephrotic syndrome in children and adolescents, as well as an important cause of renal failure in adults (62). Isolated cases of membranous nephropathy (MN) associated with CD have been documented. Giménez et al. reported a series of five children with nephrotic syndrome who developed CD. In the renal biopsy performed revealed lesions of the MCD type or mesangial glomerulonephritis with IgM deposits, without IgA deposits, whose coexistence with CD is exceptional (63). The connection between CD and MN is associated with the common autoimmune pathogenesis, proven in studies by the remission of proteinuria simultaneously with the disappearance of IgA anti-tissue transglutaminase antibodies, sometimes without immunosuppression, only with antiproteinuric therapy and a gluten-free regime (64, 65).

Mukta Mantan et al. cited a 2007 study from the Mario Negri Institute for Pharmacological Research in Milan, Italy, which examined the relationship between bread consumption and the risk of kidney cancer. Individuals with the highest bread consumption had a 94% greater likelihood of developing kidney cancer than those with the lowest bread consumption (66).

7 Distal renal tubular acidosis and celiac disease

Renal distal tubular acidosis (RTA) is a significant cause of refractory rickets and stunting in children. In rare cases, distal RTA may be autoimmune in nature, suggested by its association with other autoimmune conditions such as Sjogren's syndrome, systemic lupus erythematosus (SLE), and Hashimoto's thyroiditis (67).

The hereditary form of distal renal tubular acidosis (RTA) is primarily caused by autosomal recessive mutations in genes that encode subunits of the vacuolar H + ATPase. These mutations lead to impaired transporter function in the renal tubules (67, 68). The acquired form of distal RTA is more frequently observed in adults and is often associated with autoimmune conditions, notably Sjögren's syndrome (69, 70).

Although the association of CD with Sjögren's syndrome and distal RTA was previously reported in adults (71), the association of distal tubular acidosis with celiac disease, without other autoimmune determinations, was reported only in a study in children (67).

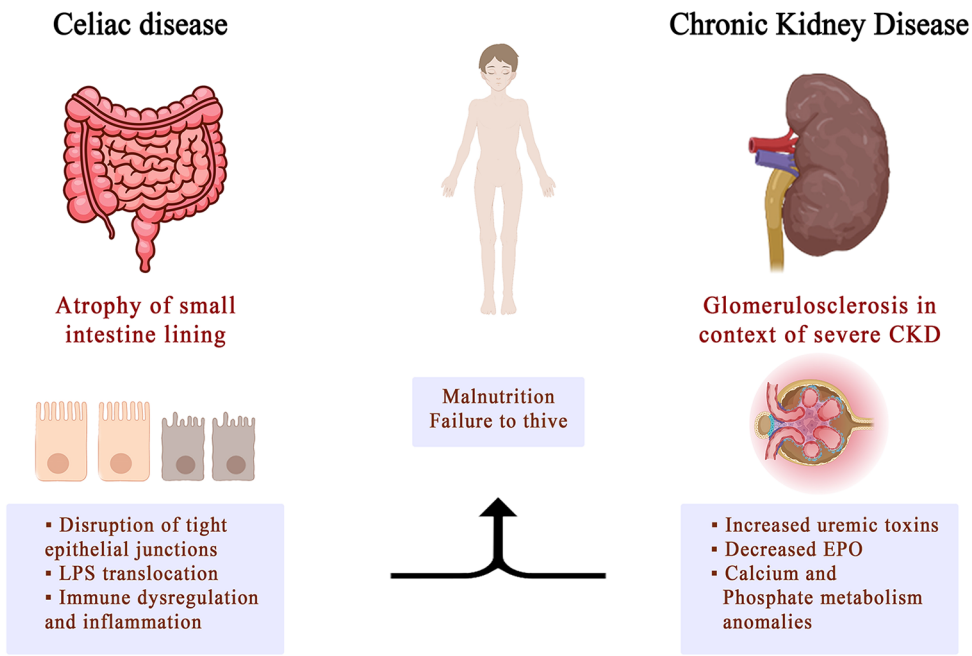

FIGURE 2
Implication of celiac disease in chronic kidney disease.

8 Urinary tract infection and celiac disease

Increased urinary tract infection (UTI) incidence in patients with CD has been attributed to an associated disorder of the urinary system, including tract motility impairment, urinary bladder dysfunction, changes in intestinal bacterial flora that predispose to urinary tract contamination, reduced immune defenses against infections, or altered immunity (72). At the same time, the reverse relationship can also be valid. A simple urinary tract infection can acutely trigger an immune reaction that causes a celiac crisis in a predisposed individual (73). Celiac crisis is the hyperacute manifestation of CD, sometimes the onset of a previously undiagnosed gluten enteropathy.

9 Final considerations

CD is often accompanied by various extraintestinal manifestations, which classifies it as a systemic disease rather than a gastrointestinal condition. Many of the extraintestinal manifestations of CD involve the kidneys. These include conditions like urolithiasis and crystal-induced kidney disease, membranoproliferative glomerulonephritis, IgA nephropathy, or an increased risk for progressing to end-stage renal disease (Figure 2).

However, there is limited knowledge about the risk of kidney disease in children with celiac disease. To date, only a few studies have investigated the risk of renal disease among patients with CD, especially adult patients, and there are no current recommendations for screening for renal involvement in these patients. More studies and pro-longed follow-up are needed to evaluate the connections between celiac chronic kidney diseases and celiac disease, especially in childhood where the experience in limited.

Author contributions

IMS: Conceptualization, Writing – original draft. IM: Supervision, Writing – review & editing. AL: Methodology, Writing – original draft. II: Conceptualization, Writing – original draft. MA: Investigation, Writing – original draft. AA: Investigation, Writing – original draft. IDM: Investigation, Visualization, Writing – review & editing. VM: Visualization, Writing – review & editing. VLL: Project administration, Writing – review & editing. AM: Methodology, Writing – original draft.

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Identification and treatment of intestinal malrotation with midgut volvulus in childhood: a multicenter retrospective study

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Background: Intestinal malrotation is a rare condition, and its delayed diagnosis can lead to fatal consequences. This study aimed to investigate the identification and treatment of malrotation in children.
Methods: Clinical data, imaging, operative findings, and early postoperative outcomes of 75 children with malrotation were retrospectively analyzed.
Results: The mean age was 6.18 ± 4.93 days and 51.26 ± 70.13 months in the neonatal group (56 patients) and non-neonatal group (19 patients), respectively. Sixty-seven patients were under the age of 1 year at the time of diagnosis. The occurrence of bilious vomiting and jaundice was significantly higher in the neonatal group (89.29%) than that in the non-neonatal group (37.5%), $p < 0.05$ and $p < 0.01$, respectively. The incidence of abnormal ultrasound (US) findings was 97.30% and 100%, respectively, and the sensitivities of the upper gastrointestinal series were 84.21% and 87.5%, respectively. Sixty-six (88%) patients had midgut volvulus, including *in utero* volvulus (two patients) and irreversible intestinal ischemia (four patients). Most neonates (89.29%) underwent open Ladd's procedure with a shorter operative time ($p < 0.01$). Reoperation was performed for postoperative complications (four patients) or missed comorbidities (two patients).
Conclusions: Non-bilious vomiting was the initial symptom in >10% of neonates and nearly 40% of non-neonates. This highlights the importance for emergency physicians and surgeons to be cautious about ruling out malrotation in patients with non-bilious vomiting. Utilizing US can obviate the need for contrast examinations owing to its higher diagnostic accuracy and rapid diagnosis and can be recommended as a first-line imaging technique. Additionally, open surgery is still an option for neonatal patients.

KEYWORDS
intestinal malrotation, midgut volvulus, Ladd's procedure, bilious vomiting, children

Abbreviations
SMA, superior mesenteric artery; SMV, superior mesenteric vein; US, ultrasound; UGI, upper gastrointestinal; MR, magnetic resonance; CT, computed tomography.

Introduction

Intestinal malrotation, a congenital rotational anomaly, refers to the abnormalities of the intestinal position resulting from non-rotation, incomplete rotation, or abnormal fixation of the embryonic gut (1). The distal duodenum does not reach the left side of the abdomen and, therefore, lacks the stability usually provided by the ligament of Treitz. It is estimated that 3.9–20 of 10,000 newborns present with intestinal malrotation (2–4), which may complicate acute, intermittent, or chronic midgut volvulus in children and adults (5). A high index of suspicion is vital for its accurate and rapid identification (5). However, acute malrotation with volvulus, a life-threatening emergency with high morbidity and mortality, is difficult to diagnose in the non-verbal pediatric populations (3, 6, 7).

Upper gastrointestinal series (UGIs) remains the gold standard for diagnosing malrotation with or without volvulus (8). However, in recent years, ultrasound (US) has been used as the first-line modality for diagnosing malrotation with volvulus (9), even during prenatal screening (10, 11). The treatment of malrotation with volvulus is challenging for emergency physicians and pediatric surgeons. Ladd's procedure is curative for malrotation with good outcomes; however, a higher rate of emergency reoperation has been reported in older children (12). Moreover, controversy remains regarding a surgical approach to Ladd's procedure, and there is no evidence that a laparoscopic Ladd's procedure is superior to open surgery, especially during the neonatal period (5, 13–17).

This study aimed to review the multicenter experience to provide useful information regarding the identification of intestinal malrotation and evidence to support the superiority of laparoscopic Ladd's procedure over open surgery or vice versa in pediatric patients.

Patients and methods

Clinical data

Clinical data from six tertiary hospitals between January 2006 and June 2022 were retrospectively reviewed. The inclusion criteria were those aged ≤18 years and had surgically confirmed intestinal malrotation. Keywords including intestinal malrotation, congenital intestinal malrotation, volvulus, duodenal stenosis or atresia, and annular pancreas were used in the digital integrated case management system. The variables, including demographics (sex, age, and birth weight), medical history, symptoms and signs, laboratory and radiological findings, surgical procedures, and outcomes (operative time, time to initial enteral feeding, length of hospital stay, and occurrence of complications) were collected and analyzed. The time to diagnosis was defined as the period from admission to the initiation of anesthesia. Based on the age at admission, the patients were divided into two groups: the neonatal group (aged ≤28 days) and the non-neonatal group (aged >28 days and ≤18 years). Based on the different surgical approaches, the patients were divided into three groups: open, laparoscopic, and laparoscopic conversion to open Ladd's procedure.

Ethical considerations

All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or National Research Committee, and the present study adhered to and was conducted according to the principles of the Declaration of Helsinki in 1964 and its subsequent amendments or comparable ethical standards. Institutional review board approval was not needed, as it is a retrospective analysis. Written informed consent was obtained from the parents/legal guardians of all children involved in the study.

Statistical analysis

All values of quantitative and qualitative variables were expressed as mean and standard deviation or as a percentage. Comparisons were made using a *t*-test or χ^2 test. SPSS software (version 20.0; SPSS, Inc., Chicago, IL, USA) was used for the statistical analysis. A *p*-value of <0.05 was considered statistically significant.

Results

Seventy-five patients met the inclusion criteria. The data for the entire cohort of patients based on age of onset are summarized in Table 1. Of the 75 patients, 56 (74.67%) belong to the neonatal group and 19 (25.33%) belong to the non-neonatal group. The mean age was 6.18 ± 4.93 days and 51.26 ± 70.13 months in the neonatal and non-neonatal groups, respectively. Approximately three-quarters of patients (76%) were male. Patients presented with vomiting (98.67%), bilious vomiting (82.67%), abdominal distension (37.33%), jaundice (29.33%), hematochezia (9.33%), and hematemesis (2.67%). Vomiting occurred in all neonates and in 94.74% of the non-neonatal patients. Of the 56 neonatal patients, 50 presented with bilious emesis and 21 had jaundice, while in the non-neonatal group, 12 presented with bile-stained emesis and 1 with jaundice ($p = 0.024$ and $p = 0.008$, respectively).

The median time to confirm malrotation was 22.5 h (2.5–480 h) in the neonatal group and 17 h (2–288 h) in the non-neonatal group ($p = 0.916$). More than 24 h was required to confirm the diagnosis in half of the patients (50.67%).

The diagnostic tools included US in 48, abdominal radiography in 39, UGIs in 27, and contrast enema in 13. Abnormal plain abdominal radiographs were observed in 30 patients, including air–fluid level, double bubble sign, intestinal gas accumulation, intestinal obstruction, and pneumoperitoneum. Air–fluid level or scant intestinal gas (Figure 1A) was found in five non-neonatal patients. UGIs revealed duodenal obstruction and/or an abnormal duodenal position of the duodenojejunal junction in 16/19 (84.21%) in the neonatal group and 7/8 (87.50%) in the non-neonatal group (Figure 1B). The contrast enema showed an abnormal position of the cecum in eight out of nine (88.89%) neonatal patients and in all four non-neonatal patients.

Abnormal US findings were found in most neonatal patients (36/37, 97.3%) and all 11 (100%) non-neonatal patients,

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TABLE 1 Comparison of clinical data of the patients.

Variable	Total	Newborns	Non-neonatal cases	*p*-value
Number of patients (*n*, %)	75	56 (74.67)	19 (25.33)	
Gender (male:female)	57:18	44:12	13:6	0.559
Mean age (days), mean (SD)		6.18 (4.93)	51.26 (70.13)	
Mean weight (kg), mean (SD)		3.05 (0.58)	19.02 (19.89)	
Initial clinical manifestation				
Emesis (*n*, %)	74 (98.67)	56 (100)	18 (94.73)	0.253
Bilious emesis (*n*, %)	62 (82.67)	50 (89.29)	12 (63.16)	0.024
Non-bilious emesis (*n*, %)	12 (16.00)	6 (10.71)	6 (31.58)	
Hematemesis (*n*, %)	2 (2.67)	1 (1.79)	1 (5.26)	0.445
Hematochezia (*n*, %)	7 (9.33)	7 (12.50)	0	0.117
Jaundice (*n*, %)	22 (29.33)	21 (37.50)	1 (5.26)	0.008
Abdominal distension (*n*, %)	28 (37.33)	22 (39.29)	6 (31.58)	0.548
Abdominal x-rays (*n*, %)	39	32 (57.14)	7 (36.84)	0.126
Abnormal findings	30	25	5	0.653
Double bubble sign	4	4	0	0.462
Air–fluid level	20	15	5	0.109
Intestinal gas accumulation	4	4	0	0.462
Pneumoperitoneum	1	1	0	0.833
Intestinal obstruction	1	1	0	0.833
UGIs (*n*, %)	27	19 (33.93)	8 (42.11)	0.521
Duodenal obstruction/DJJ abnormal position (*n*, %)	23	16 (84.21)	7 (87.50)	0.663
Contrast enema (*n*, %)	13	9 (16.07)	4 (21.05)	0.885
Abnormal position of the cecum (*n*, %)	12	8 (88.89)	4 (100)	0.692
US (*n*, %)	48	37 (66.07)	11 (57.89)	0.521
Abnormal findings	47	36 (97.30)	11 (100)	0.771
Ascites	6	4	2	
Duodenal dilatation	20	17	3	0.411
SMA-SMV inversion	35	27	8	1.000
"whirlpool" sign	24	21	3	0.071
Intestinal wall thickening	1	0	1	0.234
CT scan (*n*, %)	2	0	2 (10.53)	0.062
"whirlpool" sign	2	0	2	
Time from onset to diagnosis [median time (hours)]		22.5 (2.5–480)	17 (2–288)	
≤12 h (*n*, %)		20 (35.71)	7 (36.84)	0.916
>12–24 h (*n*, %)		7 (12.5)	3 (15.79)	
≥24 h (*n*, %)		29 (51.79)	9 (47.37)	
Intraoperative findings				
Without volvulus (*n*, %)	9	8 (14.29)	1 (5.26)	0.524
With volvulus (°) (*n*, %)	66	48 (85.71)	18 (94.74)	0.876
180	14	9	5	
270	7	5	2	
360	26	20	6	
540	7	6	1	
720	12	8	4	
Irreversible bowel ischemia	5	4	1	
Chylous ascites/cyst (*n*, %)	12	7	5	0.290
Comorbid congenital anomalies (*n*, %)	20	15 (26.79)	5 (26.32)	0.968
Annular pancreas		4 (26.67)	0	
With congenital cardiac disease		1	0	
With Down's syndrome		1	0	
Duodenal web		3 (20.00)	0	
With Meckel diverticulum		1	0	
Intestinal atresia		3 (20.00)	0	
Heterotopic pancreas		2 (13.33)	1	
Intestinal duplication malformation		0	1	
Umbilical hernia		0	1	
Hydronephrosis		0	1	
Cyst of the mesentery		0	1	
Surgical approach				0.000
Open Ladd's (*n*, %)	59	50 (89.29)	9 (47.37)	
Laparoscopic Ladd's (*n*, %)	6	1 (1.79)	5 (26.32)	
Laparoscopic conversion to open Ladd's (*n*, %)	10	5 (8.93)	5 (26.31)	

SMV, superior mesenteric vein; SMA, superior mesenteric artery; UGIs, upper gastrointestinal series; US, ultrasound; CT, computed tomography; DJJ, duodenal–jejunal junction.

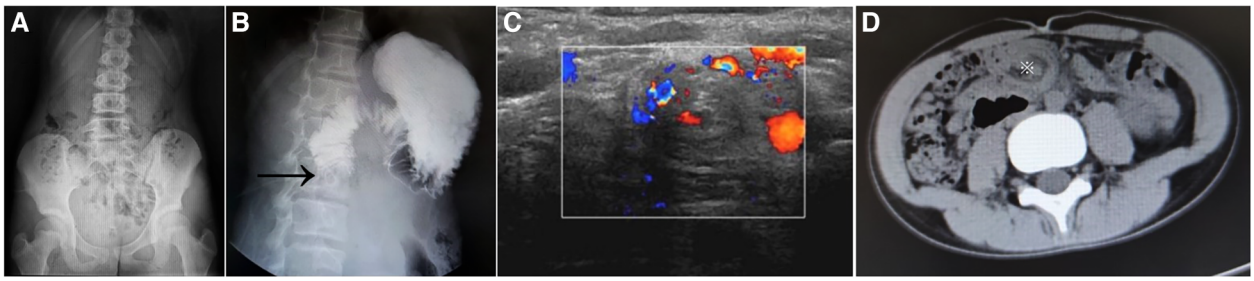

FIGURE 1
Plain abdominal film showed scant intestinal gas (A). The UGIs demonstrate obstruction of the second part of the duodenum (arrow) (B). Ultrasound shows the "whirlpool" sign, suggestive of malrotation with volvulus of 270°–360° (C). Axial CT image shows the "whirlpool" sign (asterisk) (D). UGIs, upper gastrointestinal series; CT, computed tomography.

including superior mesenteric artery–superior mesenteric vein (SMA-SMV) inversion ($n = 27$ vs. $n = 8$), "whirlpool" sign (Figure 1C) ($n = 21$ vs. $n = 3$), duodenal dilation ($n = 17$ vs. $n = 3$), and ascites ($n = 4$ vs. $n = 2$) between the neonatal and non-neonatal groups. Two non-neonatal patients showed a "whirlpool" sign at the base of the small bowel mesentery via CT scan (Figure 1D).

Open Ladd's procedure was performed in 50/56 (89.29%) of neonatal patients and 9/19 (47.37%) of non-neonatal patients, the laparoscopic procedure was performed in 1/56 (1.79%) and 5/19 (26.32%), and the remaining 10 patients required laparoscopic conversion to an open Ladd's procedure in 5/56 (8.93%) and 5/19 (26.32%), respectively. Operative time, initial postoperative feeding time, and hospital stay for the three surgical approaches are shown in Table 2. The operative duration for laparoscopic conversion to open Ladd's group (224.10 ± 61.234 min) differed significantly from that of open Ladd's (122.76 ± 65.429 min) and laparoscopic (134.00 ± 35.07 min) procedures ($p < 0.01$). However, no significant differences were detected between the open and laparoscopic Ladd's procedure groups. Similarly, there were no significant differences in the initial postoperative oral feeding time and length of hospital stay among the three surgical approach groups.

Intraoperative findings revealed 66 patients of complicated midgut volvulus (Figures 2A–C), including 48 neonates (180° in 9 patients, 270° in 5 patients, 360° in 20 patients, 540° in 6 patients, and 720° in 8 patients). Intestinal torsion of 360° was observed in one patient and 720° in two patients, which caused irreversible intestinal ischemia of 30, 35, and 80 cm in length, respectively, and required intestinal resection and primary anastomosis. Eighteen non-neonatal patients were complicated by midgut volvulus with torsions of 180° (five patients), 270° (two patients), 360° (six patients), 540° (one patient), and 720° (four patients).

Five neonates required reoperation because of early postoperative recurrent bowel volvulus (one patient), adhesive bowel obstruction (two patients), iatrogenic defect of the hepatic falciform ligament that caused internal hernia and acquired jejunal atresia (one patient), or missed coexisting annular pancreas (one patient). Postoperative recovery was uneventful in all patients.

Discussion

The midgut, perfused by the superior mesentery artery, completes its normal rotation and fixation at 10 weeks of gestation. Malrotations occur in approximately 0.2%–1% of the population (4). Abnormal gut rotation and fixation to the retroperitoneum/peritoneum lead to malrotation of the intestine, usually including non-rotation, malrotation or incomplete rotation, and inverse rotation, depending on the degree and direction of midgut rotation (18, 19). In our case series, all patients had malrotation or incomplete malrotation, which was rotated by 180° and recessed. Abnormal midgut fixation leads to a short mesenteric attachment, which makes the midgut prone to twisting (volvulus) around the SMA and SMV (4, 20–24).

Williams et al. (20) reported that the incidence of malrotation with volvulus decreases with age. In the present case series, 85.71% of neonates had midgut volvulus at the time of presentation, and the incidence was still higher in non-neonatal patients (18/19, 94.74%). Delayed diagnosis of midgut volvulus

TABLE 2 Operative time and postoperative outcome among the three groups.

Variables	Open Ladd's ($n = 59$)	Laparoscopic Ladd's ($n = 6$)	Laparoscopic conversion to open Ladd's ($n = 10$)	p-value (intergroup comparisons)
Operative time (minutes), mean (SD)	122.76 (65.429)	134.00 (35.07)	224.10 (61.234)	<0.01
Time to initial oral feeding (days), mean (SD)	4.47 (2.381)	4.50 (4.722)	3.40 (1.578)	0.221
Hospital stay (days), mean (SD)	14.51 (7.302)	14.17 (5.115)	14.40 (5.700)	0.981
In-hospital mortality, n	0	0	0	
30-day mortality, n	0	0	0	

FIGURE 2
An 8-day-old boy with bilious vomiting for 8 h, malrotation with midgut volvulus suspected by UGIs. Intraoperative views by open surgical approach show complete small bowel ischemia (black arrow) (**A**,**B**) due to a midgut torsion of 720° (blue arrow) (**B**). After torsion reduction, intestinal viability is restored (**C**), and the dilated mesenteric vein caused by venous congestion is shown (violet arrow). UGIs, upper gastrointestinal series.

usually leads to massive intestinal ischemia and necrosis, presenting with hematemesis, hematochezia, abdominal distention, peritonitis, severe shock, and multiple organ dysfunction (4, 24, 25–28). Bile-stained vomiting, usually indicating severe gut pathology, must be taken seriously by all surgeons and general pediatricians (20).

Shalaby et al. (24) reported that 82% of patients experienced midgut volvulus during emergency surgery, and its acute presentation is less common beyond the neonatal period. In this study, the most frequently associated anomalies were the annular pancreas (26.67%), duodenal web (20%), intestinal atresia (20%), and ectopic pancreas (13.33%). Three of the 15 neonates had multiple anomalies, including Meckel's diverticulum, congenital heart disease, gastrointestinal duplication cyst, and trisomy 21, similar to the results described by Kedoin (1), Shehata (29), and Azzam et al. (31). Although coexisting intestinal malrotation and enteric duplication cyst or cardiac anomalies are rare, children with gut duplication or congenital heart problems should be aware of accompanying congenital malrotation (30, 31).

Although a diagnosis of malrotation should always be considered in children with bile-stained vomiting at any age (20, 26, 32), more than 10% of neonates in our study presented with symptoms of non-bilious vomiting. Similarly, bilious vomiting occurred in only 63.16% of non-neonatal patients, and other symptoms included diarrhea, small intestinal obstruction, malnutrition, and failure to thrive, making the diagnosis of malrotation more difficult (2, 14, 22, 28, 32). These findings were similar to those of a previous meta-analysis (23).

UGIs (28, 33, 34) can help make an early accurate diagnosis in different age groups. UGI examination is associated with a sensitivity of 54%–86.5% for detecting midgut volvulus (24, 27, 34). In our case series, the sensitivities of UGIs were 84.21%–87.5%. However, some subtle abnormalities and normal variants can be misinterpreted as normal or mistaken for malrotation.

US can be a useful screening and diagnostic tool (2, 24, 35–40). Abdominal US should be performed in patients with normal duodenojejunal junction on the UGIs and persistent bilious emesis (36). In our case series, the sensitivity of diagnostic US was 97.3% and 100% in the neonatal and non-neonatal groups, respectively. If a midgut volvulus exists, abdominal US can detect "whirlpool" signs, mesenteric edema, ascites, and SMV dilation (23). If the US examination is non-diagnostic and the patient is in a non-acute condition, it is vital to repeat a contrast meal to obtain optional information to diagnose malrotation (20, 40). Moreover, experienced pediatric surgeons and radiologists should play an important role in reviewing equivocal UGIs to make decisions regarding further patient care and avoid significant delays in diagnosis.

In terms of the treatment of malrotation, Ladd's procedure is usually curative (12, 24, 41). The principles of Ladd's procedure for malrotation at any age have remained unchanged with good outcomes (24, 42). However, in older children, intermittent chronic obstruction may lead to a higher emergency reoperation rate. Ladd's procedure with duodenojejunal bypass should be considered (12).

In our case series, approximately half of the non-neonatal patients received a laparoscopic or laparoscopic conversion to open Ladd's procedure, while nearly 90% of neonatal patients underwent open surgery. Laparoscopic conversion to open Ladd's procedure required a significantly longer operative time than open or laparoscopic surgery. Our study revealed that patients, especially small babies, with a high risk of conversion to an open Ladd's procedure should be identified preoperatively using the risk analysis method in future studies (5, 41). Zhu et al. (16) reported that the laparoscopic procedure for neonates with malrotation was associated with a higher risk of recurrent volvulus than open Ladd's procedure.

In terms of the management principles of patients with acute midgut volvulus and hemodynamic instability, aggressive intravenous fluid resuscitation and urgent exploratory laparotomy should be the first choices (5, 24). In terms of reoperation, the causes for reoperation were early postoperative recurrent bowel volvulus, adhesive intestinal obstruction, missed diagnosis of coexisting annular pancreas, internal hernia, and congenital duodenal web (12, 43).

This study has several limitations. The data were collected from six medical centers to improve the sample size to some extent. The total duration of the analyses was 12 years, during which time different clinical management and outcomes were inevitably achieved due to the constant updating of clinical guidelines and imaging techniques; thus, a partial risk of bias cannot be ruled out. The homogeneity of the multicenter retrospective study will be considered in future studies. Regarding the time blocks in

patient management, several variables included time and location of initial presentation, US and UGI studies, and surgical consultation. Sabac et al. (26) reported that the median time from initial physician assessment and surgical consultation to surgical incision was over 14 h. The present study did not analyze the effects of these variables on the time to diagnosis. Predicting which patients will develop massive intestinal necrosis is difficult (44), multicenter clinical trials are needed to further assess how long a patient could stand volvulus and help create optimal strategies for malrotation (26).

In conclusion, malrotation with or without midgut volvulus is not rare and should be considered across all age groups. More than 10% of neonatal and nearly 40% of non-neonatal patients with malrotation present with non-bilious vomiting as the initial symptom. Diagnostic suspicion and interdisciplinary coordination are essential for the timely recognition and surgical treatment. Artificial intelligence and machine learning as a novel technique will be helpful in the detection and treatment of malrotation with volvulus (45, 46).

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The requirement of ethical approval was waived by Binzhou Medical University Hospital for the studies involving humans because this study is a retrospective analysis, and institutional review board approval was not needed. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

XY: Conceptualization, Data curation, Writing – original draft, Investigation, Methodology. WW: Data curation, Investigation, Writing – original draft. KW: Data curation, Investigation, Writing – original draft. JZ: Data curation, Investigation, Writing – original draft. LS: Data curation, Investigation, Writing – original draft. SJ: Data curation, Writing – original draft, Methodology. YW: Data curation, Writing – original draft, Investigation. WF: Data curation, Investigation, Writing – original draft. GD: Data curation, Investigation, Writing – original draft. TF: Data curation, Writing – original draft, Conceptualization, Project administration, Software, Supervision, Validation, Writing – review & editing. AL: Conceptualization, Methodology, Supervision, Validation, Writing – review & editing. LG: Conceptualization, Investigation, Methodology, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The etiology and differential diagnosis of "autoimmune hepatitis-like liver disease" in children: a single-center retrospective study

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Background: Children with autoimmune hepatitis (AIH) often present with symptoms similar to those of other liver diseases. This study consists of a comparison between the clinical and histological characteristics of AIH and those of other four AIH-like liver diseases [i.e., drug-induced liver injury (DILI), gene deficiency, infectious liver disease and other etiology of liver disease], as well as an evaluation of the AIH scoring system's diagnostic performance.
Methods: All children with AIH-like liver disease at our center from January 2013 to December 2022 were included. The clinical and histological characteristics of the AIH group were retrospectively analyzed and compared with those of the other four groups.
Results: A total of 208 children were included and divided into AIH group (18 patients), DILI group (38 patients), gene deficiency group (44 patients), infectious liver disease group (74 patients), and other etiology group (34 patients). The antinuclear antibodies (ANA) $\geq$ 1:320 rate was significantly higher in the AIH compared to the other four groups after multiple testing correction ($p < 0.0125$), while patients with positive antibodies to liver-kidney microsomal-1 (anti-LKM1, $n = 3$) and smooth muscle antibodies (SMA, $n = 2$) were only observed in the AIH group. The positive rates of antibodies to liver cytosol type1 (anti-LC1) and Ro52 were higher than those in the other four groups. The serum immunoglobulin G (IgG) and globulin levels, as well as the proportions of portal lymphoplasmacytic infiltration, lobular hepatitis with more than moderate interface hepatitis, and lobular hepatitis with lymphoplasmacytic infiltration, were significantly higher in the AIH group than in the other four groups after multiple testing correction ($p < 0.0125$). The cirrhosis rate in the AIH group was higher than that in the DILI and infectious liver disease groups ($p < 0.0125$). Both the simplified (AUC > 0.73) and the revised systems (AUC > 0.93) for AIH have good diagnostic performance, with the latter being superior ($p < 0.05$).

Conclusion: Positive autoantibodies (ANA ≥ 1:320 or anti-LKM1 positive, or accompanied by SMA, anti-LC1 or Ro-52 positive) and elevated serum IgG or globulin levels contribute to early recognition of AIH. The presence of lobular hepatitis with more than moderate interface hepatitis and lymphoplasmacytic infiltration contribute to the diagnosis of AIH.

KEYWORDS

children, autoimmune hepatitis, serum globulin, immunoglobulin G, autoantibodies, histology

Introduction

AIH is an inflammatory liver disorder with no clear cause which may be associated with various factors such as genetics, immunity, and the environment (including infection or exposure to toxins) (1). The diagnosis of AIH is based on histological abnormalities (e.g., interface hepatitis, lymphoplasmacytic infiltration and emperipolesis), autoantibodies, and other characteristic laboratory findings (e.g., elevated serum aspartate aminotransferase (AST) and alanine aminotransaminase (ALT), and increased IgG concentration) (2).

The diagnosis of AIH is rather challenging due to the absence of a signature diagnostic marker. AIH-related autoantibodies also appear in acute liver injury caused by other etiologies. Additionally, some liver diseases, including chronic viral hepatitis, may also lead to elevated levels of serum globulin or IgG (3). Dutch scholars have found that 40% of patients with DILI have elevated serum IgG levels, and the positive rates for ANA and antimitochondrial antibodies (AMA) are between 60% and 70%. However, the titers of these autoantibodies tend to decrease within several months (4). In a pediatric metabolic dysfunction-associated fatty liver disease (MAFLD) cohort, one-third of patients were positive for autoantibodies (5). Primary biliary cholangitis (PBC) are often accompanied by positive autoantibodies such as AMA and ANA (6). Krithiga et al. reported a case of Epstein-Barr virus hepatitis with the complication of Hodgkin's lymphoma in which the patient had likely been misdiagnosed and treated for AIH in the index presentation (7).

In this article, we define liver disease with biochemical characteristics of AIH and/or positive autoantibodies as "AIH-like liver disease", collecting patients' clinical, biochemical, immunologic and histologic data. Moreover, we will compare the clinical and histological features of AIH with those of other AIH-like liver diseases, and evaluate the diagnostic performance of the AIH scoring system.

Materials and methods

Study design and patients

This retrospective study was performed in the Department of Pediatrics at Tongji Hospital between January 2013 and December 2022. All participants met the following conditions: (1) signs of liver damage, including unexplained elevation of aminotransferases, hepatomegaly, or cirrhosis; (2) meeting one or more of the following criteria: unexplained elevation in serum globulin (>35 g/L); unexplained elevation in IgG [exceeding the upper limit of the normal range for the same age group. (0–28 days: 6.6–17.5 g/L; 29 days-6 months: 2.0–6.9 g/L; 6 months-3 years: 3.3–12.3 g/L; 3–12 years: 5.4–15.3 g/L; 12–17 years: 7.0–15.6 g/L)]; positive for at least one autoantibody; (3) diagnosis at <18 years of age.

The exclusion criteria were: (1) patients with extra-hepatic autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis at the time of initial diagnosis; (2) patients with severe systemic or extra-hepatic diseases, such as severe cardiopulmonary disease and severe sepsis; (3) patients who had received intravenous immunoglobulin therapy within the 12 weeks before initial diagnosis; (4) patients with biliary obstruction diseases such as biliary calculi and biliary atresia; (5) patients with severe clinical data deficiencies; (6) diagnosis at ≥18 years of age.

The study protocol was approved by the Medical Ethics Committee at Tongji Hospital (no.: TJ-IRB20230302), and it adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from the parents of all included patients. Clinical and laboratory data were obtained from the patients' medical records.

Diagnostic criteria for the etiology of AIH-like liver disease were: (1) AIH (2): patients conformed with the 1999 revised International Autoimmune Hepatitis Group (IAIHG) score (8) ≥16 and/or 2008 IAIHG simplified AIH score (9) ≥7, and/or histological features consistent with AIH. These included: (i) more than moderate interface hepatitis (mild: local or few portal areas destroyed; moderate: <50% of portal areas or fibrous septa destroyed; severe: >50% of portal areas or fibrous septa destroyed); (ii) lymphoplasmacytic infiltration with plasma cells accounting for ≥20% of inflammatory cells, and/or focal plasma cell clustering in the portal or lobule (defined as >5 plasma cells in one focus); (iii) hepatocellular rosettes; (iv) emperipolesis; (v) necroinflammation in the central lobules. Moreover, treatment with corticosteroid or immunomodulators and long-term follow-up (>1 year) were necessary for diagnosis. (2) DILI (10): history of using hepatotoxic drugs regardless of whether the dose reached toxic levels; recovery from liver disease after symptomatic treatment, or accepted corticosteroids therapy less than six months and no recurrence during long-term follow-up (>1 year). (3) Infectious liver disease (11): existing etiological evidence of hepatitis virus infection or other viral active infection, or existing clinical and etiological

evidence of systemic infection. (4) Gene deficiency diseases including either hepatolenticular degeneration [according to the Leipzig Diagnostic Criteria (12) and in concordance with the American Association for the Study of Liver Diseases (AASLD) guidelines (13)]. or other genetic liver diseases which can only be diagnosed by genetic testing, including progressive familial intrahepatic cholestasis (PFIC). (5) Other etiology of AIH-like liver diseases including either PBC (14) (according to the AASLD practice guidelines) or MAFLD (15): pathological or imaging evidence of intrahepatic fat accumulation and one of the following three items: excessive obesity, prediabetes or type 2 diabetes, and metabolic disorders.

Data collection

The collected information included general data (e.g., age, sex), clinical manifestations (e.g., jaundice and fatigue), laboratory examinations, imaging data (to confirm cirrhosis and rule out any obstructive liver injury etiology), pathological data, and the treatment outcomes of the children meeting the above selection criteria.

Laboratory evaluations

The laboratory evaluations included tests for pathogens (e.g., hepatitis A–E viruses, Epstein-Barr virus, cytomegalovirus), liver function markers (i.e., ALT, AST, total bilirubin (TB), direct bilirubin (DB), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), lactate dehydrogenase, serum albumin, globulin, cholesterol), blood routine, coagulation function (prothrombin time (PT), international normalized rate (INR)) and IgG.

Autoantibodies

The antibody spectrum includes ANA, anti-LKM1, AMA-M2, AMA-M2-3E, anti-LC1, antibodies to soluble liver antigen (anti-SLA), nuclear membrane glycoprotein 210 antibodies (GP210), SP100 nuclear antigen antibodies (SP100), Ro-52, and SMA. Our hospital has adopted an autoantibody kit that is compatible with the indirect immunofluorescence analyzer and immunoblotting analyzer (all from EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany). Among them, ANA and SMA are detected by indirect immunofluorescence, with a cut-off value of 1:100 [due to the different dilution systems, the detection of ANA and SMA at 1:100 in this system is equivalent to the lowest titer (1:40) in the AIH score systems]; the remaining antibodies are detected by immunoblotting (EUROLineScan color intensity 11–25 is weakly positive, 26–50 is positive).

Clinical outcome

The outcomes included four categories (1) Recovery: normalization of blood biochemical indicators, with no recurrence observed within six months following treatment cessation; (2) Chronicity: Liver function indicators remained abnormal after 6 months of the course of the disease, or there was evidence of chronic liver disease on imaging or histology; (3) Cirrhosis: Liver imaging and/or histopathological changes in liver tissue met the characteristics of cirrhosis; (4) Death.

Statistical analysis

All statistical analyses were performed with SPSS v. 26.0. The Kolmogorov–Smirnov test was used to verify whether the variables followed a normal distribution. Continuous variables with normal distributions were expressed as mean ± standard deviation and analyzed by one-way analysis of variance (ANOVA) test or student's *t*-test for pair-wise groups. Continuous variables with skewed distributions were expressed as medians [interquartile range (IQR)] and analyzed by a Kruskal–Wallis test and a Wilcoxon rank-sum test for pair-wise groups. Categorical variables were expressed as absolute number (percentage), and evaluated using either a χ^2 or Fisher's exact test. The revised and simplified systems' diagnostic accuracy was evaluated with the receiver operating characteristic (ROC) curve. The difference in the ROC curves for the revised and simplified systems were judged with a DeLong test. For each test, $p < 0.05$ was considered statistically significant. Bonferroni correction was used when multiple tests were conducted for pair-wise comparison.

Results

Basic characteristics for patients with AIH-like liver disease

A total of 208 children with AIH-like liver disease were included, among the 3,852 children with liver injury in this study. The median age was 52.5 (17.0, 98.0) months, with 104 boys and 104 girls, indicating a balanced male-to-female rate. The overall positive autoantibody rate was 84.6% (176/208), the elevated serum IgG (>1 × ULN) rate was 40.9% (85/208), and the elevated serum globulin rate (>1 × ULN) was 31.7% (66/208). The median course of identifiable liver disease at inclusion was 20.0 (8.0, 90.0) days and the median follow-up time was 23.0 (12.0, 36.0) months.

The etiology and classification of AIH-like liver disease in children

The patients were divided into 5 groups according to the diagnostic criteria for etiological factors. There were 18 patients in the AIH group, 38 patients in the DILI group, 44 patients in the gene deficiency group, 74 patients in the infectious liver disease group, and 34 patients in the other etiology group. The specific causes are shown in Figure 1.

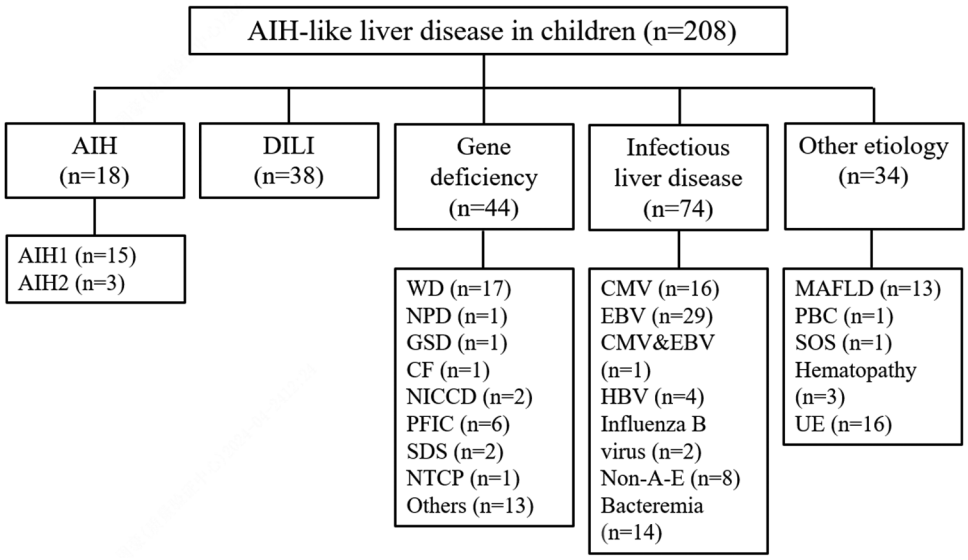

FIGURE 1
The etiology and classification of AIH-like liver disease in children. AIH, autoimmune hepatitis; DILI, drug-induced liver injury; WD, Wilson's disease; NPD, niemann-pick disease; GSD, glycogen storage disease; CF, cystic fibrosis; NICCD, neonatal intrahepatic cholestasis caused by citrin deficiency; PFIC, progressive familial intrahepatic cholestasis; SDS, shwachman-diamond syndrome; NTCP, sodium taurocholate co-transporting polypeptide deficiency; CMV, cytomegalovirus; EBV, Epstein-Barr virus; HBV, hepatitis B virus; non-A-E, non-A, non-B, non-C, non-D, non-E hepatitis; MAFLD, metabolic dysfunction-associated fatty liver disease; PBC, primary biliary cholangitis; SOS, soidal obstruction syndrome; UE, unknown etiology.

Comparison of demographic data, manifestations and autoantibodies between the AIH group and the other four groups

As shown in Table 1, there was no significant difference in age or sex among the five groups at the time of initial diagnosis ($p > 0.05$). The recognizable course of liver disease in the AIH group was significantly longer than that in the DILI group at the initial diagnosis ($p = 0.007$), but the follow-up time was significantly longer in the AIH group than in the other four groups after multiple testing correction ($p < 0.0125$).

Other than the higher proportion of jaundice in the AIH group compared to the infectious liver disease group ($p = 0.005$), there were no significant differences in the main clinical manifestations between the AIH group and the other four groups ($p > 0.0125$).

In addition, the proportion of individuals with ANA ≥ 1:320 was significantly higher in the AIH group compared to the other four groups after multiple testing correction ($p < 0.0125$), while patients with positive anti-LKM1 ($n = 3$) and SMA ($n = 2$) were only observed in the AIH group. The positive anti-LC1 rates in the AIH group were higher than those in other four groups, but this was not statistically significant after multiple testing correction ($p = 0.029$, 0.07, 0.052, and 0.037, respectively); the positive rate for Ro52 was significantly higher than that in the gene deficiency group, infectious liver disease group and the other etiology group ($p < 0.0125$). The positive rates for AMA-2M, AMA-2M-3E, anti-SLA, GP210, and SP100 were similar in each group ($p > 0.05$); the other four groups were more likely to have ANA = 1:100 and other autoantibodies which were weakly positive. All patients in AIH group showed positive autoantibodies, and there were no patient of serologically negative AIH.

Comparison of laboratory results between the AIH group and the other four groups

As shown in Table 2, during the initial diagnosis, there were no significant differences in blood routine, elevated IgM (>1 × ULN) rate or elevated IgA (>1 × ULN) rate between the AIH group and the other four groups ($p > 0.0125$); the serum IgG and globulin levels were significantly higher in the AIH group than those in the other four groups after multiple testing correction ($p < 0.0125$). There were only three patients with serum IgG levels within the normal range for their respective age groups. Two of these patients had been diagnosed with AIH-1 (ANA positive at 1:1,000 in both patients), and the remaining patient had been diagnosed with AIH-2 (anti-LKM1 positive).

TB and DB levels were significantly higher in the AIH group than those in the infectious liver disease group and the other etiology group ($p < 0.0125$); there were no significant differences in serum albumin, PT, or INR levels between the AIH group and gene deficiency group ($p > 0.0125$). However, when compared to the DILI group, the infectious liver disease group, and the other etiology group, there were significant statistical differences observed (albumin was significantly lower, PT was significantly prolonged, and INR was significantly higher, $p < 0.0125$).

Comparison of histological characteristics between the AIH group and the other four groups

As shown in Table 3, a total of 72 children received liver biopsies. The proportion of portal lymphoplasmacytic infiltration

TABLE 1 Comparison of demographic data, manifestation and autoantibodies between the AIH group and the other four groups.

Characteristics	AIH ($n = 18$)	DILI ($n = 38$)	Gene deficiency ($n = 44$)	Infectious liver disease ($n = 74$)	Other etiology ($n = 34$)	p-value
Sex, n (%)						
Female	10 (55.6)	20 (52.6)	23 (52.3)	36 (48.6)	15 (44.1)	0.918
Male	8 (44.4)	18 (47.4)	21 (47.7)	38 (51.4)	19 (55.9)	
Age (months)	56.0 (21.5, 102.3)	43.5 (16.3, 108.0)	45.5 (14.3, 99.8)	49.5 (14.5, 84.0)	74.5 (32.3, 122.0)	0.331
Recognizable course (days)	60.0 (10.0, 228.1)	12.0 (4.0, 30.0)**	75.0 (30.0, 262.5)	15.0 (7.0, 28.5)*	75.0 (17.0, 338.8)	<0.001†
Follow-uptime (months)	59.0 (27.5, 78.8)	23.5 (18.0, 35.3)**	17.0 (3.0, 29.0)**	21.0 (6.0, 36.0)**	18.0 (6.0, 34.5)**	<0.001†
Manifestation, n (%)						
Jaundice	8 (44.4)	19 (50.0)	24 (54.5)	11 (14.9)**	7 (20.6)	<0.001†
Ventosity	4 (22.2)	3 (7.9)	17 (38.6)	6 (8.1)	6 (17.6)	<0.001†
Ascites	2 (11.1)	0 (0.0)	11 (25.0)	1 (1.4)	1 (2.9)	<0.001†
Edema	1 (5.6)	1 (2.6)	12 (27.3)	1 (1.4)	0 (0.0)	<0.001†
Encephalopathy	1 (5.6)	0 (0.0)	6 (13.6)	2 (2.7)	1 (2.9)	0.034†
Fatigue	4 (22.2)	6 (15.8)	8 (18.2)	6 (8.1)	2 (5.9)	0.207
Malaise	3 (16.7)	8 (21.1)	8 (18.2)	7 (9.5)	4 (11.8)	0.463
Pruritus	1 (5.6)	4 (10.5)	4 (9.1)	2 (2.7)	0 (0.0)	0.182
Bellyache	3 (16.7)	3 (7.9)	4 (9.1)	5 (6.8)	4 (11.8)	0.720
Hemorrhage	1 (5.6)	0 (0.0)	1 (2.3)	1 (1.4)	0 (0.0)	0.491
Autoantibody positive, n (%)						
ANA = 1:100	0 (0.0)	20 (52.6)**	11 (25.0)*	31 (41.9)**	16 (47.1)**	0.001†
ANA ≥ 1:320	16 (88.9)	7 (18.4)**	11 (25.0)**	12 (16.2)**	6 (17.6)**	<0.001†
Anti-LKM1	3 (16.7)	0 (0.0)*	0 (0.0)*	0 (0.0)**	0 (0.0)*	<0.001†
SMA	2 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)*	0 (0.0)	<0.001†
Anti-LC1	3 (16.7)	0 (0.0)*	1 (2.3)	3 (4.1)	0 (0.0)*	0.014†
Ro-52	5 (27.8)	4 (10.5)	1 (2.3)**	4 (5.4)**	1 (2.9)**	0.005†
AMA-2M	1 (5.6)	1 (2.6)	2 (4.5)	0 (0.0)	0 (0.0)	0.279
AMA-2M-3E	0 (0.0)	0 (0.0)	2 (4.5)	1 (1.4)	0 (0.0)	0.367
Anti-SLA	0 (0.0)	0 (0.0)	1 (2.3)	2 (2.7)	1 (2.9)	0.817
GP210	1 (5.6)	0 (0.0)	0 (0.0)	3 (4.1)	1 (2.9)	0.451
SP100	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	–

AIH, autoimmune hepatitis; DILI, drug-induced liver injury; ANA, antinuclear antibodies; anti-LKM1, antibodies to liver-kidney microsomal-1; SMA, smooth muscle antibody; anti-LC1, antibodies to liver cytosol type1; Ro52, Ro52 antibodies; AMA, antimitochondrial antibodies; anti-SLA, antibodies to soluble liver antigen; GP210, nuclear membrane glycoprotein 210 antibodies; SP100, soluble acidic nuclear protein 100 antibodies.
*Compared to the AIH group, $p < 0.05$.
**Compared to the AIH group, $p < 0.0125$ (Bonferroni correction).
†Among the five groups, $p < 0.05$.

in the AIH group was significantly higher than that in the other 4 groups after multiple testing correction ($p < 0.0125$). The proportion of lobular hepatitis with more than moderate interface hepatitis, and the proportion of lobular hepatitis with lymphoplasmacytic infiltration were significantly higher in the AIH group than those in the other four groups ($p < 0.0125$). The proportions of lobular hepatitis with emperipolesis, hepatocellular rosettes or portal-based fibrosis were higher in the AIH group than those in the other four groups, with no significant difference after multiple testing correction ($p > 0.0125$).

The AIH scoring system's diagnostic performance

As shown in Table 4, the simplified and revised system in the AIH group were significantly higher than those in the other 4 groups ($p < 0.0125$). As shown in Figure 2, the area under the ROC curve (AUC) for each group reveals that both the simplified (AUC > 0.73) and the revised systems (AUC > 0.93) for AIH have good diagnostic performance. The difference in the area under the ROC curve suggests that the revised systems outperforms the simplified systems in terms of diagnostic accuracy, as confirmed by the Delong test ($p < 0.05$).

Comparison of treatment and outcome between the AIH group and the other four groups

All children diagnosed with AIH at our center received immunosuppressive therapy, and the proportion of those who had accepted immunosuppressive therapy in the AIH group was significantly higher than those in the other 4 groups after multiple testing correction ($p < 0.0125$). Four children with AIH received corticosteroid therapy alone. Among them, two patients were lost to follow-up (confirmed in 2013), one child was confirmed in the adult (i.e., non-pediatric) department and one child died. After enduring two surgeries for intestinal obstruction, this child developed severe malnutrition and a low

TABLE 2 Comparison of laboratory results between the AIH group and the other four groups.

Characteristic	AIH ($n = 18$)	DILI ($n = 38$)	Gene deficiency ($n = 44$)	Infectious liver disease ($n = 74$)	Other etiology ($n = 34$)	p-value
WBC ($\times 10^9$/L)	8.3 (6.6, 10.8)	7.8 (5.6, 10.5)	7.3 (5.2, 10.5)	8.1 (5.9, 11.1)	8.4 (6.3, 9.7)	0.814
Hb (g/L)	133.0 (121.0, 139.3)	125 (120.3, 131)	126 (117, 135.8)	126 (118, 132)	124 (115, 132)	0.281
PLT ($\times 10^9$/L)	272 (214.3, 324.3)	278.5 (209.5, 345.8)	234.5 (142.5, 322.3)	279 (213, 390.3)	306 (209, 342)	0.091
IgG (g/L)	22.8 ± 10.5	11.9 ± 5.7**	13.8 ± 6.2**	13.9 ± 6.4**	11.7 ± 3.1**	<0.001†
IgM > 1 ULN	2 (11.1)	0 (0.0)	14 (31.8)	9 (12.2)	3 (8.8)	0.001†
IgA > 1 ULN	2 (11.1)	0 (0.0)	13 (29.5)	10 (13.5)	1 (2.9)	<0.001†
GLB (g/L)	38.5 (31.5, 46.6)	26.6 (24.1, 30.8)**	29.5 (23.2, 38.2)**	31.8 (24.3, 36.6)**	26.8 (24.8, 33.2)**	0.002†
ALT (U/L)	313 (172.9, 686.8)	533 (168.6, 1,427.0)	280.9 (167.5, 335.8)	287.6 (134.8, 570.3)	262.5 (158.8, 484.5)	0.073
AST (U/L)	440.5 (229.3, 806.8)	679 (168.5, 1,087.3)	353.3 (224.8, 427.8)	252 (134.3, 533.8)	261.5 (121, 489.5)	0.011†
TB (μmol/L)	48.4 (13.1, 147.6)	16.6 (6.1, 121.3)	51.1 (10.7, 188.6)	9.8 (4.9, 21.2)**	8.8 (5.0, 26.1)**	<0.001†
DB (μmol/L)	38.6 (4.9, 121.1)	6.0 (2.1, 106.4)	40.0 (4.7, 143.9)	4.7 (1.8, 17.6)**	3.7 (1.8, 14.7)**	<0.001†
GGT (U/L)	104 (40.5, 153.3)	81.5 (37.8, 127.5)	135 (47.3, 194.5)	62 (27.8, 199.9)	70.5 (39.5, 137.5)	0.163
ALP (U/L)	289 (197.5, 373.8)	254 (208, 355.3)	311 (205, 467.8)	232 (170.0, 321.3)	245.5 (203, 344.5)	0.182
LDH (U/L)	339 (251.3, 565.5)	340.5 (287.8, 472.8)	307 (276.3, 414.3)	432 (329.3, 547.5)	316.5 (273.8, 376.5)	0.002†
TC (mmol/L)	3.5 (3.0, 4.1)	3.8 (3.1, 4.2)	3.9 (3.2, 4.3)	3.4 (2.9, 4.1)	3.9 (3.4, 4.6)	0.210
ALB (g/L)	32.6 ± 6.9	41.4 ± 5.0**	34.5 ± 8.8	41.1 ± 3.8**	44.0 ± 6.0**	<0.001†
PT (s)	16.2 (14.5, 18.9)	13.7 (13.0, 14.5)**	17.0 (13.2, 24.5)	13.7 (12.7, 14.6)**	13.6 (12.6, 14.8)**	<0.001†
INR	1.30 (1.1, 1.6)	1.0 (1.0, 1.1)**	1.4 (1.0, 2.3)	1.0 (1.0, 1.2)**	1.0 (0.9, 1.2)**	<0.001†

AIH, autoimmune hepatitis; DILI, drug-induced liver injury; WBC, white blood cell; Hb, hemoglobin; PLT, blood platelets; IgG, immunoglobulin G; IgA, immunoglobulin A; IgM, immunoglobulin M; GLB, serum globulin; ULN, upper limit of normal; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TB, total bilirubin; DB, direct bilirubin; GGT, gamma-glutamyl transpeptidase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; TC, total cholesterol; ALB, albumin; PT, prothrombin time; INR, international normalized rate.
*Compared to the AIH group, $p < 0.05$.
**Compared to the AIH group, $p < 0.0125$ (Bonferroni correction).
†Among the five groups, $p < 0.05$.

TABLE 3 Comparison of histological characteristics between the AIH group and the other four groups.

Characteristics	AIH ($n = 6$)	DILI ($n = 16$)	Gene deficiency ($n = 18$)	Infectious liver disease ($n = 16$)	Other etiology ($n = 16$)	p-value
Portal hepatitis, n (%)						
Lymphoplasmacytic infiltration	4 (66.7)	1 (6.3)**	1 (5.6)**	1 (6.3)**	1 (6.3)**	<0.001†
Biliary tract injury	1 (16.7)	1 (6.3)	4 (22.2)	1 (6.3)	2 (12.5)	0.593
Cholestasis	2 (33.3)	8 (50.0)	10 (55.6)	2 (12.5)	0 (0.0)	0.001†
Lobular hepatitis, n (%)						
More than moderate interface hepatitis	4 (66.7)	1 (6.3)**	0 (0.0)**	0 (0.0)**	0 (0.0)**	<0.001†
Lymphoplasmacytic infiltration	4 (66.7)	1 (6.3)**	1 (5.6)**	1 (6.3)**	1 (6.3)**	<0.001†
Rosettes	1 (16.7)	0 (0.0)	2 (11.1)	0 (0.0)	0 (0.0)	0.158
Emperipolesis	2 (33.3)	0 (0.0)*	1 (5.6)	1 (6.3)	0 (0.0)*	0.030†
Portal-based fibrosis	4 (66.7)	6 (37.5)	8 (44.4)	6 (37.5)	8 (50.0)	0.731

AIH, autoimmune hepatitis; DILI, drug-induced liver injury.
*Compared to the AIH group, $p < 0.05$.
**Compared to the AIH group, $p < 0.0125$ (Bonferroni correction).
†Among the five groups, $p < 0.05$.

blood cell count, which prevented him from tolerating immunosuppressive therapy. Nevertheless, he managed to maintain biochemical stability through corticosteroid therapy alone. The other 14 patients were treated with prednisone (1–2 mg/kg/day) combined with azathioprine (1–2 mg/kg/day). Once biochemical remission was achieved after the initial treatment (usually within 4–6 weeks), the dose of prednisone was gradually reduced, with a general maintenance dose of 5–10 mg/day. Azathioprine was maintained at the aforementioned dose, typically 1 mg/kg. Two patients relapsed and developed refractory AIH, for which they were switched to methylprednisolone (15–20 mg/day) combined with mycophenolate mofetil (CellCept: 33 mg/kg). The biochemical remission rate for children with AIH at our center is 94.4%.

As shown in Table 4, the liver disease recovery rate in the AIH group was similar to that in the gene deficiency group ($p = 1.000$), but it was lower than that in the DILI group, infectious liver disease group, and the other etiology group ($p < 0.0125$). The chronicity and cirrhosis rates for liver disease in the AIH group were similar to those in the gene deficiency group and the other etiology group ($p > 0.0125$), but were higher than those in the DILI group and the infectious liver disease group ($p < 0.0125$); the mortality rate in the AIH group was lower than that in gene deficiency group, but this was not statistically significant after multiple testing correction ($p = 0.04$).

TABLE 4 Comparison of AIH score systems, treatment and outcome between the AIH group and the other four groups.

Characteristics	AIH ($n = 18$)	DILI ($n = 38$)	Gene deficiency ($n = 44$)	Infectious liver disease ($n = 74$)	Other etiology ($n = 34$)	*p*-value
Score systems						
Simplified system	6.0 (5.5, 7.3)	4.0 (4.0, 5.0)**	4.0 (4.0, 6.0)**	4.0 (2.0, 4.0)**	4.0 (4.0, 5.0)**	<0.001†
Revised system	17.5 (16.0, 19.3)	8.0 (6.0, 10.0)**	9.0 (7.3, 12.8)**	10.0 (5.8, 13.0)**	9.5 (7.8, 12.0)**	<0.001†
Treatment, *n* (%)						
Corticosteroids	18 (100)	16 (42.1)**	13 (29.5)**	16 (21.6)**	4 (11.8)*	<0.001†
Immunomodulator	14 (77.8)	0 (0.0)**	0 (0.0)**	2 (2.7)**	0 (0.0)**	<0.001†
Outcome, *n* (%)						
Recovery	0 (0.0)	31 (81.6)**	1 (2.3)	55 (74.3)**	11 (32.4)**	<0.001†
Chronicity	18 (100)	6 (15.8)**	43 (97.7)	15 (20.3)**	22 (64.7)	<0.001†
Cirrhosis	7 (38.9)	0 (0.0)**	28 (63.6)	2 (2.7)**	4 (11.8)	<0.001†
Death	1 (5.6)	1 (2.6)	13 (29.5)*	1 (1.4)	1 (2.9)	0.008†

AIH, autoimmune hepatitis; DILI, drug-induced liver injury.
*Compared to the AIH group, $p < 0.05$.
**Compared to the AIH group, $p < 0.0125$ (Bonferroni correction).
†Among the five groups, $p < 0.05$.

A total of 17 deaths occurred. The gene deficiency group accounted for 13 deaths, of which six patients succumbed to liver failure resulting from decompensation of cirrhosis, while another six died from infectious shock triggered by pneumonia following cirrhosis. Additionally, one patient passed away due to liver transplant failure. The AIH group accounted for one death, and the reason was described above. The DILI group accounted for one death, of infectious shock complicated with aplastic anemia. Another death was in the infectious liver disease group, and due to severe hepatitis complicated by liver failure. The other etiology group had one death, of complications after liver transplant.

Recommendations for AIH diagnostic procedures

Based on the above results, we proposed AIH diagnostic procedures and clinical pathways, as shown in Figure 3.

Discussion

AIH proportion varies significantly depending on the region, age of onset and sex. The estimated prevalence rate is 1.28–15.65/100,000, and the prevalence rate among children is significantly lower than that in adults (16). Over the past 10 years, only 18 patients with AIH in children have been diagnosed at our center. We also found that other diseases may have clinical manifestations similar to those of AIH, such as DILI (38/208), gene deficiency disease (44/208) and infectious liver disease (74/208). This article retrospectively compares the differences between AIH and other AIH-like liver diseases at baseline, in hopes of providing a reference for early diagnosis of AIH in clinical practice.

The clinical manifestations of AIH in children are not specific, and may include symptoms such as fatigue, jaundice and itching. About 40% of patients with AIH show the same symptoms as acute viral hepatitis; another 40% of patients among children show signs of chronic liver disease, such as progressive fatigue, intermittent jaundice and weight loss. These patients may even have decompensated manifestations of cirrhosis such as hepatosplenomegaly, ascites and/or gastrointestinal bleeding (17, 18). Similar to other literature, our results show no significant differences in the manifestations between the AIH group and the other four groups. Only the proportion of jaundice in the AIH group is higher than that of the infectious liver disease group. The main cause is believed to be that most children with infectious diseases in this group are infected with EBV (40.5%, 30/74). Acute EBV infection typically manifests as non-jaundice hepatitis, and jaundice is rare (19).

Although positive autoantibodies are also commonly found in other liver diseases such as viral hepatitis and DILI, positive autoantibodies still strongly support diagnosis of AIH (20, 21). ANA or SMA positive and anti-LKM1 positive are characteristic autoantibodies for type 1 and type 2 AIH, respectively (22). We found that the highest ANA ≥ 1:320 rate was in the AIH group, while ANA = 1:100 and other weak positive autoantibodies were more likely to be present in the non-AIH group. Low-titer autoantibodies are found in patients with hepatolenticular degeneration, who are not directly associated with liver function impairment, steatosis and fibrosis (23). Antibodies that are more specific to autoimmune hepatitis, such as anti-LKM1, are not present in patients with MAFLD (5). This is consistent with the results of this article, with only the AIH group showing positive anti-LKM1. Other autoantibodies also contribute to the diagnosis of AIH (24). We found that the positive rates of anti-LC1 (indicating type 2 AIH) and Ro-52 in the AIH group were higher than those in the other four groups. However, they were also found in other patients, making them less specific. The results indicated that the presence of ANA ≥ 1:320, either alone or in combination with SMA or anti-LKM1 positivity, strongly supported the diagnosis of AIH. On the other hand, patients who exhibited positivity for only anti-LC1 or Ro-52 were recommended for follow-up monitoring to avoid missed diagnoses. Although anti-SLA are found in approximately 10%–20% of patients with AIH, they have a high specificity for the diagnosis of AIH (25). Interestingly, all four patients with

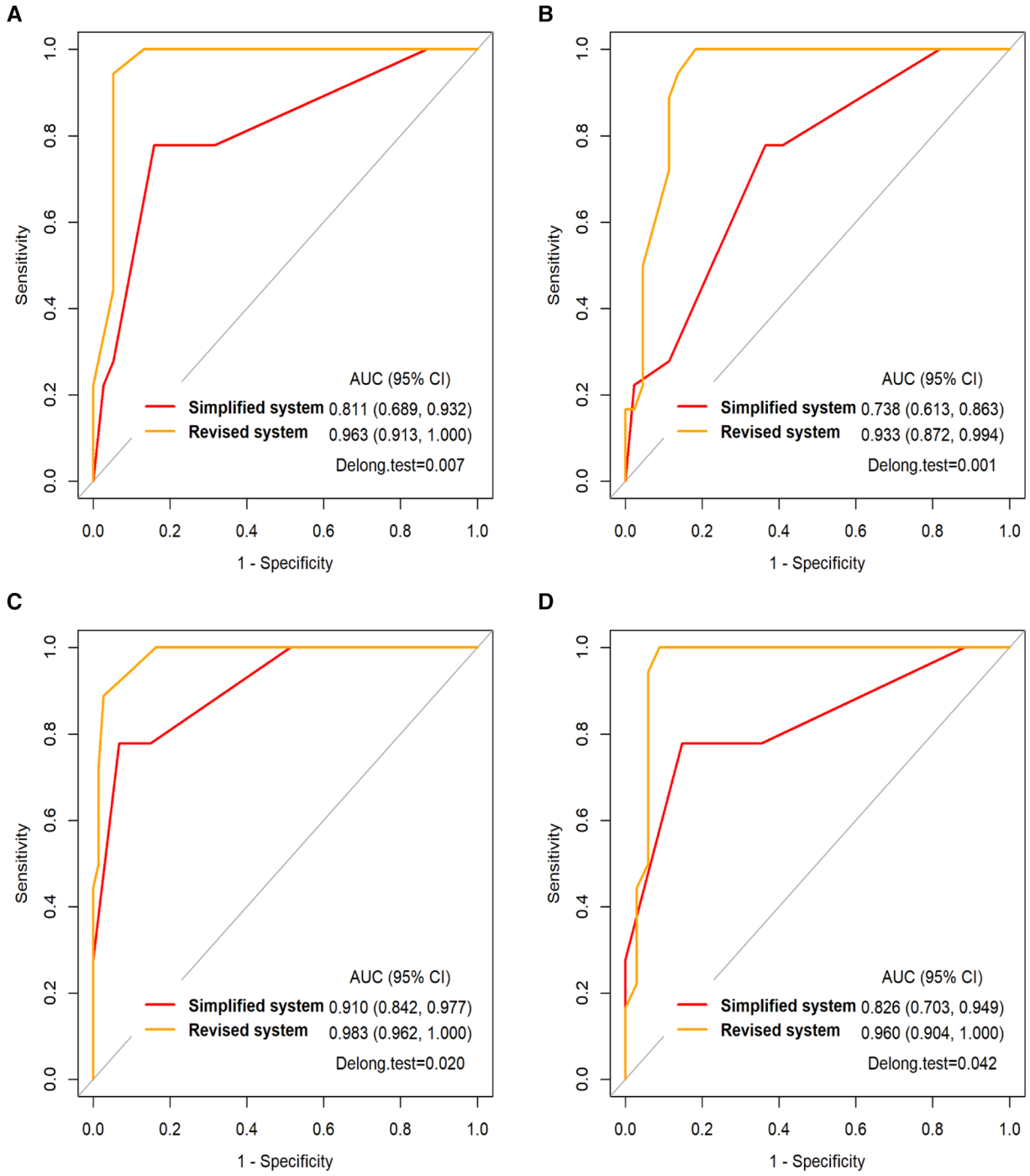

FIGURE 2
(A) the ROC curve of the simplified and revised systems for the AIH in DILI group and (B) in gene deficiency group and (C) in infectious liver disease group and (D) in other etiology group. AUC, the area under the ROC curve.

positive anti-SLA at our center were excluded from AIH. They were under three months old, and these antibodies are believed to be maternally transmitted (26). Approximately 10% of patients with AIH are seronegative AIH (27), yet our center has not encountered any patients of serologically negative AIH. This may be attributed to the fact that the diagnosis of most cases in our study is primarily based on the AIH scoring systems.

The increase in serum IgG and gamma globulin levels is an important sign of serum antibody-positive AIH (2, 24). In our study, we included the serum globulin level as a criterion for diagnosing AIH-like liver disease, as it is a routinely performed clinical test that offers early clinical insights into potential elevations in gamma globulin (28). The results showed that the levels of serum IgG and globulin in the AIH group were significantly higher than

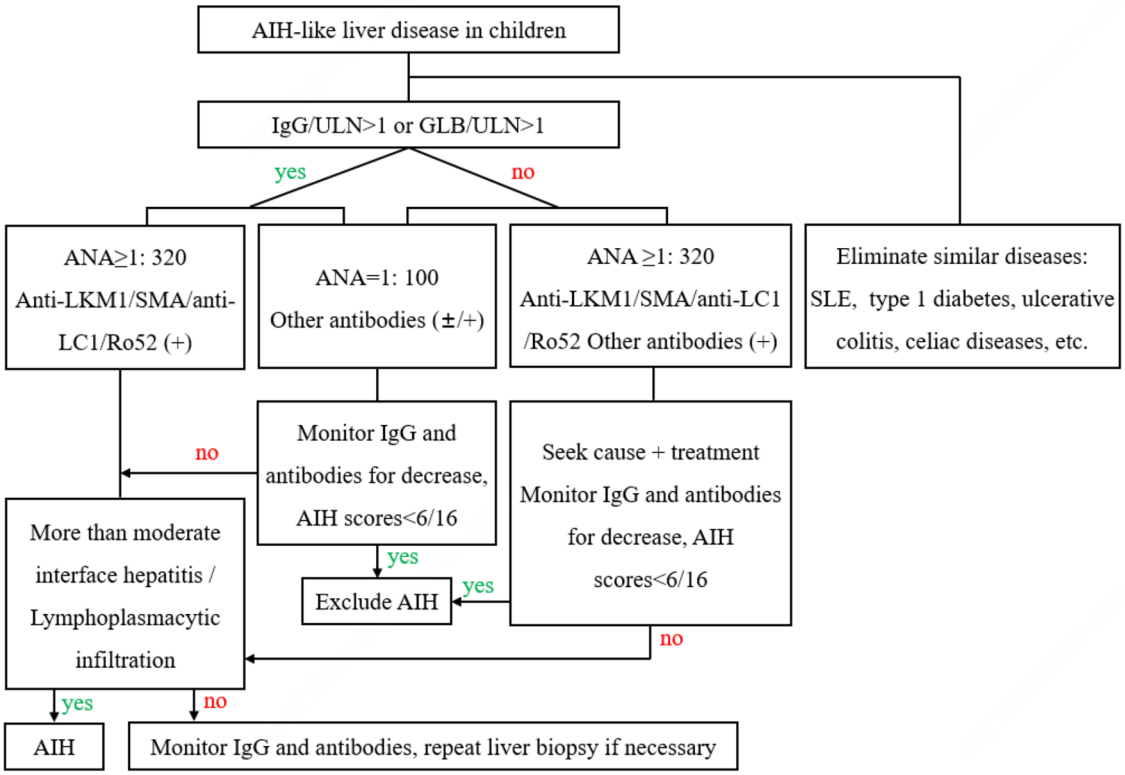

FIGURE 3
AIH diagnostic procedures and clinical pathways. AIH, autoimmune hepatitis; SLE, systemic lupus erythematosus; IgG, immunoglobulin G; GLB, seroglobulin; ULN, upper limit of normal; ANA, antinuclear antibodies; anti-LKM1, antibodies to liver-kidney microsomal1; SMA, smooth muscle antibodies; anti-LC1, antibodies to liver cytosol type1; Ro-52, Ro-52 antibodies.

those in the other four groups. Although some diseases, such as chronic viral hepatitis or cirrhosis caused by other etiologies, can also lead to increased serum globulin and IgG levels (3), some scholars have reported that the serum IgG levels in patients with AIH are significantly higher than those in non-autoimmune hepatitis and cirrhosis patients. This finding suggested that a high serum IgG level is the best indicator to distinguish autoimmune hepatitis from other AIH-like liver diseases (29). The levels of serum IgA and IgM in patients with AIH are generally normal (30). Our results showed that there was no significant difference between the AIH group and the other four groups in terms of increases in IgM and IgA rates, which is consistent with previous literature.

IgG4-related disease (IgG4-RD) is an immune-mediated chronic inflammatory disease which is accompanied by fibrosis that can affect most of the body (31). When IgG4-RD only involves the liver and/or biliary system, it needs to be differentiated from AIH. Imaging tools can help identify typical lesions of IgG4-RD, such as diffuse or focal enlargement of organs and retroperitoneal fibrosis (32). Pathologically, although IgG4-RD exhibits histological similarities to AIH, significant infiltration of IgG4-positive plasma cells is helpful for diagnosis (33, 34). Only five patients with AIH in our center have had their IgG4 levels tested, and none showed abnormal results.

Overlap syndrome AIH with primary sclerosing cholangitis (PSC) is reportedly more prevalent in childhood, affecting approximately 33% of children with autoimmune liver disease, whereas only 1.7 to 10% of adults are affected (35). In our study, two children with cholestasis and suspected of having overlap syndrome AIH with PSC had elevated GGT and ALP levels. Nevertheless, despite undergoing Magnetic Resonance Cholangiopancreatography, no specific imaging features indicative of PSC, such as bile duct stenosis or dilatation, were observed.

The levels of albumin, INR or PT reflect the synthetic function of the liver, and these are also the prognostic indicators for liver disease (36). The degree of impairment of the synthetic function of the liver (prolonged PT, elevated INR, and hypoalbuminemia) in the AIH group at our center is comparable to that in the gene deficiency group, but much more severe than that in the DILI group, infectious liver disease group, and other etiology group. Some studies have reported that patients with AIH typically show elevated aminotransferase levels, with approximately half of the patients having impaired synthetic function, namely low albumin levels and elevated international normalized rates (37), which is consistent with the findings of this study.

The main histological features of AIH are more than moderate interface hepatitis and lymphoplasmacytic infiltration (3, 38), which are not highly specific. We found that portal lymphoplasmacytic infiltration, lobular inflammation combined with more than moderate interface hepatitis and lymphoplasmacytic infiltration are the main features of AIH. Other chronic liver diseases such as DILI,

viral hepatitis, and Wilson's disease may also be comorbid with interface hepatitis (39), which is usually mild or moderate. The lobular hepatitis with lymphoplasmacytic infiltration is considered the characteristic histological feature of acute-onset AIH, which is not included in the revised and simplified IAIHG systems (2, 40). Lymphoplasmacytic infiltration is another characteristic feature of AIH (18), but only two patients showed lymphoplasmacytic infiltration in AIH group. Of course, the number of liver biopsies performed in this research center is relatively small. In the future, more patients are needed to draw more definitive conclusions.

The AIH scoring systems have had good sensitivity and specificity in most patients (41). In our study, the simplified and revised systems had good diagnostic efficiency for AIH. Some studies have shown that compared with the simplified system, the revised system has higher sensitivity (100% vs. 95%), lower specificity (73% vs. 90%), and lower accuracy (82% vs. 92%) for diagnosis (42). However, our results showed that the revised system's diagnostic accuracy was superior to that of the simplified system. Some scholars have suggested that the simplified system is more applicable to patients with typical performance, while the revised system is more applicable to patients with atypical performance, which may be the reason why the revised system's diagnostic accuracy surpassed that of the simplified system in our study (40). Only 34.6% (72/208) of the children in this study received liver biopsies at their initial visit, so most of the children did not receive scores for the liver biopsy item, which may have biased the scoring results.

During the follow-up period, none of the patients in the DILI group progressed to cirrhosis, while 38.9% of the children with AIH eventually developed cirrhosis, which is consistent with previous literature (43, 44). Many gene deficiencies reported in the literature have a tendency to progress to cirrhosis, including WD (45), This finding is consistent with our research, which demonstrated that the cirrhosis rate in the gene deficiency group similar to that in the AIH group. According to the literature, the 10-year survival rate for AIH is 79%–98%, the 20-year survival rate is 77%–90%, and the 30-year survival rate is 55% (46). The mortality rate (1/18, due to non-AIH diseases) in the AIH group was not high, which indicated that the prognosis of AIH is generally favorable, highlighting the importance of early diagnosis and effective management in maintaining a positive outcome for patients.

At present, the diagnosis, examination, and follow-up management system for adult AIH has been well established, but research on pediatric patients remains limited (47). Based on the results of this study, we proposed a diagnostic process for AIH, which may help general pediatricians to identify and diagnose AIH.

Advantages and limitations of this study

The advantage of this study lies in its extended follow-up period, with some patients being monitored for up to 10 years. However, several limitations must be acknowledged. Firstly, this study is retrospective and the number of patients with AIH is small. Secondly, the absence of radiological data poses another constraint in the study. Thirdly, the small number of patients receiving liver biopsy may have introduced some bias into the analysis of the liver histopathological characteristics and the evaluation of the AIH scoring system's efficacy.

Conclusion

The diagnosis of AIH is rather challenging. This article retrospectively analyzes the differences between AIH and other causes of AIH-like liver disease at baseline. We have proposed that ANA $\geq$ 1:320, being positive for anti-LKM1 or SMA (with anti-LC1 or Ro-52 positivity serving as auxiliary diagnostic significance), elevated serum IgG or globulin levels are helpful for early identification of AIH. Moreover, the presence of lobular hepatitis with more than moderate interface hepatitis and lymphoplasmacytic infiltration contribute to the diagnosis of AIH. The proposed diagnostic process for AIH would help general pediatricians with early recognition of AIH.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by the Medical Ethics Committee at Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

DM: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing, Software. XL: Data curation, Writing – review & editing. GA: Data curation, Funding acquisition, Writing – review & editing. WP: Funding acquisition, Investigation, Writing – review & editing. LL: Data curation, Writing – review & editing. YH: Investigation, Writing – review & editing. YiL: Investigation, Writing – review & editing. YuL: Investigation, Writing – review & editing. ZZ: Validation, Writing – review & editing. HZ: Data curation, Writing – review & editing. ZH: Data curation, Writing – review & editing. XH: Data curation, Formal Analysis, Software, Writing – review & editing. SS: Conceptualization, Supervision, Validation, Writing – review & editing, Writing – original draft.

FF: Conceptualization, Supervision, Writing – original draft, Writing – review & editing, Formal Analysis.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Development of an artificial intelligence-based multimodal model for assisting in the diagnosis of necrotizing enterocolitis in newborns: a retrospective study

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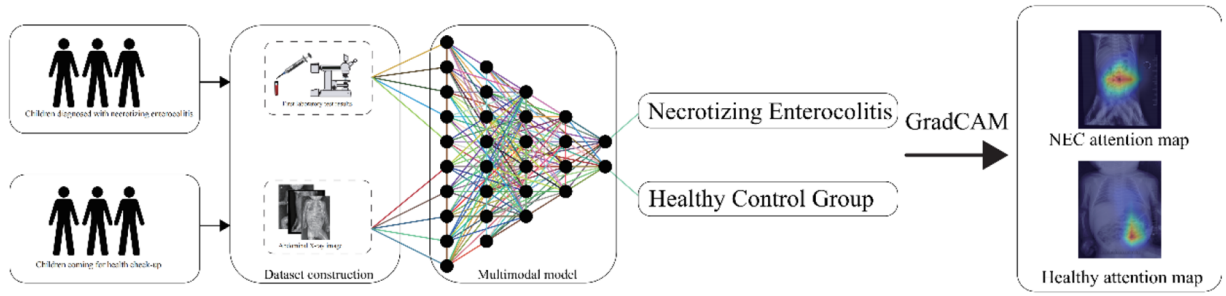
Objective: The purpose of this study is to develop a multimodal model based on artificial intelligence to assist clinical doctors in the early diagnosis of necrotizing enterocolitis in newborns.
Methods: This study is a retrospective study that collected the initial laboratory test results and abdominal x-ray image data of newborns (non-NEC, NEC) admitted to our hospital from January 2022 to January 2024.A multimodal model was developed to differentiate multimodal data, trained on the training dataset, and evaluated on the validation dataset. The interpretability was enhanced by incorporating the Gradient-weighted Class Activation Mapping (GradCAM) analysis to analyze the attention mechanism of the multimodal model, and finally compared and evaluated with clinical doctors on external datasets.
Results: The dataset constructed in this study included 11,016 laboratory examination data from 408 children and 408 image data. When applied to the validation dataset, the area under the curve was 0.91, and the accuracy was 0.94. The GradCAM analysis shows that the model's attention is focused on the fixed dilatation of the intestinal folds, intestinal wall edema, interintestinal gas, and portal venous gas. External validation demonstrated that the multimodal model had comparable accuracy to pediatric doctors with ten years of clinical experience in identification.
Conclusion: The multimodal model we developed can assist doctors in early and accurate diagnosis of NEC, providing a new approach for assisting diagnosis in underdeveloped medical areas.

KEYWORDS
artificial intelligence, necrotizing enterocolitis, multimodal model, attention analysis, computer-aided diagnosis



GRAPHICAL ABSTRACT

Introduction

Necrotizing enterocolitis (NEC) is the leading cause of neonatal death (1–3). The incidence of NEC is approximately 1%–3%, with a mortality rate of 15%–30% (4). In extremely low birth weight infants, the incidence of NEC can reach 5%–16%, with a mortality rate as high as 20%–50% (5). Surviving NEC infants often suffer from severe complications such as intestinal failure, short bowel syndrome, growth retardation, and neurological developmental disorders, which greatly affect their quality of life (6–9). Early diagnosis of NEC is crucial for improving the prognosis of affected infants. Currently, the diagnosis of NEC mainly relies on a comprehensive evaluation of clinical manifestations, laboratory tests, and imaging studies (10). However, clinical manifestations and laboratory tests lack specificity, making the integration of imaging studies particularly important. However, most clinical doctors lack systematic training in imaging, therefore heavily relying on imaging reports prepared by radiologists. In actual practice, due to various factors such as professional expertise and experience, different radiologists may provide different reports for the same patient's chest and abdomen films, which can affect the treatment decisions of clinical doctors and ultimately the health and development of the infants. Early and rapid diagnosis of NEC has always been a major challenge in intensive care clinical practice.

Multimodal deep learning models have attracted increasing attention in the field of artificial intelligence in recent years (11–14). Different forms or sources of information can all be referred to as modalities, and data composed of two or more modalities are called multimodal data. As a large amount of data of various types is generated in clinical practice, multimodal deep learning models have been widely applied and developed in the medical field. In the field of NEC-assisted diagnosis, the published studies mainly focus on the use of single-modal data such as laboratory test indicators or abdominal x-rays and ultrasound for NEC recognition and assisted diagnosis (15–18). However, these models have poor generalization, and the changes in NEC patients' blood parameters and imaging data have not been fully explored.

In order to achieve early, rapid, economical, and standardized diagnosis of NEC in clinical scenarios with imbalanced medical resources, in this study, we constructed an AI-based multimodal model and used admission laboratory test data and abdominal x-ray image data to build, train, and evaluate the model. Then, we conducted interpretability analysis and external validation with clinical doctor data, achieving the best-known performance in assisted diagnosis. This effectively assists clinical doctors in diagnosing and treating NEC in underdeveloped areas, increasing the early diagnosis rate of NEC, reducing misdiagnosis, missed diagnosis, and the occurrence of complications, and protecting the health of children.

Method

Dataset establishment

This study is a cohort study. A total of 408 newborns who were admitted to our NICU from January 2022 to January 2024 were selected as the research subjects, including 204 cases of NEC patients and 204 cases of healthy infants. All NEC patients met the diagnostic criteria for NEC. The diagnostic criteria are based on the 2020 NEC diagnostic guidelines. Figure 1 shows the flowchart of this study.

The initial blood laboratory examination indicators (12 laboratory indicators determined according to the NEC diagnosis and treatment guidelines and clinical experience of doctors) and abdominal x-ray images of the research subjects were collected as research data. Inclusion criteria for image collection: (1) The patient is diagnosed with NEC for the first time. (2) Complete laboratory examination and abdominal x-ray examination are performed before treatment. Exclusion criteria: (1) Conditions such as pneumonia, congenital heart disease, sedative use, etc., may affect the quality of abdominal x-ray image diagnosis. (2) Non-first admission children. (3) Missing laboratory test indicators exceeding 30%. (4) The presence of conditions such as pneumonia, septicemia, etc., which may affect the diagnosis of laboratory test results.

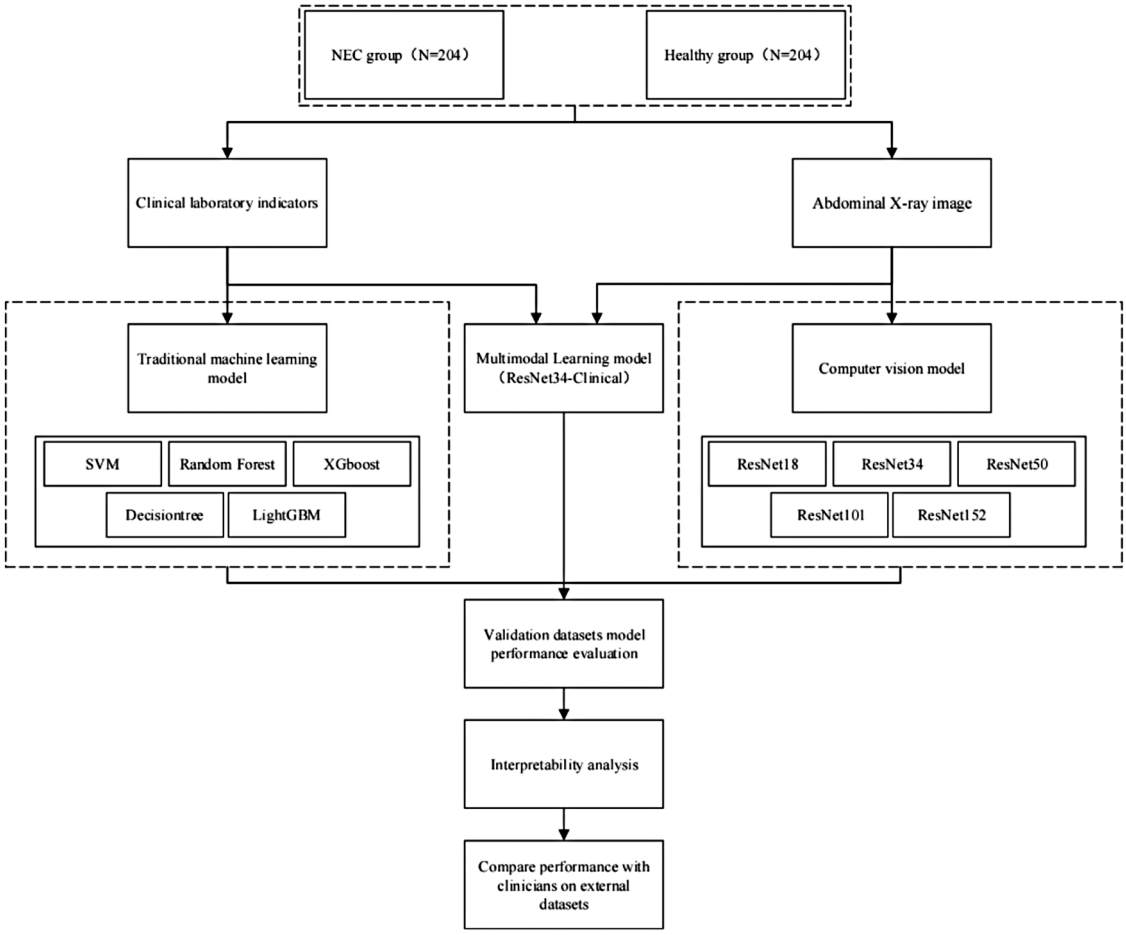


FIGURE 1
Study design flowchart.

All participants signed informed consent forms. This study was approved by the Hospital Ethics Review Committee. All data were strictly protected. All methods comply with relevant guidelines and regulations.

Data processing

A total of 408 newborns were included in this study, including 204 non-NEC newborns and 204 newborns diagnosed with NEC, gestational age ranging from 26 to 40 weeks, and an average gestational age of 33 weeks. A total of 11,016 laboratory test data of the 408 children and 408 abdominal x-ray images were analyzed. The dataset was partitioned into training and validation sets in an 8:2 ratio, comprising laboratory assay results and abdominal x-ray images from 326 to 82 children, respectively. The distribution of demographic data is shown in Table 1.

According to the NEC diagnosis and treatment guidelines, 27 laboratory examination data were included. These parameters include hematology analysis: neutrophil count, platelet count, lymphocyte count, neutrophil percentage, neutrophil-lymphocyte ratio, platelet-lymphocyte ratio, white blood cell count, and hemoglobin. In addition, C-reactive protein (CRP), procalcitonin (PCT), interleukin-6 (IL-6), and blood gas analysis, etc., were included.

TABLE 1 Comparison of clinical characteristics between the normal group and the NEC group in the cohort.

Clinical characteristic	Non-NEC (*N* = 204)	NEC (*N* = 204)	Total (*N* = 408)
Sex			
Male	100	98	198
Female	104	106	210
Gestational age			
<30	82	84	166
30–34	62	65	127
35–37	41	40	81
38–40	19	15	34

Abdominal x-ray image data were collected from newborns who met the inclusion criteria, and x-ray examinations were performed using the Carestream DRX Revolution machine. The reason for using abdominal x-ray examination in this study is that abdominal ultrasound requires a professional

ultrasonographer to achieve good results, and it is highly subjective. Abdominal x-ray examination has higher stability, can reflect the progression of NEC, and can be performed in hospitals at all levels. It has good generalizability and is also a reliable evidence recommended in clinical guidelines. In order to reduce the overfitting phenomenon during model training, image enhancement techniques such as horizontal flipping, rotation, and stretching were used to enhance the x-ray images in the dataset. All images were downsampled and converted into JPG images with a resolution of 256 × 256.

Model design

This paper proposes a multimodal classification method based on Residual Neural Network (ResNet) (19) for processing images and one-dimensional CNN for processing data. By fusing the features of images and one-dimensional data in the fully connected layer, the joint classification of multimodal data is achieved. The residual network addresses the optimization training difficulties of neural networks when the depth increases by introducing residual blocks. The laboratory test results are processed by one-dimensional CNN to construct a multimodal network that simultaneously processes laboratory and symptom images.

To evaluate the performance of the multimodal model, five single-modal residual convolutional networks (ResNet18, ResNet34, ResNet50, ResNet101, ResNet152) and separate traditional machine learning models [Support Vector Machines (SVM), Random Forest, Decision Tree, XGBoost, and LightGBM] were also designed for model training and evaluation.ResNet (Residual Network) is a type of deep neural network architecture characterized by the introduction of residual connections, allowing the network to more easily learn identity mappings during training and alleviating the problem of vanishing or exploding gradients. Traditional machine learning models typically refer to models based on statistical learning theory, which are often composed of predefined mathematical functions. The parameters of these models are adjusted through training data to minimize predefined loss functions. Deep neural networks like ResNet often have deeper hierarchical structures and are capable of solving complex tasks by learning richer feature representations, whereas traditional machine learning models tend to rely more on handcrafted features or shallow feature extractors to address relatively simple tasks. In order to reduce the training time cost of the model, we introduce the idea of transfer learning and select the ResNet34 model pre-trained on the ImageNet dataset as the benchmark model for preliminary training, and perform multi-modal model training through local unfreezing. Ten-fold cross-validation was used for validation. Adam optimizer and grid parameters were used for training. The parameters that generated the minimum loss function value in the validation dataset within 100 epochs were selected as the best-performing model.

The hyperparameters were set as Batch size 32 and learning rate 0.000001. The random seed was set as 1024. The model training, construction, and validation were performed using Pytorch (2.2.0) (20) on a computer with an AMD EPYC 7532 processor (32 cores, 64 threads, 2.4–3.3 GHz) and 4×RTX 4090 cards (24GB GDDR6X VRAM, 16,384 CUDA cores).

Model validation

In this study, the multimodal model was combined with gradient-weighted class activation mapping (GradCAM) (21, 22) for attention analysis, enhancing the interpretability of the model and the confidence of doctors. Global average pooling was applied to the last convolutional layer of the trained AI model using a classification activation map. The training weights of each output of the global average pooling layer indicate the importance of each feature map from the last convolutional layer. Then, weights were applied to the corresponding feature maps to generate significance maps. These significance maps were overlaid on the original abdominal x-ray images to visually differentiate the regions of interest prioritized by the multimodal model. To assess the model's performance, we additionally collected an external validation cohort consisting of 50 pediatric cases for prospective validation. After the diagnosis team made a definitive diagnosis, a human-machine comparison test was conducted: three doctors with advanced professional titles in neonatal ICU (unaware of the diagnosis results) and the model were asked to assess the cases based solely on laboratory tests and abdominal x-ray images.

Statistical analysis

Performance indicators, including accuracy, sensitivity, specificity, area under the curve (AUC), and F1 score, were compared between the proposed model and existing methods. All analyses were conducted using R version 4.0.0.

Results

Demographic data

A total of 408 newborns were included in this study, including 204 non-NEC newborns and 204 newborns diagnosed with NEC,

TABLE 2 Laboratory test items included in the normal group and NEC group in the dataset.

	Non-NEC (n = 204)	NEC (n = 204)	P
CRP(mg/L)	2.9	12.1	<0.001**
PCT(ng/ml)	0.7	2.34	<0.001**
Neutrophil percentage (%)	52	45	<0.001**
Platelets(10^9/L)	152	132	<0.001**
Interleukin 6 (pg/ml)	12	16	<0.001**
Leukocyte (10^9/L)	11.4	9.5	<0.001**
Transferrin (g/L)	1.2	0.7	<0.001**
Total protein (g/L)	49.6	59.1	<0.001**

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TABLE 3 Diagnostic performance of deep learning algorithms. The best performance is highlighted in bold, while the second best performance is underlined.

Model	Accuracy	Sensitivity	Specificity	Precision	F1 score
SVM	0.7978	0.7915	0.7927	0.8049	0.8172
RF	0.8032	0.7811	0.8188	0.759	0.8194
XGboost	0.7741	0.8034	0.8192	0.8083	0.7746
Decisiontree	0.8194	0.8056	0.8139	0.8053	0.7956
LightGBM	0.8051	0.7523	0.7949	0.7543	0.7612
ResNet18	0.8644	0.8949	0.8234	0.8445	0.8554
ResNet34	0.9199	0.9145	0.8668	0.8606	0.8493
ResNet50	0.8564	0.8725	0.8366	0.8408	0.8646
ResNet101	0.821	0.8257	0.8395	0.9062	0.8449
ResNet152	0.8475	0.8134	0.8662	0.9064	0.8928
ResNet34-Clinical	0.942	0.9365	0.9228	0.9302	0.9557

gestational age ranging from 26 to 40 weeks, and an average gestational age of 33 weeks. A total of 11,016 laboratory test data of the 408 children and 408 abdominal x-ray images were analyzed. The distribution of demographic data is shown in Table 1.

In order to improve the efficiency of model training and reduce the interference of irrelevant laboratory test results on model training, we conducted significance analysis on all laboratory test results (blood routine, biochemistry, immunity, etc.). The results after screening are shown in Table 2.

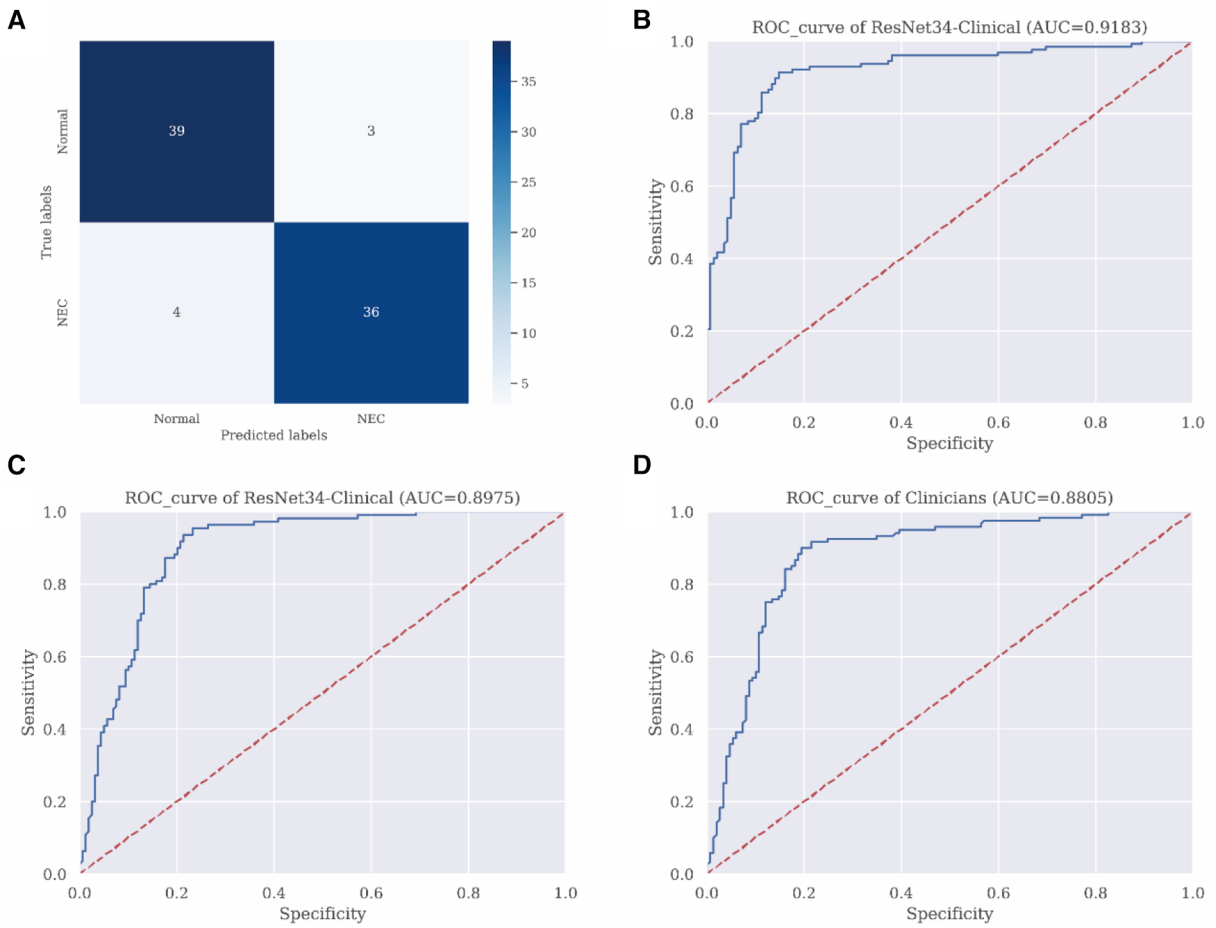

FIGURE 2
Performance evaluation of ResNet34-clinical model. (A) Confusion matrix of ResNet34-Clinical on the validation dataset. (B) Receiver operating characteristic curves of the ResNet34-Clinical model on the validation dataset. (C) Receiver operating characteristic curves of the ResNet34-Clinical model on the external independent dataset. (D) Receiver operating characteristic curves of clinical doctors on the external independent dataset.

Model evaluation

In this study, ten-fold cross-validation was used to evaluate the performance of each model, with the validation dataset being used for this purpose. Each data point included laboratory test results and abdominal x-ray image data of a newborn. Different types of data were used based on the structure of different models. Table 3 displays the diagnostic performance of each model using the validation dataset. Traditional machine learning models (SVM, RF, XGboost, Decisiontree, LightGBM) had diagnostic accuracies of 79.78%, 80.32%, 77.41%, 81.94%, and 80.51% respectively, significantly lower than the computer vision model (ResNet). The trained multimodal deep learning model (ResNet34-Clinical) exhibited the best performance in terms of accuracy, sensitivity, specificity, and F1 score, significantly outperforming both traditional machine learning models and single computer vision models. The confusion matrix of the ResNet34-Clinical model can be seen in Figure 2A.

A recall curve was plotted for the ResNet34-Clinical model, which achieved an AUC of 0.92 (Figure 2B). To assess the stability and generalizability of the model, we designed an additional external validation group of 50 cases for evaluation, comparing the model's ROC curve with the ROC curve of human clinicians. The diagnostic performance of the multimodal deep learning model (AUC = 0.83) was comparable to that of human clinicians (AUC = 0.82) (Figures 2C,D).

In order to enhance confidence and interpretability of the model in clinical scenarios, we applied the GradCAM technique to the model. After validating the abdominal x-ray images in the validation dataset, we found that the model's attention was mainly focused on fixed dilation of intestinal loops, intestinal wall edema, intussusception, and portal venous gas, which aligns with clinical experience and demonstrates the feasibility of our model. The evaluation of model attention can be seen in Figure 3.

Discussion

This study developed an artificial intelligence-based multimodal model to assist clinical doctors in early diagnosis of NEC. Among the published multimodal studies, the best performance was achieved by WENJING GAO's ResNet50 model (17), a deeper neural network, trained on a large-scale dataset (n 3= 4,535), yielding more accurate results (ROC = 0.93). In contrast, our study utilized a faster training speed, shallower neural network, ResNet34 model, achieving comparable results (ROC = 0.91) on a smaller dataset (n = 408), suggesting that lightweight models may have better applicability in medical image analysis due to limitations in data scale. To enhance clinical

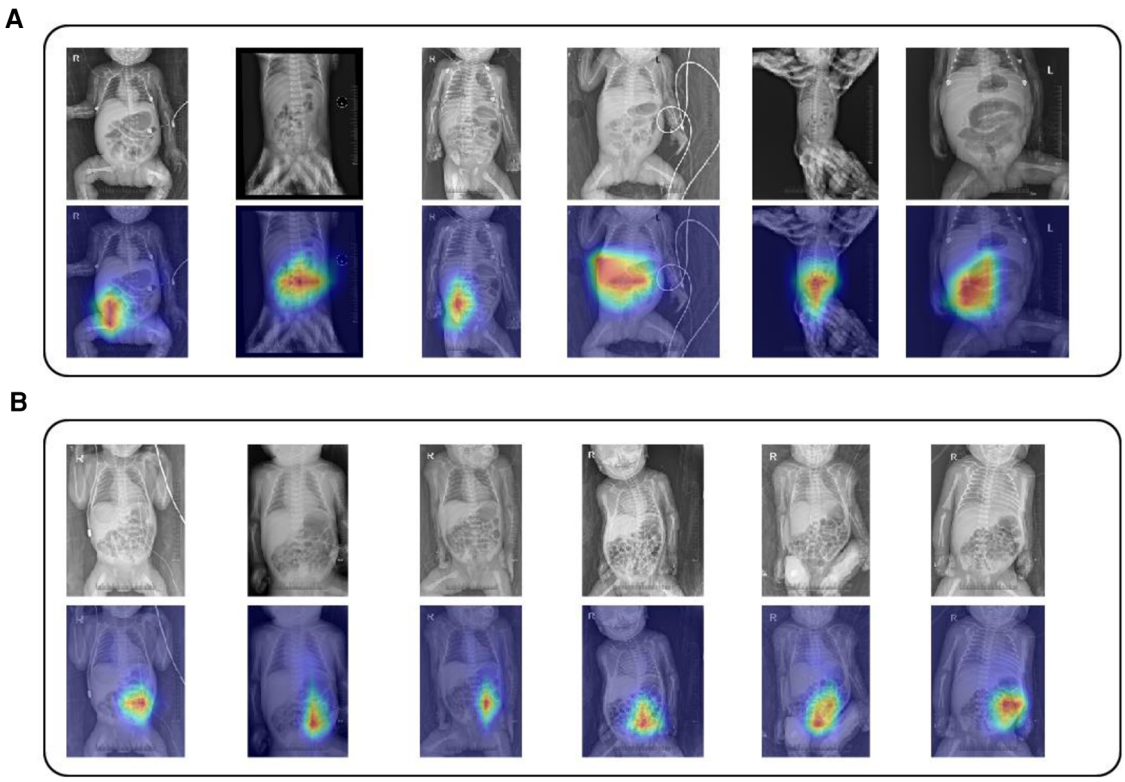

FIGURE 3
ResNet34-Clinical grad-CAM images (A) Overlaid images of abdominal x-rays and GradCAM for NEC patients. (B) Display of overlaid images of abdominal x-rays and GradCAM for non-NEC newborns. The thicker red area indicates the image portions that ResNet34-Clinical focuses on during the classification of NEC patients and healthy non-NEC newborns.

confidence in our model, we not only integrated the approach proposed by GAO, employing gradient-weighted class activation mapping to explain the model's decision process, but also conducted an additional human-machine comparative experiment with an external validation cohort, achieving promising results (ROC = 0.83). The visually significant regions identified by GradCAM are consistent with clinical experiences, including fixed dilation of intestinal loops, intestinal wall edema, intramural gas, and portal vein gas. Our multimodal model can aid clinical doctors in early and accurate diagnosis of NEC in medically underserved areas, reducing complications and even fatalities resulting from misdiagnosis.

Our multimodal model does not require special laboratory tests and can be routinely implemented for NEC diagnosis by integrating routine laboratory tests available in every hospital with standardized abdominal x-ray images. The performance of the multimodal model is significantly superior to that of other single-modal models and traditional machine learning models.

Limitations: Firstly, since this study is a single-center study, in order to ensure the generalizability of the model, we plan to validate and test the model with a larger dataset from multiple centers. Secondly, the performance of the model in this study is similar to the diagnosis accuracy of pediatric doctors with ten years of clinical experience. We plan to establish a larger-scale dataset in the next step to improve the accuracy of the multimodal model. Thirdly, for the diagnosis of NEC, abdominal ultrasound images are also crucial. Our multimodal model currently only processes laboratory results and abdominal x-ray image information. We plan to incorporate an abdominal ultrasound data processing module in the next step to fully exploit clinical data and further improve the model's performance.

In this study, we developed a multimodal model using deep learning that can assist clinical doctors in early and rapid diagnosis of NEC. This model has broad prospects for assisting in the diagnosis of NEC in medically underdeveloped areas, enabling doctors to achieve early, convenient, economical, and accurate diagnosis and treatment of NEC, better safeguarding the health of newborns.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethics Committee of Qingdao Women and Children's Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

Author contributions

KC: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. SC: Formal Analysis, Visualization, Writing – original draft. YM: Methodology, Writing – original draft. ZH: Writing – original draft. LX: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Rotavirus outbreaks in China, 1982–2021: a systematic review

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Background: Rotavirus is globally recognized as an important cause of acute gastroenteritis in young children. Whereas previous studies focused more on sporadic diarrhea, the epidemiological characteristics of rotavirus outbreaks have not been systematically understood.

Methods: This systematic review was carried out according to the Preferred Reporting Items for Systematic Review and Meta-Analysis standards, WANFANG, China National Knowledge Infrastructure (CNKI), PubMed, and Web of Science databases were searched from database inception to February 20, 2022. We used SPSS 21.0 statistical software for data analysis, RStudio1.4.1717, and ArcGIS trial version for plotting bar graphs and maps.

Results: Among 1,596 articles, 78 were included, with 92 rotavirus outbreaks and 96,128 cases. Most outbreaks (67.39%, 62/92) occurred in winter and spring. The number of rotavirus outbreaks reported in the eastern region was more than that in the western region. Outbreaks were most commonly reported in villages (33/92, 35.87%), followed by hospitals (19, 20.65%). The outbreak duration was longer in factories and workers' living places, and villages, while it was shorter in hospitals. Waterborne transmission was the main transmission mode, with the longest duration and the largest number of cases. Rotavirus groups were identified in 66 outbreaks, with 40 outbreaks (60.61%) caused by Group B rotaviruses and 26 outbreaks (39.39%) caused by Group A rotaviruses. Significant differences were found in duration, number of cases, settings, population distribution, and transmission modes between Groups A and B rotavirus outbreaks.

Conclusion: Rotavirus is an important cause of acute gastroenteritis outbreaks in China. It should also be considered in the investigation of acute gastroenteritis outbreaks, especially norovirus-negative outbreaks.

KEYWORDS

rotavirus, outbreak, epidemiological characteristics, transmission, genotypes

1 Background

Acute gastroenteritis accounts for a significant portion of global morbidity and mortality. Norovirus is the most common pathogen of acute gastroenterological outbreaks, and outbreaks caused by rotavirus are also common (1). Rotavirus is a non-enveloped virus classified within the *Reoviridae* family. The 11 segments of double-stranded RNA residing in

the core encode six viral proteins (VP1-VP4, VP6, and VP7) that make up the viral capsid, along with six non-structural proteins (NSP 1–6). According to the different VP6 antigenicity, rotavirus was divided into 10 groups (A-J). Groups A, B, C, and H infect humans and animals, while other groups only infect animals (2). Group A rotavirus is prevalent worldwide, especially among children (3). Group B rotavirus appears to have been reported only in Asia in the 1980s (4). At the same time, enteric infections caused by Group C and H rotaviruses have been rarely reported.

Previous rotavirus surveillance and studies have focused on sporadic diarrhea, especially in children under 5 years old. Many studies have reported the epidemiological characteristics, disease burden, clinical manifestation, and genotype distribution of rotavirus diarrhea among children in China and other countries (5–7). In China, sentinel surveillance of rotavirus in hospitalized children under 5 years of age with diarrhea began in 1998. In 2009, China joined the Global Rotavirus Surveillance Network. By 2021, China's rotavirus sentinel surveillance network has covered 42 sentinel hospitals in 31 provinces (autonomous regions and municipalities), and the surveillance objects have been extended to children and adults with diarrhea in outpatient and inpatient settings (8). A meta-analysis showed that the overall rate of rotavirus diarrhea in children under 5 years of age was 34.0% (95%CI: 31.3–36.8%) during 2011–2018, with differences in rotavirus detection rates among different regions in China (9). Outbreaks caused by rotavirus are also a component of measuring the overall disease burden of rotavirus. However, the epidemiological characteristics of rotavirus outbreaks have not been systematically analyzed in China. Therefore, this study collected data about rotavirus outbreaks reported in the literature from 1982 to 2021 to characterize rotavirus outbreaks in terms of temporal distribution, regional distribution, population distribution, settings, transmission modes, clinical symptoms of cases, and genotypes in China.

2 Methods

2.1 Search strategy

This systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist. We identified "rotavirus" and "outbreak" or "exposure" and "China" as the keywords, and searched for articles on PubMed, Web of Science databases, China National Knowledge Infrastructure (CNKI), and Chinese Wan Fang digital database (WANFANG) from the inception of the databases to February 20, 2022.

2.2 Selection and exclusion criteria

Studies had to meet all four of the following criteria for inclusion: (1) were published in Chinese or English; (2) three or more cases with an epidemiological association can be defined as an outbreak; (3) provided at least three of the following information: outbreak time, region, the number of cases, number of persons exposed, attack rates, settings, transmission modes, genotypes; (4) rotavirus antigen or nucleic acid could be detected in at least two patients' stool specimens, or rotavirus IgM antibodies could be detected in at least two patients' serum specimens. As in the co-infection outbreaks, if more rotavirus samples were detected in specimens than other pathogens, we supposed that rotavirus infection should be the main diarrheal pathogen in these outbreaks.

Studies should be excluded if they meet one of the following criteria: (1) were not available for full-text or valid data could not be collected from the abstract; (2) the subjects were not humans; (3) suspected rotavirus outbreaks without enough laboratory evidence; (4) were review or meta-analysis articles.

2.3 Data processing and analysis

Repeated outbreaks were counted only once, and the same information that appeared in different studies was summarized and integrated. If one article contained two or more outbreaks, each outbreak should be counted separately. The following information was extracted from each study: article information (title, geographical position, author, journal name, time of publication), outbreak terms (time, region, number of cases, number of persons exposed, attack rates, settings, transmission modes, genotypes). The definition of hospital outbreaks is that the index cases and all other cases not experience diarrhea before admission, and the outbreaks occurred in the hospital. Studies searching, selection and exclusion, and data extraction were carried out independently by two individuals, and inconsistent results were then arbitrated by the third person.

2.4 Statistical analysis

Endnote X9 was used to import and manage retrieved records. WPS Office was used to establish an epidemiological information database of rotavirus outbreaks. RStudio1.4.1717[1] and the ArcGIS trial version were used for plotting bar graphs and maps. A map of China is available at the Resource and Environment Science and Data Center.[2] SPSS 21.0 statistical software was used to conduct data analysis. A chi-square test was performed to compare the rates between different groups. The Wilcoxon rank-sum test was used to analyze differences in the number of rotavirus outbreaks between different regions, seasons, and genotypes. The Kruskal-Wallis H test was calculated to compare the differences in epidemiological characteristics between rotavirus outbreaks in different settings or by different transmission modes. Statistical significance was assumed for $p < 0.05$ (two-tailed).

3 Results

3.1 Literature screening and basic information

In total, 2,314 articles were initially identified. Of them, 1,080 were unique records, and 941 were excluded based on a title and abstract review (see Figure 1). A total of 139 full-text articles were

1 https://www.rstudio.com/products/rstudio/download/

2 https://www.resdc.cn/data.aspx?DATAID=200

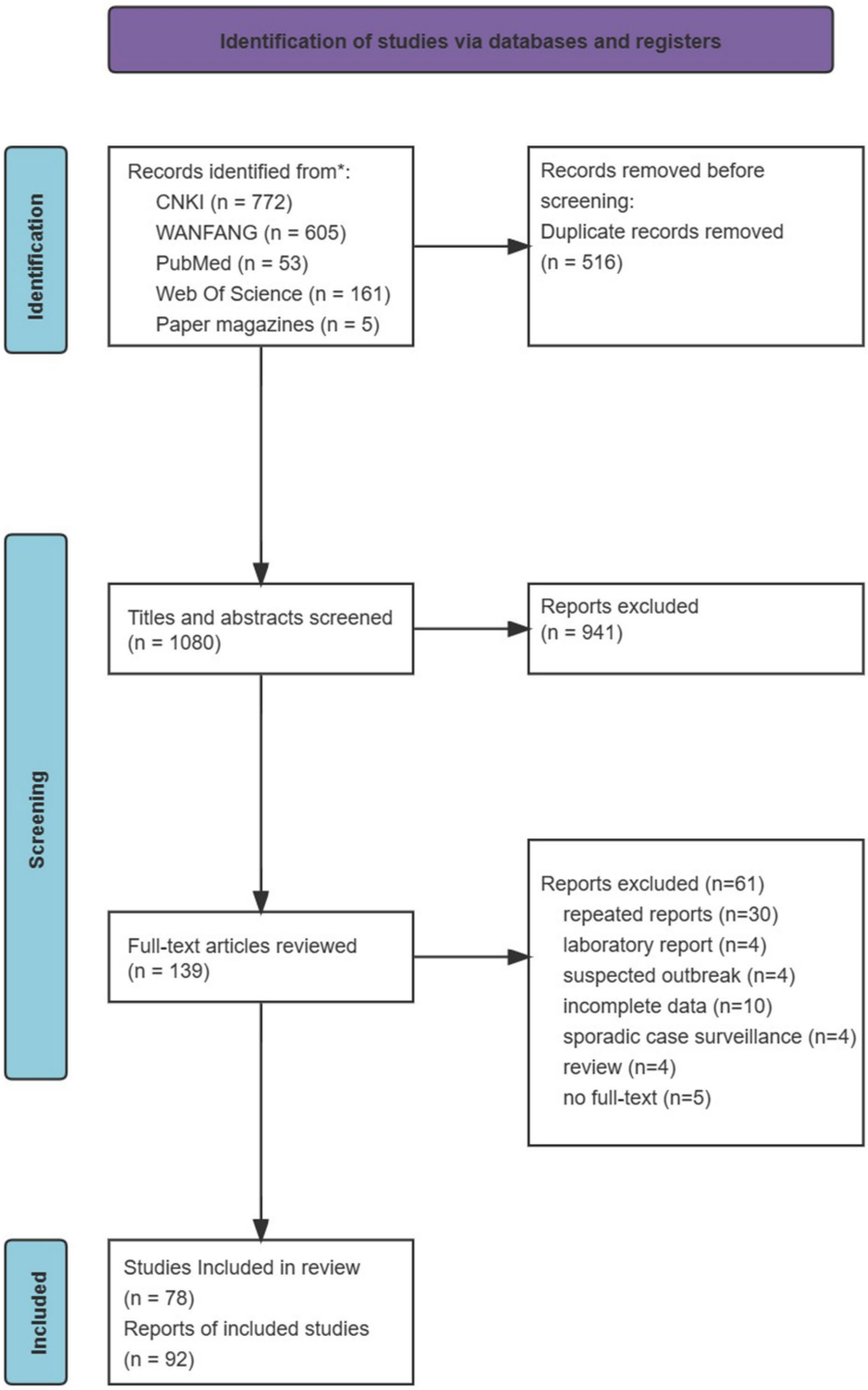

FIGURE 1
PRISMA flowcharts showing the selection process.

assessed for eligibility. From these, 30 were duplicate reports, 4 reported laboratory data only (with no epidemiological data), 4 were not identified as rotavirus outbreaks, 10 had incomplete data, 4 belonged to sporadic case surveillance, 4 were reviews, and 5 did not have full text. Finally, 78 articles were included.

A total of 92 rotavirus outbreaks were reported in the 78 included articles. The outbreaks occurred between November 1982 and March 2021. Ninety-two rotavirus outbreaks involved 96,128 cases, with a median of 140 cases per outbreak (ranging from 3 to 20,000). The duration was reported for 72 outbreaks, with a median of 18 days (ranging from 2 to 154 days). The attack rates were reported for 64 outbreaks and ranged from 0.65% to 85.34%, with a median of 17.55%. The outbreak with the fewest cases occurred in a hospital in Jiangxi province in April 2013, involving 3 cases, and lasting 3 days. The

largest reported outbreak occurred in a county in Anhui province in 1983, affecting 20,000 cases, and lasting for 4 months. It was speculated that the water source was polluted due to heavy rainfall.

3.2 Temporal distribution

A total of 91 rotavirus outbreaks were documented with year and month information. One outbreak was reported in the spring of 1983 but did not provide monthly information. The earliest outbreak occurred in November 1982, and the latest occurred in March 2021. Forty-six outbreaks (50.00%) were reported during 1982 to 1989 (see Figure 2). The number of outbreaks peaked in 1983 with 13 outbreaks. The average interval between the occurrence and publication of the 92 outbreaks was 2 years.

Rotavirus outbreaks occurred every month, with the highest number of outbreaks in April (18 outbreaks) and the lowest in September (2 outbreaks) (see Figure 3). There were 36 outbreaks in spring (March to May), 12 in summer (June to August), 18 in autumn (September to November), and 26 in winter (December to February). Most outbreaks occurred in winter and spring, accounting for 67.39% (62/92). The median duration of outbreaks (interquartile range, IQR) in the epidemic season (winter and spring) and the non-peak season (summer and autumn) were 18 (11, 32) days and 19 (7, 51) days, respectively. Statistical analysis showed no statistical significance ($W = 1{,}696.500$, $p = 0.822$) between the durations.

3.3 Regional distribution

Ninety-two outbreaks stated the occurrence areas, including 18 provinces, 2 municipalities (Beijing and Shanghai), 3 autonomous regions, and Hong Kong Special Administrative Region (see Figure 4). Guangxi Zhuang autonomous region reported the most outbreaks (n = 15), followed by Guangdong (n = 8) and Shandong (n = 7). Referring to Wu Ruijun and Zhu Baoshu's method (10), we used the "Hu line" to divide China into eastern and western regions and found that more outbreaks were reported in the east ($n = 88$) than in the west ($n = 4$) ($W = 4{,}092.00$, $p < 0.001$).

3.4 Population distribution and clinical symptoms

Information on the population distribution was available for 89 outbreaks. Among these outbreaks, 42 occurred in the general population, 28 involved only children, and 19 involved only adults. The numbers of male and female cases were reported in 42 outbreaks, with 7,520 male cases and 6,389 female cases, and the ratio of male to female was 1.18:1. The minimum ages of patients were available for 42 outbreaks, with a mean of 2 years, ranging from 30 min after birth to 20 years of age. The maximum ages were reported for 44 outbreaks, with a mean of 49 years, ranging from 9 days to 94 years of age. Age groups in the literature are inconsistent, and outbreaks mainly affected adults overall.

The most common symptom of cases was diarrhea. Diarrhea incidences were reported in 65 outbreaks, with 100% in 56 outbreaks. In the remaining 9 outbreaks, the incidences of diarrhea ranged from 45 to 85% in 6 outbreaks and was more than 90% in 3 outbreaks. Other common clinical symptoms included abdominal pain, bloating, nausea, headache, and vomiting (see Figure 5). Less common symptoms such as dizziness, fatigue, chills, and belching were also reported. The incidences of dehydration were reported in 12 outbreaks, with the median [interquartile range] being 56.45% (40.70%, 94.02%). A total of 22 deaths were reported in 5 outbreaks, with 4 cases dying from severe dehydration in 2

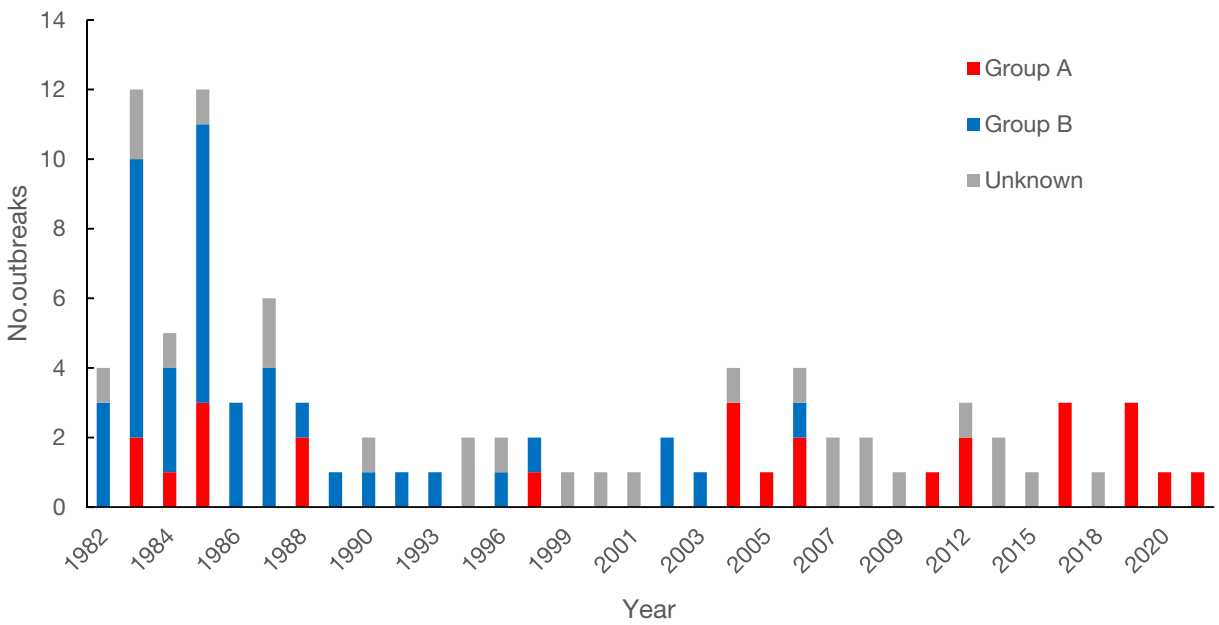

FIGURE 2
Yearly and group distribution of rotavirus outbreaks reported in China, 1982–2021.

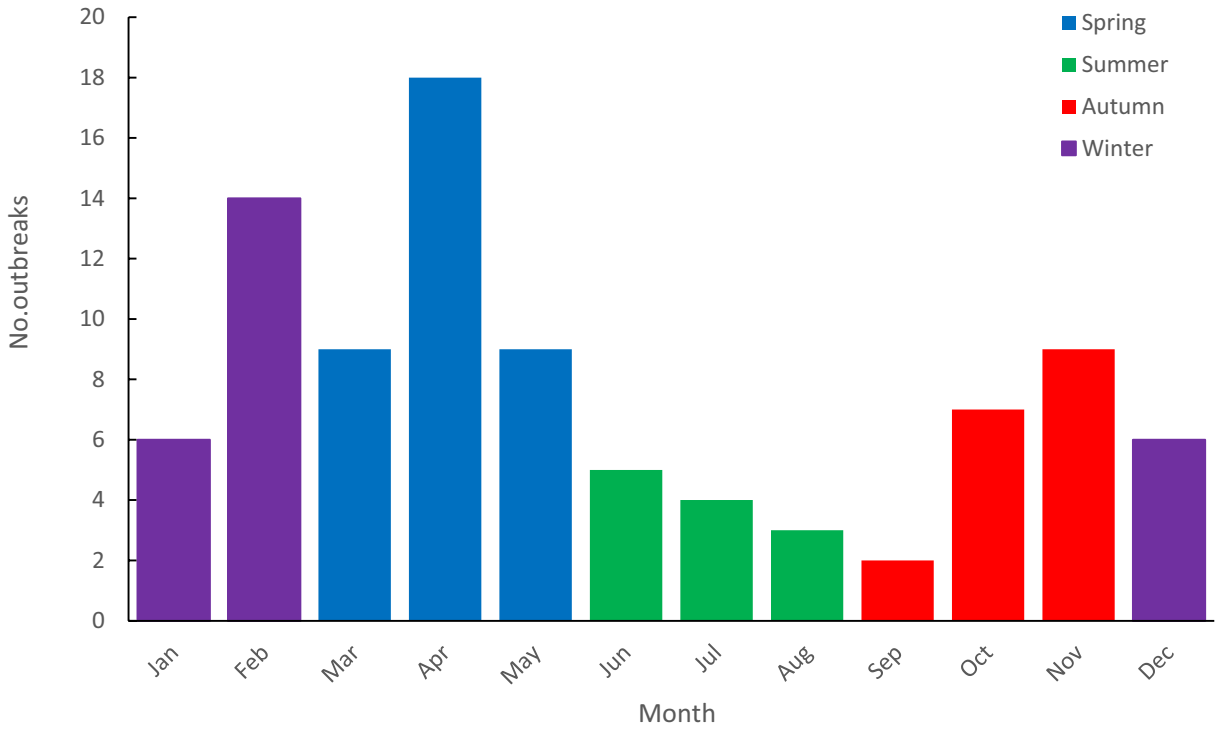

FIGURE 3
Monthly distribution of rotavirus outbreaks reported in China, 1982–2021.

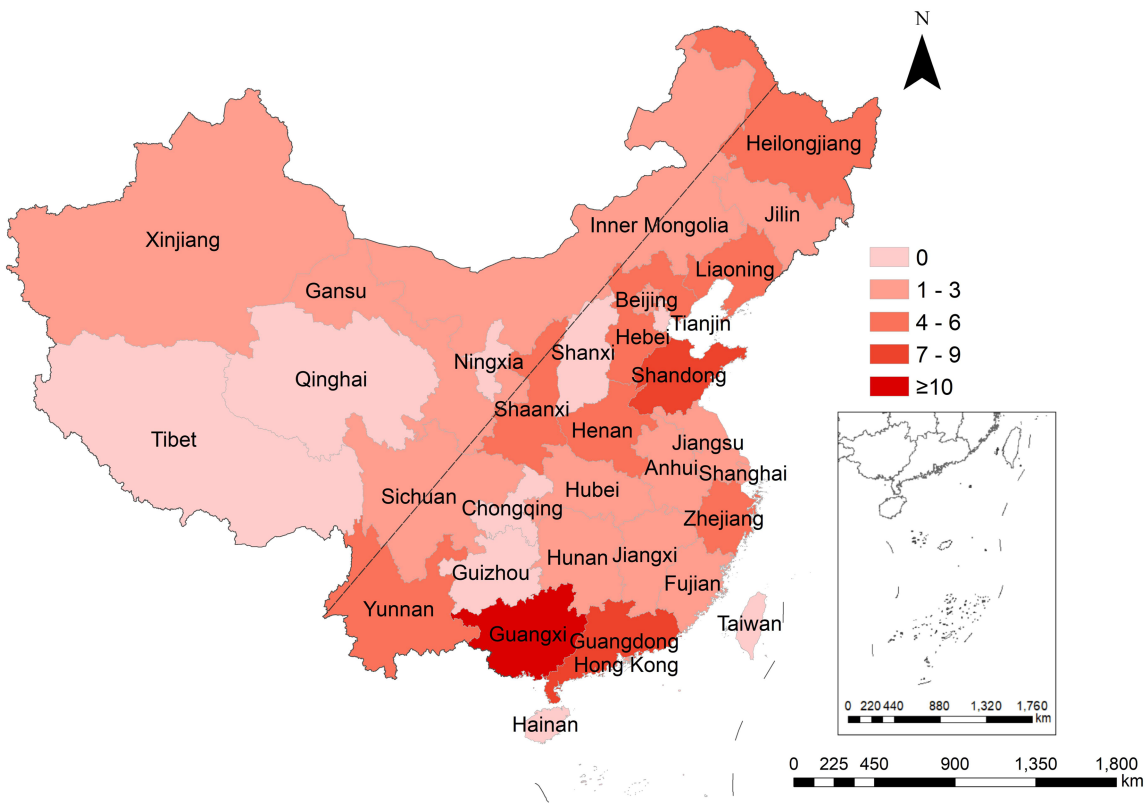

FIGURE 4
Regional distribution of rotavirus outbreaks reported in China, 1982–2021. The "Hu line" was used to divide China into eastern and western regions.

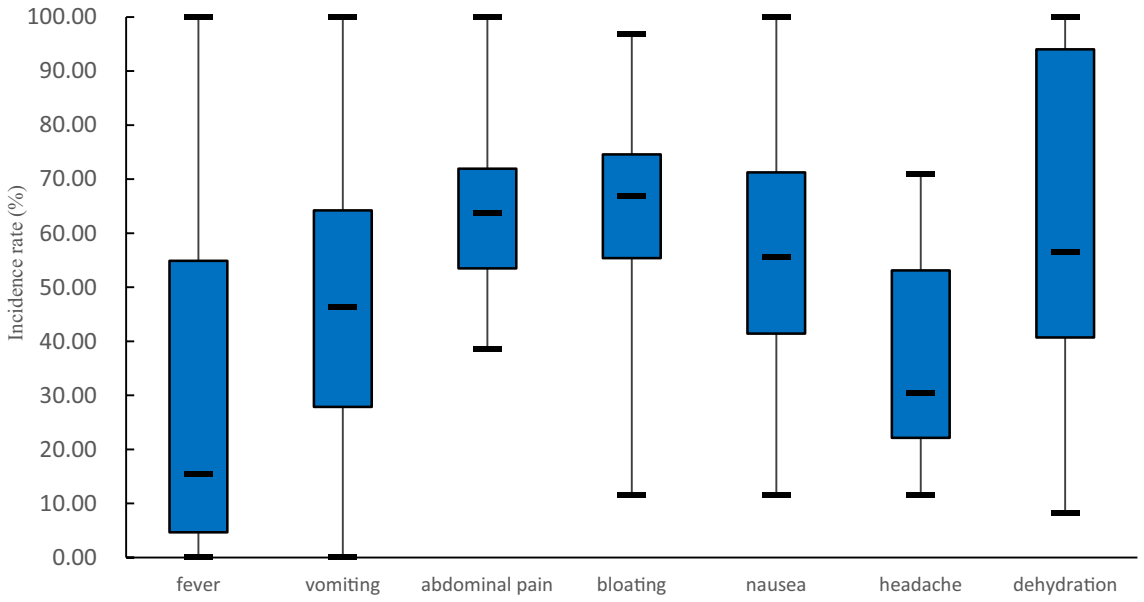

FIGURE 5
Common clinical symptoms (excluding diarrhea) of patients in rotavirus outbreaks in China, 1982–2021. The number of outbreaks involved in the analysis of each symptom is as follows: 42 in fever, 44 in vomiting, 37 in abdominal pain, 23 in bloating, 29 in nausea, 10 in headache, and 12 in dehydration.

outbreaks. The cause of death for the remaining 18 deaths was not reported. Sixteen death cases were reported in an outbreak reported in Fujian province in 1982, and the case fatality rate was 6.13% (16/261). After 2004, there have been no reported deaths in rotavirus outbreaks.

3.5 Settings and transmission modes

The majority of outbreaks occurred in villages (33/92, 35.87%). Outbreaks were also reported in hospitals (19, 20.65%), factories and workers' living places (14, 15.22%), schools (10, 10.87%), armies (6, 6.52%), communities (3, 3.26%), welfare home (1, 1.09%) and restaurants (1, 1.09%). Five outbreaks did not provide setting information.

Significant differences were found between outbreaks in different settings in terms of duration ($H = 26.997$, $p < 0.001$), and number of cases ($H = 35.726$, $p < 0.001$). No statistically significant differences were identified in outbreak attack rates ($H = 12.313$, $p = 0.055$). Outbreaks that occurred in factories and workers' living places and villages had a longer duration, while outbreaks in hospitals and armies had a shorter duration. The number of cases in factories and workers' living places was the greatest (see Table 1).

The modes of transmission were reported for 83 outbreaks. The common transmission modes were waterborne (48, 57.83%) and person-to-person (27, 32.53%). Three outbreaks (3.61%) involved foodborne transmission, three (3.61%) involved multiple transmission modes, and two (2.41%) did not exclude the possibility of respiratory transmission. In three foodborne outbreaks, one outbreak was caused by a co-infection of pathogenic *Escherichia coli* and rotavirus, and the risk factor analysis indicated that the suspected food was crayfish. The second outbreak occurred in a school, the school canteen's environmental hygiene was poor, and the plates were not cleaned in time. Combined with the clinical presentation of the case and laboratory findings, it was inferred that the outbreak was caused by an unclean diet. In another outbreak, the first case was a cafeteria worker who continued to work after becoming ill, causing diarrhea in students who had meals. Although rotavirus was not detected in the sampled food, it was presumed to be foodborne.

Waterborne transmission primarily occurred in villages (21), factories and workers' living places (13). Person-to-person transmission mainly occurred in hospitals (16), villages (4), and schools (3).

There are significant differences in outbreak duration ($H = 21.025$, $p < 0.001$) and the number of cases ($H = 34.948$, $p < 0.001$) among the different transmission modes, but there is no significant difference in attack rates ($H = 2.461$, $p = 0.482$). Waterborne outbreaks had the longest duration and highest number of cases. Person-to-person transmission outbreaks had the shortest duration and the lowest number of cases, but the highest attack rate (excluding outbreaks of unknown transmission modes).

3.6 Groups and genotypes

Rotavirus groups were identified in 66 outbreaks. Group A rotaviruses caused 26 outbreaks (39.39%), while Group B rotaviruses caused 40 outbreaks (60.61%). Genotypes were available in 9 Group A rotavirus outbreaks, including G1 (3), G2 (1), G1P[8] (1), G2P[4] (1), G4P[2] (1) and G9P[8] (2). Three G1 outbreaks occurred in 2004, 2005, and 2006, one G2 outbreak in 2004, one G1P[8] outbreak in 2006, two G2P[4] outbreaks in 1997 and 2017, respectively and two G9P[8] outbreaks in 2017 and 2019, respectively.

There are significant differences in duration ($W = 357.500$, $p < 0.001$) and number of cases ($W = 407.000$, $p < 0.001$) between

TABLE 1 Characteristics between outbreaks of different settings and transmission modes.

Groups	Outbreak duration, *N*, Median [IQR] (d)[a]	Cases, *N*, Median [IQR] (*n*)[a]	Attack rate, *N*, Median [IQR] (%)[a]
Settings			
Villages	26, 27.00 [17.50, 54.00]	33, 163.00 [63.00, 641.00]	24, 17.34 [9.70, 33.91]
Hospitals	19, 7.00 [6.00, 12.00]	19, 11.00 [6.00, 29.00]	11, 26.71 [17.78, 43.40]
Factories and workers' living place	10, 33.50[18.75, 53.50]	14, 468.00 [149.00, 4937.00]	12, 14.28 [11.47, 18.72]
Schools	8, 15.50 [9.00, 19.00]	10, 219.00 [51.00, 453.00]	8, 5.67 [2.11, 16.42]
Armies	6, 11.50 [8.25, 21.00]	6, 220.00 [22.00, 725.00]	6, 34.30 [9.52, 46.99]
Others settings	4, 14.50 [4.50, 46.25]	5, 133.00 [12.00, 345.00]	4, 41.95 [12.14, 53.46]
Unknown	4, 53.50 [22.00, 130.75]	5, 1000.00 [270.00, 11006.00]	2, 15.87 [13.00, –]
Transmission modes			
Waterborne transmission	36, 34.00 [18.00, 51.00]	48, 340.00 [149.00, 1211.00]	34, 14.97 [10.97, 33.02]
Person-to-person transmission	26, 10.00 [6.00, 17.50]	27, 18.00 [8.00, 69.00]	17, 26.03 [9.88, 35.08]
Others transmissions	8, 18.00 [7.50, 58.00]	8, 77.00 [19.00, 152.00]	6, 5.60 [1.19, 25.78]
Unknown	7, 16.00 [7.00, 61.00]	9, 98.00 [33.00, 3785.00]	5, 33.91 [2.18, 45.26]

[a]IQR, Inter Quartile Range.

Group A and B rotavirus outbreaks, but there were no significant differences in the attack rates ($W = 501.000$, $p = 0.432$). Most Group A rotavirus outbreaks occurred in hospitals and villages, while Group B rotavirus outbreaks mainly occurred in villages, factories, and workers' living places. Group A rotavirus outbreaks mainly occurred in children, and Group B occurred in the general population and adults. In Group A and Group B rotavirus outbreaks, person-to-person and waterborne transmissions were most common, respectively. There are significant differences in settings ($p < 0.001$), population distribution ($\chi^2 = 52.881$, $p < 0.001$), and transmission modes ($p < 0.001$) between Group A and B rotavirus outbreaks (see Table 2).

3.7 Co-infections

There were three outbreaks in this study as co-infections. One outbreak occurred in a village, the stool of 97 patients was examined, 61 specimens were positive for rotavirus, and 4 specimens were positive for both rotavirus and Toxigenic *Escherichia coli*. The second outbreak occurred in a unit in adults, 12 specimens were positive for rotavirus, and 5 specimens were positive for *Campylobacter jejuni*. Another outbreak occurred at a training base, 10 and 11 specimens were positive for pathogenic *Escherichia coli* and Group A rotavirus, respectively.

4 Discussion

Rotavirus is the main pathogen of sporadic acute gastroenteritis, and can also cause outbreaks of acute gastroenteritis. To get a comprehensive understanding of the rotavirus outbreaks in China, we summarized and analyzed the epidemiological characteristics of rotavirus outbreaks in China from 1982 to 2021. A total of 78 articles were included, reporting 92 rotavirus outbreaks with 96,128 cases. The median number of cases per outbreak was 140 (ranging from 3 to 20,000), and the median outbreak duration was 18 days (ranging from 2 to 154 days). The attack rates ranged from 0.65% to 85.34%, with a median of 17.55%. Waterborne transmission and person-to-person transmission were the main modes of transmission.

In this study, 46 outbreaks (50.00%) were reported between 1982 and 1989, with a peak of 13 outbreaks in 1983. This result indicates that rotavirus outbreaks are no longer a major public health issue, but they still need to be considered in the investigation of acute gastroenteritis outbreaks.

Rotavirus infections peak in winter in northern hemisphere countries and are prevalent throughout the year in subtropic countries (11, 12). In our study, rotavirus outbreaks occurred every month, with the highest incidence in winter and spring (67.39%). Rotavirus infection was negatively correlated with ambient temperature, suggesting that low temperature may be beneficial to the virus (13, 14).

The number of rotavirus outbreaks reported in the eastern region was greater than in the western region. The high population density and mobility can accelerate the spread of rotavirus (11), and humidity and temperature in the southeastern coastal areas are also important factors affecting the outbreak of rotavirus (13). In addition, the rotavirus surveillance and laboratory tests were carried out earlier in the eastern provinces (15, 16).

This study showed that rotavirus outbreaks affected all age groups. Group B rotavirus outbreaks were mainly caused by contaminated water and affected populations of all age groups. On the other hand, Group A rotavirus outbreaks were primarily caused by person-to-person transmission and affected children. The positive rate of Group B rotavirus in sporadic surveillance was extremely low (17), and no outbreaks have been reported globally in recent years. Group A rotavirus is a major cause of acute gastroenteritis in infants and young children (18–20), and the outbreaks reported abroad primarily occurred in neonatal units (21) and in adults occasionally (22). The genotypes that cause rotavirus outbreaks may be common genotypes, such as G2P[4] (22), or rare genotypes, such as G12P[11] (23). Therefore, the genotypes of pathogens are crucial for analyzing infection characteristics and outbreak tracing.

TABLE 2 Epidemiological characteristics between Group A and B rotavirus outbreaks.

Variables	Group A	Group B	w/χ^2	*p* value
Outbreak duration, *N*, Median [IQR] (d)[a]	22, 11.50 [6.00, 19.00]	28, 34.50 [19.00, 58.50]	357.500	<0.001
Cases, *N*, Median [IQR][a]	26, 28.00 [8.00, 106.00]	40, 610.00 [163.00, 2232.00]	407.000	<0.001
Attack rate, *N*, Median [IQR] (%)[a]	22, 18.03[4.94, 36.80]	31, 17.71 [11.25, 33.02]	501.000	0.432
Seasons			5.845	0.119
Spring	7	20		
Summer	8	8		
Autumn	8	5		
Winter	3	7		
Settings			—[b]	<0.001
Villages	6	20		
Hospitals	10	1		
Factories and workers' living places	1	10		
Schools	5	3		
Others	4	3		
Unknown	0	3		
Population distribution			52.881	<0.001
Children	17	0		
Adults	2	13		
General population	4	27		
Unknown	3	0		
Transmission modes			—[b]	<0.001
Waterborne transmission	6	34		
Person-to-person transmission	13	4		
Other transmission	4	0		
Unknown	3	2		

[a]IQR, Inter Quartile Range.
[b]Fisher probability method results.

Unlike norovirus, sapovirus, and astrovirus outbreaks, which occurred more frequently in nursery centers and kindergartens (24–27), rotavirus outbreaks mainly occurred in hospitals and villages. Vomiting is a common clinical symptom in children, and diarrhea is common in adults after norovirus infection (28, 29). Abdominal pain is the most common clinical symptom of astrovirus infection (24). However, diarrhea is the most prominent clinical symptom in children and adults infected with rotaviruses. Therefore, it can provide a reference for differential diagnosis.

The duration, number of cases, and attack rates of rotavirus outbreaks were significantly related to the types of settings. In this study, rotavirus outbreaks mainly occurred in villages and hospitals. Outbreaks in villages were already large when reported, caused mainly by waterborne transmission, had longer duration and greater numbers of cases, and had low attack rates due to the large number of people affected. In contrast, outbreaks in hospitals had a shorter duration, fewer cases, and higher attack rates. Nosocomial and community-acquired rotavirus outbreaks involved different settings and populations, thus influencing the attack rates and transmission modes. Due to the crowded nature of hospitals, they were mainly caused by person-to-person transmission with higher attack rates. However, due to the particularity of the hospital settings, early detection and timely professional management can shorten the duration of outbreaks and reduce the number of cases (22).

Waterborne outbreaks mainly occurred in villages before 1990, with more cases and longer duration, mostly caused by Group B rotaviruses. Due to economic development and improved water sanitation, waterborne rotavirus outbreaks are rarely reported. After 1990, rotavirus outbreaks mainly occurred in hospitals, mostly caused by Group A rotaviruses, and person-to-person transmission was most common. Hospitals have high population mobility, more sources of infection, and a large number of susceptible people, which is conducive to disease transmission. Therefore, continuous monitoring and intervention of nosocomial infection is essential.

Our study has several limitations. First, we only included rotavirus outbreaks reported in online literature, representing only a fraction of those that occurred in China. Second, the data for some indicators

were not available for all outbreaks, for example, clinical symptoms of cases were not reported in every outbreak. Third, the age of some patients was missing, and the age groups in some studies were inconsistent, making detailed age distribution of the patients' analysis impossible. Finally, most studies provided the groups of rotaviruses, but only a few studies provided rotavirus genotypes.

5 Conclusion and recommendations

This is the first systematic review based on the large number of rotavirus outbreaks in China reported in the literature. Historically, more waterborne rotavirus outbreaks have occurred in villages, with long durations and high numbers of cases. While rotavirus outbreaks have been rare in recent years, but they should still be taken into account in the investigation of acute gastroenteritis outbreaks investigation, especially for norovirus-negative outbreaks.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

YT: Formal analysis, Investigation, Writing – original draft. FY: Formal analysis, Writing – original draft. GZ: Data curation, Investigation, Writing – review & editing. CT: Investigation, Writing – original draft. XW: Data curation, Investigation, Writing – review & editing. YC: Data curation, Investigation, Writing – review & editing. HY: Writing – review & editing. LJ: Writing – review & editing. DZ: Writing – review & editing. QW: Writing – review & editing. ZG: Formal analysis, Methodology, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2024.1423573/full#supplementary-material

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Expression of activin A in liver tissue and the outcome of patients with biliary atresia

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Biliary atresia (BA) is a rare disease of unknown etiology which leads to cirrhosis and death if left untreated. The standard of care is an early hepatoportoenterostomy (HPE). Long-term follow-up is mandatory, during which most patients will require a liver transplant. Activin A belongs to the transforming growth factor-β (TGF-β) superfamily. TGF-β is a central regulator in chronic liver disease. We have studied the expression of activin A in liver tissue collected intraoperatively during the HPE. We included patients who underwent HPE in a single medical center. Clinical, ultrasonographic, and pathohistological data were collected. Activin A immunostaining was performed. Expression in the bile duct epithelium and hepatocytes was scored as either weakly positive, moderately positive, or strongly positive. Patients were then divided into three groups accordingly. We observed the outcome after the HPE at 3 months, 2 years, and at the end of follow-up. The study encompassed 37 patients. At 3 months after HPE, 92.3% of those with a weakly positive activin A reaction (group A) achieved good jaundice clearance, whereas only 44.4% of those with a moderately (group B) and 40% of those with a strongly positive reaction (group C) achieved good jaundice clearance ($p = 0.008$). Furthermore, 2 years after the HPE, 92.3% of those in group A survived with native liver (SNL), but only 33.3% of those in group B and 46.7% of those in group C had SNL ($p = 0.007$). At the end of follow-up, 83.3% of those in group A survived with native liver, as did 33.3% in group B and 40% in group C. Activin A is a valuable pathohistological predictor of the outcome of BA after an HPE.

KEYWORDS

biliary atresia, activin A, hepatoportoenterostomy, liver immunohistochemistry, survival with native liver

1 Introduction

Biliary atresia (BA) is a rare disease of unknown etiology that presents in newborn infants. It is characterized by inflammatory obliterative cholangiopathy, affecting both the intrahepatic and extrahepatic bile ducts (1–7). BA leads to the development of cirrhosis, liver failure, and death within the first 2 years of life if left untreated (1–7). The incidence of BA depends on the geographical location and is most common in Asian countries (1:5,000 live births in Taiwan), whereas in Europe the incidence is lower (1:19,000 live births in the Netherlands) (1–3). BA occurs slightly more often in females than in males (1.25:1) (1). The diagnosis is established according to the clinical

and ultrasound findings and the exclusion of other causes of conjugated hyperbilirubinemia. In most cases, it is still necessary to confirm the diagnosis with an intraoperative cholangiogram (1–3). At the time of diagnosis, the extrahepatic bile ducts are completely obliterated, whereas the intrahepatic bile ducts show signs of inflammation and fibrosis, with the accumulation of bile and variable amounts of multinuclear hepatocytes surrounding the ducts (1–3). Thus far, the only successful treatment of biliary atresia is a surgical hepatoportoenterostomy (HPE), which enables the drainage of bile from the remaining bile duct of the porta hepatis into the small intestine (1–3, 8, 9). The complications resulting from an HPE are numerous (e.g., frequent postoperative cholangitis and incomplete drainage of bile with consequent progression of cholestasis), and the disease progresses to cirrhosis of the liver in many patients with the subsequent need for transplantation even after HPE (8, 9). Biliary atresia is also the most common indication for liver transplantation in children (1–3, 8, 9).

BA can be divided into at least two clinical types: fetal and perinatal. In the fetal form, newborns develop conjugated hyperbilirubinemia and acholic stools within the first week of life, and there is no jaundice-free period. In the perinatal form, jaundice and acholic stools develop after a certain jaundice-free period (10).

Furthermore, there are more distinguishable entities such as biliary atresia splenic malformation (BASM) or cat-eye syndrome, along with cystic BA. This diversity suggests etiological and probably pathogenetic heterogeneity (11). Various causes have been proposed thus far (e.g., exposure to viral infections, genetic predisposition, or response to the bile duct epithelium's own antigens) (1, 10–18). There is also a distinct BA variant in Egyptians, which seems to be caused by an interaction between congenital aflatoxicosis in neonates and the glutathione S-transferase M1 null genotype (19). The immune system plays an important role in the development of this disease, as shown by the presence of inflammatory cells and various adhesion molecules in the bile duct area (15). There is a lingering question of whether liver histopathology can additionally help in the prediction of BA treatment outcomes (10–13).

Activin A is a member of the family of transforming growth factors (TGF-β) (20–28). It is found in various tissues and is secreted by macrophages, Th2 lymphocytes, and hepatocytes. Recent research has shown that activin A causes the inhibition of hepatocyte growth, suppresses liver regeneration, and induces hepatocyte apoptosis (20–28). Excessive secretion of activin A in the liver has thus far been detected in liver fibrosis, liver cancer, and viral hepatitis (23–28).

2 Methods

2.1 Study design

The medical records of patients who underwent an HPE at the Department of Pediatrics, Division of Gastroenterology, Hepatology and Nutrition, University Hospital Centre Zagreb, from 1986 to 2019 were reviewed retrospectively. The diagnosis of BA was established according to standard clinical, biochemical, nuclear, ultrasonographic, histologic, and operative findings. Clinical findings included conjugated hyperbilirubinemia in biochemistry analysis, history of acholic stools, dark urine, and hepatomegaly. The diagnosis was confirmed with hepatobiliary scintigraphy and a liver biopsy. We set 30 June 2021 as the end of follow-up.

2.2 Inclusion criteria

We included all children with BA who had detailed clinical information, at least 2 years of follow-up after the HPE, and wedge liver biopsy specimens in good condition in paraffin blocks (Figure 1).

2.3 Exclusion criteria

We excluded any patients with biliary atresia who underwent surgical treatment other than an HPE.

2.4 Data gathering

Data on the patient's date of birth, birth weight, sex, the date of onset of jaundice with conjugated hyperbilirubinemia and acholic stools, presence of congenital extrahepatic anomalies, the date of the HPE, and liver histology results from the biopsy obtained during the HPE were gathered, as well as the age of onset of liver cirrhosis. Diagnostic histologic findings for biliary obstruction included ductular proliferation, cellular and canalicular bile stasis, and portal edema and fibrosis. Furthermore, we gathered data including months of survival with native liver (SNL) after the HPE, the date of either liver transplantation or death, and the date of the last recorded out-patient visit for drop-outs.

2.5 Immunohistochemistry

A liver wedge biopsy was taken in all patients at the time of HPE, at the porta hepatis. Specimens were fixed in 10% formalin and then embedded in paraffin after processing the tissue. In all the biopsies, conventional stains (hematoxylin and eosin, Mallory, Gomory) were performed on 3- to 4-μm-thick paraffin sections. Hepatic fibrosis was graded on the basis of the Ishak classification: grade 0, no fibrosis; grade 1, fibrous expansion of some portal areas; grade 2, fibrous expansion of most portal areas; grade 3, fibrous expansion of most portal areas with occasional portal to portal bridging; grade 4, fibrous expansion of portal areas with marked bridging (portal to portal and portal to central); grade 5, marked bridging (portal to portal and/or portal to central) with occasional nodules–incomplete cirrhosis; and grade 6, cirrhosis (29). On all the biopsy samples, we performed antigen retrieval in a Dako PT Link device (Dako, Agilent

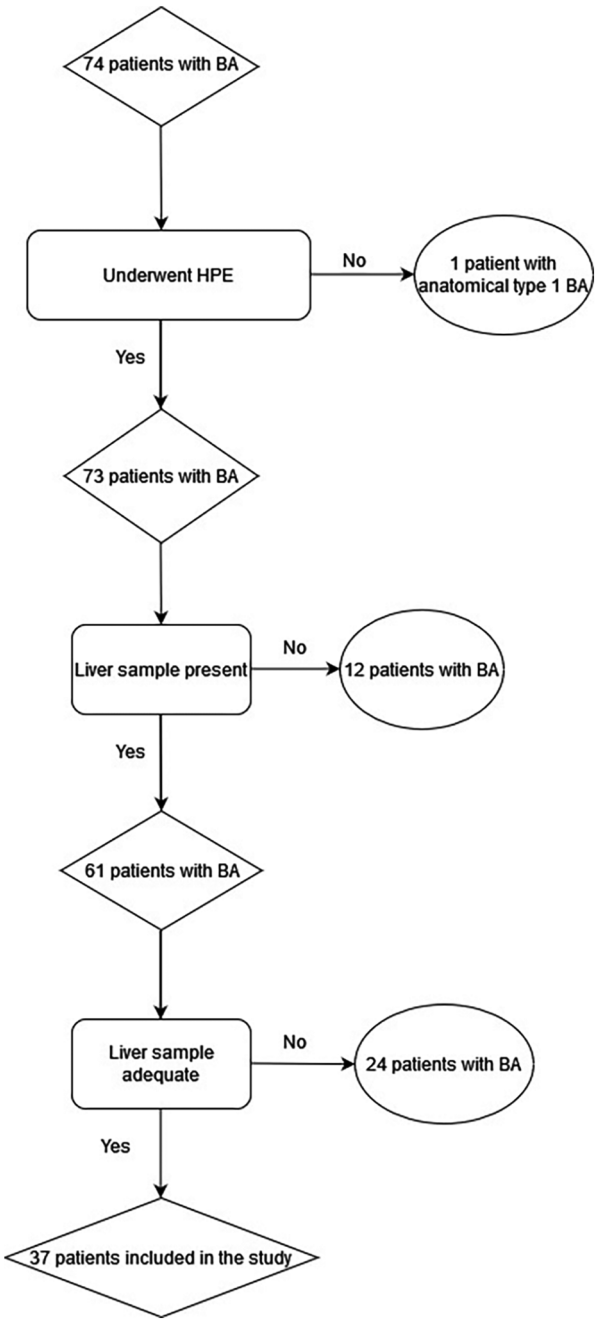

FIGURE 1
Flow chart of the sample size methodology.

Technologies, Santa Clara, CA, USA) in the appropriate buffer [Envision Flex Target retrieval solution LOW (Dako, Agilent Technologies, Santa Clara, CA, USA)]. Afterward, the samples were dyed in a Dako TechMate device (Dako, Agilent Technologies, Santa Clara, CA, USA) using the standard dyeing process. Purified rabbit polyclonal primary activin A antibody was applied to the samples at a concentration of 1:100 (RRID: AB_2125870, NBP1-30928, Novus Biologicals, Colorado, USA). Given our study duration and the viability of our paraffin-embedded samples, we chose a polyclonal antibody for the immunohistochemical (IHC) staining as it increases the chance of detecting epitopes that are still available in fixed samples with a low protein quantity (30, 31). For visualization, the EnVision FLEX kit was used (Dako, Agilent Technologies, Santa Clara, CA, USA). Finally, the specimens were contrast stained with hematoxylin and dehydrated in solutions as follows: 96% ethyl alcohol 2 × 5 min, 100% ethyl alcohol 2 × 5 min, and 100% xylene 2 × 5 min.

The samples were analyzed with a light microscope, in at least five fields of view under high magnification (×400) by one pathologist who was unaware of patient outcomes (Figure 2). The activin A expression was established using our own semiquantitative method, according to the following principle: no staining in the hepatocyte cells and biliary epithelial cells (BECs) (−); up to 10% of the hepatocytes and biliary epithelial cells have a positive reaction, weakly positive reaction (+); from 10% to 50% of hepatocytes and biliary epithelial cells positive, moderately positive reaction (++); more than 50% of hepatocytes and biliary epithelial cells positive, strongly positive reaction (+++).

2.6 Outcomes

We observed the patient outcomes at 3 months after the HPE, 2 years after the HPE, and at the study end point. The patients were divided into three groups based on their outcomes at 3 months as indicated by stool color and total bilirubin (TB). An excellent outcome was represented by the presence of colored stools and TB lower than 30 μmol/L. A good outcome was represented by the presence of colored stools and TB higher than 30 μmol/L and lower than 90 μmol/L, whereas a bad outcome was represented by the presence of either acholic stools or persistent jaundice. The outcome at 2 years after the HPE was divided into SNL or any other outcome (liver transplantation and/or death, study drop-out). Liver cirrhosis was defined when any sign of portal hypertension (inverse portal blood flow, ascites, esophageal varices, hypersplenism, or splenomegaly) was noted. The outcome at the study end point was observed as SNL, liver transplantation (LTx), or death.

3 Statistical analysis

The data analyses were performed using R software (version 4.1.2). Categorical data were descriptively presented as absolute and relative frequencies, and numerical data, depending on the distribution, as means and standard deviations or medians and interquartile ranges. The Kolmogorov–Smirnov test was used to test the normality of the distributions. Fisher's exact test was used to test differences between categorical variables, and a one-way ANOVA and the Kruskal–Wallis test for differences between numerical variables. In addition, survival estimates with native liver were based on the Kaplan–Meier method and log-rank test. P-values <0.05 were considered statistically significant.

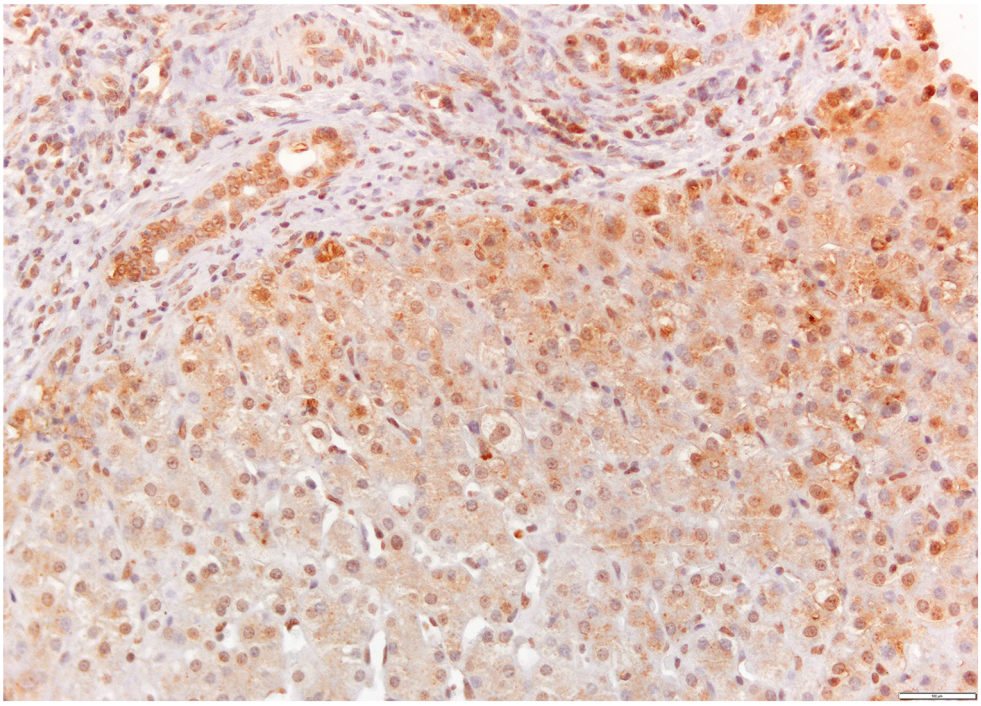

FIGURE 2
Activin A immunostaining of a liver sample from a patient with biliary atresia. The expression is present in hepatocytes and cholangiocytes in the portal space (brown-colored, ×20 magnification).

4 Results

4.1 Demographics and subgroup division

In total, 37 patients met the inclusion criteria. Nine of them were included in an earlier report summarizing the incidence and outcomes of patients with BA (32). Demographic data are presented in Tables 1, 2. Within our cohort, 13 patients had a weakly positive reaction in the activin A immunostaining (+)—group A, 9 had a moderately positive reaction (++)—group B, and 15 had a strongly positive reaction (+++)—group C.

4.2 Malformations present

There was one BASM patient in every group. There were only two patients with a single anomaly (one with ectopic pancreas and the other with pyeloureteral stenosis). Besides these two which were not readily recognized from established BA registries, the other anomalies were those usually encountered (gastroschisis, ventricular septal defect, finger malformations, atrial septal defect, and hypoganglionosis of the colon).

TABLE 1 Clinical characteristics of the study group.

Sex (%)	Male	18 (48.6)
	Female	19 (51.4)
Malformations present (%)	Yes	8 (21.62)
	No	29 (78.38)
Median age at the HPE, days (range)		76 (22–192)
Median follow-up after the HPE, years (range)		6.2 (0.6–23.8)
LTx during the follow-up (%)	Yes	12 (33.3)
	No	24 (66.6)

TABLE 2 Associated anomalies[a].

Anomaly	Total, *N* (%)
BASM	3 (8.1)
Gastrointestinal	4 (10.8)[b]
Cardiovascular	3 (8.1)[b]
Urogenital	1 (2.7)
Musculoskeletal	1 (2.7)

[a]Twelve anomalies in eight patients.
[b]BASM overlap not included.

4.3 Outcomes

Table 3 presents preoperative, pathohistological, and analyzed outcomes for each group separately. At 3 months after the HPE, more than two-thirds of the patients in group A (69.2%) achieved excellent jaundice clearance. The same outcome was noted in 11.1% of the patients in group B, and 33.3% of the patients in group C, respectively ($p = 0.007$). Likewise, 2 years after the HPE, 12 out of the 13 patients (92.3%) from group A had survived with their native liver, as had 33.3% of the patients in group B and 46.7% in group C ($p = 0.007$). At the end of the study, 10 out of the 12 patients in group A (83.3%), 3 out of the

TABLE 3 Comparison of the characteristics and outcomes of infants according to the activin A expression in the liver wedge biopsy.

		Group A	Group B	Group C	*p*-value[a]
n (%)		13 (35.1)	9 (24.3)	15 (40.6)	
Sex (%)	Female	8 (61.5)	6 (66.7)	5 (33.3)	0.239
	Male	5 (38.5)	3 (33.3)	10 (66.7)	
Anomalies (%)	No	12 (92.3)	7 (77.8)	10 (66.7)	0.307
	Yes	1 (7.7)	2 (22.2)	5 (33.3)	
Age at HPE in days [median (IQR)]		82.00 (64.00–86.00)	76.00 (39.00–106.00)	70.00 (54.00–84.50)	0.810
BA type (%)	Fetal	2 (15.4)	4 (44.4)	4 (26.7)	0.333
	Perinatal	11 (84.6)	5 (55.6)	11 (73.3)	
Ishak index [mode (range)]		4 (1–6)	5 (1–6)	6 (1–6)	0.754
Ishak index	1–4	7 (58.3)	4 (44.4)	7 (46.7)	0.835
	5–6[b]	5 (41.7)	5 (55.6)	8 (53.3)	
Age at liver cirrhosis in months [median (IQR)]		24.00 (12.25–41.75)	7.00 (7.00–15.50)	8.00 (6.75–9.00)	0.042
Number of cholangitis attacks (%)	No attacks	12 (92.3)	5 (55.6)	10 (66.7)	0.304
	1 attack	1 (7.7)	2 (22.2)	3 (20.0)	
	>1 attack	0 (0.0)	2 (22.2)	2 (13.3)	
Outcome at 3 months after HPE (%)	TB <30 μmol/L and colored stools	9 (69.2)	1 (11.1)	5 (33.3)	0.007
	TB 30–90 μmol/L and colored stools	3 (23.1)	3 (33.3)	1 (6.7)	
	TB >90 μmol/L and/or acholic stools	1 (7.7)	5 (55.6)	9 (60.0)	
Outcome 2 years after HPE (SNL, death or Tx) (%)	SNL	12 (92.3)	3 (33.3)	7 (46.7)	0.007
	Other (death or Tx)	1 (7.7)	6 (66.7)	8 (53.3)	

n, number; HPE, hepatoportoenterostomy; IQR, interquartile range; BA, biliary atresia; TB, total bilirubin; SNL, survival with native liver; Tx, transplantation.
Values are numbers for nominal variables and median (range) for continuous variables. The characteristics were compared across groups using ANOVA and the Kruskal–Wallis test for continuous variables and Fisher's exact test for categorical variables.
[a]The *p*-values in parentheses correspond to comparison between group A, group B, and group C.
[b]Ishak fibrotic stages 5 and 6 represent incomplete and complete cirrhosis.

9 patients in group B (33.3%), and 6 out of the 15 (40%) in group C survived with native liver. The log-rank test did not show a significant difference in the SNL estimate between the three groups given death and transplantation as the main events, respectively ($p = 0.136$ and $p = 0.071$). There was no significant difference in the median age at which the HPE was performed across the three groups of patients ($p = 0.810$).

4.4 Liver cirrhosis comparison

The patients in groups B and C developed signs of liver cirrhosis sooner (median age in months 7.0, IQR 7–15.5; and 8.0, IQR 6.75–9.00, respectively) than the patients in group A (median age in months 24.00, IQR 12.25–41.75) ($p = 0.042$). The mode of the Ishak index across three groups was somewhat similar [4 (1–6), 5 (1–6), and 6 (1–6), $p = 0.754$]. However, there was no significant difference between the Ishak index score of liver fibrosis and activin A expression in the liver wedge biopsies across the three patient groups ($p = 0.835$).

5 Discussion

The main finding of our study is that the patients with a moderate (group B) or strong expression of activin A (group C) on the liver wedge biopsy had worse early outcomes than those with weakly expressed activin A (group A). The patients with moderately or strongly expressed activin A also developed liver cirrhosis sooner than patients with a weak expression of activin A. Although the log-rank test did not show a significant difference in the SNL estimate across the three groups of patients, the Kaplan–Meier curves clearly show a difference with better SNL in patients with weakly expressed activin A (Figures 3a,b). An obvious concern in our study, which included only 37 patients, is the possibility that the statistical power for determining differences between these three groups was limited.

One of the known predictors of biliary atresia outcome is the age at which the HPE is performed (2, 33–35), however, in our cohort, there were no significant differences across the three groups in the median age at which the HPE was executed. Neither the type of BA nor the associated anomalies showed notable differences across the three groups of patients. Some of the variables that were previously shown to influence the outcome of BA were not part of our study design and that may be a limitation for interpreting the role of activin A as a predictor of outcome in our patients.

Activin A has been shown to inhibit mitogen-induced DNA synthesis and induce apoptosis in hepatocytes *in vitro* and *in vivo* (20). Moreover, it inhibits the proliferation and induces apoptosis of hepatocytes, contributing to the termination of liver regeneration. In rat models of liver fibrosis and cirrhosis, activin A expression is increased, and plasma activin A levels are elevated in patients suffering from acute liver failure, hepatitis, alcohol-induced cirrhosis, hepatocellular carcinoma, non-alcoholic fatty liver disease (NAFLD), and non-alcoholic steatohepatitis (NASH). Activin A also regulates the restoration of liver architecture after a partial hepatectomy by stimulating collagen production in hepatic stellate cells (HSC) and tubulogenesis of sinusoidal endothelial cells. Stimulation of

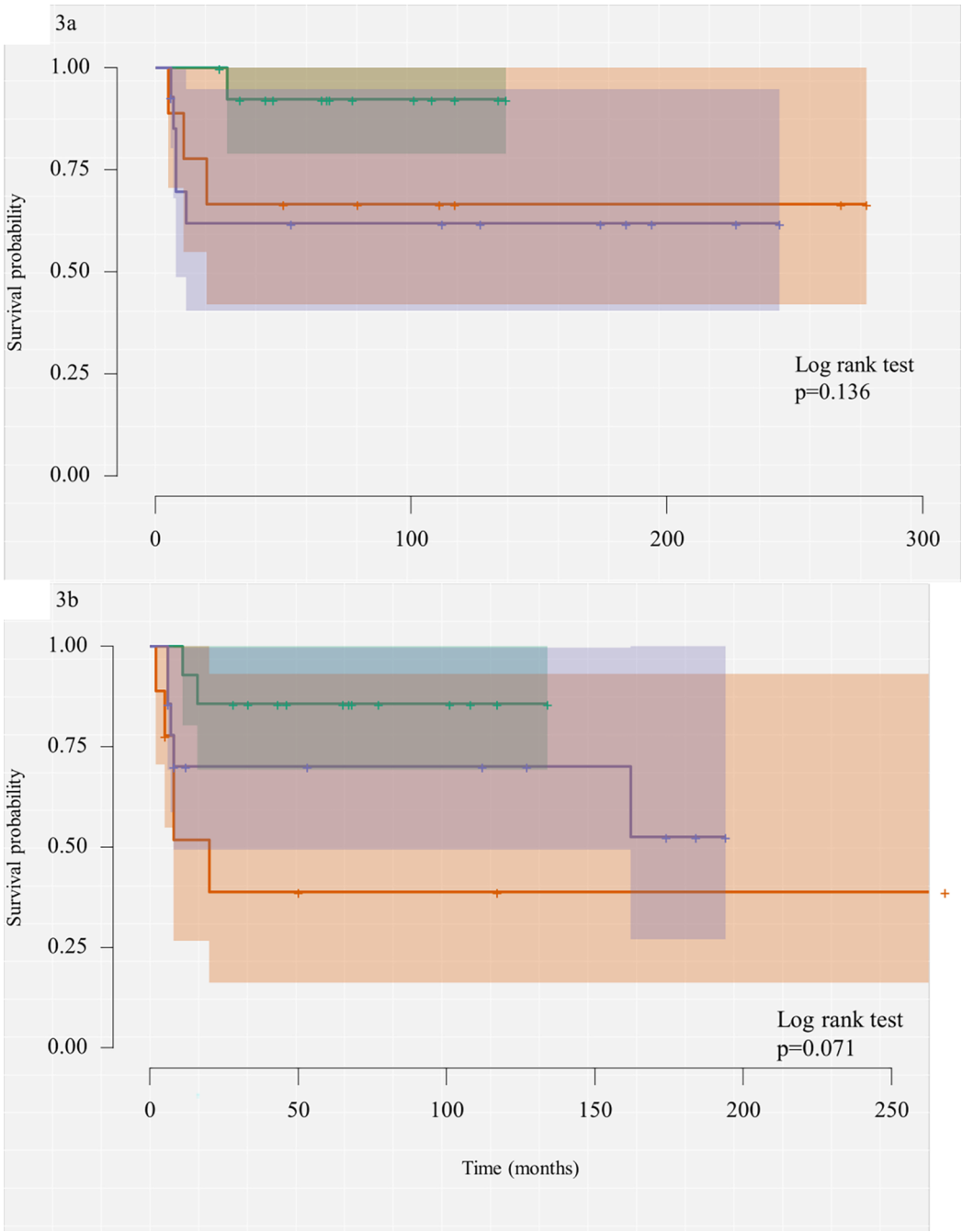

FIGURE 3
(a) Kaplan–Meier curve of the survival analysis estimating SNL probability with death as the main event. (b) Kaplan–Meier curve of the survival analysis estimating SNL probability with liver transplantation as the main event.

collagen production may also contribute to liver fibrosis (26–28, 35–37). The reports on activin A expression in biliary epithelium are conflicting as there are reports of activin A-induced inhibition of DNA synthesis in BECs and reports of increased IHC staining of BEC in liver disease (38, 39). Since both ductular proliferation and ductopenia can be present in BA, we considered activin A staining of the biliary epithelium to be equally as important as the staining of hepatocytes (40). To the best of our knowledge, there are no studies on activin A expression in the livers of patients with BA, and there is no IHC scoring system for activin A in human liver tissue, which are the main restrictions in our study. Despite a considerable amount of data in immunohistochemistry, there is still a lack of standardization, especially in the post-analytical stage, which makes comparisons of the results of different studies difficult or impossible. As we were not able to find an already established scoring system, we devised our own. We only used the number of cells stained with activin A, and given the antibody

polyclonality, we did not measure the color intensity to be more objective and to nullify the background staining. This approach has been used before in other tissues (41). There are significant connections between activin A expression and fibrotic changes in liver tissue from a liver wedge biopsy (25, 27). Our study did not show a significant connection between the Ishak classification of liver fibrosis and activin A expression. However, there was no significant difference between the three groups of patients based on the mean Ishak index at the time of the liver wedge biopsy, which showed significant fibrotic liver changes. It has been shown there is a significant effect of the presence of neutrophils in the liver tissue on the pathogenesis and outcome of patients with BA, especially the CD4+/CD8+ ratio (42). Even so, the question of the extent of the effect of liver fibrosis on the outcome of patients with BA remains. Therefore, it may be plausible that activin A expression in otherwise histologically indistinguishable tissues can serve as a more sensitive marker of fibrosis and be a part of future, more accurate assessment tools for the prediction of SNL in patients with BA.

6 Conclusion

Our results suggest that activin A expression in the liver of patients with BA before treatment might be a valuable additional tool in the complex search for more accurate predictors of outcomes after an HPE.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethical Board, University Hospital Centre Zagreb. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

PD: Conceptualization, Formal Analysis, Investigation, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. MĆ: Investigation, Methodology, Writing – review & editing. MK: Writing – original draft. MA: Formal Analysis, Writing – original draft. RG: Conceptualization, Investigation, Methodology, Supervision, Validation, Writing – review & editing. JV: Methodology, Resources, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Pyridostigmine as a therapeutic option for pediatric gastrointestinal dysmotilities in ATR-X syndrome. Case report and literature review

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Background: Alpha-thalassemia X-linked intellectual disability (ATR-X) syndrome, is a rare genetic disorder, caused by mutations in the ATRX gene. Clinical manifestations include typical facial dysmorphisms, mild-to-severe intellectual disability, hypotonia, genital anomalies, significant gastrointestinal (GI) complications, such as abdominal distension, chronic constipation, feeding difficulties, gastroesophageal reflux, and mild-to-moderate anemia secondary to alpha-thalassemia.
Case presentation: We report a patient with ATR-X syndrome suffering from gastrointestinal dysmotility and highlight the beneficial effects of pyridostigmine. Knowledge about the role and appropriate dosage of pyridostigmine in GI motility disorders is limited. To date, only nine pediatric cases involving pyridostigmine for GI dysmotility have been reported.
Conclusions: Considering current understanding about the treatment of gastrointestinal complications in patients with genetic syndromes, this case provides new insights into management of these complex clinical presentations.

KEYWORDS

gastrointestinal dysmotility, alpha thalassemia, alpha-thalassemia X-linked intellectual disability, ATR-X syndrome, pyridostigmine, constipation, therapy

1 Introduction

Alpha-thalassemia X-linked intellectual disability (ATR-X) syndrome (ATR-X, OMIM Entry#301040) is a rare disorder with complex clinical manifestations (1). The first association between alpha-thalassemia and intellectual disability was reported in 1981 by Weatherall and colleagues (2). Since the identification of the ATRX gene, located on Xq13, as causative of the ATR-X syndrome (3), more than 130 families and 200 affected individuals have been described (4) with an estimated incidence of less than 1/100,000 live-born males (5). The hallmarks of the ATR-X syndrome are distinctive craniofacial features, genital anomalies, hypotonia, and mild-to-severe developmental delay/intellectual disability (DD/ID). Craniofacial dysmorphisms include small head circumference, telecanthus or widely spaced eyes, short triangular nose, tented upper lip, and thick or everted lower lip with coarsening of the facial features over time. Genital anomalies range from hypospadias and cryptorchidism to severe hypospadias

and ambiguous genitalia. Alpha-thalassemia, observed in about 75% of affected individuals, is mild and typically does not require treatment (6). A risk of early-onset osteosarcoma has been reported in a few males with germline pathogenic variants (6). Gastrointestinal complications, as well as in many genetic syndromes (7, 8), affect most of the patients, significantly contributing to morbidity (6). Recurrent vomiting or regurgitation is a common finding and seems likely to be a manifestation of a more generalised gut dysmotility. Aspiration is a common cause of death in early childhood. Excessive drooling, aerophagia, food refusal, and feeding-associated distress in these children are common. Constipation is also frequent and might be a major issue in some patients (9, 10). Notably, pseudo-volvulus, ultra-short Hirschprung disease and colonic hypoganglionosis have been reported too (9). The primary aim of this paper is to identify new therapeutic strategies for ATR-X syndrome-related GI complications, suggesting pyridostigmine as a viable alternative to the commonly utilized drug classes.

2 Case report

The patient is a 10-year-old caucasian male, born at 39 weeks' gestation following a pregnancy complicated by oligohydramnios, with a spontaneous vaginal delivery. His parents are non-consanguineous, and the family pedigree revealed no significant genetic history. Hypotonia was noted during the perinatal period. At one year of age, molecular analysis of the ATRX gene identified a pathogenic hemizygous *c.736C > T* variant. His clinical phenotype includes characteristic facial dysmorphisms (microcephaly, low-set hairline, telecanthus, a small triangular nose with anteverted nostrils, and a carp-shaped mouth with full lips), psychomotor delay, epilepsy, moderate bilateral hearing impairment, and laryngomalacia without impact on respiratory function. He was admitted to our department with significant gastrointestinal issues, including chronic gastroesophageal reflux (GER), dysphagia, and severe, persistent constipation. He had recently been hospitalized three times due to the worsening of his GI symptoms. Abdominal x-ray revealed gastroparesis and severe intestinal loop distension (Figure 1). Furthermore, barium enema showed a sub-stenotic region in the rectosigmoid area. Treatment with macrogol and enemas was ineffective. Persistent, severe abdominal pain and distension led to distress and agitation. After cardiac evaluation, he was started on oral pyridostigmine at a dose of 30 mg/day (1.6 mg/kg/day), gradually increased to 60 mg/day (3.2 mg/kg/day) within 20 days. This approach avoided the need for surgery, notably improved the patient's bowel habit, and reduced abdominal discomfort, leading to a significant decrease in irritability and an overall enhancement in his quality of life. He continued on a maintenance dose of oral pyridostigmine at 45 mg/day, and after a year of sustained treatment, his gastrointestinal symptoms fully resolved, with no recurrence of abdominal distension, fecal stasis, nighttime awakenings, or significant side effects.

FIGURE 1
Upright plain abdominal x-ray showing massive dilation of the intestinal loops.

3 Discussion

Gastrointestinal manifestations are common findings in ATR-X syndrome, such as feeding problems, vomiting, abdominal distension, and chronic constipation (10). According to Leon and colleagues 30% of patients suffer from GI complications (4). In 2006 Martuciello et al. reviewed 128 cases of ATR-X syndrome patients with GI manifestations, most commonly gastroesophageal reflux (GER), drooling and constipation. Patient's refusal for food is relatively common among these patients and at times leads to dehydration. Intestinal malrotation was a rare finding in Martucciello's review but it was the cause of death in two out of four patients described (10). Watanabe and colleagues investigated the gastroesophageal function of a child with ATR-X syndrome, presenting with reduced esophageal clearance and gastric volvulus. Laparoscopic anterior gastropexy was conducted, and a button PEG-J was inserted, resulting in a better nutritional management and quality of life (11). In 2015 Horesh et al. reported a patient with recurrent large bowel volvulus, who kept on suffering from food refusal, abdominal distension, and dehydration after subtotal colectomy (1). Management of GI motility disorders is challenging, with limited medical and surgical options. Pyridostigmine, an acetylcholinesterase inhibitor (CI), may be considered in such patients when other treatments have not been beneficial. CIs are well known drugs, mainly used in general anesthesia and in the symptomatic treatment of patients with myasthenia gravis (12). In the GI tract, the inhibition of choline acetyl esterase enzyme increases the availability of

TABLE 1 Published studies on the effects of pyridostigmine in pediatric gastrointestinal dysmotilities. Adapted and updated from Di Nardo et al. 2019 (13).

Authors	Patients *n*	Age	Clinical features	Pyridostigmine dose	Outcome	Side effects
Boybeyi et al. (14)	1	3 y	Neuropathic PIPO presenting with abdominal distension, intolerance to oral feeding, constipation	30 mg/kg/day	Resolution of abdominal distension, improved oral intake, increased bowel movements	None
Manini et al. (15)	4	18 y	Abdominal distension, intolerance to enteral feeds and bilious emesis	0,3 mg/kg/day	Decreased abdominal distension, increased enteral calories, decreased TPN	None
		7 y	Chronic abdominal distension, constipation, feeding intolerance, and vomiting	4 mg/kg/day decreased to 0,75 mg/kg/day	Decrease abdominal distension, increased bowel movement frequency, resolved vomiting with better tube feeding tolerance	None
		11 y	Ileus with abdominal distension, vomiting, abdominal pain, decreased stool output	1,7 mg/kg/day increased to 1,1 mg/kg/day	Immediate improvement in distension, pain and vomiting	Abdominal pain and cramps
		10 y	Abdominal pain, vomiting, abdominal distension	1,1 mg/kg/day increased to 2,2 mg/kg/day	Improvement in vomiting and abdominal distension	None
Choudury et al. (16)	1	9 y	Myopathic PIPO presenting with abdominal distension and pain, vomiting and constipation	0,5 mg/kg twice per day, increased to 1 mg/kg twice per day	Reduction of abdominal distension and gastric drainage, tolerance of enteral feeding	None
Di Nardo et al. (13)	1	2 y 4 m	Constipation, food refusal, abdominal distension	2 mg/kg twice per day, increased to 3 mg/kg twice per day	Disappearance of vomiting, reduction of abdominal distension, occurrence of spontaneous bowel movements	None
Lee et al. (17)	2	11 y	Myopathic PIPO presenting with congenital myotonic dystrophy, small bowel and colonic distension, megacystis	150 mg/day	Reduction in length of hospital stay and need of parenteral nutrition	None
		5 y		7 mg/kg/day		

acetylcholine at the neuro-neuronal synaptic cleft within the enteric nervous system as well as at neuromuscular junctions (13). Little is known about the role and dosage of pyridostigmine in pediatric GI motility disorders. In 2009, Boybeyi and colleagues reported a 3-year-old patient with pediatric intestinal pseudo-obstruction (PIPO), successfully treated with pyridostigmine 30 mg/Kg/day (14). Manini et al. presented a case series of four pediatric patients with abdominal distension and other dysmotilities. Administration of pyridostigmine markedly improved GI symptoms, with 100% success rate and with side effects (abdominal pain and cramps) in one case only (15). Similar results were obtained by Choudury, who reported the case of a 9-year-old girl with a long-term history of constipation and intermittent abdominal distension, since the age of 3 years (16). Different types of laxatives were used, including polyethylene glycol, lactulose, senna, sodium picosulfate and docusate sodium, with no definitive effect. She was started on enteral pyridostigmine at a dose of 0.5 mg/Kg twice per day and gradually increased to 1 mg/kg twice per day. An abdominal x-ray confirmed her clinical improvement. Lee et al. described the use of pyridostigmine in two children with chronic intestinal pseudo-obstruction harbouring ACTG2 gene variants (17). After 10 days of intravenous neostigmine (0.5 mg in 50 ml normal saline) at 0.5 mg per hour, the treatment was switched to oral pyridostigmine at a dose of 180 mg/day (case 1) and 7 mg/kg/day (case 2) without side effects. In response to pyridostigmine treatment, the length of hospital stay and dependency on parenteral nutrition were reduced in both patients (17). In 2019 a 2-year and 4-month- old girl was presented by Di Nardo and colleagues (13). Chronic constipation, food refusal, poor weight and height gain were her main symptoms. Pyridostigmine 2 mg/kg/twice per day, then increased to 3 mg/kg twice per day, led to improvement of gut motility with reduction of abdominal distension, disappearance of vomiting and, occurrence of spontaneous bowel movements (13) (Table 1).

4 Conclusions

ATR-X syndrome is characterized by a wide range of severe gastrointestinal symptoms, including abdominal distension and chronic constipation. The therapeutic potential of pyridostigmine in this context is a significant finding. The present paper adds information to the few experiences currently available in literature. According to our experience and reported cases, pyridostigmine may be a suitable alternative when first-line treatments are ineffective, as they were in our case. Its off-label use seems to be further supported by a low side-effect profile; of the nine patients documented in the literature, only one experienced minor side effects (abdominal pain and cramps), with no adverse effects observed in the remaining cases or in ours. Optimal dosage, long-term efficacy, and side effects of pyridostigmine in pediatric GI motility disorders remain an area for future research. A deeper understanding of GI complications in ATR-X syndrome is the goal to determine the most effective pyridostigmine dosage for these patients.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

FC: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. CS: Investigation, Writing – review & editing. MC: Investigation, Writing – review & editing. MM: Investigation, Writing – review & editing. RC: Data curation, Writing – review & editing, Investigation. TF: Data curation, Writing – review & editing. SS: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Efficacy of Tui Na in idiopathic constipation in children with cerebral palsy: a randomized controlled clinical trial

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Objective: To investigate the efficacy and potential adverse events of Tuina therapy for idiopathic constipation in children with cerebral palsy (CP).
Methods: A total of 60 CP children with idiopathic constipation were enrolled and randomly divided into Tuina and control groups. The control group was treated with basic treatment and 12 sessions of lactulose oral solution, whereas the Tuina group received basic treatment and 12 sessions of infantile Tuina treatment. The following parameters were compared: the Bristol stool form scale (BSFS), the Constipation Assessment Scale (CAS) and the improvement in constipation. In addition, adverse effects were recorded.
Results: At 4 weeks after the final treatment, the percentage of infants whose constipation improved was 23 (76.7%) in the Tuina group and 21 (70.0%) in the control group ($P = 0.771$). Initially, the CAS score, weekly bowel movements and proportion of infants with bowel evacuation ≥2 h were comparable between the two groups ($P > 0.05$). At 4 weeks after the final treatment, the CAS score, weekly bowel movements and proportion of infants with bowel evacuation ≥2 h all significantly improved ($P < 0.05$) compared with those in the initial situation. However, no difference was found in either group at 4 weeks after the final treatment. No serious adverse reactions (such as diarrhea, abdominal pain, vomiting, subcutaneous redness, skin breakage, or syncope) were recorded.
Conclusions: Tuina was as effective as medical care in addition to basic treatment for both groups. The results of this study suggest that Tuina, as a nonpharmacological therapy, may be helpful as an alternative treatment for constipation. More advanced research and large-sample studies should be conducted.

KEYWORDS
tuina, idiopathic constipation, children, cerebral palsy, effect

Introduction

Cerebral palsy (CP) is a nonprogressive neurologic condition that manifests as movement and muscle tone impairments due to neurological developmental abnormalities. It affects approximately 2.5 per 1,000 births worldwide (1). Children with CP suffer from highly reduced mobility and subsequent constipation. In addition, most

children with CP who are constipated may also have other systemic symptoms, such as sleep disturbances (including prolonged sleep onset, shortened sleep duration, and easy awakening) and emotional irritability (2). As the most common problem, with a high incidence rate of 75%, constipation usually results from the intake of several components, including reduced fiber and liquid intake, mobility reduction, damage to the central nervous system, and therapeutic drugs (3–5). In addition, it worsens as the severity of CP increases (3).

To date, no consensus exists on the treatment of constipation in children with CP (6). The treatment mainly focuses on improving the symptoms of constipation, such as lifestyle guidance, pharmacological therapy and nonpharmacological therapy (7). Imanieh M.H. et al. compared three different therapeutic methods and suggested that effective and safe agents are still important treatmen choices in the treatment of chronic constipation in CP children (8). Veugelers R. et al. conducted a cross-sectional observational study and reported that children with severe generalized cerebral palsy often experienced constipation. Laxatives are common medicines, but their dosing is frequently inadequate to prevent symptoms (3). In addition, García Contreras A.A. et al. suggested the use of synbiotics, prebiotics, and probiotics to improve stool characteristics, such as a history of painful defecation (9). However, discomfort associated with anal administration, high drug dependence, and poor long-term efficacy are unavoidable.

As an important alternative and nonpharmacological treatment method to traditional Chinese medicine, Tui Na has shown effective therapeutic effects on constipation (10). However, to our knowledge, few studies have drawn accurate conclusions. The effect of Tui Na on constipation remains controversial and poorly documented.

In the present study, we conducted a randomized controlled clinical trial to investigate the efficacy of Tuina therapy for on idiopathic constipation in children with CP. In addition, we examined the tolerance and safety of Tui Na therapy via adverse effects.

Materials and methods

This clinical trial was designed and approved by the Ethics Review Committee of The First Hospital of Hunan University of Chinese Medicine (approval number: HN-LL-KY-2022-010-01). Written informed consent was obtained from the parents of all the participants. The study was registered with the China Clinical Trial Registry on April 18, 2022 (registration number: ChiCTR2200058879).

From June 2022 to June 2023, a total of 60 CP children with idiopathic constipation, ranging in age from 16 to 98 (average, 42.70 ± 17.81) months, were enrolled. All the children in the group met the diagnostic criteria for CP and the Rome III criteria for constipation, and no constipation-related therapies were administered in the month before the study (11). All the children were randomly divided into Tuina and control groups ($n = 30$). The control group was subjected to basic treatment and 12 sessions of lactulose oral solution, whereas the Tuina group received basic treatment and 12 sessions of Liu's infantile Tuina treatment. No differences were found in terms of age, sex, body mass index (BMI), duration of disease, or other accompanying conditions. ($P > 0.05$, Table 1).

Children were excluded if they had organic lesions of the rectum or sigmoid colon or congenital anomalies (such as anal stenosis and intestinal stricture), if they had skin breakage or local infection, if they had constipation due to previous surgery, or if their parents were unwilling to participate in the study.

Therapy regimen

All the children received the same basic treatment for dietary adjustment and increased activity. The Tuina was performed by the same experienced Tuina therapist, who was a major in Tuina.

Control group

The control group was given a total of 12 sessions of lactulose oral solution (Abbott Biologicals B.V. Netherlands) strictly (3 sessions per week for 4 weeks, with follow-up at 2 weeks and 4 weeks after the end of treatment), and the dose was adjusted according to the age of the child.

Tuina group

All participants will receive Tuina treatment three times a week (adjusted by initial local condition assessment) and continuously for four weeks. Each Tuina session lasts 20 min. If participants were older than 3 years, 50 times more added for each additional year.

The Tuina regimen includes four steps as follows (all the acupoints are listed in Table 2):

TABLE 1 Baseline characteristics of all patients.

		Tuina group	Control group	*P* value
Age (m)		49.60 ± 15.06	39.43 ± 22.368	0.281
Gender	Male	21	16	0.288
	Female	9	14	
BMI (kg/m2)		14.67 ± 0.97	14.41 ± 0.95	0.772
Duration(m)		32.93 ± 13.80	26.23 ± 14.83	0.923
Clinical pattern	Tetraplegia	13	14	0.703
	Diplegia	13	11	
	Hemiplegia	4	5	
Stool frequency/wk				
1		18	17	0.822
2		10	11	
3		2	1	
Bristol stool scale				
1		23	22	0.945
2		5	6	
3		2	2	

TABLE 2 Tuina acupoints, locations, methods and times.

Acupoints	Location
Tianmen	Located on the face, from the middle of the eyebrows to the front hairline in a straight line
Kangong	Located on the face, from the center of the eyebrow to the tip of the eyebrow in a horizontal line
Taiyang (temple)	Located on the face, in the depression behind the eyebrows
Baihui (DU20)	Located on the head, 5 cun above the middle of the front hairline
Sishencong (EX-HN 1)	Located on the head, 1 cun anterior, posterior and lateral to Baihui,4 acpoints totally
Fengchi (GB 20)	Located on the nape, below the occipital bone, in the depression between the superior sternocleidomastoid muscle and the superior trapezius muscle
Fengfu (GV 16)	Located on the nape, 1 cun directly above the midpoint of the posterior hairline, in the depression below the external occipital protuberance
Yingxiang (LI 20)	Located on the face, 0.5 cun away from the nose, in the nasolabial groove
Zongjin	Located on the palmar side, at the midpoint of the transverse wrist at the back of the palm
Yinyang	Located on both sides of the Zongjin, the side of the little finger is yin and the side of the thumb is yang
Pi jing (spleen meridian)	Located on the threaded surface at the end of the thumb
Gan jing (liver meridian)	Located on the threaded surface at the end of the index finger
Xin jing (heart meridian)	Located on the threaded surface at the end of the middle finger
Fei jing (lung meridian)	Located on the threaded surface at the end of the ring finger
Shen jing (kidney meridian)	Located on the threaded surface at the end of the little finger
Da chang (large intestine)	Located at the radial aspect of the index finger, in a straight line from the tip of the index finger to the first web space
Banmen	Located in the plane of the thenar eminence of the hand
Zhongwan (RN12)	Located on the anterior midline, 4 cun above the umbilicus
Fu (abdomen)	Located in the upper abdomen
Gui Wei	Located at the end of the coccyx
Qi jie gu (seven segmantal bones)	Located in the lumbosacral region, in a straight line from the fourth lumbar vertebra to the end of the coccyx
Ji (spine)	Located on the posterior midline, in a straight line from the first thoracic vertebra to the end of the caudal vertebra
Zusanli (ST 36)	Located in the calf, 3 cun directly below Dubi, and one finger-breadth lateral to the anterior border of the tibia
Jianjing (GB21)	Located on the shoulder, at the midpoint of the line joining Dazhui and acromion

Step 1: The child lay supine or sat while the therapist manipulated the Tianmen acupoint 48 times, parted-pushed the Kangong acupoint 48 times, straight pushed the Taiyang acupoint 48 times, pinched the great tendon 24 times, and broke up Yin Yang (parting-pushing from the central transverse crease of the wrist) 24 times.

Step 2: The child lies supine or sits while the therapist tonifies the spleen meridian 400 times, clears the liver meridian 200 times, tonifies the heart meridian 300 times, then clears the heart meridian 150 times, tonifies the lung meridian 300 times, and tonifies the kidney meridian 400 times.

Step 3: The children lay supine while the therapist kneaded the Baihui acupoint, Si Shencong acupoint, bilateral Fengchi acupoint, and bilateral Yingxiang 100 times each. The therapist then cleared away the Dachang acupoint 150 times, kneaded the Zusanli acupoint 80 times, and kneaded the Tanzhong acupoint and Tiansu acupoint 100 times. The children were subsequently changed to a prone position. The therapist kneaded the Pi Shu acupoint, Shen Shu acupoint, and Dachang Shu acupoint 100 times each. The therapist then manipulated the Qijiegu acupoint 60 times, kneaded the Guiwei acupoint 150 times and pinched the entire spinous process 5 times.

Step 4: The children lied prone or sat while the therapist kneaded Jianjing 2 times.

Parameters

All assessments were completed by an independent data assessor who was blinded to group allocation. At the initial diagnosis, the researchers would record the child's demographics (name, sex, age, height, weight, and duration), previous diagnostic and treatment history, and complete stool and serum collection. The mothers recorded stool frequency, consistency and the time spent on bowel care each day in a 4-week bowel diary. All follow-up indicators were measured at the initial visit and 4 weeks after the final treatment.

Bristol stool form scale (BSFS) (12): Stool properties are classified into 7 classes: types 4 and 5 are normal stools, whereas types 1, 2, 3, 6, and 7 are abnormal stools. The parents were asked to choose the BSS stool type to represent their children's stools.

Constipation Assessment Scale (CAS): The CAS is used to evaluate the severity of constipation through self-reported items. The total score ranges from 0 (no constipation) to 16 (severe constipation). Parents or caregivers were asked to answer this questionnaire (13).

Constipation improvement was defined as follows: (1) the defecations reached 3 times a week, and the stool consistency had softened (3<BSFS<6); (2) the frequency of stool evacuation attempts (more than 2 h per day) decreased (14).

Adverse effects, such as adverse reactions such as diarrhea, abdominal pain, vomiting, subcutaneous redness, skin breakage, and syncope, were also recorded.

Sample size calculation

The sample size was estimated via G*Power. On the basis of our preliminary study, a significant difference (5 points) in CAS

(alpha 0.05, power 0.80) was detected. Given an expected dropout rate of 10%, at least 30 patients were to be enrolled in each group.

Statistical analysis

All the data were analyzed via SPSS (version 25.0; IBM Corp, Armonk, NY, USA). The X^2 test will be used to compare the rates of the count data; the measurement data will be expressed as the means ± standard deviations (x ± s). Independent samples t tests were used for comparisons between groups when normality and equal variances were met; the independent samples Mann–Whitney U test was used if the overall data did not meet normality. $P < 0.05$ was considered statistically significant.

Results

At 4 weeks after the final treatment, the percentage of infants with improved constipation was 23 (76.7%) in the Tuina group and 21 (70.0%) in the control group ($P = 0.771$).

Initially, no difference was observed in the CAS score between the treatment groups: 10.47 ± 2.27 vs. 11.76 ± 2.24 ($P = 0{,}671$, Table 3). The stool frequency in the Tuina group was 17 once per week and 12 twice per week, whereas in the control group it was 16 once per week and 12 twice per week ($P = 0.550$, Table 3). The mean number of weekly bowel movements in both groups was 1.45 ± 0.58 vs. 1.31 ± 0.55 per week. In addition, the number of infants who underwent bowel evacuation >2 h/d was comparable between the two groups ($P = 0.795$, Table 3).

At 4 weeks after the final treatment, the CAS score and bowel evacuation in both groups decreased significantly ($P < 0.05$). The number of weekly bowel movements increased significantly ($P < 0.001$). However, there was no difference in the CAS score or weekly bowel movements between the treatment groups after treatment: 3.63 ± 2.29 vs. 3.76 ± 1.89, 3.98 ± 0.55 vs. 3.98 ± 0.65 ($P = 0{,}671$, 0.217, respectively; Table 3).

The proportion of infants with more than 2 h per day of painful bowel evacuation decreased significantly in both groups ($P = 0{,}671$ and 0.217, respectively; Table 3). However, no significant difference was found between the groups 4 weeks after the final treatment.

No serious adverse reactions (such as diarrhea, abdominal pain, vomiting, subcutaneous redness, skin breakage, or syncope) were reported.

Discussion

In the present study, our findings confirmed that Tuina was an effective therapy for children with CP with constipation. No serious complications were found throughout the entire treatment process.

As a basic therapy, lifestyle guidance emphasizes interventions in the diet, exercise, and bowel habits of the child. Early satiety and poor feeding often occur in approximately 41% of patients with CP, which contributes to undernutrition. To date, there is ongoing debate regarding the recommendation of dietary fiber and fluid intake due to the inadequacy of the study design. The American Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) did not support fiber consumption to prevent constipation (15). However, lower fiber and fluid intake also worsens this condition (4). García Contreras A.A. et al. conducted an analytical cross-sectional study and reported that low fiber and fluid intake and the use of anticonvulsant polytherapy usually resulted in harder stools and lower defecation. They recommended that daily fiber and fluid intake are important for improving constipation (16). At our institute, we still agree with the hypothesis that appropriate fiber and fluid intake can improve constipation. We advised each child with dietary adjustment. Considering the diversity of participants, well-designed high-quality randomized controlled trials are urgently needed to determine the recommendation and appropriate quantity for CP children with constipation.

In children with CP, ambulatory function is often positively correlated with the extent of brain damage. Improving brain function may enhance the brain's ability to regulate the gastrointestinal system, playing a key role in treating constipation in children with CP. Most associations are found between constipation and neurologic diseases and the spine due to damage to the central nervous system. Deterioration of the neurological function of the

TABLE 3 Comparison of stool frequency and consistency between the tuina group and the control group.

Variables	Tuina group		Control group		*P1*[a]	*P2*[b]
	Before	After	Before	After		
Bristol stool scale						
≤2	29	2	28	3	0.550	0.643
≥3	1	28	2	27		
Before/after[c]	<0.001		<0.001			
Bowel movements/wk	1.45 ± 0.58	3.98 ± 0.55	1.31 ± 0.55	3.98 ± 0.65	0.144	0.217
Before/after[c]	<0.001		<0.001			
Bowel evacuation >2 h/d	17	7	18	9	0.795	0.771
Before/after[c]	0.038		0.002			

[a]Comparison between both groups before treatment.
[b]Comparison between both groups after treatment.
[c]Comparison within the same group before and after intervention.

brain in children with CP is positively correlated with the severity of constipation (17). Disruption of the neural modulation of colonic motility plays a crucial role in the process of constipation (18). Damage to the early central nervous system includes a series of adaptive neuroplasticity processes. Earlier interdisciplinary intervention can improve brain plasticity and improve an individual's condition (19). The acupoints Baihui, Sishencong and Fengchi are pressed to enhance brain function and improve intelligence, which regulate the qi and blood of the Du meridian, and the acupoints Baihui, Sishencong, and Fengchi are pressed to enhance brain function and improve intelligence, regulating the effects of mind regulation and brain development in the theory of traditional Chinese medicine. Our study revealed comparable efficacy between infantile Tuina and oral lactulose treatment for idiopathic constipation in children with CP. At 4 weeks after the final treatment, the percentage of infants with improved constipation was 23 (76.7%) in the Tuina group and 21 (70.0%) in the control group ($P = 0.771$). The tina of the head can promote blood circulation and improve the blood supply to the brain, which partially compensates for damaged brain tissue functions by accelerating the development of the nervous system.

Chronic disability and decreased motor activity due to muscle weakness in the gastrointestinal system are among the most common problems and are associated with the severity of the disorder (20). Deficiencies in abdominal, intestinal, and anal muscle strength may also trigger defecation disorders. A delay in transit time at the entire or segmental colon was associated with constipation in patients with CP. Tuina is a nonpharmacological therapy via performing manipulations on the body surface. The theoretical basis originates from the special meridian-acupoint theory of traditional Chinese medicine. Tuina can induce autonomic responses via cutaneo-visceral reflexes to improve constipation (21).

Children with CP the hypotonic type exhibit delayed motor development and reduced motor activity. Abdominal massage can promote gastrointestinal motility and has a direct therapeutic effect on constipation. In this study, the application of abdominal manipulation was emphasized, and a combination of rubbing, holding, kneading and vibrating techniques was applied directly to the abdomen of the child. The abdomen serves as the body's projection area for the stomach and intestines. External pressure stimulates the intestines, causing deformation or displacement, which promotes intestinal content transmission and facilitates defecation.Moreover, gentle and powerful manipulation of the abdomen can reflexively regulate the central system, reduce the excitability of the descending colon and rectal parasympathetic nerves, increase peristalsis of the descending colon and rectum, and create favorable conditions for defecation (22). The improvement in muscle strength not only increases daily activity and promotes intestinal peristalsis but also enhances the contraction of the abdominal muscles, anal muscles and intestinal wall muscles and increases abdominal pressure, all of which can accelerate fecal excretion and reduce the symptoms of constipation in children. In addition, stretching exercises could reduce spasticity, improve joint mobility, and enhance functional independence. These improvements increase the motility of the gut and relieve constipation by inducing parasympathetic stimulation (23).

Limitations

There were several limitations in the present study. First, this was a small sample size study, and the follow-up time was only 4 weeks. However, the sample size was calculated via G*Power. Future studies should include a larger and more diverse population to improve external validity, including the addition of a sham Tuina group. Second, the age of the enrolled children was 16–98 (average, 42.70 ± 17.81) months old. Some results came from their parents due to incomplete comprehension and expression skills. Third, the Tuina in this study was performed by the same skilled therapist, but some variability in the standardization of stimulation intensity by manual manipulation is inevitable. A longer observation period is needed to achieve the long-term efficacy and recurrence rates of constipation in Tui Na.

In conclusion, constipation is a highly prevalent disease of the gastrointestinal tract in children with CP, and Tuina is as effective as medical care in addition to basic treatment. The results of this study suggest that Tui Na may serve as a viable alternative treatment for constipation.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Ethics Review Committee of The First Hospital of Hunan University of Chinese Medicine. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

Author contributions

HW: Data curation, Formal Analysis, Writing – original draft. BC: Investigation, Methodology, Writing – original draft. QL: Data curation, Project administration, Validation, Writing – original draft. QY: Data curation, Formal Analysis, Resources, Writing – original draft. JM: Investigation, Methodology, Project administration, Writing – original draft. QM: Investigation, Methodology, Project administration, Writing – original draft. XY: Investigation, Methodology, Project administration, Writing – original draft. YW: Investigation, Methodology, Project administration, Writing – original draft. YL: Investigation, Methodology, Project administration, Writing – original draft. ZC: Investigation, Methodology, Project administration,

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Publisher's note

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Unpacking the dangers of super absorbent polymer water beads: an *in vitro* analysis

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Objectives: This study aims to investigate the risk of pediatric bowel obstruction from various types of super absorbent polymer (SAP) beads in different liquid media, explore treatment options, and develop a clinical decision-making algorithm for healthcare providers.
Methods: Three experiments were conducted involving the measurement of SAP beads' expansion in different liquid media. The first experiment examined the expansion of beads in tap water, gastric fluid, and small intestine fluid. The second compared the expansion of beads from six manufacturers in water. The third tested the effect of varying concentrations of Polyethylene Glycol 3350 on bead expansion.
Results: The study found that SAP beads reached their largest size in water, with significant size-dependent and solution-specific effects on their expansion. Large beads had a β of 12.67 (95% CI: 10.25–15.1; $p < 0.001$) compared to small beads. Gastric acid reduced expansion with a β of −7.01 (95% CI: −9.67 to 4.35; $p < 0.001$) and alkaline solution with a β of −3.88 (95% CI: −6.54 to 1.23; $p = 0.002$) compared to water. Treatment solutions containing high concentrations of polyethylene glycol (PEG) 3350 effectively reduced the size of the beads ($p < 0.001$).
Conclusions: This research highlights the importance of understanding the characteristics and risks associated with SAP beads to mitigate the dangers they pose to pediatric populations. Our findings underscore the need for standardized management of SAP bead ingestion, which could improve patient outcomes while reducing unnecessary diagnostic and therapeutic interventions. Further research and clinical validation of these strategies *in vivo* are essential to develop safe and efficient protocols for managing SAP bead ingestions

KEYWORDS
pediatric bowel obstruction, foreign body ingestions, super absorbent polymer beads, water beads, polyethylene glycol 3350

Introduction

The *Pediatrics Journal* cites an incidence rate of 17.9 foreign body ingestions per 10,000 children under six years old in the United States (US) (1). The demographic most frequently affected by these incidents includes children aged one year (21.3%) and boys (52.9%), with toys accounting for nearly 10% of cases. Super absorbent polymer (SAP) beads are particularly concerning, as they have been associated with bowel obstruction in several case studies (2). Initially created in the 1960s by the US

Department of Agriculture for soil water conservation, SAPs have since permeated mainstream toy production. When ingested, these beads can continue to expand by absorbing water in the small intestine, potentially causing bowel obstruction in the duodenal sweep or ileocecum. Recent cases of intestinal obstruction tied to SAPs have sparked serious concerns, prompting major retailers to remove such products from their shelves (3). However, due to their commercial appeal, similar beads continue to be marketed to adults as decorative items. From 2007 to 2022, there were over 8,000 estimated visits to US emergency departments linked to water beads, with these visits more than doubling in the last 2 years of the study. This increase occurred despite product recalls and existing voluntary safety standards, indicating that current prevention strategies are insufficient (4). Further emphasizing the rising problem, a study using the National Electronic Injury Surveillance System (NEISS) identified 226 water bead injuries from 2013 to 2023, with 66% involving ingestion. Children under age 2 years accounted for 29% of injuries, and multiple water beads were involved in 56% of cases. A significant uptrend in injury frequency was observed after 2020, with 7% of cases requiring escalation of care. These findings highlight the increasing prevalence and significant harm associated with water bead injuries, particularly affecting children under 5 years of age (5).

This study aims to investigate the potential risk of pediatric bowel obstruction posed by different types of SAP beads in various liquid media. We also seek to explore possible treatments and develop a clinical decision-making algorithm to aid healthcare providers when dealing with cases of children ingesting these polymers. The urgency of this research is emphasized by the significant adverse outcomes that can result from improper management of these ingestions. Establishing a standardized, evidence-based approach to handling ingested superabsorbent polymers could prove to be an invaluable tool for clinicians. By potentially decreasing the morbidity and mortality rates associated with these ingestions, this research underscores the importance and necessity of understanding and addressing this issue.

Materials and methods

This study was organized around three meticulously planned experiments. The diameters of the super absorbent polymers were measured in millimeters using an Esydon® Digital Caliper (ASIN: B0C86NSBDK).

The first experiment aimed to determine the effect of three different control solutions: tap water, simulated gastric fluid, and simulated small intestine fluid, on the expansion of water beads. The simulated small intestine fluid was prepared by buffering 180 ml of lactated ringer fluid with an osmolality of 265 mOsm/kg to achieve a pH of 7.5–8.0. The simulated gastric fluid was purchased from Carolina Biologics Company®, composed of water (99%), pepsin (0.5%), hydrogen chloride (0.22%), and Thymol (0.1%). With an approximate total molarity of HCl of 0.0603 M the pH of the 180 ml solution of simulated gastric fluid is approximately 1.22 and an osmolarity of 127.4 mOsm/kg. Super absorbent polymer beads were obtained from six major manufacturers: Leeche®, Ainolway®, Yiquo®, Babiya®, Waterballz®, and Orbeez®. The beads were categorized into small (<3 mm), medium (3–7 mm), and large (>7 mm) groups and measured in their dry state using an Esydon Digital Caliper®. Six beads from each group were submerged in 180 ml of each of the three control solutions and three beads out of those from each group were measured at 2, 6, 12, and 24 h. Three beads from each control solution were transferred to three different treatment solutions containing 17 grams of PEG 3350, 6 grams of psyllium fiber, and 10 grams of Saccharin, each mixed into 180 ml of water. These beads were then measured again after 24 h.

The second experiment involved a comparative study of water beads from six major manufacturers: Ainolway®, Cosmos®, and Magic Labs® (manufacturers of smaller beads), and Babiya®, Leeche®, and Yiquduo® (manufacturers of larger beads). All water beads were made from the same material, sodium polyacrylate gel. Ten beads from each manufacturer were measured and then immersed in 180 ml of water. Subsequent measurements were taken at intervals of 2, 12, and 24 h. The aim was to provide a direct comparison between the different manufacturers and improve measurement reliability.

The third experiment focused on the effect of varying concentrations of polyethylene glycol (PEG) 3350 on the maximum expansion size of large water beads (>7 mm). The beads were initially placed in 180 ml of simulated gastric juice, then transferred to 180 ml of alkaline fluid to simulate the pH change in the small intestine. Ten beads were immersed in each of the eight different PEG concentrations: 17 g, 34 g, 51 g, 68 g, and 85 g in 90 ml and 180 ml of water respectively. Measurements were taken at 0, 2, 12, and 24 h.

All analyses conducted were descriptive, employing summary statistics such as median and interquartile range for continuous variables, and frequency and proportion for categorical variables. Differences between groups were evaluated using nonparametric methodologies like the Kruskal–Wallis test for continuous variables and Fisher's exact test for categorical variables. Linear mixed modeling was utilized to assess changes in bead sizes over time and provide distribution estimates. Graphical representations of changes in bead sizes were shown using line and bar graphs. All analyses were performed using R software version 4.3.0 (R Core Team, Vienna, Austria), considering a p-value <0.05 as statistically significant.

Results

Experiment 1: effect of solution type on diameter over time

In the first experiment, water beads were categorized by size and exposed to different control solutions to observe their expansion over time. The expansion characteristics of super absorbent polymer beads were evaluated for small (<3 mm), medium (3–7 mm), and large (>7 mm) bead sizes across a range

of control and treatment solutions. Measurements were taken at multiple time points (0, 2, 6, 12, and 24 h) to assess the dynamics of bead size changes under different conditions. Overall, the results revealed significant variation in the expansion potential of the beads depending on their size and the solution in which they were immersed.

The initial mean diameters prior to submersion were 1.97 mm, 5.48 mm, and 8.17 mm for small, medium, and large beads, respectively. At 24 h, the small beads expanded to a maximum size of 10.6 mm [3.43–10.6 mm], with a mean size of 8.35 mm (SD ± 2.79 mm). The medium beads reached a maximum size of 27.73 mm [14.7–27.7 mm], with a mean size of 21.8 mm (SD ± 5.28 mm). The large beads reached a maximum size of 32.4 mm [16.6–32.4 mm], with a mean size of 25.7 mm (SD ± 15.4 mm).

Small beads (<3 mm) expanded modestly in simulated gastric fluid, growing from 2.23 mm (SD = 0.225, range: 2.00–2.60 mm) to 3.43 mm (SD = 0.666, range: 3.00–4.20 mm) after 24 h. In contrast, they expanded significantly in tap water, reaching 10.6 mm (SD = 0.808, range: 9.70–11.3 mm) after 24 h. Simulated small intestine fluid also supported moderate growth to 7.00 mm (SD = 1.14, range: 6.20–8.30 mm) after 24 h. When transitioned to treatment solutions, PEG-treated beads expanded to 8.40 mm (SD = 1.61, range: 6.90–10.1 mm), while psyllium-treated beads grew further to 10.5 mm (SD = 1.47, range: 9.60–12.2 mm). Splenda-treated beads exhibited comparable growth, reaching 10.2 mm (SD = 0.513, range: 9.80–10.8 mm). Across all conditions, the overall mean size of small beads at 24 h was 8.36 mm (SD = 2.79, range: 3.00–12.2 mm).

Medium (3–7 mm) and large (>7 mm) beads exhibited greater expansion potential. Medium beads in tap water grew from 5.48 mm (SD = 0.223, range: 5.30–5.80 mm) to 27.7 mm (SD = 1.46, range: 26.2–29.1 mm) at 24 h, while those in simulated gastric fluid reached 14.7 mm (SD = 0.208, range: 14.5–14.9 mm). Simulated small intestine fluid facilitated significant expansion to 22.1 mm (SD = 0.265, range: 21.8–22.3 mm). For large beads, the most substantial growth occurred in tap water, where they expanded from 8.17 mm (SD = 0.758, range: 7.10–9.20 mm) to 32.4 mm (SD = 34.4, range: 9.50–72.0 mm). In simulated gastric fluid, large beads grew to 16.0 mm (SD = 5.93, range: 9.20–19.9 mm), and in simulated small intestine fluid, to 29.8 mm (SD = 17.8, range: 9.20–40.2 mm). Among the treatment solutions, saccharin-treated medium and large beads achieved the largest final diameters of 24.3 mm (SD = 2.30, range: 22.0–26.6 mm) and 31.4 mm (SD = 0.400, range: 31.0–31.8 mm), respectively. Overall, medium and large beads showed remarkable expansion across most conditions, particularly in tap water and saccharin solutions. See Table 1.

In summary, the results highlight that the expansion capacity of super absorbent polymer beads is influenced by both bead size and solution type. Small beads showed limited growth in acidic conditions but significant expansion in psyllium and saccharin solutions. Medium and large beads demonstrated remarkable expansion, particularly in tap water and simulated small intestine fluid. Among the treatment solutions, saccharin consistently facilitated the greatest bead growth, particularly for large beads. These findings emphasize the importance of solution composition in influencing the hydration and expansion dynamics of super absorbent polymers.

In all solutions, a statistically significant correlation was found between the dry size of the bead and the maximum diameter it could reach upon hydration regardless of the solution type. Multivariable Linear mixed modeling further quantified the effect of bead size, liquid type, and time on expansion. Medium beads showed a significant increase in size with a coefficient of 8.25 (95% CI: 4.38–12.13; $p < 0.001$) and large beads with a β of 11.87 (95% CI: 8.07–15.68; $p < 0.001$) compared to small beads. For liquid type, gastric acid significantly reduced expansion with a β of −5.16 (95% CI: −8.9 to −1.42; $p < 0.001$) and alkalotic solution with a β of −2.21 (95% CI: −5.94 to 1.53; $p = 0.002$) compared to water. Time also played a significant role, with the 24-h mark showing the largest increase in size with a β of 12.6 (95% CI: 10.09–15.12; $p < 0.001$). These results demonstrate a size-dependent and solution-specific impact on the expansion of water beads, with larger sizes expanding more significantly over time and certain solutions like gastric acid limiting expansion. The maximum diameter attained by the water beads varied significantly across the samples, ranging to 72 mm.Unsurprisingly, a statistically significant correlation was found between the dry size of the bead and the maximum diameter it could reach upon hydration.

After 2 h of exposure to the initial control solutions, three water beads were randomly selected from each group and introduced to three unique treatment solutions: PEG 3350 (17 grams in 6 oz), fiber (3 grams in 6 oz), and hyperosmolar Splenda water (1 gram in 6 oz). Our observations showed that the water beads exhibited the least growth in the PEG 3350 solution. Specifically, the small beads expanded to a mean diameter of 8.4 mm (SD ± 1.61) the medium beads to 16.8 mm (SD: ±5.93), and the large beads to 16.5 mm (SD: ±7.2) in the 17-gram PEG 3350 solution. The detailed data of experiment 1 are outlined in Table 1 and Figure 1. This experiment provides important insights into the variability in water bead expansion across different sizes and solution types and emphasizes the predictive value of the bead's dry size.

Experiment 2: analysis of bead characteristics on the expansion of SAP beads

In the second experiment, the expansion of water beads from six different brands—AINOLWAY, BABIYA, COSMOS, LEECHE, MAGIC LABS, and YIQUO—was compared over 72 h when submerged in water. The initial mean diameters of the beads varied significantly across brands, ranging from 2.05 to 7.41 mm, with a p-value <0.001, indicating a statistically significant difference.

Over 24 h, the beads expanded, with BABIYA showing the most substantial growth (mean diameter 33.8 mm), and AINOLWAY the least (mean diameter 7.55 mm). This trend continued through 48 and 72 h, with BABIYA beads maintaining

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TABLE 1 Size-dependent effect on maximum growth.

Immersion fluid	Water				
Time when measured	0 h	2 h	6 h	12 h	24 h
	(*N* = 6)	(*N* = 3)	(*N* = 3)	(*N* = 3)	(*N* = 3)
Small beads: Size (mm)					
Mean (SD)	1.97 (0.463)	9.21 (0.788)	10.0 (0.947)	10.1 (0.925)	10.6 (0.808)
Median[Min, Max]	1.80 [1.70, 2.90]	8.93 [8.60, 10.1]	9.69 [9.30, 11.1]	9.72 [9.50, 11.2]	10.7 [9.70, 11.3]
Medium beads: Size (mm)					
Mean (SD)	5.48 (0.223)	15.4 (0.900)	18.0 (2.80)	24.0 (1.01)	27.7 (1.46)
Median[Min, Max]	5.40 [5.30, 5.80]	15.4 [14.5, 16.3]	18.9 [14.9, 20.3]	23.9 [23.1, 25.1]	27.9 [26.2, 29.1]
Large beads: Size (mm)					
Mean (SD)	8.17 (0.758)	19.9 (17.4)	26.3 (26.0)	29.3 (29.4)	32.4 (34.4)
Median[Min, Max]	8.30 [7.10, 9.20]	10.1 [9.50, 40.0]	13.1 [9.50, 56.2]	15.3 [9.50, 63.0]	15.7 [9.50, 72.0]
Immersion fluid	Gastric Acid				
Time when measured	0 h	2 h	6 h	12 h	24 h
	(*N* = 6)	(*N* = 3)	(*N* = 3)	(*N* = 3)	(*N* = 3)
Small beads: Size (mm)					
Mean (SD)	2.23 (0.225)	2.07 (0.0577)	3.47 (0.473)	3.30 (0.800)	3.43 (0.666)
Median[Min, Max]	2.15 [2.00, 2.60]	2.10 [2.00, 2.10]	3.30 [3.10, 4.00]	3.30 [2.50, 4.10]	3.10 [3.00, 4.20]
Medium beads: Size (mm)					
Mean (SD)	5.62 (0.264)	11.4 (2.21)	12.8 (2.65)	13.7 (2.12)	14.7 (0.208)
Median[Min, Max]	5.70 [5.20, 5.90]	10.4 [9.80, 13.9]	12.9 [10.1, 15.4]	14.7 [11.3, 15.2]	14.8 [14.5, 14.9]
Large beads: Size (mm)					
Mean (SD)	8.15 (0.589)	11.6 (3.52)	11.6 (3.59)	12.7 (3.55)	16.0 (5.93)
Median[Min, Max]	8.15 [7.40, 9.00]	9.85 [9.20, 15.6]	9.80 [9.20, 15.7]	12.6 [9.20, 16.3]	19.0 [9.20, 19.9]
Immersion fluid	LR (alkalotic)				
Time when measured	0 h	2 h	6 h	12 h	24 h
	(*N* = 6)	(*N* = 3)	(*N* = 3)	(*N* = 3)	(*N* = 3)
Small beads: Size (mm)					
Mean (SD)	2.15 (0.217)	6.35 (1.07)	6.65 (1.31)	7.05 (1.40)	7.00 (1.14)
Median[Min, Max]	2.10 [1.90, 2.50]	6.70 [5.14, 7.20]	7.30 [5.14, 7.50]	7.30 [5.54, 8.30]	6.50 [6.20, 8.30]
Medium beads: Size (mm)					
Mean (SD)	5.50 (0.228)	12.3 (0.854)	14.1 (1.82)	18.0 (3.35)	22.1 (0.265)
Median[Min, Max]	5.50 [5.20, 5.80]	12.2 [11.5, 13.2]	15.1 [12.0, 15.2]	16.2 [16.0, 21.9]	22.2 [21.8, 22.3]
Large beads: Size (mm)					
Mean (SD)	8.47 (0.468)	16.4 (7.35)	21.6 (12.0)	22.9 (12.4)	29.8 (17.8)
Median[Min, Max]	8.30 [8.10, 9.30]	16.4 [9.10, 23.8]	22.8 [9.10, 33.0]	26.5 [9.10, 33.0]	40.0 [9.20, 40.2]
Immersion fluid	PEG	Psyllium	Splenda		
Time when measured	24 h	24 h	24 h		
	(*N* = 3)	(*N* = 3)	(*N* = 3)		
Small beads: Size (mm)					
Mean (SD)	8.40 (1.61)	10.5 (1.47)	10.2 (0.513)		
Median[Min, Max]	8.20 [6.90, 10.1]	9.70 [9.60, 12.2]	10.1 [9.80, 10.8]		
Medium beads: Size (mm)					
Mean (SD)	16.8 (5.93)	25.0 (2.27)	24.3 (2.30)		
Median[Min, Max]	16.1 [11.3, 23.1]	24.3 [23.1, 27.5]	24.3 [22.0, 26.6]		
Large beads: Size (mm)					
Mean (SD)	16.5 (7.20)	28.4 (1.80)	31.4 (0.400)		
Median[Min, Max]	16.6 [9.20, 23.6]	28.4 [26.6, 30.2]	31.4 [31.0, 31.8]		

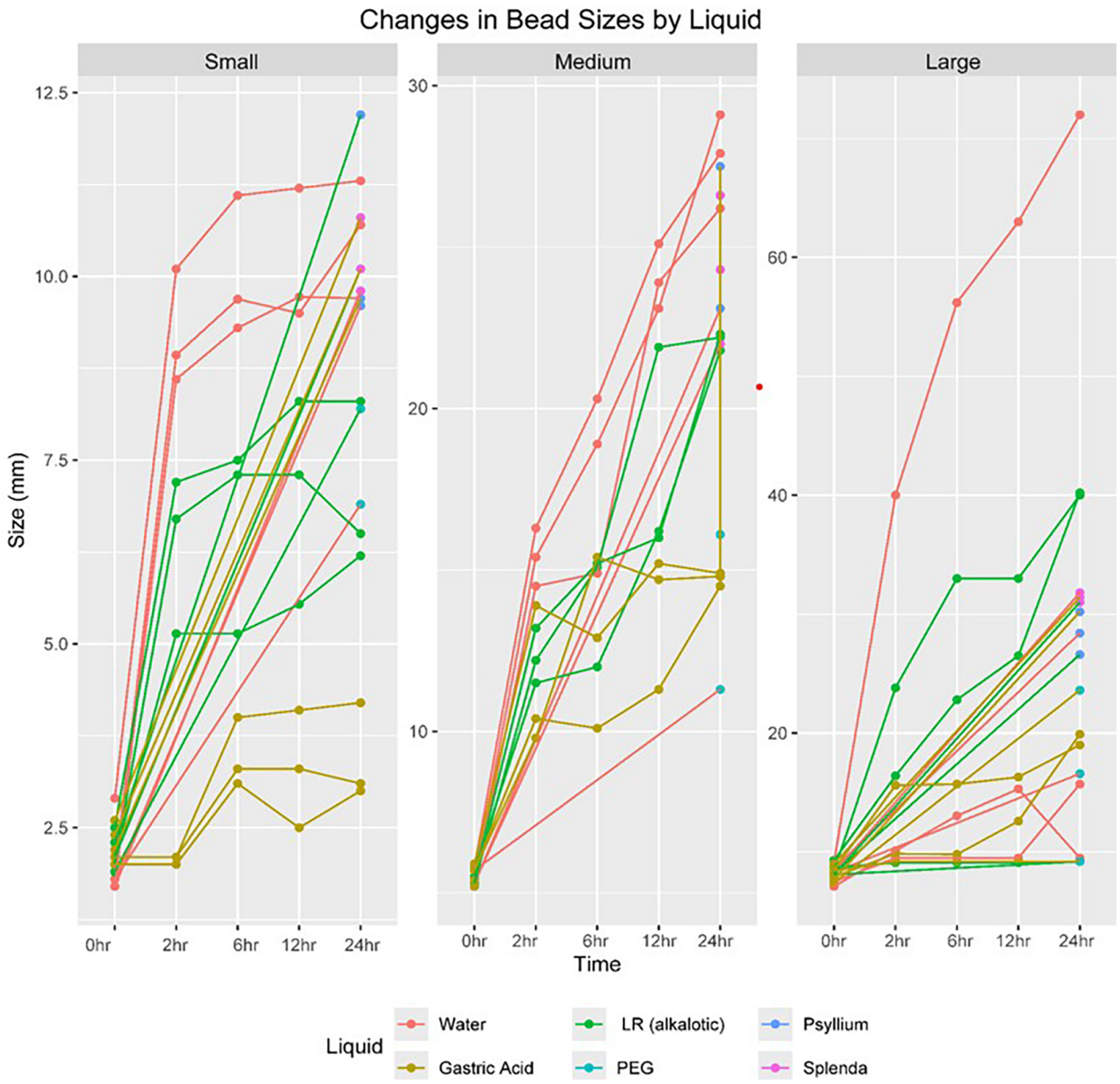

FIGURE 1
Graphical representation of the size dependent effect on maximum growth.

the largest size and AINOLWAY the smallest. The overall mean diameters at 48 and 72 h across all brands were 20.2 mm and 21.4 mm, respectively, both with p-values <0.001, affirming the significant differences in expansion among brands. Linear mixed model analysis provided a detailed examination of the time and brand effects on bead expansion. The time effect was significant at all measured points, with the greatest expansion observed at 72 h ($\beta = 16.57$; 95% CI: 14.63–18.5; $p < 0.001$). When looking at brand effects, BABIYA and YIQUO showed the largest size increases with β of 24.4 and 23.29, respectively ($p < 0.001$ for both). COSMOS and MAGIC LABS had smaller, yet significant, size increases (β of 3.39 and 2.89; $p = 0.003$ and $p = 0.008$, respectively). LEECHE also showed a substantial increase in size ($\beta = 17.4$; $p < 0.001$). The complete results are referenced in Table 2 and Figure 2 and demonstrate that both the time elapsed and the brand of water beads significantly affect the degree of expansion, with some brands showing markedly more substantial growth than others.

Experiment 3: analysis of PEG 3350 effect on the expansion of SAP beads

In Experiment 3, the diameter expansion of water beads was assessed at 24 and 48 h after soaking in various concentrations of polyethylene glycol (PEG) 3350. Beads were initially conditioned in simulated gastric acid followed by an alkaline solution before exposure to the PEG solutions.

TABLE 2 Product-related effect on maximum growth.

SAP Brand	AINOLWAY	BABIYA	COSMOS	LEECHE	MAGIC LABS	YIQUO	Overall	*P*-value
	(*N* = 10)	(*N* = 10)	(*N* = 10)	(*N* = 10)	(*N* = 10)	(*N* = 10)	(*N* = 60)	
0 h								Kruskal–Wallis test
Mean (SD)	2.15 (0.0850)	7.25 (0.484)	2.54 (0.0474)	7.41 (0.567)	2.05 (0.0850)	7.36 (1.07)	4.79 (2.62)	<0.001
Median [Min, Max]	2.15 [2.00, 2.30]	7.25 [6.40, 7.90]	2.50 [2.50, 2.60]	7.60 [6.40, 8.20]	2.05 [1.90, 2.20]	7.15 [6.20, 10.0]	4.40 [1.90, 10.0]	
Median (IQR)	2.15 (2.10, 2.20)	7.25 (7.00, 7.60)	2.50 (2.50, 2.59)	7.60 (6.98, 7.78)	2.05 (2.00, 2.10)	7.15 (6.75, 7.68)	4.40 (2.20, 7.23)	
24 h								Kruskal–Wallis test
Mean (SD)	7.55 (0.805)	33.8 (2.68)	9.36 (0.560)	23.5 (3.20)	4.57 (0.767)	29.8 (5.06)	18.1 (11.8)	<0.001
Median [Min, Max]	7.40 [6.50, 8.70]	33.9 [29.4, 36.7]	9.20 [8.70, 10.4]	23.2 [20.1, 29.8]	4.45 [3.00, 5.80]	28.7 [24.9, 41.4]	15.3 [3.00, 41.4]	
Median (IQR)	7.40 (6.93, 8.25)	33.9 (31.8,36.5)	9.20 (9.03, 9.68)	23.2 (20.9,24.3)	4.45 (4.30, 5.05)	28.7 (25.9,32.4)	15.3 (7.55, 28.9)	
48 h								Kruskal–Wallis test
Mean (SD)	3.61 (0.428)	36.0 (2.41)	9.18 (0.487)	27.8 (4.53)	9.27 (0.695)	35.6 (5.59)	20.2 (13.7)	<0.001
Median [Min, Max]	3.75 [3.00, 4.20]	36.4 [30.5, 38.7]	9.30 [8.50, 10.0]	26.5 [22.0, 34.8]	9.35 [7.90, 10.2]	33.9 [28.1, 47.7]	16.1 [3.00, 47.7]	
Median (IQR)	3.75 (3.23, 3.88)	36.4 (36.3,36.8)	9.30 (8.80, 9.48)	26.5 (25.3,31.6)	9.35 (8.98, 9.55)	33.9 (32.0,38.5)	16.1 (8.80, 33.7)	
72 h								Kruskal–Wallis test
Mean (SD)	3.60 (0.462)	37.5 (2.67)	9.39 (0.606)	27.8 (4.86)	12.6 (0.680)	37.3 (5.21)	21.4 (13.9)	<0.001
Median [Min, Max]	3.75 [3.00, 4.20]	37.7 [32.4, 41.5]	9.45 [8.40, 10.1]	25.9 [23.0, 37.5]	12.5 [11.7, 14.2]	36.4 [29.9, 49.6]	18.6 [3.00, 49.6]	
Median (IQR)	3.75 (3.13, 3.88)	37.7 (36.5,39.4)	9.45 (8.95, 9.93)	25.9 (24.8,29.1)	12.5 (12.2,12.8)	36.4 (35.1,37.9)	18.6 (9.48, 35.6)	

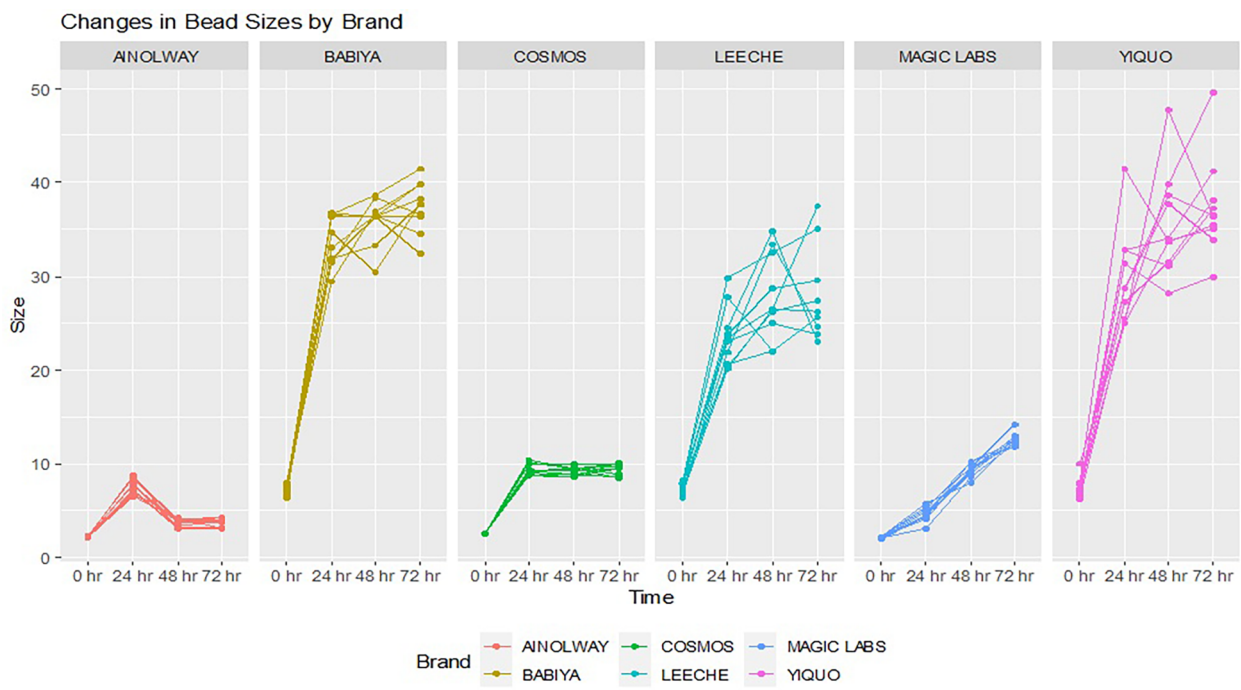

FIGURE 2
Graphical representation of the product related effect on maximum growth.

At the 24-h mark, a significant expansion was observed across all PEG concentrations when compared to the baseline (Kruskal–Wallis test, $p < 0.001$). Beads in the 17 g of PEG in 3 oz of water solution had a mean diameter of 17.9 mm (SD: 1.89); median (IQR): 17.5 (17.1,17.8) and those in 17 g of PEG in 6 oz of water demonstrated a larger mean diameter of 21.8 mm (SD: 1.74); median (IQR): 22.1 (20.3,22.6). The smallest mean diameter of 10.4 mm (SD: 0.531) median (IQR): 10.4 (10.1,10.8) was found in the 51 g PEG in 3 oz of water group, while the 34 g in 6 oz solution resulted in a relatively larger mean diameter of 19.4 mm (SD 1.68); median (IQR): 18.9 (18.5,19.5).

At the 48-h interval, the increase in diameter persisted with statistical significance (Kruskal–Wallis test, $p < 0.001$). The mean diameter in the 17 g PEG in 6 oz water group was 23.6 mm (SD

1.91); median (IQR): 23.7 (21.8, 25.3), reflecting the continued swelling of beads. The 51 g PEG in 3 oz water group maintained the trend of smaller bead sizes with a mean diameter of 10.9 mm (SD 0.829); median (IQR): 10.9 (10.1, 11.6).

In the multivariable analysis, time within groups was a significant factor for diameter expansion. The 48-h time point was associated with an increased bead diameter [mean increase = 1.12, 95% CI (0.7, 1.54), $p < 0.001$] compared to at 24 h. In univariate analysis, time was not a significant factor at 24 h [mean increase = 1.1, 95% CI (−0.22, 2.5), $p = 0.1$], suggesting that other variables may contribute to diameter changes at this time point. Between-group comparisons showed that concentration of PEG was significantly associated with bead size. The largest decreases were observed in high concentrations of PEG with smaller volumes of water, indicating a decrease in bead size. Specifically, 51 g of PEG in 3 oz of water had a significant shrinkage effect [mean decrease of 12.07 mm, 95% CI (−13.02, −11.12), $p < 0.001$] compared to 17 g of PEG in 6 oz of water. The least shrinkage effect within the high concentration groups was observed with 34 g of PEG in 6 oz of water [mean decrease of 2.23 mm, 95% CI (−3.18, −1.29), $p < 0.001$] compared to 17 g of PEG in 6 oz of water. Complete data can be referenced in Figure 3, Tables 3, 4.

Discussion

Our findings demonstrate a potential risk of obstruction in patients who ingest SAP beads larger than 3 mm in dry size, which can expand to sizes greater than 25 mm. This contrasts with previous studies that did not identify SAP beads as a risk, since they did not observe expansion to sizes posing an obstruction risk (6–8). However, these studies did not consider important characteristics that may influence the expansion to larger sizes, which can lead to serious consequences, including fatalities as reported in previous case studies (2). These studies did not account for the dry size of the water beads, which is a crucial determinant of their expansion potential. Our research specifically addresses this gap by thoroughly investigating how beads with different initial dry sizes respond in various liquid environments, thereby providing a clearer understanding of which beads pose the greatest risk. Additionally, prior studies did not consider the concentration of polyethylene glycol (PEG) 3350, commonly known as MiraLAX, which our study has shown can effectively reduce bead size. By exploring the impact of varying concentrations of MiraLAX, we provide valuable insights into potential therapeutic strategies for managing SAP bead ingestions.

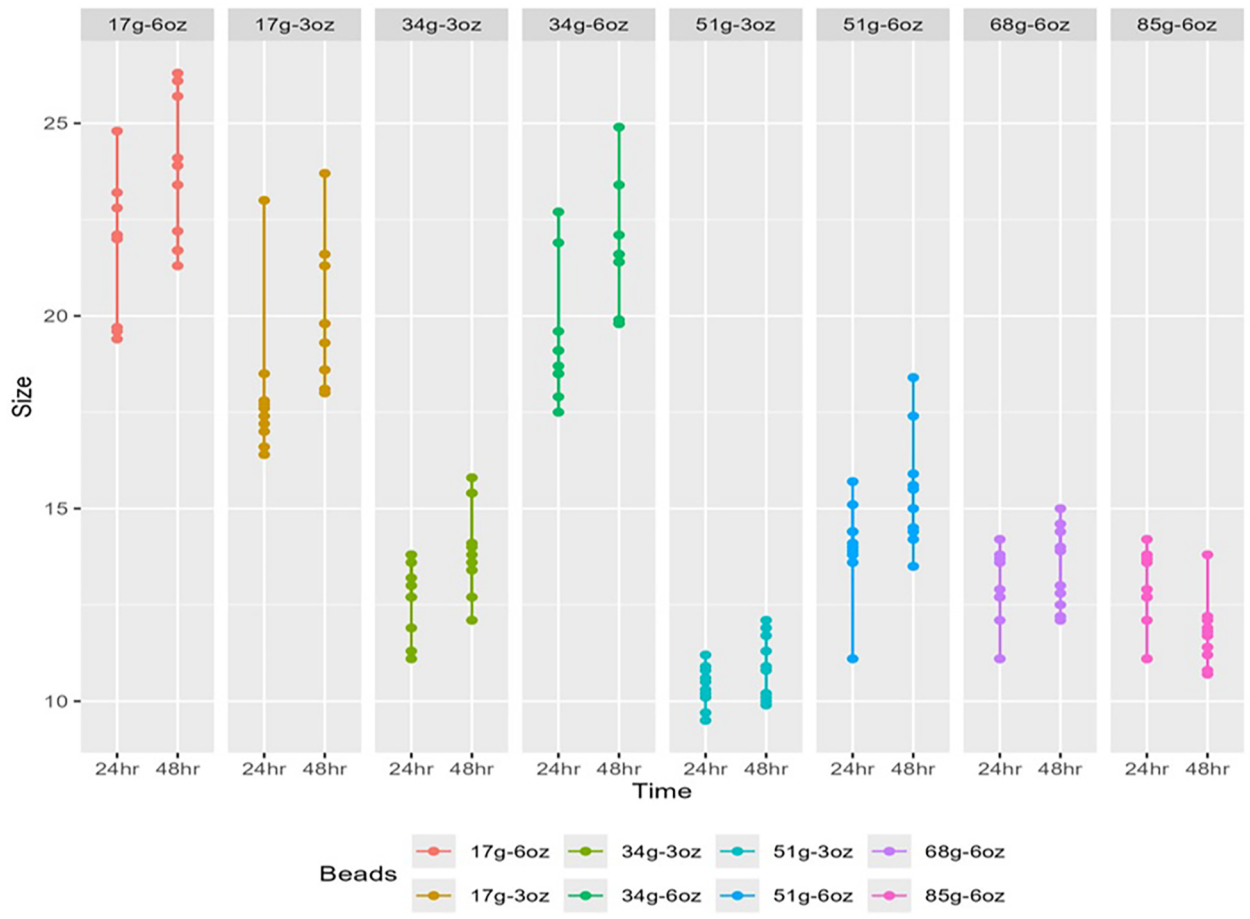

FIGURE 3
Graphical representation of the PEG 3350 related effect on maximum growth.

TABLE 3 PEG 3350 related effect on maximum growth.

Details of immersion fluid	17 g-3oz (*N* = 10)	34 g-3oz (*N* = 10)	51 g-3oz (*N* = 10)	Overall (*N* = 80)	*P*-value
24 h					Kruskal–Wallis test
Mean (SD)	17.9 (1.89)	12.7 (0.985)	10.4 (0.531)	15.2 (3.95)	<0.001
Median [Min, Max]	17.5 [16.4, 23.0]	12.9 [11.1, 13.8]	10.4 [9.50, 11.2]	13.8 [9.50, 24.8]	
Median (IQR)	17.5 (17.1,17.8)	12.9 (12.1,13.5)	10.4 (10.1,10.8)	13.8 (12.6,18.1)	
48 h					Kruskal–Wallis test
Mean (SD)	19.9 (1.82)	14.0 (1.20)	10.9 (0.829)	16.3 (4.68)	<0.001
Median [Min, Max]	19.6 [18.0, 23.7]	13.9 [12.1, 15.8]	10.9 [9.90, 12.1]	14.8 [9.90, 26.3]	
Median (IQR)	19.6 (18.6,20.9)	13.9 (13.5,15.1)	10.9 (10.1,11.6)	14.8 (12.2,20.3)	
Details of immersion fluid	**17 g-6oz (*N* = 10)**	**34 g-6oz (*N* = 10)**	**51 g-6oz (*N* = 10)**	**68 g-6oz (*N* = 10)**	**85 g-6oz (*N* = 10)**
24 h					
Mean (SD)	21.8 (1.74)	19.4 (1.68)	14.0 (1.20)	12.8 (1.09)	12.8 (1.09)
Median [Min, Max]	22.1 [19.4, 24.8]	18.9 [17.5, 22.7]	14.1 [11.1, 15.7]	12.8 [11.1, 14.2]	12.8 [11.1, 14.2]
Median (IQR)	22.1 (20.3,22.6)	18.9 (18.5,19.5)	14.1 (13.8,14.4)	12.8 (12.3,13.7)	12.8 (12.3,13.7)
48 h					
Mean (SD)	23.6 (1.91)	21.6 (1.63)	15.4 (1.50)	13.5 (1.06)	11.8 (0.881)
Median [Min, Max]	23.7 [21.3, 26.3]	21.5 [19.8, 24.9]	15.3 [13.5, 18.4]	13.5 [12.1, 15.0]	11.8 [10.7, 13.8]
Median (IQR)	23.7 (21.8,25.3)	21.5 (20.3,22.0)	15.3 (14.4,15.8)	13.5 (12.6,14.3)	11.8 (11.3,12.1)

TABLE 4 Linear mixed model using time, size type and type of liquid as predictors.

Experiment 1			Experiment 2			Experiment 3		
Characteristic	β (95% CI)	*p*-value	Characteristic	β (95% CI)	*p*-value		Multivariable	
Time (Within group)			Time (Within group)			Characteristic	β (95% CI)	*p*-value
0 h	Ref		0 h	Ref		Time (Within Group)		
2 h	6.02 (3.5–8.53)	0.001	24 h	13.31 (11.38–15.25)	<0.001	24 h	Ref	
6 h	8.24 (5.72–10.75)	<0.001	48 h	15.44 (13.5–17.37)	<0.001	48 h	1.12 (0.7,1.54)	<0.001
12 h	10.08 (7.57–12.6)	<0.001	72 h	16.57 (14.63–18.5)	<0.001			
24 h	12.6 (10.09–15.12)	<0.001				Dilutant (Between Group)		
						3oz	Ref	
Type (Between group)			Brand (Between group)			6oz	5 (4.36, 5.64)	<0.001
Small	Ref		AINOLWAY	Ref				
Medium	8.25 (4.38–12.13)	<0.001	BABIYA	24.4 (22.03–26.77)	<0.001	Grams of PEG (Between Group)		
Large	11.87 (8.07–15.68)	<0.001	COSMOS	3.39 (1.02–5.76)	0.003	17 g	Ref	
			LEECHE	17.4 (15.03–19.77)	<0.001	34 g	−3.88 (−4.66, −3.1)	<0.001
Liquid (Between group)			MAGIC LABS	2.89 (0.52–5.26)	0.008	51 g	−8.12 (−8.9, −7.34)	<0.001
Water	Ref		YIQUO	23.29 (20.92–25.66)	<0.001	68 g	−10.18 (−11.19, −9.17)	<0.001
Gastric Acid	−5.16 (−8.9 to 1.42)	0.003				85 g	−11.03 (−12.03, −10.02)	<0.001
LR (alkalotic)	−2.21 (−5.94 to 1.53)	0.124						
PEG	−5.93 (−11.00 to 0.86)	0.011						
Psyllium	1.07 (−4.00 to 6.14)	0.339						
Splenda	1.49 (−3.58 to 6.56)	0.282						

Moreover, previous research often lacked detailed information regarding the number and types of beads studied, leading to inconsistencies and limitations in the applicability of their findings. Our study addresses these omissions by clearly specifying the types and quantities of beads examined, along with their brand differences, which allows for a more comprehensive and reliable assessment of the risks associated with SAP bead ingestions.

Interestingly, not all SAP beads pose the same level of risk. This underscores the critical need for effective management and screening strategies, as current methods to mitigate obstruction risks are either unstructured or invasive. The *in vitro* data from this study helps identify the potential for size expansion associated with the dry size of the bead, the type of bead, and the different solutions that the SAP beads are exposed to. Notably, the larger the dry bead size the greater the potential for expansion to a size that could lead to obstruction. The beads appear to have the most rapid growth in the first 24 h, with the large SAP beads reaching a size >25 mm as early as 6 h. The beads did not exceed >20 mm in the gastric acid even at 24 h. This may suggest the reduced expansion of SAP beads in the

stomach and that the SAPs will pass through the pylorus. This could also mean that antacids, histamine inhibitors, and proton pump inhibitors may influence the size of the SAP beads *in vivo* as well. *in vitro* data does not take into account the gastric motility and other fluids that may influence pH and osmolality such as refluxed bile, mucus and retained food/liquids. Polyethylene Glycol 3350 (PEG 3350) had the most significant impact on the potential growth of the SAP beads *in vitro*. In cases of SAP ingestion, PEG 3350 may be used as a potential treatment for patients, such as in other foreign body ingestions, provided they are on parenteral fluids and are closely monitored in a hospital setting (9). Even modest doses of PEG 3350 (17 grams in 6oz) led to a statistically significant limitation in the maximum expansion of SAP beads. As shown in the *in vitro* data, reducing water in the PEG 3350 solution results in an increase in the mass concentration that can enhance the osmotic effect in the gastrointestinal tract due to the higher number of PEG 3350 molecules retaining water. The more concentrated the greater the effect of PEG 3350. These findings have implications for the use of PEG 3350 in applications that require control over the expansion of hydrogel materials.

A proposed algorithm is included in Figure 4 based on the data gathered from this study and previous studies. This algorithm could serve as a potential guideline to help emergency department physicians and hospitalists develop an effective approach to patients who present after ingestion of SAP beads. It is crucial to obtain a history detailing the type of bead, when the bead was ingested, and if any significant risk factors pose a greater risk for obstruction. Garnering this information can help determine whether the patient needs hospitalization, imaging, or invasive intervention. x-ray imaging can be helpful in identifying obstructions, but are unlikely to detect SAP beads that are radiolucent. Ultrasound may be a more effective imaging technique for detecting water beads that have expanded without exposing patients to significant radiation. This was shown in a previous case series that looked at different imaging studies used to identify SAP beads (10). The recommended dosing for PEG 3350 is based on NAPGHAN guidelines and other studies that suggest safe doses as high as 2 g/kg (11, 12). Higher concentrations may increase the risk of dehydration, thus we recommend parenteral fluids. Regarding the safety of using more osmolar solutions than standard recommendations, this is not dissimilar to using

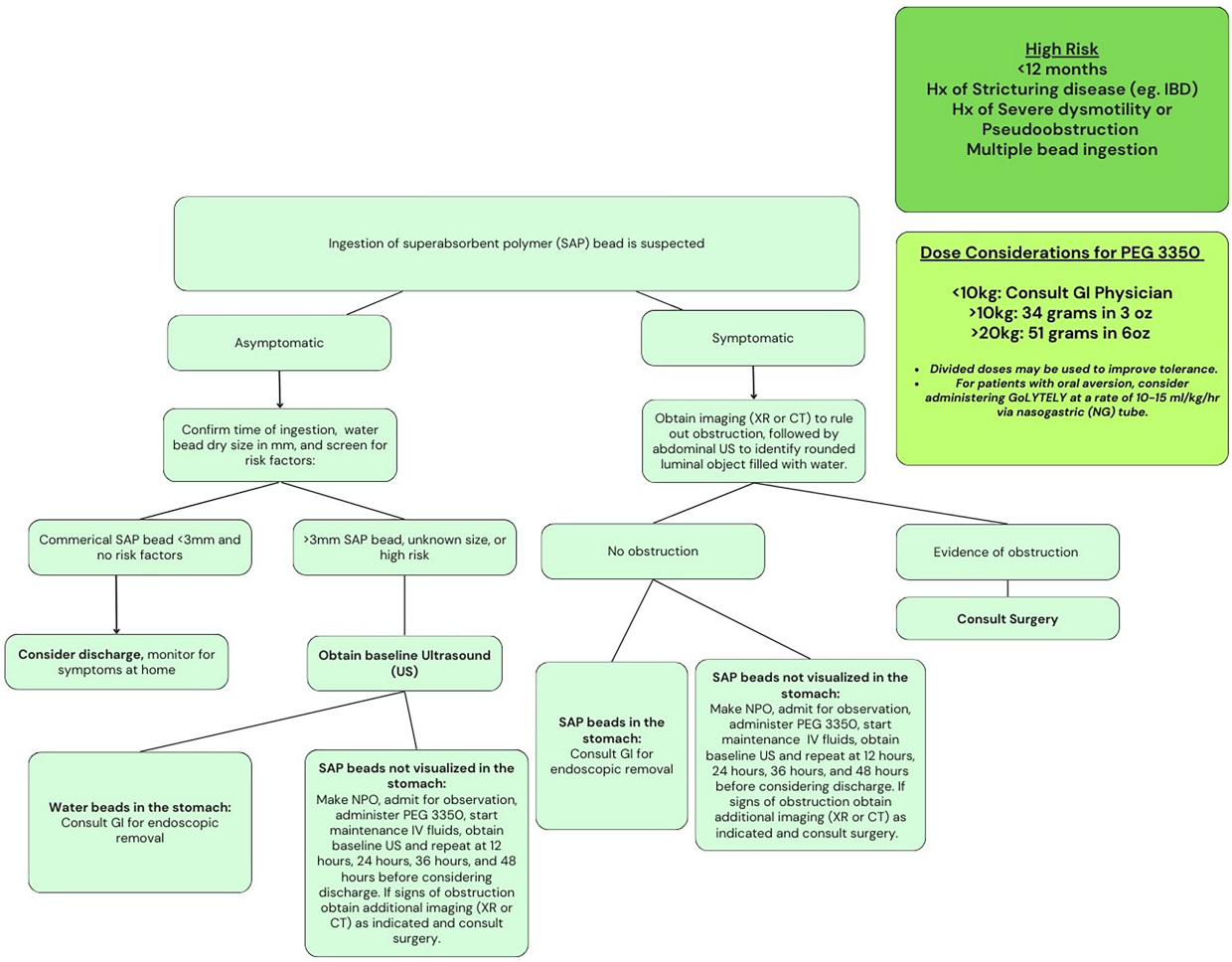

FIGURE 4
Proposed algorithm.

GoLYTLEY which has a significantly higher osmolality than the both standard doses of PEG 3350 and the concentrated maximum doses PEG 3350 recommended. 34 grams in 3oz is approximately 114.4 mOsm/L and 51 in 6oz is approximately 85.8 mOsm/L compared to 300 mOsm/L with GoLYTELY.

Limitations

Our study was conducted in an *in vitro* setting under controlled laboratory conditions, which inherently differ from the dynamic and variable physiological environment encountered in pediatric patients. Unlike real-world clinical scenarios, *in vitro* experiments do not account for the continuous gastric motility, secretion patterns, and the complex interplay of various endogenous fluids that may influence pH and osmolality. These include refluxed bile, mucus, and residual food or liquid content within the stomach, all of which can impact the dissolution, interaction, and potential effects of ingested materials. Additionally, the chemical properties of the different water beads are poorly defined, as there are no safety data sheets available. The properties of these beads are limited to the understanding that the gel is based on sodium polyacrylate. A more comprehensive understanding of other variables related to the properties of the beads could further help stratify risk.

Future studies should aim to simulate more accurately the *in vivo* environment by incorporating dynamic models that mimic gastric and intestinal conditions, such as immersing SAP beads in gastric acid followed by exposure to intestinal juices and studying this independently. Efforts to simulate peristalsis and the influences of other intestinal fluids, potentially through the use of a porcine intestine model, could provide a more comprehensive understanding of the behavior of SAP beads within the gastrointestinal tract. This approach, combined with a deeper investigation into the chemical properties of the beads, would help to bridge the gap between *in vitro* findings and their real-world clinical implications.

Conclusions

This study highlights the significant expansion potential of super absorbent polymer (SAP) water beads in various liquid environments, underscoring the associated risks in pediatric ingestions. Our findings indicate that bead size and the composition of the surrounding fluid are critical factors influencing expansion. Beads with a dry size greater than 3 mm demonstrated substantial growth, particularly in water, posing a significant risk as they can expand to obstructive diameters exceeding 25 mm within a short timeframe. In contrast, beads smaller than 3 mm pose minimal risk. Gastric acid may limit expansion, whereas alkaline solutions seem to allow continued growth, presumably increasing the risk of intestinal obstruction post-gastric emptying. Differences in expansion between brands suggest that variations in the chemical properties of SAP gels used by manufacturers may also contribute to these differences. Lastly, our data suggest that higher concentrations of polyethylene glycol (PEG) 3350 can effectively reduce bead size, presenting a potential therapeutic strategy when patients are adequately monitored in a hospital setting.

The rapid and significant expansion of larger beads, coupled with the rise in ingestion cases, underscores the need for early identification and intervention to mitigate ingestion hazards. Given the lack of current guidance, further research is necessary to develop a safe and standardized approach to SAP ingestions in a clinical setting. Importantly, the *in vitro* data provided by this study could be crucial in better understanding the risk of SAP ingestions, ultimately guiding the development of safer, more efficient, and less invasive management strategies.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

JH: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. JM: Writing – original draft, Writing – review & editing. AB: Data curation, Formal Analysis, Methodology, Software, Writing – original draft, Writing – review & editing. RN: Conceptualization, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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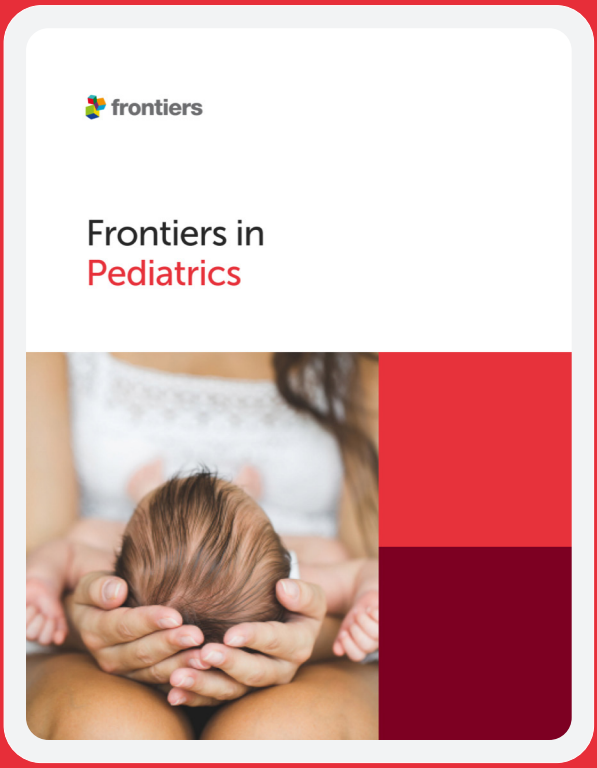

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