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EDITORIAL

Regenerative medicine using dental pulp stem cells for liver diseases

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Abstract

Acute liver failure is a refractory disease and its prognosis, if not treated using liver transplantation, is extremely poor. It is a good candidate for regenerative medicine, where stem cell-based therapies play a central role. Mesenchymal stem cells (MSCs) are known to differentiate into multiple cell lineages including hepatocytes. Autologous cell transplant without any foreign gene induction is feasible using MSCs, thereby avoiding possible risks of tumorigenesis and immune rejection. Dental pulp also contains an MSC population that differentiates into hepatocytes. A point worthy of special mention is that dental pulp can be obtained from deciduous teeth during childhood and can be subsequently harvested when necessary after deposition in a tooth bank. MSCs have not only a regenerative capacity but also act in an anti-inflammatory manner *via* paracrine mechanisms. Promising efficacies and difficulties with the use of MSC derived from teeth are summarized in this review.

Key words: Dental pulp; Mesenchymal stem cell; Regenerative medicine; Liver disease; Tooth bank

Core tip: Dental pulp contains a mesenchymal stem cell population that has a similar gene expression pattern to that of the bone marrow and differentiates into cells of multi-cellular lineages. There have been several reports showing hepatic differentiation of this stem cell population in the presence of specific growth factors in serum-free culture medium. Their self-renewal and high proliferative capacities verify their stem-cell character and suggest that they are a promising cell source of regenerative medicine for refractory liver diseases. Currently, these cells are in the stage of animal studies to prove the efficacy and safety of dental pulp stem cell-

based medicine for liver diseases.

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INTRODUCTION

The liver has a remarkable regenerative capacity in both physiological and pathological situations. However, this regenerative capacity is still insufficient to compensate for the functions of end-stage liver cirrhosis and fulminant hepatic failure, and prognosis of these diseases is extremely poor. Orthotropic liver transplantation is currently the only way to save patients in these critical situations; however, chronic donor shortage, post-operative severe complications, cost-effectiveness, and ethical issues always limit its application[1].

There has always been a high expectancy that the remarkable regenerative capacities of stem cells will be used to treat intractable diseases and improve their prognosis. Currently, regenerative medicine using induced pluripotent stem cells (iPSCs) is attracting the most clinical attention[2]. The first clinical trial of a retina pigment epithelium cell transplant derived from iPSCs for the treatment of age-related macular degeneration was conducted in Japan in 2014[3]. In another study, Takebe *et al*[4] succeeded in creating artificial liver buds using iPS cells.

However, because iPS cells do not exist in nature and are obtained artificially by inducing foreign genes or proteins, unexpected tumorigenesis and immunological rejections are always clinical concerns when using these cells. It has also been suggested that the induced genes might affect the expression of cellular genes[5].

Mesenchymal stem cells (MSCs) are pluripotent cells that differentiate into variety of cell types. In particular, MSC from dental pulp (MSC-DP) has attracted clinical attention because they are easily obtained from extracted wisdom teeth or even from the deciduous teeth of children. This is in contrast to the collection of bone marrow MSCs for which a painful medical procedure is needed. MSC-DP have a marked proliferative capacity and can be passaged scores of times without losing their stem cell properties[6]. Thus, this cellular resource is considered to be a promising source of cells for regenerative medicine that could be applicable to a variety of impaired organs, including diseased livers[7]. In this review, recent experimental development of MSC-DP therapy for liver diseases is summarized.

MSCS AND THEIR APPLICATION TO REGENERATIVE MEDICINE

The recent developments in regenerative medicine using stem cells have been outstanding. Application of autologous tissue stem cells to treat injured organs is the ideal method of regenerative medicine because, unlike the use of iPS cells, these methods do not require the induction of foreign genes or proteins, which possibly decreases the risk of tumorigenesis. Additionally, it does not involve critical ethical issues such as those encountered with embryonic stem cell (ES cell) therapies. Organ stem cells reside in almost all tissues and have the abilities of self-renewal and multi-lineage differentiation. They include hematopoietic stem cells (HSCs), MSC, neural stem cells, and skin and gut stem cells. These cells are relatively easily obtained by low-invasive procedures such as bone marrow aspiration, by using operative material or even by re-using discarded tissues such as umbilical cord or teeth. In particular, it is expected that the tooth bank will be used as a practical source of cells for regenerative medicine in the near future[8,9].

MSCs have been most extensively studied using bone-marrow stem cells. Pittenger *et al*[10] reported the multilineage potential of monolayer-cultured MSCs derived from bone marrow. MSCs exist in the stromal cells of bone marrow where they represent only 0.001%-0.1% of the total population of nucleated cells[10,11]. They are adherent cells that show high proliferative potential in the presence of bFGF and hence, a homogeneous clone can be obtained by cell cloning[12]. MSCs were shown to differentiate into multiple lineages such as neurons, muscle, skin cells, and hepatocytes. They are positive for CD44, CD73, CD90, CD105, CD271, and STRO-1 and negative for hematopoietic cell markers such as CD34 and CD45[12].

Although hepatocytes have previously been considered to differentiate from endodermal cells, they have now been found to differentiate even from non-endodermal cells. Research involving the differentiation of MSCs into hepatocytes has mainly used MSCs from bone marrow. Lagasse *et al*[13] transplanted HSCs into a model mouse of tyrosinemia and found that they engrafted in liver and improved of liver function. Krause *et al*[14] showed that a single HSC clone not only reconstituted bone marrow but also differentiated into lung, skin, liver, and gut cells. Schwartz *et al*[15] reported the culture of MSC derived from bone marrow in the presence of FGF-4 and HGF and showed that these MSCs developed the capacity to produce albumin and urea, which indicated the presence of progenitor cells of hepatocytes.

Subsequently, it was shown that such features were not limited to MSCs from bone marrow; MSCs from adipose tissue and placenta were also shown to differentiate into hepatocytes[16,17].

LIVER REGENERATION STUDIES USING STEM CELLS

Terai *et al*[18] administered bone marrow cells derived

from GFP-labelled mice to carbon tetrachloride-induced liver injury model mice and found that these bone-marrow cells engrafted in the injured liver, resulting in the absorption of fibrosis and the improvement of prognosis. Based on these experimental results, clinical trials of autologous bone marrow cells for end-stage liver cirrhosis patients started in November, 2011, in Japan[19]. Many other clinical trials of regenerative treatment for end-stage liver cirrhosis using HSCs have also been reported. Pai *et al*[20] reported the improvement in the liver function of alcoholic cirrhotic patients who were administered CD34-positive cells that were induced by G-CSF treatment.

While general anesthesia is needed to obtain a sufficient number of bone marrow cells for treatment, MSCs can be expanded from a small volume of bone marrow fluid because of their high proliferative capacity under simple culture conditions. MSCs have also been applied to the treatment of ischemic heart disease, cerebral infarction, and neurological or autoimmune disorders *via* the production of growth factors and cytokines, which stimulate the repair of injured tissues[21]. Several clinical trials using MSCs for decompensated liver cirrhosis have also been reported since 2007[22]. However, not all of these clinical trials showed efficacy of this treatment[23].

MSCS DERIVED FROM DENTAL PULP

Dental pulp is surrounded by dentin and is located in an enclosed space that connects with the external space through the apical foramen. Dental pulp has a strong capacity for repairing worn-down or carious teeth by producing dentin. Bone tissues are occasionally produced in the healing process of dental pulp. Dental pulp polyps are formed as granuloma tissues when squamous epithelium is formed that covers nerves that are exposed due to dental caries. These phenomena suggest that dental pulp has the capacity to develop into cells of multiple lineages, forming both bone and squamous epithelium.

Dental pulp is a mesenchymal tissue derived from dental papillae. Dental pulp cells have been reported to express bone markers similar to those expressed by osteoblasts[24]. Gronthos *et al*[25] were the first to report the presence of MSC-DP. They showed that dental pulp cells from adult teeth became clonogenic and rapidly proliferated under culture conditions. The cells formed densely calcified nodules under osteo-inductive culture conditions and also formed dentin/pulp-like complexes when conjugated with hydroxyapatite/tricalcium phosphate, which revealed their stem cell characters. They further showed that MSC-DP also displayed a multi-lineage capacity, differentiating into adipocytes and neural cells, which seemed to be irrelevant to tooth function[26]. The gene expression profiles of MSC-DP were shown to be similar to those of osteoblasts or bone marrow stromal stem cells[27].

Because MSC-DP are positive for STRO-1 and most STRO-1-positive MSC-DP are positive for pericyte-associated antigen, MSC-DP are considered to have originated from perivascular cell populations[28]. Although the first MSCs were obtained from adult teeth, MSCs have also been derived from human exfoliated deciduous teeth (SHED), periodontal ligament[29], apical papillae of immature permanent teeth[30], or periapical cysts[31]. In particular, SHED have a distinct capacity by virtue of higher proliferative potential than adult teeth with a multi-lineage differentiation capacity[32]. SHED are easily applicable to a cell banking source such as that used for umbilical cord because of the low ethical hurdles and the fact that the concept of the re-use of discarded tissues is easily acceptable to the general public[33]. Recent studies have shown that MSC-DP might induce immune regulatory mechanism of the host and have indicated the possibility of the application of MSC-DP to clinical practice[34,35].

DIFFERENTIATION OF DP-MSC INTO HEPATOCYTES AND REGENERATIVE MEDICINE

The above information suggested that MSC-DP may be a promising cell resource for regenerative medicine for various organs. Ishkitiev *et al*[36] were the first to report that MSC-DP differentiated into hepatocyte-like cells. They cultured SHED in the presence of HGF, dexamethasone, and oncostatin, and found that they transformed into a hepatocyte-like shape and produced IGF-1 and albumin. They also identified the presence of urea in the culture medium, which suggested the possibility that the urea cycle was functioning in these cells. They purified CD117-positive cells from MSC-DP using magnetic cell sorting and succeeded in inducing hepatic differentiation of these cells in serum-free medium with a high efficacy[37]. Since these cells still maintained stem cell markers such as embryonic (nanog), mesenchymal (CD44H), endodermal (nestin, CK19), ectodermal (p63), and mesodermal (SPARC, alkaline phosphatase, STRO-1) even after 70 passages, they may be applicable as a solid cell resource for regenerative medicine that can be obtained in sufficient cell numbers[37]. The efficacy of MSC-DP in differentiating into hepatocytes was as high as that of bone marrow-MSC[38]. When incubated with hydrogen sulphide, MSC-DP acquired more characteristic features of hepatocytes, showing a higher urea metabolism and glycogen synthesis[39]. Hepatocytes that were differentiated from MSC-DP repopulated the cirrhotic livers of rats and were shown to improve liver function and survival of the animals[7]. Yamaza *et al*[40] reported that transplanted SHED ameliorated liver dysfunction and improved inflammation and fibrosis in carbon tetrachloride - induced liver fibrosis model mice.

TOOTH BANK FOR REGENERATIVE MEDICINE

There is emerging interest in using MSC-DP as a clinical resource of cells for regenerative medicine for myocardial infarction[41], rheumatoid arthritis[42], diabetes mellitus[43], Parkinsonism[44], Alzheimer diseases[45], and refractory muscle diseases[46]. SHED derived from primary teeth are immature and have higher potential stem cell characteristics than adult-derived cells in terms of their proliferative capacity[32]. The benefits of SHED cell banking are as follows:

Minimum immunological rejection since the cells are derived from an autologous source: Cell banking is possible at a very young age, long before illness manifests; the cells are obtained painlessly; low cost compared to umbilical cord cells; low ethical hurdles.

SHED are suitable for obtaining cells of multiple lineages such as cells of connective tissue, teeth, nerve, liver, and pancreas, while umbilical cord MSCs are suitable for obtaining HSCs.

A large number of SHED cells can be obtained because of their high proliferative capacity and cloning of cells derived from a single MSC clone is possible.

MSC-DP is covered by enamel tissue and has little exposure to external radiation, which is related to a lowered risk of carcinogenesis of the graft.

A tooth bank for the storage of MSC-DP from deciduous teeth has been established by public-private collaboration[33]. However, many practical problems remain to be solved such as cost-benefit issues based on the balance of the risk of suffering diseases with the cost of long-term storage and harvest of cells, safety, and ethical concerns.

FUTURE DIRECTIONS

It has been reported that engrafted MSC do not actually transdifferentiate into specific cell lineages, but instead fuse with host cells using their plasticity[47,48]. MSCs are less potent than ES cells. In addition, MSC-DP, similar to MSCs in general, not only contribute to tissue repair as an actual source of regeneration, but they also elaborate immunomodulatory or anti-inflammatory functions that may affect the local environment of transplanted tissues[34,35,49]. There have been studies that showed that the conditioned medium of MSC cultures exerted immunomodulatory effects through paracrine mechanisms, that were mediated by extracellular vesicles such as exosomes produced by these cultured cells[50-52]. Moreover, MSCs were reported to improve the levels of liver injury and attenuate fibrosis in animal models[53,54]. These tissue repair effects of MSC-DP that occur through MSC-DP mediated paracrine mechanisms should be elucidated in parallel with studies to clarify the capacity of MSC-DP-derived hepatocytes as a substantial source of repopulating hepatocytes for fatal liver diseases.

Takebe *et al*[4] recently proposed the concept of "organ buds" instead of organ and cell transplantation. They obtained a liver bud by co-culturing hepatocytes derived from iPS cells with MSCs and vascular endothelial cells. That study indicated the possibility that MSC might not be a central player in regenerative medicine, providing a substantial hepatic function, but might instead be a supporting player in the development of regenerating organ. Based on this concept, these researchers recently showed that MSCs contributed the formation of an organ bud by providing MSC-dependent cytoskeletal contraction force[55].

These effects of MSC-DP on promotion of damaged-liver tissue repair through a paracrine mechanism or by an auxiliary force, should be elucidated in future studies.

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FIELD OF VISION

Tryptophan: A gut microbiota-derived metabolites regulating inflammation

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Abstract

Inflammatory bowel diseases (IBD), which comprise Crohn's disease and ulcerative colitis, are chronic intestinal disorders with an increased prevalence and incidence over the last decade in many different regions over the world. The etiology of IBD is still not well defined, but evidence suggest that it results from perturbation of the homeostasis between the intestinal microbiota and the mucosal immune system, with the involvement of both genetic and environmental factors. Genome wide association studies, which involve large-scale genome-wide screening of potential polymorphism, have identified several mutations associated with IBD. Among them, *Card9*, a gene encoding an adapter molecule involved in innate immune response to fungi (*via* type C-lectin sensing) through the activation of IL-22 signaling pathway, has been identified as one IBD susceptible genes. Dietary compounds, which represent a source of energy and metabolites for gut bacteria, are also appreciated to be important actors in the etiology of IBD, for example by altering gut microbiota composition and by regulating the generation of short chain fatty acids. A noteworthy study published in the June 2016 issue of *Nature Medicine* by Lamas and colleagues investigates the interaction between *Card9* and the gut microbiota in the generation of the microbiota-derived tryptophan metabolite. This study highlights the role of tryptophan in dampening intestinal inflammation in susceptible hosts.

Key words: Intestinal inflammation; Tryptophan; Microbiota

Core tip: A noteworthy article published in Nature Medicine by Lamas and colleagues highlights the role of tryptophan, a microbiota-derived metabolite, in reducing inflammation in the gut. This commentary puts in perspective the main results from this study.

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COMMENTARY ON HOT TOPICS

The human intestinal tract harbors a complex community including 100 trillion of microbes, referred as intestinal microbiota. This diverse microbial ecosystem provides benefits to the host, essentially through its role in energy metabolism and immunity. However, perturbations of gut microbiota (termed dysbiosis) is associated with several disorders, including inflammatory bowel disease (IBD) and metabolic syndrome (obesity-associated diseases)[1]. IBD arise as a complex interaction between host genetic factors, mucosal immune system, intestinal dysbiosis, and environmental factors among which dietary compounds being increasingly appreciated in the onset of inflammatory related disorders. Unraveling the complex crosstalk between these factors arise as a challenge for the understanding and treatment of these disorders. A study published in the June 2016 issue of Nature Medicine by Lamas *et al*[2] made significant progress in this area by investigating how a gene predisposing to IBD (*Card9*, encoding the caspase recruitment domain-containing protein 9) leads to a colitogenic microbiota by impairing its ability to generate tryptophan-derived metabolite.

In their study, the authors reported that the deletion of *Card9* gene, a central component of the innate antifungal immune response, render mice more prone to chemically-induced colitis by dextran sulfate sodium (DSS)[2]. This report strengthens previous studies conducted by others and identifying CARD9 as a gene predisposing to IBD in humans[3-5]. Lamas *et al*[2] also demonstrated that *Card9* knockout mice (*Card9*$^{-/-}$) display alteration of immune-related signaling pathways in the colon, with a strong decrease in interleukin-22 (IL-22) production. The authors evidenced a shift in the bacterial communities and alterations in the composition of the fungal microbiota in Card9$^{-/-}$ mice. Complex inter-kingdom relationships exist in the gut microbiota, suggesting a possible role of CARD9 in shaping the bacterial and fungal communities and required to control fungi during colitis. To decipher the mechanism of such colitis susceptibility and the involvement of gut microbiota in the onset of colitis, the authors use a model of microbial transplantation to germ-free recipient animals, and showed that transfer of colitic-associated microbiota of *Card9*$^{-/-}$ susceptible hosts were sufficient to transferred colitis susceptibility and IL-22 cytokine production impairment in germ-free wild type (*Card9* sufficient) recipients. Those data strengthen the essential role played by the intestinal microbiota, bacteria but also fungi, in triggering intestinal inflammation following Card9 impairment[2].

Further analysis revealed that the colitic-associated microbiota of *Card9*$^{-/-}$ mice is characterized by the absence of bacteria metabolizing tryptophan (an essential amino acid, whose intake is through the diet) into indoles derivatives, such as *Lactobacillus reuteri* and *Allobaculum* sp. Indoles derivatives are ligands for the aryl hydrocarbon receptor (AHR) that can drives local production of IL-22 by innate lymphoid cells and T-cells[6]. Importantly, the authors described that the treatment of *Card9*$^{-/-}$ susceptible animals with an AHR agonist [(*i.e.,* 6-Formylindolo(3,2-b) carbazole named FICZ] was sufficient to restore a normal level of IL-22 production and to protect mice from DSS-induced colitis. Previous studies focusing on the amino acid tryptophan demonstrated that mice fed with a low-tryptophan diet became susceptible to chemically induced inflammation[7] and, conversely, mice or piglets fed with a tryptophan supplemented diet have a reduced inflammation and a decreased severity of DSS-induced colitis[8,9].

As a therapeutic strategy, the authors next postulated that altering the intestinal microbiota in genetically susceptible host so as to increase its ability to generate AHR ligands could protect from intestinal inflammation. Thus, the authors demonstrated that supplementation with three commensal *Lactobacilllus* strains with high tryptophan-metabolic activities was sufficient to restore intestinal IL-22 production and to reverse the colitis susceptibility observed in susceptible *Card9*$^{-/-}$ mice. While previous studies have highlighted how diet can affect the microbiota in a detrimental way, such as the consumption of milk-fat-derived diet that lead to a bloom of pathobiont (*i.e., Bilophila wadsworthia*) and colitis in Il10$^{-/-}$ mice[10]; the study from Lamas *et al*[2] is a good example of the positive interplay between diet and the intestinal microbiota leading to the generation of microbial metabolites that play a central role in the protection against intestinal inflammation.

Finally, in their study, Lamas *et al*[2] further corroborated the results obtained in mice with the analysis of samples from IBD patients, and demonstrated that such patients have a reduced fecal AHR activity and fecal levels of tryptophan. The authors showed that these reductions correlate with *CARD9* polymorphism. These important findings consolidate the prominent role of dietary components and microbial-generated metabolites in mediating inflammation-related disorders. Tryptophan appears to be an important amino acid in IBD patients since they have lower levels of serum and fecal tryptophan compared to healthy subjects[2,11]. In light of the close relationship occurring between the intestinal microbiota and dietary intake, such data further highlight the need of controlling both macro- and micro-nutrients consumption in IBD patients with genetic predisposition.

In the same issue of Nature Medicine, an additional study by Rothhammer *et al*[12] also expand the substantial effect of tryptophan in regulating inflammation, by focusing their study on the central nervous system

(CNS), and providing evidence on the significant role of the bidirectional communication between the gut microbiota and the brain. The authors found that mice fed with a tryptophan-deficient diet have exacerbated CNS inflammation, corroborating the results from Lamas *et al*[2]. These two reports support a potential probiotic strategy, wherein tryptophan-catabolizing *Lactobacillus* strains able to enhance AHR activity that can further beneficially impact the immune system through IL-22 production. Further exploration of possible manipulations of the gut microbiota through dietary modulations by a tryptophan-enriched diet or by re-shaping the microbiota *via* targeting specific populations of bacteria, for example by favoring the tryptophan-producing bacteria or by reducing its pro-inflammatory potential, will provide novel insights into the development of individual targeted approaches that can be harnessed to prevent and/or treat IBD patients.

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REVIEW

Pathogenic mechanisms of pancreatitis

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Abstract

Pancreatitis is inflammation of pancreas and caused by a number of factors including pancreatic duct obstruction, alcoholism, and mutation in the cationic trypsinogen gene. Pancreatitis is represented as acute pancreatitis with acute inflammatory responses and; chronic pancreatitis characterized by marked stroma formation with a high number of infiltrating granulocytes (such as neutrophils, eosinophils), monocytes, macrophages and pancreatic stellate cells (PSCs). These inflammatory cells are known to play a central role in initiating and promoting inflammation including pancreatic fibrosis, *i.e.*, a major risk factor for pancreatic cancer. A number of inflammatory cytokines are known to involve in promoting pancreatic pathogenesis that lead pancreatic fibrosis. Pancreatic fibrosis is a dynamic phenomenon that requires an intricate network of several autocrine and paracrine signaling pathways. In this review, we have provided the details of various cytokines and molecular mechanistic pathways (*i.e.*, Transforming growth factor-β/SMAD, mitogen-activated protein kinases, Rho kinase, Janus kinase/signal transducers and activators, and phosphatidylinositol 3 kinase) that have a critical role in the activation of PSCs to promote chronic pancreatitis and trigger the phenomenon of pancreatic fibrogenesis. In this review of literature, we discuss the involvement of several pro-inflammatory and anti-inflammatory cytokines, such as in interleukin (IL)-1, IL-1β, IL-6, IL-8 IL-10, IL-18, IL-33 and tumor necrosis factor-α, in the pathogenesis of disease. Our review also highlights the significance of several experimental animal models that have an important role in dissecting the mechanistic pathways operating in the development of chronic pancreatitis, including pancreatic fibrosis. Additionally, we provided several intermediary molecules that are involved in major signaling pathways that might provide target molecules for future therapeutic treatment strategies for pancreatic pathogenesis.

Key words: Pancreatitis; Pancreatic stellate cells; Transforming growth factor-β/SMAD; Janus kinase/signal transducers and activators; Mitogen-activated protein kinases

Core tip: Pancreatitis is an acute or chronic inflammatory disease of the pancreas and characterized by destruction of acinar cells, which lead activation of several inflammatory cells like macrophages and granulocytes which secrete number of pro-inflammatory cytokines. These pro-inflammatory cytokines activate pancreatic stellate cells, *i.e.*, the key cells of pancreatic fibrosis. Various molecular signaling pathways (*i.e.*, transforming growth factor-β/SMAD, mitogen-activated protein kinases, Rho kinase, Janus kinase/signal transducers and activators, and phosphatidylinositol 3 kinase) are known to have critical role in the activation of pancreatic stellate cells in chronic pancreatitis and development of pancreatic fibrosis that lead to the pancreatic carcinoma.

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INTRODUCTION

Pancreatitis is a disease defined as acute or chronic inflammatory process of the pancreas characterized by premature activation of digestive enzymes within the pancreatic acinar cells and causing pancreatic autodigestion[1]. In pancreatitis, a local inflammatory process initiated by release of pro- and anti-inflammatory cytokines and chemokines recruits granulocytes, monocytes and lymphocytes[2]. Annual incidence of acute pancreatitis varies from 13 to 45 per 100000 people in United States[3], whereas chronic pancreatitis ranges from 4.4 to 11.9 per 100000 per year, with a higher occurrence in Japan as compared to the United States[4-7]. Men are up to 1.5 times more likely to have chronic pancreatitis compared to women in the United States[7]. In 2009, there were 19724 admissions for chronic pancreatitis in the United States, with associated annual hospitalization costs of $172 million[5,8]. However, the pathogenesis of chronic pancreatitis is not fully understood, but it is believed that repeated episode of acute damage lead chronic pancreatitis. Recurrent pancreatic injury leads to scarring and remodeling that promotes fibrosis as well as calcification, and these calcifications develop into stones found within the tissue or pancreatic duct[5,9] (Figure 1). The main causes of pancreatitis are; obstruction in the main pancreatic duct, gallstones, alcohol misuse, smoking, hypercalcemia, hyperparathyroidism, drugs like valproate, thiazide toxicity, and genetic mutation[10-12]. During pancreatic injury, atrophic acinar cells activate several inflammatory key players like macrophages and granulocytes which release a number of pro-inflammatory cytokines [*i.e.,* interleukin (IL)-1, IL-6, IL-8, IL-18, IL-33, and tumor necrosis factor (TNF)-α]. These pro-inflammatory cytokines further activate pancreatic stellate cells (PSCs) to promote chronic pancreatitis[13]. The detail of each cytokine involved in the pathogenesis of pancreatitis has been described independently.

PRO-INFLAMMATORY CYTOKINES

IL-1

Induction of IL-1 has been reported in acute pancreatitis and numerous reports implicated the role of IL-1, and IL-1 receptor (IL-1R) in the pancreatic pathogenesis[14-18]. Interestingly, it has been shown that IL-1R gene-deficient mice or treatment with IL-1 receptor antagonist (rhIL-1Ra) attenuates cerulein-induced chronic pancreatitis in mice[16]. IL-1 converting enzyme (ICE) is responsible for the secretion of IL-1β from pro-IL-1β and experimental pancreatitis was significantly attenuated by pre-treatment with an ICE inactivator (VE-13045), resulting in reduced histological grading of pancreatitis and mortality. These findings were further supported by using ICE-knock out mice or intraperitoneal (i.p.) injection of ICE-inhibitor[19]. Additionally, IL-1β is also believed to play a role in the pathogenesis of pancreatitis. An elevated serum level of IL-1β has been associated with the development of acute pancreatitis[20]. Recently, Xu *et al*[20] have revealed that IL-1β can induce trypsin activation and decreases the cellular viability of pancreatic acinar cells. These effects depend on impaired autophagy *via* intracellular calcium changes. Ca^{2+} signaling may be a promising therapeutic target for the treatment of pancreatitis[20].

IL-6

IL-6 is a very important pro-inflammatory cytokine involved in inflammation and immune responses[21]. An important role of IL-6 has been shown in the development of acute and chronic pancreatitis as well as in pancreatic cancer. IL-6 mediates its action *via* gp130 protein and leads activation of Janus kinase/signal transducers and activators (JAK/STAT) signaling pathway[21]. Reported data have revealed that patients with pancreatitis indicated high serum levels of IL-6 as compare to healthy individuals[22,23]. *In vitro* studies have shown enhanced secretion of IL-6 from human pancreatic peri-acinar myofibroblast cells in the presence of several inflammatory mediators (*i.e.,* TNF-α, IL-17, IL-1beta) and growth factors (*i.e.,* fibroblast growth factor-2) and this data further supports the crucial role of IL-6 in the pathogenesis of acute pancreatitis[24,25]. Interestingly, neutralization of IL-6 by anti-IL-6 antibody therapy revealed suppression of STAT-3 activation in pancreatic acinar cells and consequently reduces the severity of acute pancreatitis[26]. The abnormal expression and deregulation of IL-6 in pancreatitis suggested that IL-6 serves as a valuable early marker for pancreatitis.

IL-8

IL-8, known as chemokine (C-X-C motif) ligand 8 or

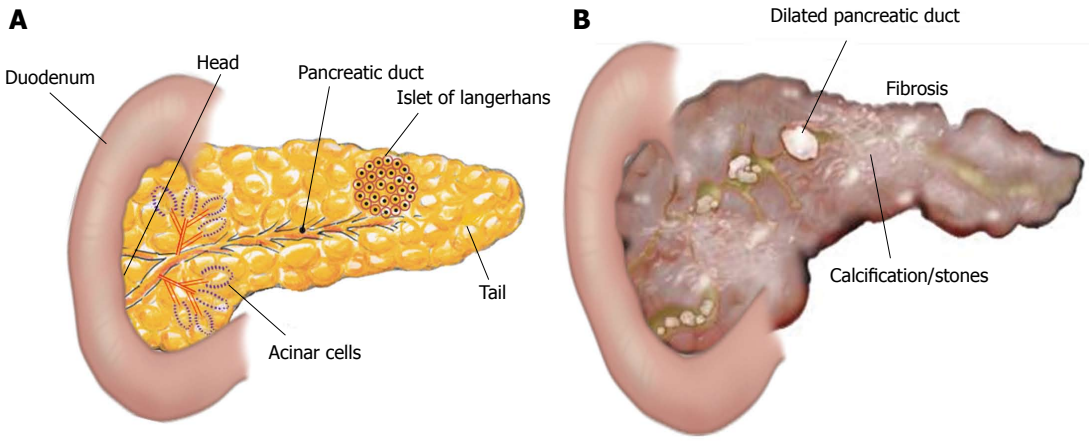

Figure 1 Structure of pancreas. A: The pancreas is a leaf-like structure and has two types of cells: Exocrine cells, that include acinar pancreatic duct cells, and endocrine cells, that include islets of Langerhans; B: The inflammatory process in the pancreas promotes fibrosis (scarring of tissue), calcifications or stones, and dilated pancreatic duct.

CXCL8, acts as a potent chemo-attractor of neutrophils and affects neutrophil function during onset of inflammatory responses by regulating the trafficking of various types of leukocytes through interaction with transmembrane receptors. IL-8 is produced by several types of cells such as monocytes/macrophages and epithelial cells[27,28]. Systemic complications of acute pancreatitis are associated with higher levels of IL-8[29-31]. Induction of IL-8 was also reported in a patient with aggravation of pancreatitis which suggests that IL-8 takes part in the pathogenesis of pancreatitis[32]. Severity of acute pancreatitis is associated with polymorphisms of the *IL-8* gene[33]. However, the mechanism of IL-8 mediated severity of acute pancreatitis is not yet well understood and requires further study in this area.

IL-18

Induction of IL-18 is now identified in a number of disorders, such as autoimmunity[34], cutaneous[35] and allergen-induced allergic responses[36]. IL-18 is a member of IL-1 family cytokine and implicated in numerous aspects of the innate and adaptive immune system, with some analogy to IL-1β[37]. Evidences indicate that IL-18 is induced in the blood of acute[38] and chronic pancreatitis patients[39,40]. Furthermore, higher serum level of IL-18 was also reported during mild and severe forms of acute pancreatitis compared to healthy controls[41]. Additionally, the induced IL-18 level was also reported in taurocholic acid and endotoxin-induced acute pancreatitis in rat[42]. Interestingly, IL-18 along with IL-12 induces severe acute pancreatitis in obese mice[43]. Notably, it is also reported that the IL-18 has an important role in the progression of disease from acute to chronic stages[40]. Overall, IL-18 seems to be released early during the course of acute pancreatitis and may act as a key immunomodulator of the inflammatory response in severe pancreatitis and associated fibrosis. However, the mechanistic pathway of IL-18-induced chronic pancreatic pathogenesis is yet not understood.

IL-33

IL-33, a new member of the IL-1 superfamily of cytokine, binds to a complex of the ST2L/IL1 receptor accessory protein (IL1RAcP), which mediates its function[44]. Several investigations suggest a crucial role of IL-33 in the pathogenesis of chronic pancreatitis and possibly pancreatic cancer[45,46]. IL-33 was found to activate acinar cell pro-inflammatory pathways and to exacerbate acute pancreatic inflammation in mice[47]. However, the activated PSCs express IL-33 in the nucleus and regulate the platelet-derived growth factor (PDGF)-induced proliferation in PSCs[48]. IL-33 also acts as a pro-inflammatory cytokine and modulates its receptor gene expression in Colo357 cells, *i.e.,* human pancreatic carcinoma cells[45]. IL-33 and its receptor complex (ST2L and IL1RAcP) constitute a novel signaling system; therefore, this pathway may be important in promoting acute and chronic pancreatitis. Additionally, a role for IL-33 in the stimulation, proliferation and migration of pancreatic myofibroblasts is also reported[46].

TNF-α

TNF-α is a pleiotropic cytokine and acts as a central regulator of inflammation[49,50]. It is mainly secreted by monocytes and macrophages but is also released by pancreatic acinar cells after an inflammatory trigger[51-54]. A number of studies have revealed TNF-α plays an essential role in the pathogenesis of pancreatitis and contributes inflammatory responses to disease pathogenesis[51-53,55]. An *in vitro*-based study indicates cultured pancreatic acinar cells are able to produce, release, and respond to TNF-α[56], leading to the activation of nuclear factor-kappa B (NFκB); interestingly, inhibition of NFκB activity decreases the inflammatory response during experimental pancreatitis[57,58]. Serum levels of TNF-α have not been considered to be a good indicator of disease severity because the liver is able

to rapidly clear TNF-α before it reaches the general circulation; therefore, it is often difficult to detect TNF-α in the serum of acute pancreatitis patients[59]. One study indicates that TNF-α levels were higher in acute pancreatitis as compared to the chronic form of the disease, but its concentration did not correlate with the severity of disease[60]. In contrast to this, a recent study has shown levels of TNF-α are also increased in patients with chronic pancreatitis and the concentration of TNF-α coordinately increases in advanced chronic pancreatitis[61]. Furthermore, TNF-α mediates its effect by two surface receptors, TNF-α receptor 1 (TNFR1), or p55, and TNFR2, or p75, and both receptors are expressed in the pancreas[54,62]. Interestingly, genetic deletion of TNFR1 prevents the activity of TNF-α and revealed beneficial effects on symptom severity and mortality in cerulein-induced pancreatitis[63].

ANTI-INFLAMMATORY CYTOKINES

IL-10

IL-10 is produced by a number of activated immune cells like monocytes/macrophages, Treg, and Th1 cells[64,65]. IL-10 gene deficient mice showed more inflammatory responses and lung injury during acute pancreatitis and chronic pancreatitis[66,67]. Pre-treatment of IL-10 agonist (*i.e.,* IT 9302) was found to reduce lung injury and mortality in a rabbit pancreatitis model[68]. Plasma IL-10 level was found to correlate with the severity of pancreatitis and could be used as a marker for severity prediction[22,69]. Initial studies based on several rodent models of acute pancreatitis revealed a protective role of IL-10 by reducing the production of inflammatory cytokines from macrophages and also diminished the level of serum amylase, serum lipase, edema, necrosis and hemorrhage[70-72]. However, recombinant IL-10 treatment in human pancreatitis has given mix responses[73]. In summary, IL-10 holds the promise of a global attenuation of the cytokine response, and more work is needed to establish its beneficial use in pancreatitis.

GRANULOCYTES INFILTRATION IS CRITICAL IN THE PATHOGENESIS OF CHRONIC PANCREATITIS

Granulocytes infiltration in the pancreas is implicated in the initiation and progression of pancreatic inflammation. The major granulocytes identified in acute and chronic pancreatitis patients are neutrophils and eosinophils. Neutrophils play a crucial role in acute inflammatory pancreatitis, are attracted to the site of injury by the help of chemokines such as CXCL8 in humans as well as CXCL1 in mouse, and further regulate the immune responses. Neutrophils remain in the blood circulation and have a very short life of approximately 24 h[74]. However, in an inflammatory condition they became activated and their lifespan is prolonged for several days, during which they control inflammatory responses and activate several pro-inflammatory mediators[75]. Trypsinogen activation is the key step for progression of pancreatitis; and a report suggested that initial trypsinogen activation is not regulated by neutrophils, whereas later activation of trypsinogen during pancreatitis is dependent on neutrophils[76]. In addition, several cases have been reported in the literature indicating the presence of increased number of eosinophils in patients with pancreatitis and termed this condition as "Eosinophilic Pancreatitis"[77,78]. Eosinophilic pancreatitis is a rarely occurring disorder and reports indicate that eosinophilic pancreatitis is frequently diagnosed only after "false positive" pancreatic resection for suspected pancreatic tumor and mimic pancreatic neoplasm[78,79]. The first report of peripheral blood eosinophilia in a patient with chronic relapsing pancreatitis with pleural effusion was published by Juniper[80] in 1955 and thereafter, several evidences came in the literature[81-84]. Tokoo *et al*[81] performed a study of 122 patients with chronic pancreatitis and found marked eosinophilia in approximately 21 cases (17.2%). All of the affected patients were males; no females were found affected. Endocrine pancreatic function was normal in the chronic pancreatitis patients with eosinophilia, whereas marked exocrine pancreatic dysfunction was observed in these patients. The eosinophilia of chronic pancreatitis has been frequently developed in association with severe damage to adjacent organs (pleural effusion, pericarditis, and ascites), as well as an association with pancreatic pseudocyst. This finding suggests that there may be a close correlation between marked eosinophilia and severe tissue injury during acute exacerbations of chronic pancreatitis[81]. Another study revealed 28 cases (15.6%) of chronic pancreatitis with eosinophilia among 180 chronic pancreatitis patients and the ratio of male to female patients was 8.3:1. The occurrence of eosinophilia during the course of chronic pancreatitis might be responsible for the progression of pancreatic inflammation and fibrosis[82]. Additionally, reports indicate that peripheral eosinophilia, allergic disorders, and pancreatic eosinophil infiltration have been associated with autoimmune pancreatitis[83,84]. Diagnosis and treatment of eosinophilic pancreatitis is important as it promotes pancreatic fibrosis and neoplasm.

EXPERIMENTAL TOOLS TO DISSECT THE MECHANISM THAT PROMOTES PANCREATITIS

Pathogenesis of pancreatitis is essentially understood by using experimental animal models, because of the anatomical location of the pancreas and the difficulty in procuring human tissue at different stages of the inflammatory process. Several animal models are reported to understand the pathogenesis of pancreatitis, which enable us to develop more effective treatment therapies to improve the quality of life of patients suffering

from pancreatitis-associated complications. In brief, we summarize some experimental models used for understanding the disease initiation and progression.

Cerulein-induced pancreatitis model

The most widely used acute and chronic pancreatitis model, the cerulein-induced model is a highly reproducible and economical model in rats and mice[85-87]. Acute pancreatitis can be induced by intraperitoneal (*i.p.*) injection of cerulein (5 μg/kg per hour in rats and 50 μg/kg in mice) several times at hourly intervals, and repeated doses of cerulein can induce chronic pancreatitis[88,89]. Cerulein is an analog of cholecystokinin[90] and induces the secretion of digestive pancreatic enzymes from pancreatic acinar cells like amylase and lipase. Cerulein treatment further causes infiltration of inflammatory cells within the pancreas, pancreatic edema, and acinar cells vacuolization that are comparable to acute pancreatitis in humans. Cerulein-induced pancreatitis model has been considered as a representative model of mild acute pancreatitis of human.

L-arginine-induced pancreatitis model

Another experimental and reproducible pancreatitis model is L-arginine-induced model. This model is also widely used to study the pathophysiology of acute necrotizing pancreatitis to produce acinar cells necrosis. Initially, Mizunuma *et al*[91] and Tani *et al*[92] have demonstrated *i.p.* administration of excessive doses of L-arginine (500 mg/100 g body weight) in rat caused damage of pancreatic acinar cells. A single *i.p.* dose of 500 mg/100 g revealed necrosis in 70%-80% of acinar cells within 3 d[91,92]. Since, these observations, the L-arginine-induced acute pancreatitis rat model has been used by several investigators[93,94].

Bile salt-induced pancreatitis model

The first experimental biliary acute pancreatitis model was established by Bernard in 1856 *via* retrograde injection of bile and olive oil into the pancreas of a canine[95]. Since then, various bile salts such as sodium chenodeoxycholate[96], sodium taurocholate, sodium glycodeoxycholic acid[97], sodium-taurodeoxycholate and taurolithocholic acid 3-sulphate have been reported to induce acute pancreatitis in different animal models. Among these bile salts, the taurine-conjugated bile salt sodium taurocholate was the most widely used and best characterized chemical for the induction of acute pancreatitis[98]. Furthermore, a choline-deficient, ethionine-supplemented diet model is another established model to study the pathogenesis of acute and chronic pancreatitis[99,100].

Pancreatic duct ligation model

In the rat model of pancreatitis, bile reflux was first implicated in the disease pathogenesis and termed as biliary pancreatitis[101,102]. Biliary pancreatitis develops from obstruction by gallstone or bile reflux into the pancreatic duct, which causes induction of acute pancreatitis. The rat model shows that due to high pancreatic duct pressure, pancreatic juice refluxes into the bile duct in the presence of ampullary orifice obstruction, resulting in pancreatic edema, inflammatory cell infiltration, increased amylase production[103]. Chronic pancreatitis develops in these mice with time that includes atrophy, loss of acinar cells, and fibrosis[103,104].

Alcohol-induced pancreatitis model

Alcohol is another accountable factor for the pathogenesis of pancreatitis, and it has been used to trigger chronic pancreatitis in animal models[85,105,106]. Lieber and DeCarli have investigated the effects of ethanol on several organs by giving repeated feedings of ethanol as a part of the diet to rats and baboons[107] and the animals developed fatty liver disease, alcoholic hepatitis, and later on cirrhosis. Undesirably, alcohol ingestion alone did not induce chronic pancreatitis despite long experimental durations. However, the combination of alcohol with various agents such as cerulein or lipopolysaccharide exacerbated pancreatitis and resulted in fibrosis[108]. Activation of pancreatic stellate cells and fibrosis has been observed in the rat given isocaloric Lieber-DeCarli liquid diets along with alcohol for up to 10 wk and challenged with 1 or 3 repeated doses of lipopolysaccharide[109]. Alcohol-induced pancreatic damage is thought to be mediated by its metabolites, which activates ROS to cause acinar cells injury and activate pancreatic stellate cells, leading to fibrosis[110].

SNARE proteins mediating basolateral exocytosis in alcohol-induced pancreatitis

The important role of SNAREs [soluble NSF (N-ethylmaleimide-sensitive fusion proteins) attachment proteins receptors] mediating basolateral exocytosis in alcohol-induced pancreatic injury has been reported[111]. SNARE proteins are of two types (1) t-SNAREs present on the target membrane, and (2) v-SNAREs, positioned on the membrane of vesicles. The t-SNAREs, syntaxin and synaptosome-associated proteins, together make a SNARE complex which binds to v-SNAREs and triggers the fusion of vesicle and target membranes. In the pancreas, this leads to release of zymogen granules into the ducts for transport to the duodenum for their activation[112]. Additionally, a study indicates ethanol/cholecystokinin-evoked pancreatic acinar basolateral exocytosis has been mediated *via* protein kinase C alpha phosphorylation of Munc18c, which enables Syntaxin-4 to become receptive in forming a SNARE complex in the basolateral plasma membrane. The authors also considered this phenomenon as an operating mechanism contributing to alcoholic pancreatitis[111]. Importantly, displacement of Munc18c from the pancreatic acinar basal membrane surface has been observed in tissue samples from a patient suffering from alcohol-induced chronic pancreatitis[113].

CHRONIC PANCREATITIS LEADS FIBROSIS AND PANCREATIC CANCER

Chronic pancreatitis develops fibrosis and it is the common pathological characteristic feature and major risk factor for pancreatic cancer[114]. Recent data has shown 48960 new cases of pancreatic cancer arise and 40560 deaths occur annually in the United States because of pancreatic cancer[115]. Chronic pancreatitis is a long-standing inflammation of the pancreas that often leads to permanent damage of pancreas and serious complications, including pancreatic cancer. Chronic pancreatitis is characterized by marked stroma formation with an increased number of infiltrating macrophages and stellate cells, which are believed to play a central role in triggering inflammation and disease progression. The treatment of chronic pancreatitis and pancreatic cancer remains problematic as tissue becomes fibrotic due to injury that triggers several inflammatory, cellular as well as molecular signaling cascades that lead to formation and deposition of extra cellular matrix (ECM) at the site of injury. Several key cells are known to be involved in the process of fibrogenesis, such as inflammatory cells (*e.g.*, macrophages and T cells), epithelial cells, fibrogenic effector cells, and endothelial cells. There are different types of effector cells in different organs, such as fibroblasts, myofibroblasts, and fibrocytes[116]. Among these cells, fibroblasts and myofibroblasts are the key cells in fibrosis and are responsible for secretion of ECM[117]. However, the function of fibrocytes is similar to the fibroblasts but to a lesser extent. Apart from this, macrophages have a more indirect contribution to fibrosis through their roles in chronic inflammation by producing a wide range of cytokines such as, transforming growth factor-β (TGF-β), PDGF, fibroblast growth factor 2 (FGF2) and insulin-like growth factor 1, all of them have pro-fibrotic effects on fibroblasts[118,119]. If fibrogenic processes persist for long time, parenchymal scarring, cellular dysfunction and organ failure take place[116]. Fibrosis is becoming a global problem and it can be of various types depending upon the tissue where it happened, such as cardiac, hepatic, renal, pulmonary, skin, liver and pancreatic fibrosis, *etc*. Fibrosis is an irreversible process and most of the drugs are not effective to treat fibrosis. Restriction of the progression of fibrogenesis might be a promising approach for the treatment of several fibrotic diseases.

Herein, our focus is on pancreatic fibrosis that happens during repeated injury to the pancreas. The normal pancreas has two major functions: (1) exocrine; and (2) endocrine. Exocrine pancreas comprises more than 95% of the pancreatic mass and consists of two types of pancreatic cells: (1) acinar cells, which produce digestive enzymes; and (2) ductal cells lining pancreatic ducts, which secrete a watery fluid to transport the digestive enzymes into the intestine. Endocrine pancreas mainly consists of the islets of Langerhans, which secrete insulin and other hormones[5,120] (Figure 1).

Development of fibrosis is a dynamic phenomenon that requires an intricate network of several autocrine and paracrine signaling pathways[116]. In this process, ECM formation takes place in the interstitial spaces and in areas where the exocrine compartment, mainly acinar cells are damaged[121,122]. Pancreatic injury activates acinar cells, macrophages and neutrophils which induces pro-inflammatory cytokines (IL-1, IL-6, and IL-8), chemokines (monocyte chemoattractant protein-1, macrophage inflammatory protein-1) and growth factors, which further activate quiescent PSCs[2]. The available facts suggest that these activated PSCs are the main cells in the development of fibrosis during chronic pancreatitis *via* secretion of TGF-β, FGF and COX-2 which leads to synthesis of ECM[123,124]. A schematic mechanistic pathway involved in the progression of chronic pancreatitis is shown below in Figure 2.

Furthermore, activated PSCs have the ability to synthesize and secrete several matrix proteins, matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases, thus indicating that PSCs have dual functions to regulate the physiology of the exocrine pancreas, *i.e.,* they can synthesize as well as degrade the extracellular matrix[125,126]. This indicates that PSCs have the ability to make a balance between fibrogenesis and matrix degradation to regulate the health of pancreatic tissue; that is, conservation of normal architecture or development of progressive fibrosis. Fibrosis is a complex process and the mechanism of pancreatic fibrosis is still not well understood. Due to pancreatic fibrosis, a number of therapies in pancreatic cancer have failed. In our standing, for proposing or designing any therapeutic strategy for chronic pancreatitis or pancreatic cancer, the mechanism of fibrosis development in the pancreas is important. Herein, we provide a summary of various molecular signaling pathways [*i.e.,* TGF-β/SMAD, mitogen-activated protein kinase (MAPK), Rho kinase, JAK/STAT, and phosphatidylinositol 3 kinase (PI3K)] that have been shown to play a critical role in the activation of PSCs during chronic pancreatitis and trigger the phenomenon of fibrogenesis in pancreas (Figure 3).

TGF-β1/SMAD PATHWAY

TGF-β is a multipotent cytokine and exists in three isoforms (TGF-β1, TGF-β2 and TGF-β3) in mammals and plays an integral role in regulating immune responses, cell growth, cell differentiation and apoptosis[127,128]. TGF-β mediates its downstream signaling by binding to its specific receptors and triggers the activation of several SMAD proteins, which acts as chief transducers of the signal from the receptors to the nucleus. The receptor-regulated SMADs (R-SMADs), SMAD-2 and SMAD-3, are directly phosphorylated by the TGF-β1 receptor and make a complex with the common mediator SMAD (*i.e.,* co-SMAD; SMAD-4) that

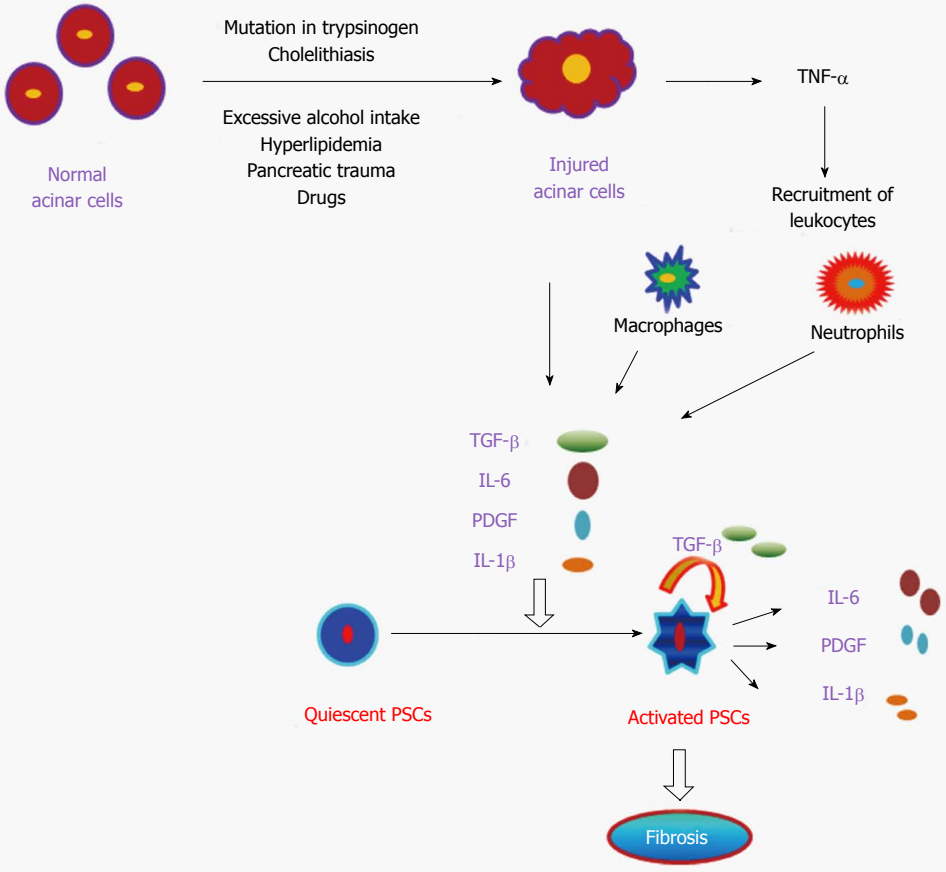

Figure 2 Pathogenesis of pancreatitis. Diagrammatic representation of the onset of pancreatitis by damaged pancreatic acinar cells which in turn activates quiescent pancreatic stellate cells (PSCs) to become activated PSCs and promote subsequent fibrosis of pancreas. TNF-α: Tumor necrosis factor-α; TGF-β: Transforming growth factor-β; PDGF: Platelet-derived growth factor; IL: Interleukin.

translocate into the nucleus and activates the transcription of target genes[127,129,130]. Earlier studies have confirmed the involvement of TGF-β in the pathogenesis of acute pancreatitis, chronic pancreatitis and development of fibrosis[131-136]. PSCs play a key role in triggering pancreatic fibrosis and interestingly TGF-β was found to regulate activation and proliferation of PSCs in an autocrine manner *via* involvement of SMAD-2, SMAD-3 and ERK pathways[137,138]. Amelioration of pancreatic fibrosis in cerulein-treated mice was observed with defective TGF-β signaling by over-expressing a dominant-negative mutant form of TGF-β type 2 receptor (pS2-dnR Ⅱ) only in the pancreas under control of pS2/TFF1 promoter[139]. Subsequent study has revealed suppression of TGF-β signaling halts cerulein-induced pancreatitis[140]. These studies indicate a functional TGF-β signaling pathway might be required for cerulein to induce acute pancreatitis in these mice[139,140]. In contrast, deactivation of TGF-β signaling induces autoimmune pancreatitis in mice, indicating the important role of TGF-β either in maintaining immune homeostasis and suppressing autoimmunity or in preserving the integrity of pancreatic acinar cells[141]. Transgenic mice with an S100A4/fibroblast-specific protein 1 Cre-mediated conditional knockout of TGF-β type 2 receptor spontaneously developed autoimmune pancreatitis in 6 wk. This indicates autoimmune pancreatitis resulted from loss of TGF-β signaling in S100A4-positive dendritic cells[142].

Plenty of evidence suggests the involvement of TGF-β in pancreatic fibrosis, however TGF-α was also found to increase the proliferation as well as migration of PSCs *via* up-regulation of MMP-1, which might contribute to the pathogenesis of chronic pancreatitis[143]. A recent report has shown loss of SMAD-4 synergizes with TGF-α over-expression in promoting pancreatic metaplasia, PanIN development, and fibrosis[144]. Furthermore, a higher level of TGF-β1 during pancreatic inflammation triggers the deregulation of the microRNA-217-SIRT1 pathway and then promotes EMT and subsequent fibrosis in the pancreas[145]. Although, TGF-β still remains elusive in terms of our understanding of its multifunctional modes of action and TGF-β also activates SMAD-independent signaling pathways including MAPK pathways and phosphoinositide (PI) 3-kinase[129,146,147] but the detailed mechanisms are not well understood.

MAPK

MAPK are of three types, ERK, JNK, and p-38, and play an important role in a variety of cellular processes, including cell proliferation, cell survival, apoptosis, and cytokine production[148]. In alcohol-induced pancreatic injury, ethanol and its metabolite acetaldehyde were

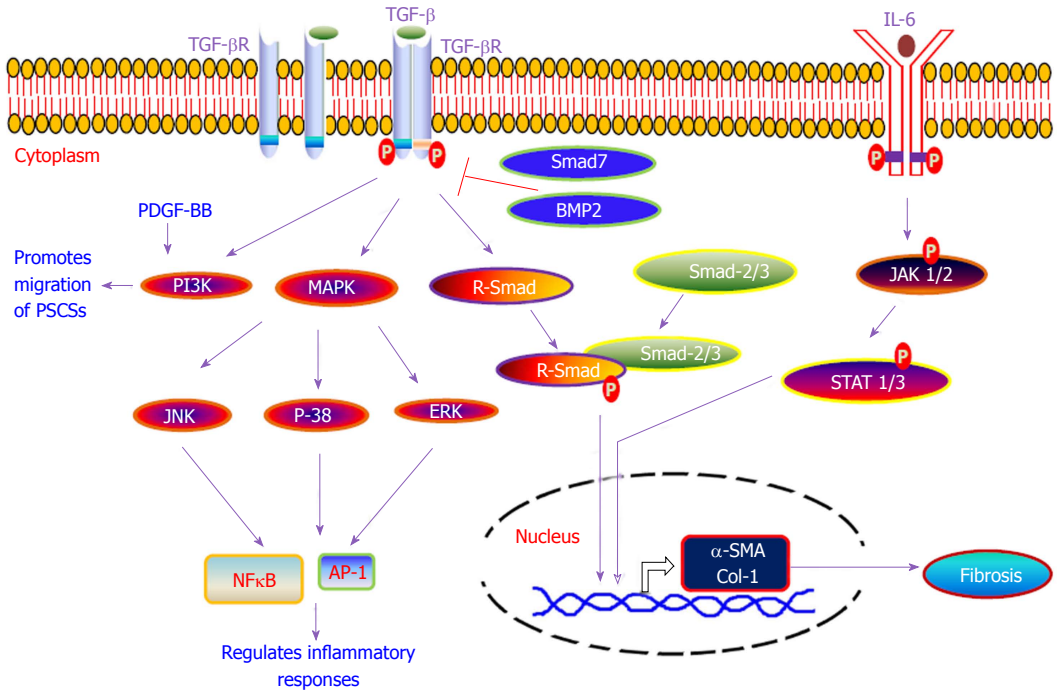

Figure 3 Various signaling pathways involved in the development of pancreatic fibrosis. Diagrammatic representation of various molecular signaling pathways which are involved in the development of pancreatic fibrosis. TGF-β: Transforming growth factor-β; PDGF: Platelet-derived growth factor; IL: Interleukin; MAPK: Mitogen-activated protein kinase; PI3K: Phosphatidylinositol 3 kinase; AP-1: Activator protein-1; NFκB: Nuclear factor kappa B.

found to induce activator protein-1 (AP-1) and MAPK signaling in PSCs[149,150]. Furthermore, CX3CL1 is a chemokine that serves as an adhesion molecule as well as a migration factor, and was elevated in patients with alcoholic chronic pancreatitis[151]. A recent report indicates ethanol induces CX3CL1 release *via* ERK activation in PSCs[152]. However, H_2O_2 induces oxidative stress, AP-1, MAP-kinase pathway and expression of α (I) procollagen in PSCs[153]. Apart from this, PDGF induces rapid activation of Raf-1, ERK 1/2, and AP-1 protein and further indicates a correlation between ERK activity and PSC activation[154]. Furthermore, the involvement of protease-activated receptor-2 (PAR-2) was also found in the pathogenesis of pancreatitis and PAR-2 agonists increased collagen synthesis *via* activation of JNK and p-38 MAP kinase pathways in PSCs, suggesting the role of PAR-2 during induction of pancreatic fibrosis[155]. PD98059 is an inhibitor of MAP/ERK kinase-1 (MEK-1) pathway and was able to protect against cerulein-induced acute pancreatitis in mice[156]. Apart from this, angiotensin II-treated PSCs start proliferation and increase DNA synthesis *via* an epidermal growth factor receptor transactivation-ERK activation pathway, indicating the possible role of angiotensin Ⅱ in development of pancreatic fibrosis[157,158]. Taken together, these studies have broadened our knowledge to understand the role of the MAP kinase signaling pathway in the development of pancreatitis-associated fibrosis, but still the existence of several molecular signaling pathways which may cross-talk to each other have an important role in the development of fibrosis and need to be explored further.

RHO KINASE PATHWAY

In chronic pancreatitis, activation of PSCs and induced stress fiber formation suggest the reorganization of cytoskeletal proteins is involved in this disease process[159]. The Rho family proteins RhoA, Rac and Cdc42 are considered the core molecules that induce stress fiber formation and regulate cellular adherence by remodeling of the cytoskeleton in response to external signals[160,161]. Further, the inhibition of Rho A signaling diminished the endothelial hyper-permeability that was induced by sera from severe acute pancreatitis patients with lung injury *via* inhibiting F-actin aggregates[162]. Inhibitors of Rho kinase such as Y-27632 and HA-1077 (fasudil) block activity of PSCs, *via* reducing α-SMA, proliferation, chemotaxis, and type Ⅰ collagen production in culture-activated PSCs[163]. During cerulein-induced pancreatitis in mice, Y-27632 caused induction of serum amylase levels, higher interstitial edema and vacuolization at 12-18 h after the first injection of cerulein. Y-27632 in turn inhibited the recovery of protein expression of ROCK-Ⅱ at 18 h after the first cerulein injection. These results indicate that RhoA and ROCK-Ⅱ accumulate in normal CCK-stimulated pancreatic enzyme secretion and prevent cerulein-induced acute pancreatitis[164]. Rho-kinase signaling was found to regulate trypsinogen activation and its release from the pancreatic acinar cells during acute pancreatitis and subsequent CXC chemokine formation, neutrophil infiltration and tissue injury[165]. Thus, these results indicate that Rho-kinase may serve as a novel molecular target for future treatment of acute pancreatitis, but there is need for

vast effort to understand the Rho-Kinase signaling in pancreatitis. This area opens up a new avenue for future research.

JAK/STAT SIGNALING PATHWAY

The JAK/STAT signaling pathway regulates several cellular functions such as cell proliferation, differentiation, and inflammatory responses[166-168]. IL-6 is a well-known pro-inflammatory cytokine and mediates its action *via* JAK/STAT signaling pathway[21] and plays a crucial role in the progression of pancreatitis. Various reports have indicated higher serum levels of IL-6 in patients with pancreatitis as compared to healthy individuals[22,23]. Furthermore, an *in-vitro* study also indicates induced secretion of IL-6 from the human pancreatic peri-acinar myofibroblast cells under the influence of several inflammatory mediators, such as TNF-α, IL-17, IL-1β and FGF-2; this data further indicates the crucial role of IL-6 in the pathogenesis of acute pancreatitis[24,25]. Interestingly, blockade of IL-6 using anti-IL-6 antibody suppresses STAT-3 activation in the pancreatic acinar cells and consequently diminishes the severity of acute pancreatitis by induction of pancreatic acinar cell apoptosis[26]. Apart from this, another report suggests that PDGF induces the proliferation of PSCs[169] by activating the JAK-2/STAT-3 pathway[155]. The inhibition of JAK-1/STAT-1 improves the severity of cerulein-stimulated pancreatic injury by inhibiting the activation of NFκB, and this indicates that activation of JAK-1/STAT-1 is involved in the early events of pancreatic injury[170]. Still, a better understanding of the JAK/STAT signaling pathway is required to know its role in the proliferation of PSCs and progression of fibrosis in chronic pancreatitis.

PI3K-AKT PATHWAY

PI3K-Akt is a major intracellular signaling pathway that belongs to a family of lipid and protein kinases. When growth factors bind to membrane bound receptor tyrosine kinase, it activates PI3K and its downstream regulators Akt and mTOR and regulates several aspects such as cell growth, survival, apoptosis and inflammation[171-173]. Earlier, it has been shown the PI3K pathway inhibitor wortmannin reduces the intra pancreatic activation of trypsinogen in acinar cells[174] and decreases inflammatory cytokines in severe acute pancreatitis in rats[175]. These reports suggest involvement of the PI3K pathway in the pathogenesis of acute pancreatitis. PI3Kγ is an isoform of PI3K known to regulate pathologic responses of the pancreatic acinar cells during pancreatitis[176]. The role of PI3Kγ was studied in two different models of acute pancreatitis, cerulein and choline-deficient/ethionine-supplemented diet. Mice lacking the *PI3Kγ* gene are protected from acinar cell injury/necrosis and show reduced severity of acute pancreatitis, indicating PI3K inhibitors may provide a possible therapy for acute pancreatitis[172,177].

CLINICAL CHARACTERISTICS AND DIAGNOSIS OF PANCREATITIS

The major clinical characteristics of pancreatitis are abdominal pain localized to the upper-to-middle abdomen, abdominal distension, nausea, fever, flank pain, vomiting, back pain, jaundice, hematemesis, melena diarrhea with foul-smelling, oily bowel movements and weight loss[178]. Abdominal pain is the most common symptom found in 50% to 80% of cases, and it is the major cause for hospitalizations of patients related to pancreatitis. Although the pancreatic pain is low in the abdomen, following food intake it worsens and becomes localized to the epigastric area[179]. Ammann *et al*[180] have identified two types of pancreatic pain (type A and type B) on the basis of natural history of alcoholic chronic pancreatitis. In type A pain, there are short (< 10 d) episodes of acute pain with long pain-free periods, whereas type B pain persists for a longer period of time (1-2 mo) with intervals of intense pain. Type A pain is experienced more often and is typically easier to treat. Several serum-based biomarkers have been identified for the diagnosis of acute pancreatitis such as amylase, lipase and trypsinogen[181]. In acute pancreatitis, the level of amylase (glycoside hydrolase) is rapidly induced within 4 to 6 h of disease onset, remains high for 3 to 4 d and sensitivity decreases with time from onset[182-184]. Higher levels of lipase have been found during the onset of acute pancreatitis, and it is more specific and sensitive than amylase for detecting acute pancreatitis because serum level of lipase remain elevated for around 2 wk before it returns to the normal level[183,185]. The sensitivity and specificity of amylase is about 63.6% and 99.4%, whereas sensitivity and specificity of lipase were 95.5% and 99.2%, respectively[186,187]. Pancreatic lipase is four times more active than amylase and it is less affected by exocrine pancreatic deficiency occurring in patients with chronic pancreatitis[183,188]. Trypsinogen is the inactive form of the enzyme trypsin and is cleaved by duodenal enterokinase to produce the active enzyme trypsin and trypsinogen activated peptide[183,189]. Normally trypsinogen is secreted in very low levels from pancreatic acinar cells but during pancreatitis secreted trypsinogen enzyme moves into the systemic circulation due to increased vascular permeability, and consequently there is increased clearance in the urine. During the onset of disease, trypsinogen concentration is elevated in the serum as well as urine and declines to normal level within 3 to 5 d[183,185,190].

CURRENT TREATMENTS STRATEGY

The first-line of treatment involves fasting along with intravenous fluids if the pancreatitis is very painful and this help the pancreas to rest and recover. Depending on the underlying cause of pancreatitis, management may vary to address the specific cause. Currently, several medications and treatment options are available

such as analgesics likes paracetamol or non-steroidal anti-inflammatory drugs or both followed by tramadol, perhaps coupled with a neuroleptic antidepressant. Another option is steroid therapy in which prednisolone is used for the treatment of autoimmune pancreatitis[191,192]. Furthermore, micronutrient therapy seems to be promising and it includes vitamin C, E, B6, B12, folic acid, methionine, and β-carotene. Braganza *et al*[10] have revealed that micronutrient therapy is designed to supply methyl and thiol moieties, which are helpful to restrict the generation of reactive oxygen species and deactivate pro-inflammatory oxidation products, reduce mast cell degranulation, decrease necrosis of pancreatic acinar cells and lessen pro-fibrotic induction. The outcome of these six clinical trial-based studies revealed that micronutrient therapy controls the pain and curbs attacks in patients suffering with chronic pancreatitis[193-199]. If pancreatitis is due to an obstructing gallstone, surgical intervention may be needed to remove the gallstone. Intervention may also be required to treat a pseudocyst or surgically remove the part of affected pancreas. Micronutrient treatment seems to substantially reduce the need for surgery.

CONCLUSION

The current review provides a comprehensive understanding of the development of chronic pancreatitis and the role of cells and cytokines involved in promoting pathogenesis. Briefly, we have discussed disease characteristics, molecular mechanisms involved in pancreatitis, the role of granulocytes such as neutrophils and eosinophils, the details of associated cytokines and chemokines implicated in the progression along with major signaling pathways such as TGF-β/SMAD, MAP kinase, PI3K, Rho kinase, and JAK/STAT that are crucial in the development of pancreatic fibrosis following pancreatic injury. This review will help to understand the intricate process of several autocrine and paracrine pathways involved in pancreatitis pathogenesis including remodeling. We provided details regarding the disease that might be useful for investigators to focus on, and cells and their associated mediators that might be helpful for future strategies for diagnostic and therapeutic interventions in the treatment of pancreatitis.

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REVIEW

Psychotropic drugs and liver disease: A critical review of pharmacokinetics and liver toxicity

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Abstract

The liver is the organ by which the majority of substances are metabolized, including psychotropic drugs. There are several pharmacokinetic changes in end-stage liver disease that can interfere with the metabolization of psychotropic drugs. This fact is particularly true in drugs with extensive first-pass metabolism, highly protein bound drugs and drugs depending on phase I hepatic metabolic reactions. Psychopharmacological agents are also associated with a risk of hepatotoxicity. The evidence is insufficient for definite conclusions regarding the prevalence and severity of psychiatric drug-induced liver injury. High-risk psychotropics are not advised when there is pre-existing liver disease, and after starting a psychotropic agent in a patient with hepatic impairment, frequent liver function/lesion monitoring is advised. The authors carefully review the pharmacokinetic disturbances induced by end-stage liver disease and the potential of psychopharmacological agents for liver toxicity.

Key words: Liver; Toxicity; Psychotropic drugs; Pharmacokinetics; Hepatic disease

Core tip: The liver is the organ by which the majority of substances are metabolized, including psychotropic drugs. There are several pharmacokinetic changes in end-stage liver disease that can interfere with the metabolization of psychotropic drugs. The evidence is insufficient for definite conclusions regarding the

prevalence and severity of psychiatric drug-induced liver injury. High-risk psychotropics are not advised when there is pre-existing liver disease, and after starting a psychotropic agent in a patient with hepatic impairment, frequent liver function/lesion monitoring is advised.

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INTRODUCTION

Among all of the organs in the human body, the liver performs the greatest number of functions. The liver's multiple activities are important and have impacts on all body systems, including the nervous system. It is also in the liver that most of the substances that we ingest are metabolized, including drugs.

Liver failure occurs when large parts of the liver become damaged beyond repair, and the liver is no longer able to function. Drug-induced liver injury (DILI) is the 4th most important cause of liver disease in Western countries[1]. The incidence of DILI is between 1/10000 and 1/100000 patients-years[2,3].

The drugs used in psychiatry and neurology are the second most important group of drugs implicated in hepatotoxicity, after anti-infectious drugs[4]. The hepatic reserve is reduced in patients with cirrhosis or chronic hepatic failure, and when DILI occurs in such patients, it can be more severe[5]. Therefore, high-risk drugs should be contraindicated in cases of pre-existing liver disease[6].

Conversely, liver failure interferes with different stages of drug pharmacokinetics: Absorption, metabolism, distribution and elimination. Therefore, it affects drug concentrations, duration of action, and effectiveness. It is essential to be aware of these processes and consequent changes in the circulating concentrations of psychiatric drugs to prevent drug toxicity.

Psychiatric symptoms in patients with end-stage liver disease can occur due to co-existing psychological or physiologic processes (*e.g.*, liver failure, encephalopathy, adjustment reactions to the stress of severe medical illness, *etc.*). All of these situations must be treated, not only with psychological interventions but also with psychotropic drugs. In these cases, patients with end-stage liver disease require special concern because they are medically vulnerable and are at increased risk for medication-induced adverse reactions.

The purpose of this paper is to review the evidence regarding fundamental pharmacokinetic alterations caused by end stage liver disease as well as the potential for liver toxicity with psychopharmacological agents. In our review, we analyse the evidence for DILI, severe liver injury leading to death or liver transplantation, abnormalities of liver function tests in clinical trials and hepatotoxicity. Finally, we provide several recommendations and directions regarding the psychotropic drugs that require special attention and how to minimize the risks of liver toxicity.

PHARMACOKINETIC CHANGES IN END-STAGE LIVER DISEASE

Liver failure can affect some aspects of medication pharmacokinetics, ranging from absorption to distribution and elimination. We discuss the most important pharmacokinetic processes that might lead to increased drug concentrations in liver disease patients.

Distribution

In end-stage liver disease, a great part of the blood in the portal vein escapes from the liver and flows straight into the systemic circulation (by means of portosystemic shunts). This process is due to intra- and extra-hepatic shunts that can occur in these patients. Therapeutic shunts (surgical and angiographic) can also be used to alleviate portal hypertension[7].

These shunts can affect first-pass metabolism by diminishing liver perfusion. In these cases, less drug passes through the liver before systemic distribution. Consequently, there is an elevation in drug concentrations in the blood. This effect is particularly important for drugs with extensive first-pass metabolism (Table 1). The pharmacokinetics of other psychotropic drugs, such as diazepam and paroxetine, with less affinity for liver enzymes, are not as influenced by first-pass metabolism[8].

Although olanzapine has great first-pass metabolism, it is mostly metabolized by second-phase liver metabolic processes (preserved in liver disease), so it might not be an important factor for this particular drug[9].

Protein binding

More than 80% of psychiatric drugs are bound to plasma proteins, such as lipoproteins, alpha$_1$-acid-glycoprotein and albumin. Some psychotropic drugs, such as fluoxetine, aripiprazole and diazepam, are highly protein bound. Nevertheless, there are some psychotropic drugs that minimally bind to proteins, such as venlafaxine, lithium, topiramate, gabapentin[10], pregabalin, methylphenidate and memantine[11-17].

The cirrhotic liver produces a smaller quantity of albumin and alpha1-acid-glycoprotein, which is conducive to an increased concentration of free active drug in the blood[18,19].

This increase is particularly important for highly protein-bound drugs, such as benzodiazepines (particularly diazepam, which is more than 99% protein

Table 1 Psychotropic drugs with extensive first-pass metabolism[10-16]

Tricyclic antidepressants - first-pass metabolism greater than 50% after oral administration
SNRI antidepressants - venlafaxine
SSRI antidepressants - sertraline
NRI antidepressants - bupropion
Typical antipsychotics - chloropromazine
Atypical antipsychotics - olanzapine (40%), quetiapine

SSRI: Selective serotonin reuptake inhibitors; SNRI: Serotonin and norepinephrine reuptake inhibitors; NRI: Norepinephrine reuptake inhibitors.

bound)[20]. Therefore, in cirrhosis, the side effects that result from the administration of these drugs, such as sedation, can be more severe.

Metabolism

Some psychotropic drugs are water-soluble and are directly removed from the circulation in the urine and bile, which is the case with lithium, gabapentin, and topiramate[10]. However, all of the other psychotropic drugs are lipid soluble and must be metabolized in the liver, where they undergo some chemical changes and become more soluble. Only then can they be excreted in the urine or bile.

The metabolic reactions that take place in the liver can occur in two main phases[19]. In phase Ⅰ, cytochrome P-450 enzymes (monooxygenases) are responsible for the hydrolysis, oxidation, dealkylation or reduction of the molecule. Most of the time, these reactions decrease the pharmacological activity of the substrate. However, drugs are sometimes metabolized into active metabolites, which is the case with some benzodiazepines (such as diazepam, chlordiazepoxide), tricyclic antidepressants (such as amitriptyline and imipramine) and antipsychotics (such as chlorpromazine, thioridazine, risperidone)[10,21]. In phase Ⅱ, liver enzymes are responsible for the conjugation of the drug with an endogenous molecule, such as glucuronic acid, sulphate, amino acids, acetate or glutathione. This process renders the original molecule more hydrophilic[19], and in most of the cases, it eliminates all of the pharmacological activity.

Conjugation with glucuronic acid (glucuronidation) is normally preserved in liver disease[21]. Therefore, it might be beneficial to select a psychiatric drug that only requires glucuronidation (and does not require a phase Ⅰ reaction), which is the case with temazepam, oxazepam, and lorazepam[8,9,19]. Olanzapine also requires almost only glucuronidation in its metabolization[9].

Fluid status

Although it is believed that water-soluble drugs, such as lithium, are safe to use in liver disease patients, there some aspects that must be considered.

In fact, it is not easy to maintain therapeutic serum levels of drugs such as lithium with the changes in fluid status that can occur in liver disease patients. These changes can be due to possibly abnormal renal haemodynamics (which often occur in liver disease patients) but also to any sudden change in fluid status that can occur due to some therapeutic procedures (such as paracentesis, extreme diuresis, or diarrhoea induced in the treatment of liver encephalopathy).

If the total volume of body fluid is suddenly reduced, the regular therapeutic drug level can become critically toxic. Therefore, when using these types of drugs (such as lithium) in patients with cirrhosis, a strict coordination is mandatory between the different medical specialists that assist the patient[10,17].

DILI

DILI can be classified depending on different criteria: Underlying injury; pathophysiological mechanism; clinical evolution; and severity of the lesion. Each of these criteria are reviewed.

Underlying liver injury

DILI can be classified into three main categories according to the pattern of liver injury (*i.e.*, hepatocellular and cholestatic or mixed). Hepatocellular injury accounts for 90% of drug-induced hepatotoxicity and is associated with abnormally high serum alanine aminotransferase (ALT) titres, with a small or no increase in alkaline phosphatase (ALP) titres; an associated high serum bilirubin level, found in cases of severe hepatocellular damage, is a marker for poor prognosis[22]. Cholestatic liver injury is associated with high serum ALP titres only slightly higher than normal ALT levels; serum bilirubin concentrations might also be high. In cases of mixed injury, both ALT and ALP levels are abnormally high.

Another type of lesion is steatosis. This reaction is generally chronic and occurs with gradual and increased fat accumulation in the liver (especially triglycerides), which can be caused by different situations, including the use of certain drugs. In drug-induced steatosis (almost always reversible), benign macrovacuolar steatosis can become steatohepatitis and cirrhosis in some cases[23].

Steatosis can occur with exposure to some antipsychotics (*e.g.*, clozapine, olanzapine) and antiepileptics (*e.g.*, valproate)[23-28]. Less frequently, steatosis can be microvesicular, consistent with a more serious form of fat deposition in the hepatocytes, associated with more severe and acute clinical consequences (*i.e.*, valproate or Reye's syndrome).

Pathophysiological types of DILI

Two pathophysiological types of DILI have been identified.

The more common type is idiosyncratic, dose independent and unpredictable[29]. It is the consequence either of immune-mediated liver damage (immunoallergic idiosyncratic DILI) or of direct cellular injury (metabolic idiosyncratic DILI)[30]. A hypersensitivity syndrome (fever,

rash, eosinophilia, auto-antibodies) and a short latency period (1-6 wk)[30] suggest immune-mediated hepatic injury, whereas the absence of any hypersensitivity syndrome and a longer latency period (1 mo to 1 year) suggest an idiosyncratic metabolic mechanism[31]. Intrinsic DILI, related to drug accumulation, has also been described; it is dose dependent and predictable and has generally been observed during preclinical and clinical trials, leading to early drug withdrawal.

Clinical evolution (acute/chronic)

DILI can be acute or chronic, depending on clinical presentation. Acute DILI is the most common form of DILI, accounting for 10% of all cases of acute hepatitis. Histologically, it can present as acute hepatitis, cholestatic injury, a mixed pattern or acute steatosis. Chronic DILI is defined as persistence of abnormal liver enzymes for > 6 mo, and it accounts for 10% of DILI cases, more often following acute cholestasis. It can resemble other causes of chronic liver disease, such as autoimmune hepatitis or alcoholic liver disease[32].

Severity of DILI

Regarding its severity, DILI can be mild, severe and fatal.

According to the Drug-Induced Liver Injury Network (DILIN), in mild DILI, there is elevation of ALT and/or alkaline phosphatase, but no important increases in bilirubin and no impairment of coagulation. In severe DILI, there is elevation of ALT and/or alkaline phosphatase, bilirubin is also increased, and one or more of the following exists: Extended jaundice for more than three months; and liver or other organ failure (induced by the drug). In fatal DILI, death occurs if the patient does not undergo liver transplantation[33].

The available data show that all psychotropic agents are associated with a risk of hepatotoxicity[34]. Most of the cases of DILI are mild, and liver tests normalize after drug withdrawal. Nevertheless, sometimes the consequences are very severe, leading to death or liver transplantation.

The most important means of assessing the potential for a psychotropic drug to cause severe or fatal hepatic injury is to review the published case reports. Nevertheless, there is no way to determine incidence rates, and the inexistence of case reports cannot be interpreted as the medication being free of risk regarding severe or fatal DILI. Conversely, the risks with different medications cannot be compared by this methodology because they are prescribed in different rates, and they have existed for different periods of time. For example, the probability of having case reports for older drugs is much higher than for newer ones[35].

Another problem is that, in many cases of reported DILI for a certain drug, the patient has co-medications and several medical co-morbidities.

Detection of DILI during premarketing clinical trials is a difficult challenge because of the small numbers of patients treated and the short duration of the majority of clinical trials (6-12 wk) relative to the latency of DILI[36,37].

Antidepressants

Antidepressant-associated DILI is generally of the hepatocellular type and less frequently of the cholestatic or mixed type[31-34]. Concerning pathophysiology, it can be immunoallergic or metabolic. Various biological and clinical presentations are possible, ranging from isolated increases in liver enzyme levels to loss of hepatocellular function, acute liver failure, and death[38].

Based on severity and frequency of liver injuries reported for the different antidepressants, Voican classified the agents as high risk and lower risk. High-risk agents include tricyclic antidepressants (imipramine, amitriptyline) and nefazodone (which has been withdrawn from the market in several countries, due to 55 severe cases of DILI reported, including 20 deaths), as well as venlafaxine, duloxetine, sertraline, bupropion, trazodone, and agomelatine[22,38-42].

Drugs with apparently lower risks are citalopram, escitalopram, paroxetine and fluvoxamine[38,43].

Gahr *et al*[44] confirmed the results of Voican's comprehensive review using an innovative method. They calculated and compared reporting odds ratios, based on the number of adverse drug reactions related to hepatic disorders/total number of adverse drug reaction among several antidepressants[44].

Regarding agomelatine (AGM), there is disagreement between the pervasive idea that this antidepressant might have a great risk of liver toxicity and the availability of published data providing this evidence perhaps because of the short life of this antidepressant[44].

However, in a recent EMA (European Medicines Agency) post-authorization opinion, AGM was reported to be associated with a high hepatotoxic risk, and some limitations on its use were suggested. Clinical trials have shown a higher prevalence of increased ALT in patients treated with AGM (1.34% on AGM 25 mg/d, 2.51 on AGM 50 mg/d), compared to placebo (0.5%). Moreover, since the marketing authorization for AGM in 2009, several cases of severe liver injury-associated with AGM have been reported[6,30].

These cases indicate that AGM should be avoided in patients with pre-existing liver function compromise. Furthermore, it is recommended by the company responsible for this drug that regular laboratory analysis be performed in cases of prescription of AGM. If there is treatment-associated elevation of liver enzymes, AGM should be rapidly discontinued. Patients of female sex, who are older than 50 years of age, and who are polymedicated can have increased risk of liver toxicity related to AGM, although there is still only scarce regarding these matters. More studies are expected in this field, and they could likely affect the actual recommendations regarding AGM[6,44]. Table 2 summarizes the data on

Table 2 Antidepressants and liver toxicity

	Epidemiology	Type of lesion	Mechanism
Tricyclic antidepressants			
Imipramine	ALT transient elevation-20%[45] Cholestatic jaundice: 0.5%-1%[2] DILI: 4/100000 patient-years[2,46] Fatal/Trxp DILI: 1[47]	Hepatocellular, cholestatic	Immuno-allergic
Amitriptiline	ALT transient elevation-10%[45] Abnormal LFT: 3%[48] Fatal/Trxp DILI: 1[39]	Hepatocellular, cholestatic	Immuno-allergic
Clomipramine	Severe DILI: 2 reports[42,49]	Hepatocellular	Immuno-allergic
MAO inibitors			
Moclobemide	Abnormal LFT: 3%[50] Fatal DILI: 1[51]	Hepatocellular, cholestatic	Immuno-allergic
Serotonin-norepinephrine reuptake inhibitors			
Venlafaxine	ALT > 3ULN: 0.4%[52] Severe DILI: 6[53-56] Fatal DILI/Trxp: 1[57]	Hepatocellular, cholestatic	Immuno-allergic, metabolic
Duloxetine	ALT > 3ULN: 1.1%[58] ALT > 5ULN: 0.6%[59] DILI: 26.2/100000 patient-years[60,61] Severe DILI-7[5] Fatal/Trxp DILI: 13[60]	Hepatocellular, cholestatic, mixed	Immuno-allergic, metabolic
Serotonin-reuptake inhibitors			
Sertraline	ALT > 3ULN: 0.5%-1.3%[46] DILI: 1.28/100000 patient-years[46] Severe DILI: 4[62-65] Fatal/Trxp DILI: 2[66,67]	Hepatocellular, cholestatic, mixed	Immuno-allergic, metabolic
Paroxetine	ALT > 3ULN: 1%[46] Severe DILI: 4[68-71]	Hepatocellular, cholestatic, chronic hepatitis	Metabolic
Fluoxetine	ALT > 3ULN: 0.5%[46] Severe DILI: 6[72-77]	Hepatocellular, cholestatic, chronic hepatitis	Metabolic
Fluvoxamine	Unknown[38] DILI: 3[78-80]	Hepatocellular	Metabolic
Citalopram, escitalopram	No difference in LFT *vs* placebo[81,82]	?	?
Other antidepressants			
Nefazodone	DILI: 28.96/10000 patient-years[38] Severe DILI-35[83] Fatal-20[83]	Hepatocellular, cholestatic, mixed	Metabolic
Trazodone	ALT > 3 unknown[38] Severe DILI-7[84] Fatal/Trxp DILI-2[57,85]	Hepatocellular, cholestatic	Immuno-allergic
Bupropion	ALT > 3ULN: 0.1%-1%[86] Severe DILI: 3[86-88] Fatal/Trxp DILI: 2[89,90]	?	?
Agomelatine	ALT > 3ULN: 1.4% (25 mg/d) ALT > 3ULN: 2.5% (50 mg/d)[6,91] Severe DILI: 6 reports[92,93] Fatal/Trxp DILI: 1[94]	Hepatocellular	
Mirtazapine	ALT > 3ULN: 2%[95] Severe DILI 2: reports[96]		

DILI: Drug-induced liver injury; ALT: Alanine aminotransferase; LFT: Liver function tests; ULN: Upper normal limit.

hepatotoxicity of the main antidepressant drugs.

Antipsychotics

Cytochrome P450 (in the liver) is responsible for the metabolization of most antipsychotics (excluding sulpiride, amisulpride, and paliperidone)[97,98]. Antipsychotics can induce liver injury by means of three main mechanisms: Hepatocellular, cholestatic and steatosis.

Typical antipsychotics: The risk of hepatotoxicity with chlorpromazine is well established[34].

The main mechanism by which chlorpromazine and other phenothiazines induce cholestatic disease remains unclear. The existence of eosinophilia and rash during its early onset (frequently 1 mo) and that there is not a dose relationship for its toxicity reveal that the mechanism could be some type of hypersensitivity. Nevertheless, some authors have indicated that its toxicity might be related to an idiosyncratic metabolic reaction that depends on individual sensitivity[2]. The bile duct can be the most affected, and as a consequence, a severe ductopenic syndrome can occur[2].

Table 3 Antipsychotics and liver toxicity

	Epidemiology	Type of lesion	Mechanism
Typical			
Cloropromazine	Jaundice: 0.16%-0.3%[99] Severe DILI: > 350[100,101,115-124] Fatal Injury: 8[102-109]	Cholestatic	Immuno-allergic
Haloperidol	ALT > 3ULN: 2%[110] Severe DILI: 1[111]	Cholestatic	Immuno-allergic
Atypical			
Clozapine	ALT > 3ULN: 15%[125] Severe DILI: 16[126-140] Fatal Injury: 2[141,142]	Hepatocellular Cholestatic Chronic esteatosis	Immuno-allergic Chronic estatosis
Olanzapine	ALT > 3ULN: 6%[143] Severe DILI: 7[139,144-149]	Hepatocellular Cholestatic Chronic esteatosis	Immuno-allergic, Chronic estatosis
Risperidone	ALT > 3ULN: 3%[150] Severe DILI: 13[150-162]	Hepatocellular Cholestatic Chronic esteatosis	Immuno-allergic Chronic estatosis
Quetiapine	ALT > 3ULN: 0%[143] Severe DILI: 3[151,163,164] Fatal injury: 2[165,166]	?	?
Ziprasidone	Not reported Severe DILI: 1[167]	?	?
Aripiprazole	Not reported		
Amissulpride	Not reported		

DILI: Drug-induced liver injury; ALT: Alanine aminotransferase; ULN: Upper normal limit.

A study that reviewed prescriptions in the United Kingdom between 1985 and 1991 showed a total incidence of chlorpromazine jaundice of 0.16% (more elevated in patients who were older than 70 years old, 0.3%)[99].

Severe DILI was reported in more than 350 cases[100,101], and fatal injury in 8 cases[102-109].

Haloperidol, while structurally similar to the phenothiazines, rarely causes severe liver compromise. When it occurs, the mechanisms of liver toxicity are similar to those of phenothiazines (cholestatic lesions)[2]. A frequency of elevated liver enzymes of 2%[110] was reported, but only 1 case of severe DILI was reported[111].

Atypical antipsychotics: Atypical antipsychotics rarely induce severe liver toxicity. Nevertheless, asymptomatic increases in the levels of liver enzymes and bilirubin are not uncommon when using these psychotropic drugs. In most cases, the laboratory changes appear after 6 wk of treatment, and they tend to disappear and not worsen[35].

The type of hepatic lesion associated with antipsychotics can follow a primary hepatocellular pattern; therefore, the main change in laboratory tests seems to be an elevation in aminotransferases[35]. Nonalcoholic fatty liver disease can also be associated with treatment with atypical antipsychotics *via* metabolic syndrome, which they can induce[112].

Hence, many authors have advocated that it is important to assess liver function tests before initiating treatment with atypical antipsychotics, and subsequently, routine control of aminotransferases must be performed. Checking every year (and 6/6 mo in the case of clozapine) has been recommended[113]. In patients with heavy use of alcohol or other substances, more frequent control might be necessary. In this latter group of patients, it is also recommended to be more careful with slight changes in laboratory tests. If signs of liver compromise (*e.g.*, jaundice, pruritus, nausea, anorexia, *etc.*) are present, laboratory tests should be assessed at once.

The antipsychotic should be stopped if there is an asymptomatic increase in aminotransferases higher than 3 times the maximum level of normal (aminotransferases are sensitive marker of liver injury)[114].

It is necessary to pay special attention to patients with pre-existing hepatic disease or patients treated with other drugs that can be aggressive to the liver. Because the majority of atypical antipsychotics are relatively new, there still are no long-term hepatic follow-ups with some of these drugs. Therefore, new evidence might appear in longer controlled studies regarding the frequency of and risk factors for liver damage[108].

In his comprehensive review, Marwick stated that LFT abnormalities in adults receiving regular antipsychotics are "common, early, mild, and often transient"[35]. Severe or fatal DILI is very rare. Chlorpromazine is the antipsychotic most associated with severe liver toxicity and therefore should not be used in patients with pre-existing liver dysfunction[35]. Among the atypical antipsychotics clozapine, is the antipsychotic most associated with LFT abnormalities, and aripiprazole, ziprasidone and amisulpride might be associated with

Table 4 Mood stabilizers and benzodiazepines and liver toxicity

	Epidemiology	Type of lesion	Mechanism
Antiepileptics			
Carbamazepine	Transient ALT, AST, GGT elevations: 61% patients 1%-22%[3] DILI: 1%[170]	Hepatocellular, cholestatic	++Hypersensitivity -- Metabolic
Valproate	Transient ALT, AST elevations: 10%-15% patients[170] Hyperrubillirubinemia-44%[170] DILI: 3%-44%[173] Fatal DILI: 0.02% (0.2% children < 2a)[1]	Hepatocellular	Metabolic (Toxic metabolites through w-oxidation) Statosis
Lamotrigine	Transient ALT, AST elevations < 1% Rare hepatotoxicity[170] (4 severe DILI)[174]	Hepatocellular	Metabolic
Topiramate	Transient ALT, AST elevations < 1%[1] Rare hepatotoxicity (2 severe DILI)[174]	Hepatocellular	Metabolic
Gabapentine; pregabaline	Rare hepatotoxicity[1]	?	?
Benzodiazepines			
Chlordiazepoxide, diazepam, flurazepam	Rare hepatotoxicity[171,172]	Cholestatic	Hypersensitivity
Litium			
	Very rare hepatotoxicity[1]	?	?

DILI: Drug-induced liver injury; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase.

Table 5 Pharmacokinetic changes caused by end-stage liver disease: Psychotropic drugs that require special attention

Avoid drugs with extensive first-pass metabolism	Avoid Tricyclic Antidepressants (first-pass metabolism 50%), venlafaxine, sertraline, bupropion, chlorpromazine, quetiapine
Avoid highly protein bound drugs	Avoid most psychotropic drugs (specially fluoxetine, aripiprazole and benzodiazepines). Except: Venlafaxine, lithium, topiramate, a gabapentin, a pregabalin, memantine
Avoid drugs depending on phase I hepatic metabolic reactions	Preferable: Lithium, gabapentin, topiramate, amisulpride (depending mainly on renal excretion) and some benzodiazepines (oxazepam, temazepam, lorazepam) that depend on phase II reaction or glucuronidation, which is preserved in cirrhosis

fewer LFT abnormalities. Table 3 summarizes the data about the hepatotoxicity of the main antipsychotics.

Mood stabilizers and benzodiazepines

The overall incidence of the hepatotoxicity of antiepileptics has been estimated at 1/26000 to 1/36000. The most used antiepileptic drugs in psychiatry are valproate, carbamazepine, topiramate, lamotrigine and gabapentin. Of these drugs, Valproate is associated with the greatest risk of potential liver toxicity. Gabapentin and pregabalin are the safest[129].

Valproate hepatotoxicity is generally idiosyncratic. The period of treatment before the onset of the injury can range from 3 d to 2 years. The absence of hypersensitivity symptoms, the morphology of the DILI and the slow onset suggest that the idiosyncrasy is metabolic. It is more common in infants and children[129].

Transient elevations of aminotransferases can be present in 10%-15% of patients and hyperbilirubinemia in up to 44%. Therapy can be continued as long the elevations in aminotransferases are less than 3 times the ULN. Sometimes, normalization of liver tests occurs likely because of adaptation[168]. Regarding carbamazepine, hepatic adverse events are frequent but are most represented by transient asymptomatic elevations in liver tests (ALT, AST, GGT).

Severe liver damage caused by carbamazepine is infrequent, but it has a very typical presentation. One to eight weeks after beginning treatment with this drug, a hypersensitivity syndrome occurs, with fever, rash, facial oedema, lymph node enlargement, and leucocytosis (with eosinophilia)[1,169].

Less frequently, carbamazepine-induced DILI can occur without immuno-allergic characteristics. In these cases, the resulting clinical syndrome has a late onset (up to 6 mo after initiating treatment)[1,169].

Hypersensitivity is noted in up to 10% of patients. Hepatic adverse events have been reported to constitute 10% of all hypersensitivity reactions, for a total incidence of DILI due to carbamazepine hypersensitivity reactions of 1%[170].

Elevations in occur in less than 1% of patients on lamotrigine. Hepatotoxicity is rare and idiosyncratic, and it typically exhibits a hepatocellular pattern of injury[170]. The same outcome occurs with topiramate[1].

Benzodiazepine-induced liver damage is rare, with few cases reported in the literature, generally with a cholestatic pattern[171,172].

Long-term treatment with lithium can, in some cases, induce some LFT abnormalities. These changes are generally temporary and asymptomatic, reverting even if treatment continues. In cases of lithium over-

dose, these LFT changes can be marked, although the damage is much more severe in other organs, such as the kidney[1]. Table 4 summarizes the data about the hepatotoxicity of the main mood stabilizers and benzodiazepines.

CONCLUSION AND GENERAL RECOMMENDATIONS

The available data on psychotropic drug-induced hepatic toxicity are mostly from reported cases and, to a lesser extent, from the results of clinical trials and other studies, especially for the most recent drugs. It is therefore difficult to draw conclusions about the prevalence and severity of DILI.

Regarding pharmacokinetic changes in end-stage liver disease, there are some psychotropic drugs that require special attention, as shown in Table 5.

It is likely that all psychopharmacological agents are associated with a risk of hepatotoxicity. However, the evidence is insufficient for rigorous conclusions to be drawn about the prevalence and severity of psychiatric DILI[175].

Hepatic reserve is reduced in patients with cirrhosis or chronic hepatic failure, and when DILI occurs in such patients, it can be more severe[5,176]. Therefore, high-risk drugs should be contraindicated in cases of pre-existing liver disease[6] (based on comprehensive reviews).

Before starting a psychotropic agent, baseline laboratory testing (*e.g.*, LFT, ALT) is recommended[113,177]. If liver disease is present, it is preferable to use psychotropic drugs with minimal liver metabolism (*e.g.*, topiramate, sulpiride and amisulpride)[35]. High-risk psychotropic agents (referred to in comprehensive reviews, see above) are not advised when there is pre-existing liver disease. After starting a psychotropic agent in a patient with hepatic impairment, frequent liver function/lesion monitoring is advised[113].

If a patient has normal laboratory tests (*e.g.*, LFT, ALT) before initiating treatment, there is no clear unanimity regarding the frequency of analysis re-assessment. Laboratory tests with ALT > 3ULN or ALP > 2ULN are considered sensitive markers for liver damage, and in these cases, the psychotropic agent should be stopped[35,114].

After starting a psychotropic agent, patients should be counselled to report signs and symptoms of liver dysfunction that could be associated with the use of their drug, including weight loss/decreased appetite, gastrointestinal problems or changes, dark (*i.e.*, tea-coloured) urine, yellowing of eyes (*i.e.*, jaundice), weakness, or unexplained/increasing fatigue. Other signs and symptoms include pruritus, clay-coloured stools, muscle pain, and increased confusion. Some of these conditions are already associated with chronic hepatitis infection, so it is important to emphasize observations of new-onset signs and symptoms. Patients and/or their caretakers should be encouraged to report these observations to their clinicians should they occur at any time after starting a psychotropic agent. Prompt discontinuation of the suspected agent at symptom onset might decrease the likelihood of worsening progression, which can lead to permanent liver damage[83].

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MINIREVIEWS

Update on clinical and research application of fecal biomarkers for gastrointestinal diseases

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Abstract

Gastrointestinal (GI) diseases comprise a large spectrum of clinical conditions ranging from indigestion to inflammatory bowel diseases (IBDs) and carcinomas. Endoscopy is the usual method employed to diagnose these condition. Another noninvasive way to assess and diagnose GI conditions are fecal biomarkers. Fecal biomarkers provide information regarding a specific disease process and are perhaps more acceptable to clinicians and patients alike because of their non-invasivity compared to endoscopy. Aim of this review was to evaluate the current status of the fecal biomarkers in clinical and research for in GI diseases. Multiple types of fecal biomarkers are discussed in this review including; markers to assess IBD, which are released as a results of an inflammatory insults to intestinal epithelia such as antimicrobial peptides (lactoferrin) or inflammation related proteins (calprotectin). While markers related to function of digestion are primarily related to partially digested food or mucosal proteins such as abnormal amount of fecal fat α1-antitrypsin, elastase and secretary IgA. The upcoming fecal biomarker like M2 pyruvate kinase and neutrophil gelatinase associated lipocalin are discussed as well. Apart from above mention, the fecal biomarkers under exploration for possible clinical use in future are also discussed. These include cathelicidins, osteoprotegerin, β-glucuronidase, Eosinophil proteins, *etc*.

Key words: Biomarkers; Gastrointestinal diseases; Inflammation; Lactoferrin; Calprotectin

Core tip: There is a general inclination of clinicians as well as pathologists' to consider fecal biomarkers due to its non-invasivity. There are multiple types of fecal biomarkers in clinical use and under exploration for potential clinical use in future. It includes biomarkers for evaluating inflammatory bowel disease (*e.g.*, calprotectin, lactoferrin), for evaluating colorectal cancer, malabsorption and eosinophilic protein for allergic gastrointestinal diseases. In this review we have analyzed the current status in terms of their practical utilization of fecal

biomarkers with established indications and those which are under various stages of investigation.

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INTRODUCTION

A biomarker is an endogenous or exogenous substance measured in blood, plasma or urine whose levels correlate with disease occurrence or severity. Biomarkers are used to distinct a pathological condition from a physiological state and also monitoring treatment and disease progression. There is a general inclination of clinicians as well as pathologists' to consider fecal biomarkers due to its non-invasive nature with likely acceptability to the patient. Fecal biomarkers can be subdivided into following types based on their clinical application.

Markers of inflammatory bowel disease

These include inflammation related proteins, released during an inflammatory process in the gastrointestinal (GI) tract (*e.g.*, calprotectin) or antimicrobial peptides (*e.g.*, lactoferrin).

Biomarker of colorectal cancer

These are found in undifferentiated tissues and cells with increased expression in rapid turnover of such cells, *e.g.*, M2-pyruvate kinase.

Biomarkers for evaluation of malabsorption

Multiple markers are identified; most are undigested food particles like fecal fat globules, enzymes like α1-antitrypsin and elastase for malabsorption assessment.

Biomarker for GI allergic diseases

Eosinophil related proteins are either released by or related to eosinophils and have application in assessing allergic and parasitic infestation of GI tract.

Biomarkers of gut health

This is an interesting group of biomarkers which with point toward the overall health of the gut mucosa. These biomarkers assess the integrity of gut barrier proteins and microbial fermentation products which are produced while fermentation of dietary particles by bacteria produces various chemicals, few of which such as short chain fatty acids, are used as biomarkers.

Different types of fecal biomarkers for GI diseases in clinical use and under investigations are shown in Tables 1 and 2. Although evidence for the newer markers is growing, currently only few fecal biomarkers have achieved a place in routine clinical practice notably calprotectin is on top of that list. Out of the multiple fecal biomarkers with emerging roles in clinical use for GI diseases, only some are extensively studied for their clinical and diagnostic utilities. In this review we aim to provide an overview of current status of fecal biomarkers for GI diseases with established value in clinical and potential for future use.

METHODOLOGY

A search of databases PubMed, MEDLINE and Google Scholars was performed using the search terms "fecal biomarkers" and "gastrointestinal disease biomarkers". We selected articles written in English, published since 1990 in peer-reviewed journals, excluding reviews. The articles were then reviewed by two pathologists and one gastroenterologist keeping in view the ideology behind this review and relevant articles selected. This review is divided in two parts, in part one we have aimed to include diagnostic accuracies for the established markers in clinical use and in second part the clinical applications of fecal biomarkers under investigation for GI diseases are discussed.

Markers of inflammatory bowel disease

Calprotectin (S100A8/S100A9) and S100 A12 proteins: The S100 proteins are a family of calcium-binding proteins specifically linked to innate immune functions by their expression in phagocytes, monocytes, macrophages and granulocytes. The calprotectin is a heterodimer of S100A8 and S100A9. These proteins are released by cells of innate immunity and GI epithelial cells in condition of inflammation. They limit the growth of bacteria and fungi by sequestering manganese and zinc.

Calprotectin is a marker to diagnose or monitor inflammatory bowel disease (IBD), presently considered a gold standard and also included in clinical practice guidelines. It is reported to perform better than S100-A12 in diagnosing IBD and its levels correlate with the severity of IBD. Calprotectin is observed to perform better in predicting ulcerative colitis then Chron's disease[1,2]. Meta-analysis have reported that calprotectin perform better in adults (sensitivity 93% and specificity 96%) than children (sensitivity 92% and specificity 76%)[3]. In contrast there are conflicting studies regarding diagnostic utility of the S100A12 as an inflammatory marker and it have moderate performance compared to other inflammatory markers[4-6].

Calprotectin is resistant to bacterial degradation in the gut and is stable in stool for up to one week at room temperature and is readily measured using immunochemical techniques. Limitations and diagnostic accuracies are conversed in Table 1.

Lactoferrin: Lactoferrin, an iron binding glycoprotein secreted in body fluids and produced by neutrophils,

Table 1 Fecal biomarkers for gastrointestinal diseases in clinical use with established diagnostic accuracies

S#	Name	Indication	Limitations	Sensitivity	Specificity
Biomarkers of IBD					
1	Calprotectin	Distinguishing functional from organic bowel disease and predicting relapse in IBD	Disease nonspecific Affected by age, comorbidities, NSAIDs use	70%-100%	70%-100%
2	S100 proteins	Inflammatory marker for IBD	Day to day variations Miss low level inflammatory activity	60%-67%	70%-90%
3	Lactoferrin	Markers of inflammation, Distinguish between IBS and IBD	Nonspecific marker of inflammation Raised in breastfeeding infants Cannot predict low level inflammation	67%-87%	90%-100%
Biomarker of cell turnover					
4	M2-PK	Screening of gastrointestinal tract cancers	Also raised in inflammation	67%-93%	88%-92%
Biomarkers of digestion and malabsorption					
5	Elastase-1 (e1)	Pancreatic insufficiency	Low specificity, also affected by other intestinal disorders	100%	96%
6	Fecal fat	Liver damage, hypolipidemic drugs, impaired gallbladder function, Celiac disease, Small bowel bacterial overgrowth	Cannot predict severity of disease Cannot be performed in diarrhea Not accurate or specific test	70%-94%	80%-99%
7	A1-antitrypsin	Protein-Losing Enteropathy, Whipple lipodystrophy, gastric carcinoma, intestinal lymphangiectasia	Nonspecific marker. Levels affected by inflammation	60%-78%	80%-85%

IBD: Inflammatory bowel disease; IBS: Irritable bowel syndrome; NSAID: Nonsteroidal anti-inflammatory drug.

mononuclear phagocytes and epithelial cells, Figure 1. It limits the growth of bacteria by limited availability of iron and causes direct damage to bacterial cell membrane leading to its bactericidal activity. This glycoprotein is stable in feces as it is resistant to proteolysis and can be measured by immunochemical methods. These glycoproteins are released in excess amounts by neutrophils and phagocytic cells after inflammation, making it a unique marker of inflammation[7]. Commercial assay for fecal lactoferrin measurement are now available based on immunochemical methods.

This is considered a good marker for evaluating IBD subjects, while evaluation as marker to distinguish between IBD and irritable bowel syndrome (IBS) there remains question marks, due to differences in results reported by different studies[8-11].

Cathelicidins: These are small cationic antimicrobial peptides like defensins and are produced by neutrophils and epithelial cells of GI tract, released upon stimulation of these cells during infection. These peptides exhibit antimicrobial activity against GI pathogens, gram-negative and positive bacteria by disrupting microbial membrane integrity, Table 2. These peptides play a vital role in maintaining the balance between the GI luminal bacteria and antibacterial peptides, which is crucial for a healthy GI tract. Studies have reported this balance is disturbed in various disease states. However the role of these peptides as the cause or consequence of disease state is still unknown. They could participate in the development of different disorders ranging from inflammation to cancer.

Schauber *et al*[12] reported that colonic expression of cathelicidin is increased in ulcerative colitis but not Crohn's disease. A study looking at cathelicidin role in Escherichia coli O157:H7 infection in mice and subsequent renal damage found that its deficiency was associated with severe infection and renal damage[13]. Another study by Sarker *et al*[14] reported that antimicrobial peptides cathelicidins expressions were decreased in rectal and colonic epithelia in shigellosis infection in rabbits along with decreased expression in epithelia of lung and trachea, a sign of systemic infection. They also observed the treatment with phenylbutyrate counteracted the decreased expression of cathelicidins in such patients offering a potential antimicrobial activity against shigella infection[14].

Osteoprotegerin: Osteoprotegerin is member of the tumor necrosis factor receptor superfamily. It binds to the receptor activator of nuclear factor kappa B ligand (RANKL), which in turn has pro-inflammatory properties, Table 2. A study by Nahidi *et al*[15] examining the role of osteoprotegerin in pathogenesis of IBD reported that it induced gut barrier deformities; increased permeability and decreased integrity of cell membrane along with loss of tight junctions; indicating that osteoprotegerin has pro-inflammatory effect and may contribute in pathogenesis of IBD[15]. However the complete understanding of its function in IBDs needs further evaluation.

Beta-glucuronidase: Beta-glucuronidases enzymes secreted by lysosomes of colonocytes and certain bacteria, *e.g*., *E. coli*, belong to glycosidase family of enzymes. This enzyme catalyzes the complex dietary carbohydrates, like glycosaminoglycansheparan sulfate. They also deconjugate variety of drugs, toxins,

Table 2 Fecal biomarkers under investigation for evaluating gastrointestinal diseases

S#	Name	Source	Function	Indication	Limitations
Biomarkers of inflammatory bowel disease					
1	Cathelicidins	Secreted by Neutrophils, keratinocytes and epithelial cells of gastrointestinal tract, respiratory tract, urogenital tract	Antibacterial activity, modulate inflammation by altering cytokine response, chemoattraction of inflammatory cells in diseased tissues	Marker of inflammation (IBD) and Shigellosis	Antimicrobial peptides so also increased in GI infections
2	Osteoprotegerin	Member of the TNF receptor superfamily	Binds to RANKL and blocks its interaction with RANK	Marker of inflammation (IBD)	Plays a role in bone metabolism so levels are increased in bone diseases
3	Beta-glucuronidase	Produced by colonocytes Also produced by anaerobic gut bacteria (particularly *E. coli*)	Enzyme that breaks down complex carbohydrates Deconjugate glucuronide molecules from a variety of toxins, carcinogens, hormones, and drugs	Marker of inflammation (IBD)	False results in cases of GI bacterial infection
4	Neutrophil Gelatinase Associated lipocalin	Member of the lipocalin family, secreted by neutrophils	Immunomodulation. Attaches to and neutralizes bacterial formylpepetides	Marker of inflammation (IBD)	Also increased in GI infections like enterocolitis
Eosinophil related proteins					
5	Eosinophil Protein X	When lamina propria is damaged, eosinophils migrate into the gut lumen Released by eosinophil; contribute to ongoing inflammation and tissue destruction	Marker of Eosinophil activity, Allergic and Parasitic influences	IgE-mediated food allergy Intestinal parasitic infection IBD	Also increased in GI inflammation
Biomarker of cell turnover					
6	Defensins	Expressed by neutrophils, epithelial and mucosal lining cell in small and large intestine	Antimicrobial peptide	Markers of colorectal cancer	Also raised in inflammation
Biomarkers of gut health					
7	Fecal secretory IgA	Secreted from mucosal surfaces	Gut epithelial barrier; Defense against the entry of enteric toxins and pathogenic organisms Development of immune tolerance of normal commensal gut organisms	Evaluate immunological response to intestinal pathogens Colorectal cancer	Cannot be used in subjects with immunoglobulin deficiency
8	SCFAs	Products of fermentation by colonic microbial flora; common ones are propionate, acetate, and butyrate	Provides 60%-70% of colonocytes energy requirements Lower colonic pH	Marker of inflammation (IBD)	< 5% of SCFA produced is excreted in stool Also levels altered by diet and rate of transit

SCFAs: Short-chain fatty acids; TNF: Tumor necrosis factor; IBD: Inflammatory bowel disease; GI: Gastrointestinal; RANK: Receptor activator of nuclear factor kappa B ligand.

hormones and also bilirubin in gut and are considered culprit of breast milk related jaundice in neonates, Table 2.

A study by Mroczynska *et al*[16], done on IBD and healthy children, reported that beta-glucuronidase activity was decreased by two times in children with IBD compared to healthy group. While in another study Manoj *et al*[17] reported that reduction in activity of intestinal as well as decreased levels of fecal beta-glucuronidase by using dietary fibers isolated from coconut or black gram may potentially play a role in preventing the formation of colon tumors induced by the carcinogen 1,2-dimethylhydrazine. These debatable findings warrant that further researchs is needed to completely understand chemical basis of beta-glucuronidase function.

Neutrophil gelatinase associated lipocalin: These proteins are released by neutrophils in response to some bacterial peptides (formylpeptides), which initiate bacterial protein synthesis. The neutrophil gelatinase associated lipocalin (NGAL) released into the gut lumen then binds with bacterial peptide and neutralizes it, stopping bacterial protein synthesis[18].

NGAL is another important inflammation related fecal marker under investigation for potential clinical utility. Serum and urinary NGAL are considered established markers for acute kidney injury and few studies have shown its levels are elevated in IBD but the levels don't correlate with disease severity[19]. Recently multiple studies have shown that fecal NGAL levels are raised in subjects with IBD and its levels are significantly associated with disease activity and severity[20-22].

Fecal biomarker of colorectal cancer

Pyruvate kinase: M2-pyruvate kinase is a dimer of pyruvate kinase; an enzyme involved in glycolysis pathway and plays an important role in tumor metabolism. It has increased expression in undifferentiated tissues and cells with in rapid turnover cells.

Its main role is in predicting GI cancers, both bleeding and non-bleeding types. Studies have reported

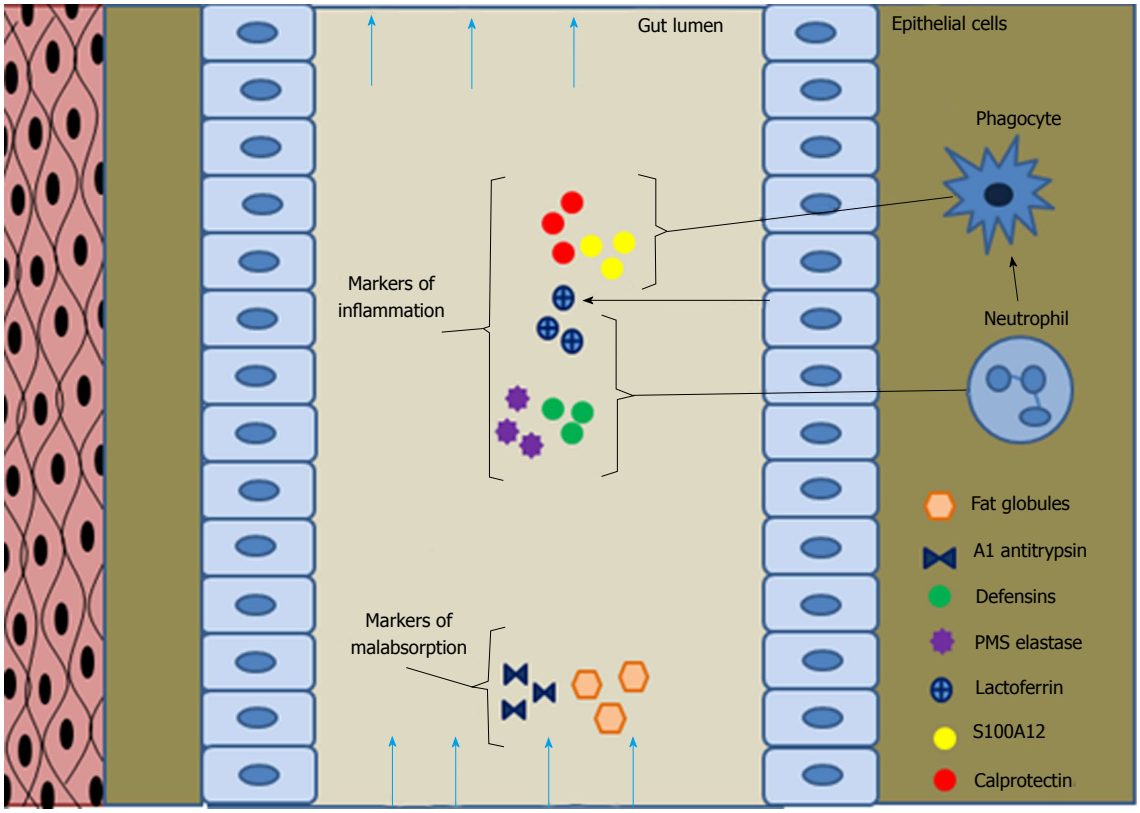

Figure 1 Overview of the potential source and clinical utility of various fecal biomarkers in clinical use.

it to be a marker in predicting colorectal cancers with good diagnostic accuracy, sensitivity and specificity of 93% and 97% respectively, Table 1[23]. Currently it is being used for monitoring colorectal cancer subjects after treatment. Along with it, levels of M2-Pk are also elevated in breast, lung, ovarian, and thyroid cancers[24]. One of its important limitations is that its levels are also increased in inflammation, so it should be used with caution in inflammatory conditions[25].

Defensins: Defensins are small cationic antimicrobial peptides, classified into alpha and beta defensins on basis of their disulfide bond and sizes. These are expressed by neutrophils, epithelial and mucosal lining cell in small and large intestine, Figure 1. They play an important role in innate immunity; antimicrobial activity against bacteria, fungi and some enveloped viruses and the expression is induced by the pro-inflammatory cytokines and also through microorganisms.

As the name implies they were considered as markers of infectious and inflammatory GI diseases. However there is now accumulating evidence suggesting defensins as an evolving marker for evaluating colorectal cancers but there are controversial findings[26-28]. Studies have also reported it to be an important marker for colorectal cancer. A study by Layton *et al*[29] presented at American Association of Cancer Research 104th Annual Meeting in 2013 reported that β-defensins 1 was expressed in colon tissue samples of normal subjects while this expression was lost in subjects with colorectal cancer. While another study by Melle *et al*[30], reported that α-defensins are expressed more in colonic epithelium of patients with colorectal cancer than in normal epithelium, establishing defensins potential role as a tumor markers[30]. So there remains a question mark regarding utility of this biomarker for evaluating colorectal cancer.

Biomarkers for evaluation of malabsorption

Elastase-1(e1) and PMN elastase: Serum elastase is a protease present in pancreatic secretion reaches the colon without being metabolized and is not affected by intestinal transit times or pancreatic enzyme replacement therapy, Figure 1. Elastase hydrolyzes denatured hemoglobin, casein, fibrin and albumin. It is a known biomarker for assessing exocrine pancreatic insufficiency, been in use for more than 3 decades. While its deficiency is associated with development of pulmonary emphysema and excess release results in hemorrhage due to vascular injury of acute pancreatic necrosis. Serum Elastase e1 levels are used for the diagnosis of acute or chronic pancreatitis, pancreatic insufficiency with good diagnostic accuracy[31], Table 1.

Another type of elastase enzymes, the polymorphonuclear elastase (PMN-elastase) is secreted by neutrophils in response to inflammation[32]. A study assessed the performance of calprotectin, lactoferrin and PMN-elastase in assessing IBD severity and differentiating between IBS and IBD, found that all these markers were able to differentiate active IBD

from inactive IBD as well as from IBS with diagnostic accuracies for lactoferrin, calprotectin and PMN-elastase of 80%, 80% and 74%[33].

Fecal fat: Excess fat in the stool (steatorrhea) is often the first sign of fat malabsorption. This can be due to a number of factors, including chronic pancreatitis with or without stone obstruction, cystic fibrosis, neoplasia, Whipple disease, regional enteritis, tuberculous enteritis, celiac disease, or the atrophy of malnutrition, Table 1.

The fecal fat assessment is done by microscopy after sudan stain and is largely considered non-specific as it is affected by diet, discrepancies in sample collection, qualitative reporting and assay variation leading to lower diagnostic accuracy. To overcome these hurdles a new quantitative fecal fat microscopic method was introduced by Fine *et al*[34] in 2000, reported to have improve diagnostic accuracy; sensitivity of 94% and a specificity of 95% compared to the traditional method sensitivity and specificity of 76% and 99%, respectively. In this method they microscopically counted the fat globules of different diameter ranges (0-5 μm, 6-10 μm, 11-20 μm, 21-40 μm, 41-80 μm, and > 80 μm) in five high-power fields and the average number of each size range fat globules present were multiplied by the size-range midpoint. All products were then added to get a single fecal fat droplet total size number product. They reported that results obtained by this method correlates well with chemically measured fecal fat output and has a high diagnostic accuracy.

Alpha-1-antitrypsin: Alpha-1-antitrypsin a protease inhibitor is produced by the liver, macrophages, and intestinal epithelium and is resistant to degradation by digestive enzymes. Therefore offers utility for use as a biomarker in assessing the proteins loss distal to the pylorus. Protein loss is associated in certain GI conditions such as gastroenteritis and sprue. Alpha-1-antitrypsin can readily be measured by using commercially available assays, Table 1.

Fecal alpha-1-antitrypsin clearance has been a marker of clinical disease severity in IBDs for many years[35]. Although α1-antitrypsin deficiency is more often associated with lung and liver pathologies, α1-antitrypsin deficient patients with concomitant IBD have been shown to develop more aggressive disease and rapid progression requiring surgery[36]. In a study by Becker *et al*[37] it was found that individual fecal α1-antitrypsin can predict prognosis in IBD patients.

Biomarker for GI allergic diseases

Eosinophil protein X: When lamina propria is damaged, eosinophils migrate into the gut lumen and multiple eosinophil granules related proteins are released, Table 2. These proteins contribute towards ongoing inflammation and tissue destruction associated with eosinophil related diseases like allergic diseases esophagitis, colitis, celiac disease, intestinal parasitic infections and IgE-mediated food allergy[38]. There is multiple eosinophil proteins including major basic protein, eosinophil cationic protein, eosinophil derived neurotoxin and eosinophil peroxidase associated with eosinophilic activity during inflammation[39,40]. This biomarker is however nonspecific and requires further studies to understand its role in eosinophil related disease pathology.

Biomarkers of gut health

Short-chain fatty acids: These are fatty acids with 1-6 carbon atoms, common ones are propionate, acetate, and butyrate produced as a results of metabolism of polysaccharides, oligosaccharides, peptides and glycoproteins by bacterial fermentation, absorbed by portal circulation and are an important energy source for colonic cells[41,42]. They lower the gut pH by regulating fluid and electrolyte uptake *via* activation of apical Na^+/H^+ exchange receptor[43]. These Short-chain fatty acids (SCFAs) are considered markers of colonic health and are known to have anti-inflammatory properties, Table 2. A study by Ohigashi *et al*[44], comparing colorectal carcinoma, adenoma and non-adenomatous subjects reported that compared to rest, subjects with carcinoma had decreased SCFA levels and altered microbial environment and pH.

Fecal secretory IgA: Immunoglobulin-A (IgA) are secreted by mucous membranes and as the name implies are antibodies important for mucosal immunity. These antibodies only form 15% for all immunoglobulins and in dimeric form called secretory IgA. This immunoglobin forms a defense against enteric toxins and pathogenic organisms. Secretory IgA is mainly secreted in mucosal secretions like tears, saliva, sweat, genitourinary tract, GI tract, prostate and respiratory epithelium.

Fecal secretory IgA is a part of mucosal barrier against infections and is also know to inhibit inflammation playing a protective role; therefore it is considered as a marker of gut health[45]. This biomarker is used to assess intestinal infections, coeliac disease and food allergies (Table 2)[46,47]. Few studies have also evaluated its clinical utility as an alternate marker of IBD but its use for these diseases is limited due to non-specific nature of this molecule.

DISCUSSION

The currently used diagnostic tools for identifying GI diseases are endoscopic procedures. Endoscopies are costly, invasive, time consuming, and also require patient preparation. Most of the time endoscopic procedure also required sedation especially in pediatrics patients. Interpretation of an endoscopic report is also subjective and opinions of two experts can differ at time. Generally speaking non-invasive approaches like serological test, urinary, fecal or salivary biomarkers are logically more acceptable to patients. Fecal biomarkers are now increasingly being used and the development of

sensitive and specific immunochemical techniques have led to its increased utility.

Newer biomarkers with established diagnostic utilities in clinical use include lactoferrin, defensins and S100 proteins especially calprotectin. Calprotectin and lactoferrin are now also included in clinical practice guidelines in the management of IBD. However the clinical application of these biomarkers is well established for IBD but validation studies are still needed to understand their role in other GI pathologies. Also the reference cut offs used by each study is different, so there is need to standardize the assays and reference cutoffs of the established markers to clearly distinct diseased from non-diseased states. With more research to increase our understanding regarding roles of these biomarkers in GI health and disease, there is the potential for few more markers such as cathelicidins to be incorporated into clinical practice in near future.

Currently, apart from the fecal markers of inflammation there is not enough literature regarding fecal biomarkers clinical utility in other GI diseases or health. For example eosinophilic proteins have the potential to be used as disease markers for allergic states and parasitic infestations; very common in developing country. But these markers require more studies to better understand their roles in diseased states. Another advantage of these markers will be that they will provide more insight into the cause of disease. Furthermore as we are in an era of preventive medicine markers which can pick early changes in gut health are required so the patients screened out before developing a diseased state. In conclusion development of fecal biomarker and establishment of their clinical and diagnostic utilities is a developing field with a lot of promise, but we still need more research to validate these findings.

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ORIGINAL ARTICLE

Basic Study

Plecanatide-mediated activation of guanylate cyclase-C suppresses inflammation-induced colorectal carcinogenesis in Apc$^{+/Min\text{-}FCCC}$ mice

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Abstract

AIM

To evaluate the effect of orally administered plecanatide on colorectal dysplasia in Apc$^{+/Min\text{-}FCCC}$ mice with dextran sodium sulfate (DSS)-induced inflammation.

METHODS

Inflammation driven colorectal carcinogenesis was induced in Apc$^{+/Min\text{-}FCCC}$ mice by administering DSS in their drinking water. Mice were fed a diet supplemented with plecanatide (0-20 ppm) and its effect on the multiplicity of histopathologically confirmed polypoid,

flat and indeterminate dysplasia was evaluated. Plecanatide-mediated activation of guanylate cyclase-C (GC-C) signaling was assessed in colon tissues by measuring cyclic guanosine monophosphate (cGMP) by ELISA, protein kinase G-II and vasodilator stimulated phosphoprotein by immunoblotting. Ki-67, c-myc and cyclin D1 were used as markers of proliferation. Cellular levels and localization of β-catenin in colon tissues were assessed by immunoblotting and immunohistochemistry, respectively. Uroguanylin (UG) and GC-C transcript levels were measured by quantitative reverse transcription polymerase chain reaction (RT-PCR). A mouse cytokine array panel was used to detect cytokines in the supernatant of colon explant cultures.

RESULTS

Oral treatment of $Apc^{+/Min\text{-}FCCC}$ mice with plecanatide produced a statistically significant reduction in the formation of inflammation-driven polypoid, flat and indeterminate dysplasias. This anti-carcinogenic activity of plecanatide was accompanied by activation of cGMP/GC-C signaling mediated inhibition of Wnt/β-catenin signaling and reduced proliferation. Plecanatide also decreased secretion of pro-inflammatory cytokines (IL-6, IL-1 TNF), chemokines (MIP-1, IP-10) and growth factors (GCSF and GMCSF) from colon explants derived from mice with acute DSS-induced inflammation. The effect of plecanatide-mediated inhibition of inflammation/dysplasia on endogenous expression of UG and GC-C transcripts was measured in intestinal tissues. Although GC-C expression was not altered appreciably, a statistically significant increase in the level of UG transcripts was detected in the proximal small intestine and colon, potentially due to a reduction in intestinal inflammation and/or neoplasia. Taken together, these results suggest that reductions in endogenous UG, accompanied by dysregulation in GC-C signaling, may be an early event in inflammation-promoted colorectal neoplasia; an event that can potentially be ameliorated by prophylactic intervention with plecanatide.

CONCLUSION

This study provides the first evidence that orally administered plecanatide reduces the multiplicity of inflammation-driven colonic dysplasia in mice, demonstrating the utility for developing GC-C agonists as chemopreventive agents.

Key words: Guanylate cyclase-C; Uroguanylin; Plecanatide; Inflammation; Colorectal cancer

Core tip: Plecanatide, an analog of human uroguanylin, binds and activates guanylate cyclase-C signaling to produce its anti-tumorigenic activity. This study provides the first evidence that oral treatment with plecanatide inhibits inflammation-driven colorectal carcinogenesis. The potential mechanism in $Apc^{+/Min\text{-}FCCC}$ mice appears to be agonist-mediated activation of guanylate cyclase-C signaling, resulting in inhibition of Wnt/β-catenin pathway and downregulation of pro-inflammatory cytokines and growth factors.

Chang WCL, Masih S, Thadi A, Patwa V, Joshi A, Cooper HS, Palejwala VA, Clapper ML, Shailubhai K. Plecanatide-mediated activation of guanylate cyclase-C suppresses inflammation-induced colorectal carcinogenesis in $Apc^{+/Min\text{-}FCCC}$ mice. *World J Gastrointest Pharmacol Ther* 2017; 8(1): 47-59 Available from: URL: http://www.wjgnet.com/2150-5349/full/v8/i1/47.htm DOI: http://dx.doi.org/10.4292/wjgpt.v8.i1.47

INTRODUCTION

Colorectal cancer (CRC) ranks third among newly diagnosed cancers in the United States and is the third most common cause of cancer mortality[1]. Clinical studies indicate that patients with long-standing inflammatory bowel disease (IBD) have a 2 to 8-fold increased risk of developing CRC as compared to the general population[2]. Although the precise etiology underlying inflammation-promoted CRC remains unclear, emerging data suggest that chronic inflammation, oxidative stress and excessive production of cytokines, chemokines and growth factors by infiltrating immune cells, may eventually lead to the development of dysplasia[3-6]. Like sporadic CRC, development of IBD-promoted carcinogenesis follows a sequential progression of disease from low-grade to high-grade dysplasia, and eventually CRC[7]. Prophylactic intervention with 5-aminosalicylate (5-ASA) is widely considered as a promising chemopreventive strategy[8]. However, additional case-controlled studies with larger numbers of patients are needed to further elucidate the chemopreventive utility of 5-ASA in IBD-promoted CRC. Conceptually, chronic prophylactic intervention with an orally safe and mucosally active agent that not only suppresses inflammation but also regulates renewal of the gastrointestinal (GI) mucosa is desirable.

Uroguanylin (UG) and guanylin (GN), endogenous natriuretic peptides, are agonists of guanylate cyclase-C (GC-C) that are structurally similar to bacterial enterotoxin (ST), secreted by the pathogenic *Escherichia coli* (*E. coli*) responsible for traveler's diarrhea[9]. Binding of these peptides to GC-C on the apical surface of epithelial cells lining the GI tract stimulates intracellular production of cyclic guanosine monophosphate (cGMP), resulting in activation of cGMP-dependent protein kinase G-II (PKG-II) and cystic fibrosis transmembrane conductance regulator. This leads to enhanced transepithelial efflux of Cl^- and HCO_3^-, inhibition of Na^+ absorption and passive secretion of water into the intestinal lumen; a process essential for a normal bowel movement[10]. Thus, a major physiological function of UG and GN is to regulate ion and fluid homeostasis in the GI tract[10-12].

GC-C signaling also plays a key physiological role in regulating the proliferative index of epithelial cells and maintaining the integrity of the GI mucosa[10,13,14].

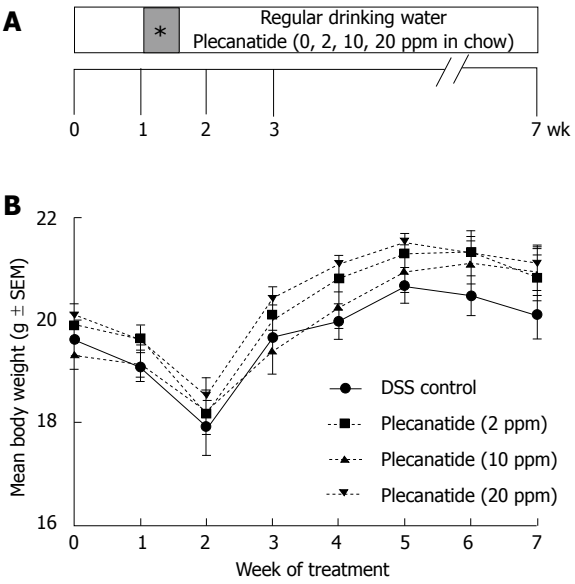

Figure 1 Inflammation-driven colorectal carcinogenesis in Apc$^{+/Min-FCCC}$ mice. A: Outline depicting the experimental design of the animal study. Female Apc$^{+/Min-FCCC}$ mice (n = 23/group) were randomized into four treatment groups: DSS alone (vehicle control) or DSS plus diet supplemented with 2, 10 or 20 ppm plecanatide. One week later, all animals were administered 2% DSS in the drinking water for 4 d (shaded box with asterisk) and regular water for the remainder of the study. At the time of euthanasia, (7 wk of study), the entire colon were fixed in formalin for histopathological evaluation; B: Body weights of Apc$^{+/Min-FCCC}$ mice treated with either DSS alone or DSS plus a diet supplemented with indicated concentrations of plecanatide (n = 20-23/group). Body weights were obtained weekly, and DSS was administered to all animals on days 7-10 of study. DSS: Dextran sodium sulfate.

Thus, disruption of GC-C signaling, due to reduced production of UG and/or GN, could potentially lead to neoplastic transformation. Indeed, transcript levels of both UG and GN are reduced dramatically in colon polyps and adenocarcinomas[13]. Furthermore, dietary supplementation of Apc$^{+/Min}$ mice with human UG not only inhibits polyp formation but also delays tumor progression[13]. Recent studies performed using GC-C and UG knockout mice further illustrate the involvement of GC-C signaling in the maintenance of homeostatic intestinal barrier function, gut permeability and intestinal epithelial cell proliferation[15]. Recently, we demonstrated that oral treatment with GC-C agonists such as plecanatide or dolcanatide effectively ameliorated GI inflammation in acute and chronic models of experimental colitis in murine models[16]. Thus, treatment with UG to overcome this deficiency may represent a promising approach for the prevention of inflammation-driven CRC.

The present study provides the first evidence to demonstrate that oral treatment with plecanatide effectively suppresses the formation of inflammation-driven CRC in Apc$^{+/Min-FCCC}$ mice.

MATERIALS AND METHODS

Materials

DSS (molecular weight 30000-40000) was purchased from MP Biomedicals (Solon, OH). All other chemicals and reagents were obtained from commercial vendors. Plecanatide (H-Asn^{1}-Asp^{2}-Glu^{3}-Cys^{4}-Glu^{5}-Leu^{6}-Cys^{7}-Val^{8}-Asn^{9}-Val^{10}-Ala^{11}-Cys^{12}-Thr^{13}-Gly^{14}-Cys^{15}-Leu^{16}-OH; Disulfide bond between Cys^{4} and Cys^{12}; Cys^{7} and Cys^{15}) was synthesized for this study according to procedures described previously[17].

Animals

Female Apc$^{+/Min-FCCC}$ (C57BL/6J) mice (7-wk-old, n = 92) were obtained from the Laboratory Animal Facility at Fox Chase Cancer Center (FCCC)[18]. Mice were genotyped for a point mutation in codon 850 of the *Apc* gene[19]. Animals were maintained in a temperature- and humidity-controlled room and received Teklad Global 2018SX diet (Harlan Teklad, Madison, WI) and drinking water *ad libitum*. All animal protocols were approved by the Institutional Animal Care and Use Committee at FCCC (IACUC # 08-4). Drug-supplemented diet was prepared as described previously[13].

Methods

Experimental design: Inflammation-promoted colorectal neoplasia was induced by administering 2% dextran sodium sulfate (DSS) to female Apc$^{+/Min-FCCC}$ mice in the drinking water as outlined in Figure 1A. At treatment week 0 (8 wk of age), mice were randomized to one of four experimental groups: DSS treatment alone (no plecanatide treatment (control)) or DSS plus 2, 10 or 20 ppm plecanatide in the diet (n = 23/group). All animals were administered DSS for 4 d beginning at treatment week 1 and received regular water for the remainder of the study (Figure 1A). At the end of the study, the entire small intestine and colon were excised, cut longitudinally and rinsed with saline. An equivalent strip of the small intestine and colon from each animal was snap frozen for molecular analysis of UG and GC-C transcript levels by quantitative reverse-transcription polymerase chain reaction (RT-PCR). The remainder of the colon was fixed in 10% formalin overnight, cross-sectioned at 2 mm intervals and processed for histopathological review.

Histopathological analyses: Sections stained with H and E were histopathologically evaluated for neoplasia in a blinded manner, as described previously[20]. All classifications were based on standardized morphology and nomenclature for the human pathology of inflammation-promoted colorectal neoplasia[21]. A diagnosis of carcinoma was assigned when neoplastic glands had invaded into the muscularis mucosae or beyond. Any dysplasia or cancer exhibiting an elevated growth pattern was considered polypoid. Non-polypoid (flat) lesions were elevated less than 2-fold above the adjacent non-neoplastic colorectal mucosa. Lesions that could not be readily classified as either polypoid or non-polypoid were categorized as indeterminate.

Immunohistochemistry: Ki-67 was selected as a biomarker of cell proliferation. Antigen retrieval was

performed prior to staining in a Ventana Benchmark XT automated stainer (Tucson, AZ). All buffers and washes were per standard XT protocol. For Ki-67 staining, sections were incubated with Ki-67 primary antibody (1:1500 dilution; Vector Laboratories, Inc., Burlingame, CA) for one hour at room temperature. Negative controls were processed with rabbit IgG at approximately the same protein concentration as the primary antibody. Staining was detected using a rabbit secondary antibody kit (Vector Laboratories, Inc.) according to the manufacturer's instructions. All sections were counterstained with lite hematoxylin. Only cells with nuclear staining of Ki-67 were considered positive. The number of Ki-67 positive cells in dysplasias (2 fields/dysplasia, 600 ×) and non-neoplastic colonic crypts (20 crypt columns/animal, 600 ×) were counted and recorded as a Ki-67 labeling index (number of positive cells/total number of cells evaluated). The rate of proliferation of each tumor was established and the mean rate of all tumors in the treatment group was calculated.

The cellular localization of β-catenin was determined using specific polyclonal antibodies (1:4000 dilution; Sigma, St Louis, MO). Negative controls were processed with rabbit IgG. Staining was detected using a goat anti-rabbit secondary antibody kit (Vector Laboratories, Inc.) as per the manufacturer's instructions. Sections were counterstained with lite hematoxylin. The number of tumor cells with nuclear localization of β-catenin was counted and expressed as a percentage of the total number of tumor cells per field (400 ×).

Immunoblotting: One centimeter long colon tissues from 6 animals per group were pooled and homogenized in RIPA buffer (10 mmol/L Tris, pH 7.2; 150 mmol/L NaCl, 1% sodium deoxycholate, 1% triton × 100, 0.1% SDS, 0.1 mmol/L Na_3VO_4; 50 mmol/L NaF), containing a protease inhibitor cocktail (Boehringer Mannheim, GmbH, Germany). The homogenate was centrifuged at 12000 g for 15 min at 4 ℃ and the supernatant was used as crude tissue lysate. The crude lysates (approximately 50 g protein) were subjected to 10% SDS-PAGE under reducing conditions, followed by immunoblotting with antibodies specific for β-catenin (1:4000 dilution, Sigma), PKG-Ⅱ (1:200, dilution Santa Cruz Biotechnology, Inc., CA), glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 1:10000 dilution, Ambion), GC-C (1:500, Santa Cruz Biotechnology Inc., CA), phospho-VASP and c-myc (1:1000, Cell Signaling, MA, United States), cyclin D1 (1:1000, Abcam, MA, United States), and β-actin (1:1000, Chemicon, CA). Blots were developed using the ECL plus Western Blotting Detection System (GE Healthcare, Buckinghamshire, United Kingdom) or LiCor blotting system. The resulting images were analyzed using the FluorChem E system (Cell Biosciences, Santa Clara, CA).

Measurement of cyclic GMP in tissue lysates: Colon tissues (1 cm) from 6 animals per group were pooled to prepare crude tissue lysates. The levels of cGMP were determined using an ELISA kit (Cayman Chemical Co., Ann Arbor, MI)[13]. The protein concentration of the lysates was determined using the Pierce BCA protein assay (Thermo Fisher Scientific, Rockford, IL). Results were normalized per mg protein and expressed as mean pmol ± SEM.

Quantification of UG and GC-C transcript levels by RT-PCR: Representative areas of the proximal and distal small intestine and colon were randomly selected from DSS control and DSS + plecanatide-treated mice (n = 4-5/group) for analysis. Total RNA was extracted from 5-10 mg of tissue using the RNAqueous®-4PCR Kit (Ambion, Austin, TX) and quantified using a Nanodrop2000 (Thermo Fisher, Waltham, MA). Total RNA (1 μg) was reverse-transcribed to cDNA using a High Capacity RNA-to-cDNA Kit (Applied Biosystems, Carlsbad, CA), according to the manufacturer's instructions. Quantitative RT-PCR amplification and analysis were carried out using a LightCycler480 (Roche, Basel, Switzerland), UG and GC-C specific TaqMan reagents (Integrated DNA Technologies, Coralville, IA) and RT-PCR Master Mix (Roche, Basel, Switzerland). All amplification reactions (20 L total volume) were performed in duplicate with 10 ng cDNA (based on input RNA) and subjected to 35 PCR cycles using parameters set by the manufacturer. GAPDH was used as an endogenous control. The data generated were analyzed and expressed as target gene expression relative to endogenous control, using the comparative Ct method and the $2^{-\Delta\Delta Ct}$ formula. Results are expressed as fold change in relative levels of UG or GC-C transcripts per segment of small intestine or colon of plecanatide-treated mice, as compared to those of control mice treated with only DSS.

DSS treatment to induce acute inflammation in $Apc^{+/Min\text{-}FCCC}$ mice: An independent study was designed to evaluate the effect of plecanatide on GC-C signaling and cytokine expression during the acute phase of colonic inflammation. $Apc^{+/Min\text{-}FCCC}$ mice (n = 12; 6-8 wk old) were administered 2% DSS in the drinking water for 4 d followed by 3 d of regular water. Starting on day 1, six animals received an oral gavage of plecanatide (2.5 mg/kg body weight) daily while the other six were administered vehicle (0.9% sodium chloride solution, Sigma, St. Louis, MO). Vehicle control animals (n = 6) received regular drinking water (no DSS) and were administered an oral gavage of saline daily. It should be noted that the amount of plecanatide delivered by a single oral gavage at this dose is similar to that ingested daily when animals were administered a diet supplemented with 10 ppm plecanatide in the main tumorigenesis study. Mice were euthanized on day 7, and the entire colon was excised. Part of the colon tissue from each animal was snap frozen for analysis of intracellular cGMP and determination of the expression of GC-C, PKG-Ⅱ, p-VASP and β-actin by immunoblot.

The remaining tissue was used immediately for explant cultures as described below.

Explant culture: Tissues were washed in PBS containing 100 units of penicillin, 0.1 mg streptomycin and 0.25 μg amphotericin B per milliliter (1 x antibiotic and anti-mycotic solution; Sigma, St. Louis, MO) and cut into 1 cm pieces. Tissue explants were cultured in a 24-well plate overnight in RPMI 1640 media (Mediatech, Manassas, VA) in the absence or presence of 1 μmol/L plecanatide at 37 ℃ in a CO_2 incubator. After the incubation period, explants were snap frozen and stored for analysis of cyclin D1, c-myc and β-actin expression by immunoblot.

Cytokine analysis: Expression of select mouse cytokines was detected in the spent media of pooled explant cultures (n = 6/pool) using a mouse Proteome Profiler Panel A kit array panel (R and D Systems, Minneapolis, MN). The mean intensities of the dot blots were calculated using ImageJ software (NIH).

Statistical analysis

Wilcoxon 2-sample test and analysis of variance (ANOVA) were used to compare differences between groups such as body weight, lesion multiplicity, and lesion type. Student's t test was used to evaluate differences in cGMP, Ki-67 labeling index, expression of total and nuclear β-catenin, cytokines, PKG-Ⅱ protein, UG and GC-C transcript levels.

RESULTS

Plecanatide reduced inflammation-promoted dysplasia in Apc$^{+/Min\text{-}FCCC}$ mice

All experimental treatments were well tolerated. The rate of survival was: 91% for mice in the control group, 83% for mice administered 2 ppm plecanatide, and 96% for mice receiving diet supplemented with either 10 or 20 ppm plecanatide. As observed previously in this model[22], body weights declined immediately following DSS treatment and increased gradually thereafter in all groups (Figure 1B). The body weights of mice treated with DSS + plecanatide increased more rapidly than did those of mice receiving DSS + vehicle. Tissues were also scored for the degree of inflammation (data not shown). Consistent with our previous experience in this animal model, the inflammation scores were generally low, most likely due to anticipated resolution of the colonic inflammation by the end of the study.

The effect of plecanatide on the multiplicity of polypoid, flat, indeterminate and total colonic dysplasias was determined (Figure 2). While the multiplicity of all morphological subtypes of colon lesions in mice treated with 2 ppm plecanatide did not differ from that of controls, a reduction in the multiplicity of each of the subtypes of dysplasia was observed in mice treated with higher doses of the drug. For example, 10 and 20 ppm plecanatide reduced polypoid dysplasias (Figure 2A), with a statistically significant reduction (approximately 40%) observed at a dose of 20 ppm, as compared to the control group (P = 0.05). A similar reduction in the multiplicity of non-polypoid/flat (P = 0.041) and indeterminate (P = 0.05) dysplasias was observed post treatment, but only in mice receiving 10 ppm plecanatide (Figure 2B and C). Surprisingly, the higher dose of plecanatide (20 ppm) did not produce an appreciable reduction in the multiplicity of either flat or indeterminate colonic dysplasias. A statistically significant reduction (P = 0.028) in total colonic dysplasia was observed in mice administered 10 ppm of plecanatide in the diet (Figure 2D). As previously observed with oral UG treatment[13], plecanatide also reduced the multiplicity of small intestinal tumors (data not shown). Since suppression of polyp formation by oral treatment with GC-C agonists has now been well-established[13,23], this study focused only the effect of orally administered plecanatide on the multiplicity of colonic dysplasias. Therefore, subsequent analyses were performed on colon tissues from mice treated with 10 ppm plecanatide.

Plecanatide mediated activation of GC-C signaling

Although UG, GN, and other GC-C related agonists are known to stimulate production of cGMP *via* activation of GC-C, in epithelial cells lining the GI tract, and cultured T84 and Caco-2 cells[12,24,25], it was of interest to evaluate if orally administered plecanatide could stimulate cGMP production within the murine colon. Acute inflammation was induced in mice as described in the Materials and Methods section. Colon tissues (1 cm piece) from 6 mice within a treatment group were pooled, homogenized, and crude lysates were used to measure cGMP. A significant reduction in the cGMP levels was observed in colon tissues from DSS-treated animals as compared to vehicle controls (P = 0.01). Oral treatment with plecanatide (2.5 mg/kg) completely restored the DSS-mediated reduction in cGMP levels (Figure 3A).

PKG-Ⅱ, a cGMP-dependent protein kinase, is activated by cGMP following activation of GC-C by its agonists. PKG-Ⅱ is expressed on epithelial cells lining the GI tract and undergoes auto-phosphorylation upon activation of GC-C signaling[26]. Colon tissues from mice with DSS-induced inflammation were homogenized and lysates were subjected to immunoblotting with antibodies specific for phosphorylated-vasodilator-stimulated phosphoprotein (p-VASP), PKG-Ⅱ and GC-C (Figure 3B). The p-VASP antibody detects only Ser^{239} phosphorylated VASP. Activated PKG-Ⅱ expression, seen on blots as a partially resolved doublet, was considerably higher in colon tissues from mice treated with both DSS and plecanatide as compared to those treated with only DSS. The level of p-VASP was also much higher in colon tissues from DSS + plecanatide treated mice as compared to that observed in tissues from DSS treated mice. Colonic expression of GC-C was comparable among all

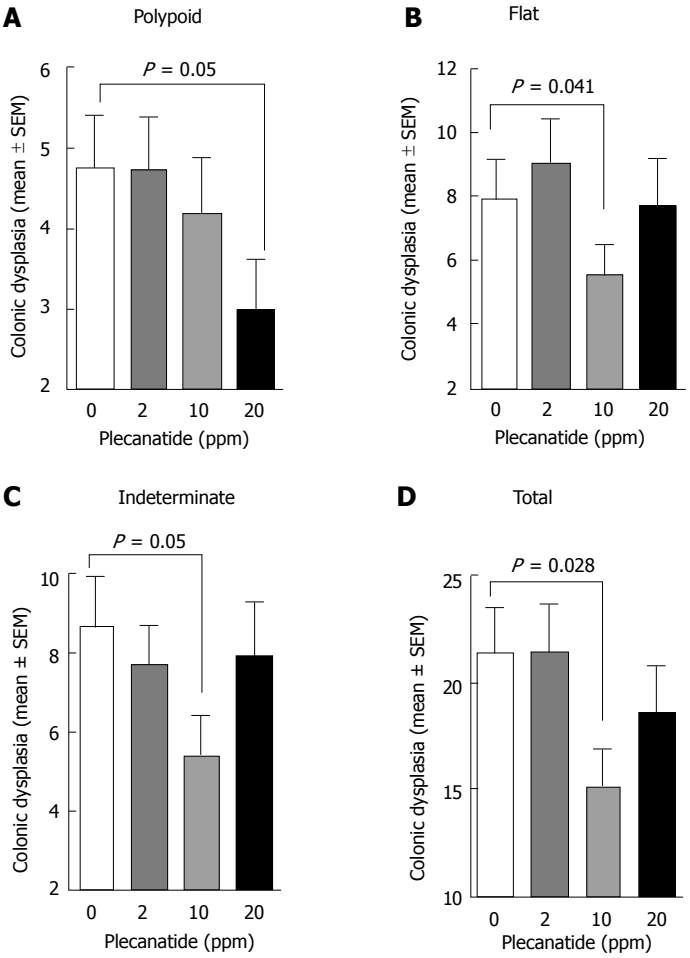

Figure 2 Treatment with plecanatide inhibits inflammation-associated colonic dysplasia in dextran sodium sulfate-treated Apc$^{+/Min\text{-}FCCC}$ mice. Analyses revealed the number of pathologically confirmed polypoid (A), flat (B), indeterminate (C) and total (D) dysplasias within the colon of DSS-treated mice following administration (7 wk) of either control diet or diet supplemented with varying doses of plecanatide (n = 23/group). Wilcoxon 2-sample test and analysis of variance (ANOVA) were used to compare the multiplicity of dysplasias in independent groups. A P value $\leqslant$ 0.05 was considered statistically significant. DSS: Dextran sodium sulfate

cohorts, irrespective of the treatment group (Figure 3B). Collectively, these results suggest that GC-C signaling is activated in the colon by orally administered plecanatide.

Plecanatide reduces proliferation of colonic epithelial cells

Immunohistochemical staining of Ki-67 was performed using non-neoplastic (normal) and neoplastic mucosa from the colons of mice treated with DSS or DSS + plecanatide (10 ppm). As depicted in Figure 4A, plecanatide reduced the number of Ki-67 positive epithelial cells in both the non-neoplastic and neoplastic mucosa. However, the reduction in Ki-67 labeling only achieved significance in the neoplastic mucosa (P < 0.001). Similarly, although the number of caspase-3 positive cells was increased in both non-neoplastic and neoplastic colon tissue, the elevation was significant (P = 0.05) only in plecanatide treated non-neoplastic tissue (data not shown). It should be noted that endogenous UG expression and GC-C signaling-mediated regulation of epithelial cell homeostasis is not altered in the normal epithelium. In addition, the rate of cell proliferation is also much higher in tumor tissue as compared to the normal epithelium. Thus, it is possible that oral treatment with plecanatide has a more pronounced inhibitory effect on proliferation in tumor tissue than in the normal epithelium. To further confirm the anti-proliferative activity, crude lysates of colon tissues from mice treated with vehicle, DSS and DSS + plecanatide (2.5 mg/kg) were examined by immunoblotting with antibodies specific for c-myc and cyclin D1 (markers of proliferation) (Figure 4B). Plecanatide reduced levels of c-myc and phosphorylated cyclin D1 (slower moving band) in colon tissues as compared to treatment with either vehicle or DSS alone. Taken together, these results suggest that plecanatide reduces proliferation of epithelial cells lining the GI mucosa.

A statistically significant reduction in the levels of total β-catenin was observed in colon tissues from mice administered 10 ppm plecanatide in the diet as compared to control DSS-treated mice. The densitometry of the blot is shown in Figure 5A. Consistent with our previous report[18], immunohistochemical staining revealed membranous localization of β-catenin within the non-neoplastic colonic mucosa. Strong cytoplasmic and nuclear staining of β-catenin staining was observed in colonic dysplasias from DSS-treated mice (panel Ⅰ, Figure 5B). Treatment with plecanatide (10 ppm) reduced nuclear staining of β-catenin, with a concomitant increase in its localization to the membrane of neoplastic cells (Figure 5B panel Ⅱ). The densitometric analysis revealed that plecanatide treatment reduced β-catenin levels in the nucleus by 41% (P = 0.02) as compared to analogous neoplastic regions within the

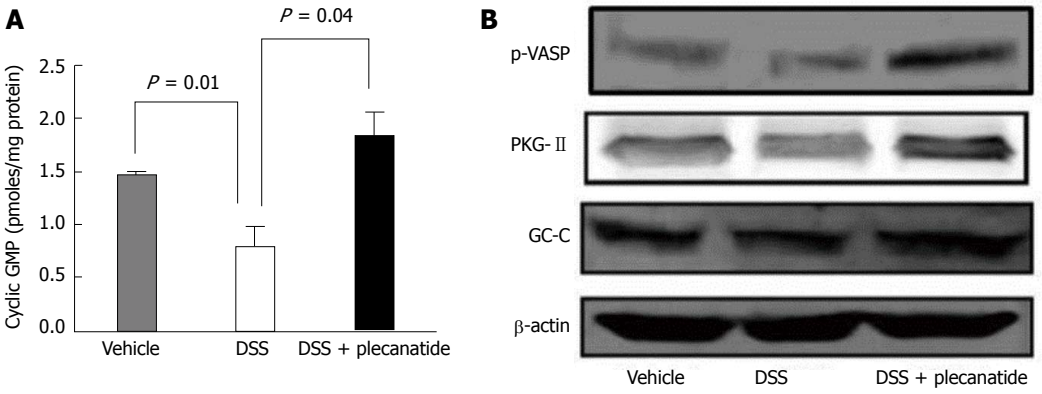

Figure 3 Orally administered plecanatide activates guanylate cyclase-C signaling within the colon. Effect of plecanatide on (A) stimulation of cGMP production and (B) expression of phosphorylated VASP, PKG-Ⅱ and GC-C in colon tissues from Apc$^{+/Min-FCCC}$ mice with DSS-induced acute inflammation (n = 6/group). Mice with acute inflammation were administered plecanatide (2.5 mg/kg) by oral gavage; a dose equivalent to that ingested daily by animals fed a diet supplemented with 10 ppm plecanatide in the main tumorigenesis study. Colon tissue (1 cm) from 6 animals per group was pooled to prepare cell lysates. Intracellular cGMP levels depicted in (A) are expressed as pmoles/mg protein ± SEM. Student t test was used to evaluate differences in cGMP between treatment groups. P values ≤ 0.05 were considered statistically significant. Representative Western blot analyses of phospho-VASP, PKG-Ⅱ and GC-C were performed using appropriate antibodies. To demonstrate equivalent protein loading for each condition, membranes were probed subsequently with β-actin antibody. GC-C: Guanylate cyclase-C; GMP: Guanosine monophosphate; p-VASP: Phospho-vasodilator-stimulated phosphoprotein; PKG-Ⅱ: Protein kinase G-Ⅱ.

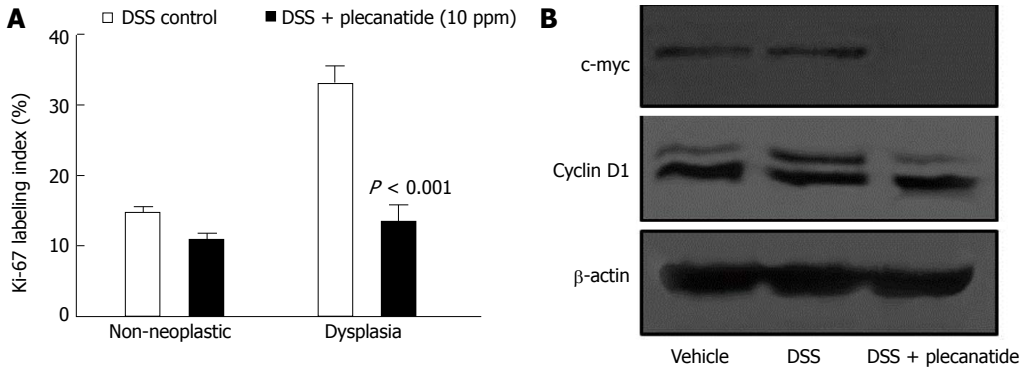

Figure 4 Effect of plecanatide on markers of proliferation in colonic epithelial cells from Apc$^{+/Min-FCCC}$ mice with dextran sodium sulfate-induced inflammation. A: Normal (non-neoplastic) and neoplastic colon tissues from mice treated with DSS only and DSS + plecanatide (10 ppm) were stained with antibodies specific for Ki-67. Nuclear staining of Ki-67 (positive) was recorded as a labeling index (number of positive cells/total number of cells evaluated; mean ± SEM). Statistical comparisons between DSS control and DSS plus plecanatide-treated groups (n = 7-9 mice per group) were performed using the Student t test. A P value ≤ 0.05 was considered significant; B: Colon tissue (1 cm) from 6 animals per group was pooled to prepare cell lysates. A representative immunoblot demonstrating the effect of plecanatide on expression of c-Myc and cyclin D1 is shown. β-actin was used to normalize protein loading. DSS: Dextran sodium sulfate.

colons of DSS-treated mice (Figure 5B, panel Ⅲ).

Plecanatide treatment restores UG expression

Consistent with previous reports that expression of UG is reduced dramatically in inflamed tissues from colitic mice and in colon biopsies from IBD patients[13,27], UG transcript levels were also reduced significantly in intestinal tissues following DSS treatment as compared to those of vehicle-treated mice (data not shown). Since administration of plecanatide reduces GI inflammation[16] and the multiplicity of colonic dysplasias in colitic mice, it was important to determine if plecanatide treatment increased transcript levels of UG and GC-C following amelioration of GI inflammation in intestinal tissues from Apc$^{+/Min-FCCC}$ mice. UG and GC-C expression was determined in the proximal and distal segments of the small intestine and colon by quantitative RT-PCR (Figure 6A). UG expression was increased significantly within the proximal small intestine and proximal colon of animals receiving oral plecanatide. A similar increase in UG expression was observed in the distal small intestine, but did not achieve statistical significance. It should be noted that the relative expression of UG is known to be extremely low in the distal colon as compared to that in the proximal region of the small intestine[24]. Thus, accurate quantitative measurement of UG expression in the distal colon segment may be compromised by low endogenous levels. No appreciable change in relative levels of GC-C transcripts was observed in either the small intestine or colon following plecanatide treatment (Figure 6B).

Plecanatide downregulates pro-inflammatory cytokines in colon explants

Induction of colonic inflammation with DSS is known to increase the production of proinflammatory cytokines

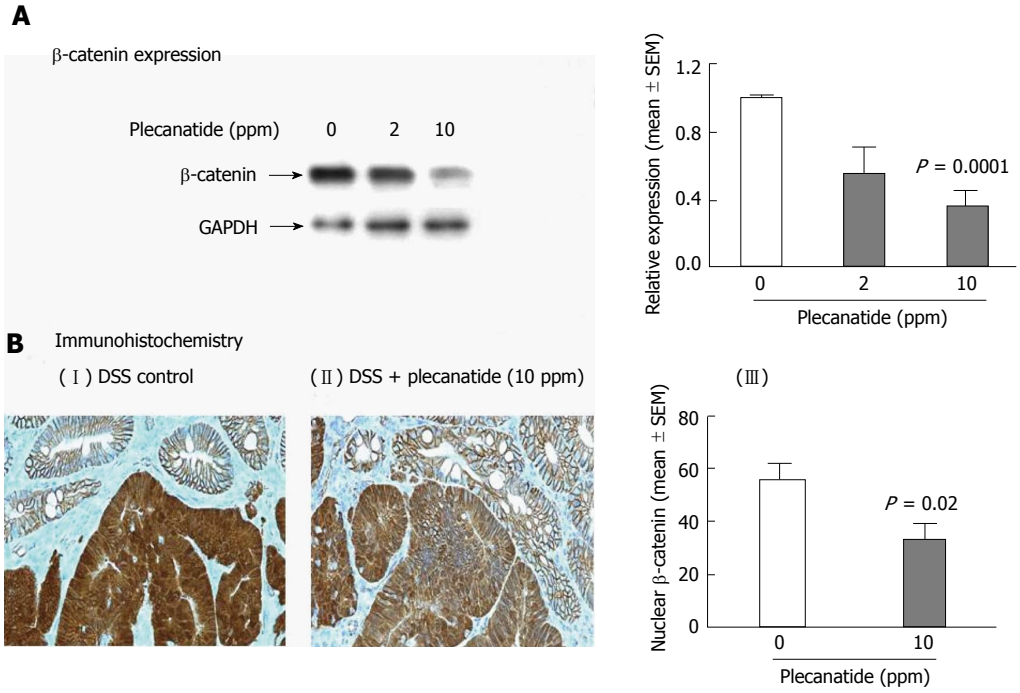

Figure 5 Total β-catenin expression is reduced within the colon of Apc$^{+/Min-FCCC}$ mice treated with dextran sodium sulfate plus plecanatide. A: Western blot analysis and the associated densitometric quantification of levels of total β-catenin expression (mean ± SEM) within the colon; B: Immunohistochemical localization of β-catenin within the colonic mucosa. Membranous localization of β-catenin was observed within the normal colonic mucosa irrespective of the treatment group, while cytoplasmic and nuclear β-catenin staining predominant in adenomas from DSS-treated mice (panel Ⅰ). Plecanatide treatment caused a significant reduction in nuclear staining of β-catenin in dysplasias, while the cell membranes exhibited enhanced protein localization (panel Ⅱ). The number of tumor cells with nuclear localization of β-catenin was counted in distal colon tumors (n = 7-9 mice/group) and expressed as a percentage of the total number of tumor cells per 400 X field (panel Ⅲ). Statistical comparisons between DSS control and DSS plus plecanatide-treated groups were performed using the Student's t test. A P value of ≤ 0.05 was considered significant. DSS: Dextran sodium sulfate; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; .

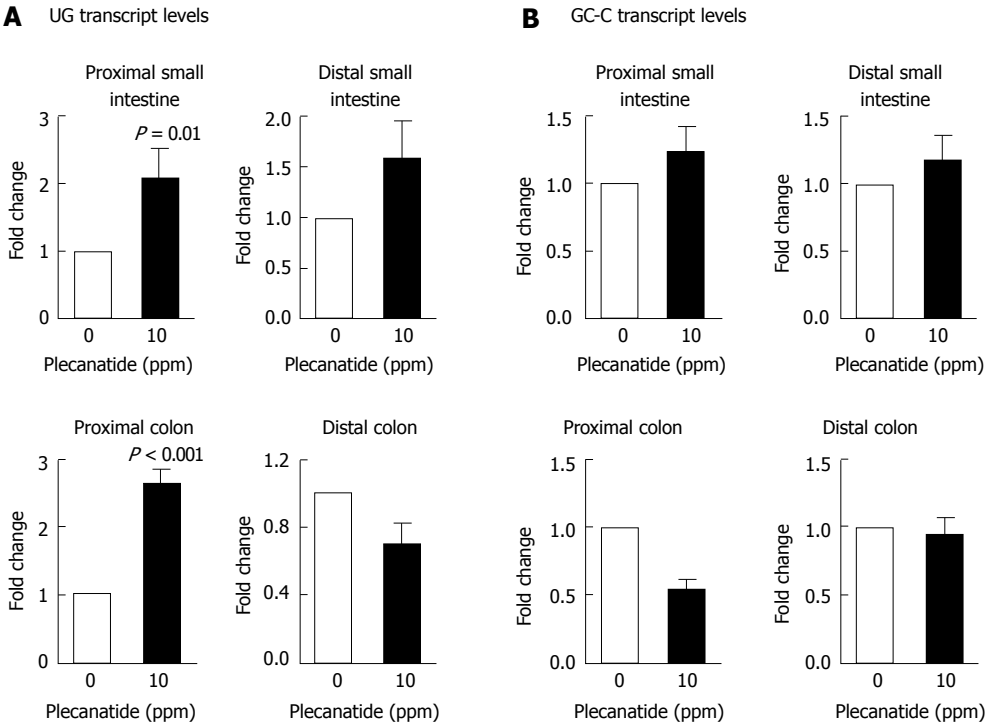

Figure 6 Effect of plecanatide on expression of uroguanylin and guanylate cyclase-C. The relative levels of UG (A) and GC-C (B) transcripts in the small intestine and colon of DSS-treated and DSS + plecanatide treated Apc$^{+/Min-FCCC}$ mice (n = 5-6/group) were determined by quantitative RT-PCR and normalized to those of GAPDH in the same sample. Transcript levels are expressed as fold change (mean ± SEM) as compared to control samples treated with only DSS. Student's t-test was used to evaluate statistical differences between DSS control and plecanatide-treated mice. A P value of ≤ 0.05 was considered significant. GC-C: Guanylate cyclase-C; UG: Uroguanylin; DSS: Dextran sodium sulfate.

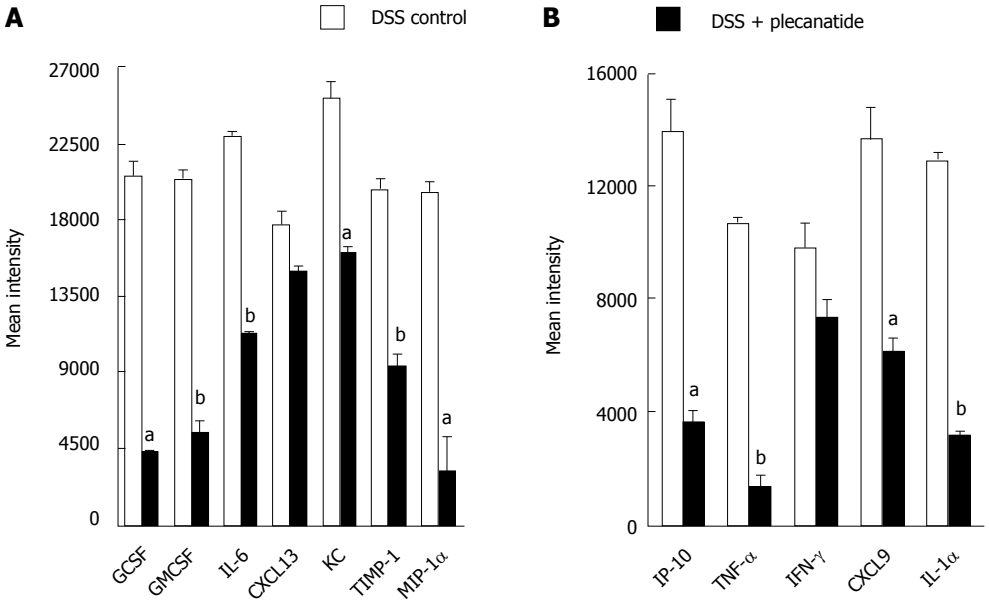

Figure 7 Effect of plecanatide on cytokine expression. Expression of cytokines, chemokines and growth factors was measured in the spent media of colonic explant cultures derived from mice treated with DSS only or DSS + plecanatide. Murine cytokines, chemokines and growth factors were measured in pooled supernatants (n = 6/pool) using membranes coated with specific capture antibodies (Proteome Profiler Panel A kit; R and D Systems, Minneapolis, MN). Immunoblots were scanned using Image J software. The data represent the mean intensity of cytokine/chemokine levels in colonic samples derived from untreated and plecanatide-treated mice. Student's t test was used to evaluate statistical differences in the secretion of cytokines/chemokines between DSS control and DSS + plecanatide treated samples. ^{a}P value $\leqslant$ 0.05, $^{b}P \leqslant$ 0.01; *vs* DSS control.

and growth factors in mice[28]. Thus the effect of plecanatide treatment on activation of GC-C signaling and on secretion of select pro-inflammatory cytokines, chemokines and growth factors was examined in colon explants from Apc$^{+/Min\text{-}FCCC}$ mice with acute inflammation. Consistent with the *in vivo* results shown in Figure 3, plecanatide treatment activated GC-C signaling, increased cGMP production and restored levels of PKG-II in colon explants (data not shown), confirming that orally administered plecanatide is able to activate GC-C signaling within the colon. Next, we determined the effect of plecanatide treatment on the secretion of cytokines and growth factors in explant cultures of colon tissues from mice in the above study. Analysis of the supernatant from pooled explant cultures using a mouse cytokine array revealed a significant reduction in secretion of pro-inflammatory cytokines (IL-1α, IL-6 and TNFalpha), chemokines (IP-10, MIP-1α, KC and CXCL9) and growth factors (GCSF and GMCSF) in DSS + plecanatide treated mice as compared to mice treated with DSS alone (Figure 7). These data are consistent with the anti-inflammatory activity of plecanatide in experimental models of murine colitis[16].

DISCUSSION

This is the first study to demonstrate that oral treatment with plecanatide reduces the multiplicity of DSS-promoted colonic dysplasias in mice; a well-established model for studying inflammation-associated colorectal carcinogenesis[29]. Results presented here demonstrate that Apc$^{+/Min\text{-}FCCC}$ mice treated with DSS exhibit an increased multiplicity of colonic dysplasias and that oral treatment with plecanatide produces a statistically significant reduction in inflammation-induced dysplasia in these mice. Although the multiplicity of all morphological subtypes of colonic dysplasias was reduced in mice exposed to plecanatide, the dose required to achieve tumor inhibition varied. For example, plecanatide reduced the formation of polypoid dysplasias in a dose-dependent manner, with an approximate 40% reduction observed at 20 ppm. On the other hand, a similar magnitude of reduction in the multiplicity of flat and indeterminate colonic dysplasias was observed at 10 ppm, with no appreciable inhibition recorded at a higher dose (20 ppm). The chemopreventive effect of NSAIDs and cyclooxygenase-2 inhibitors against inflammation-driven colorectal carcinogenesis has been evaluated in several animal models including Apc$^{+/Min}$ mice. While the reduction in colonic tumors in these studies was approximately 50%[29], it is noteworthy that the observed approximately 40% reduction in dysplasia following plecanatide treatment, is comparable to the result obtained from a meta-analysis of 5-ASA in a clinical setting (37%-49% reduction in neoplasia)[30]. Our results, indicating a differential response of lesion types to plecanatide, are consistent with previous observations suggesting that flat and polypoid dysplasias arise *via* distinct genetic mechanisms[31,32] and respond differently to prophylactic therapies[8]. In addition, a differential response of polypoid and flat dysplasias to celecoxib (Celebrex®) has also been observed[32].

The lack of an observed plecanatide dose response

is consistent with the results of several animal studies examining the effect of plecanatide and dolcanatide (another UG analog) on amelioration of colitis in mice[16]. Orally administered plecanatide or dolcanatide activates GC-C receptors and produces fluid distention only in the duodenum and jejunum[33], suggesting that they act primarily in the proximal intestine to stimulate fluid secretion. Therefore, a higher dose of plecanatide may lead to excess fluid production in the proximal intestine, resulting in dilution of the orally administered plecanatide prior to reaching the colon segments. It should also be noted that plecanatide acts locally by binding to GC-C expressed on epithelial cells lining the luminal surface of the GI tract and its systemic absorption is not needed to produce a pharmacological effect. Thus, lack of a dose response could also be due to saturation of the GC-C that is available on the luminal surface of the GI mucosa. In addition, results from animal studies conducted by this group with orally administered 5-ASA demonstrate that higher doses do not confer greater protection from the formation of colitis-associated tumors[8]. Thus, development and optimization of a new formulation of plecanatide that bypasses the proximal intestine is warranted to not only decrease fluid secretion, but also enhance its chemoprotective activity. A longer duration of exposure to plecanatide would also allow more time for the colonic mucosa to heal and could potentially enhance chemopreventive response to treatment.

Activation of GC-C signaling by its ligands is associated with an increase in cGMP, a decrease in cyclin D1, delayed cell cycle progression and reduced DNA synthesis[34-36]. Importantly, oral administration of cGMP restored crypt proliferative homeostasis and reduced proliferation (Ki-67 positive cells) in the crypts, but not in the villi of *Gucy2c*$^{-/-}$ mice[36]. In addition, normal functioning of GC-C signaling appears to also regulate the balance between proliferation and differentiation in the intestinal epithelium[37]. In this context, GC-C signaling plays a key role in organizing the crypt-surface axis, restricting the depth of the crypts and the number of proliferating cells and regulating the rate of cell cycle progression through the G1-S transition[23]. Consistent with these findings, UG and *E. coli* ST inhibit the proliferation of T84 and Caco-2 colon carcinoma cells[13,14]. Data from the present study demonstrating that plecanatide reduced levels of β-catenin, c-myc and cyclin D1 as well as nuclear and total β-catenin provide further support for these findings. Of relevance, treatment with dolcanatide also reduced transcript levels of c-myc, cyclin D1 and Birc5 (survivin) in T84 cells[38]. This study suggests that orally administered plecanatide may act *via* cGMP/GC-C signaling to mediate downregulation of Wnt/β-catenin signaling within the colon.

The ability of plecanatide to retard the progression of inflammation-associated colorectal neoplasia represents an extension of our prior findings, demonstrating that oral treatment with UG suppressed polyp formation in Apc$^{+/Min}$ mice[13] and plecanatide ameliorated colitis in mice[16]. It should be noted that the expression of UG is reduced significantly in inflamed tissue from IBD patients as well as in colon tissue from mice with colitis[13,27,39]. These findings suggest that loss of UG expression, in the presence of key mutations in the *APC* gene, results in dysregulation of GC-C signaling and in downstream activation of the Wnt/β-catenin pathway. The resulting transactivation of genes responsible for hyperproliferation and anti-apoptotic mechanisms may be the quintessential events during the early stages of neoplastic transformation in colonocytes. In this context, silencing of GC-C signaling is typically associated with early loss of APC heterozygosity and subsequent AKT-mediated inhibition of apoptosis; a potential trigger for neoplastic transformation in colonocytes[23,36]. These reports also suggest that loss of GC-C signaling could be associated with increased susceptibility to intestinal carcinogenesis in mice. However, the possibility that the observed reduction in polyp formation occurs *via* a non-GC-C mechanism[40] cannot be completely ruled out. Transcription of the GC-C gene can be regulated by β-catenin/TCF signaling[40]. Interestingly, treatment with *E.coli* ST peptide stimulated duodenal HCO_3^- secretion, *albeit* at a reduced level, in GC-C$^{-/-}$ mice[41], suggesting the existence of a non-GC-C mechanism, possibly involving a UG/ST receptor yet to be identified.

A known human kindred mutation that causes altered expression of GC-C and presents clinically as bowel dysfunction in Norwegian families has been reported[42]. This GC-C "gain-of-function" mutation in infants of these families leads to chronic diarrheal diseases, often accompanied by electrolyte imbalance, dehydration, metabolic acidosis and ileal inflammation. Two additional kindred mutations in *GUCY2C* were reported in two unrelated Bedouin families, where the "loss-of-function" of GC-C was associated with meconium ileus[43]. Although there is no mention of an association of these kindred mutations with increased susceptibility to colon cancer, deregulated GC-C signaling early in life may be the key event that increases susceptibility to intestinal inflammation and eventually colon cancer.

The precise cause for downregulation of UG and GN and early dysregulation of GC-C signaling during inflammation and colon carcinogenesis remains largely unexplored. Nevertheless, it is known that the genes encoding endogenous GC-C ligands UG and GN are located on chromosome 4 in mice and 1p34-35 in humans, a region lost frequently during human colon carcinogenesis[44-46]. Our results, albeit preliminary, suggest that the level of UG transcripts in the small intestine and proximal colon increase following treatment with plecanatide. One possible explanation for the restoration of UG expression is the mucosal healing that eventually follows inhibition of inflammation and/or colorectal dysplasia. It should be noted that UG transcripts levels were measured in intestinal tissue samples comprised of both neoplastic and adjacent

normal tissue. Since the expression of UG is lost in colon polyps and tumors but not in the surrounding normal colonic mucosa[13], measurement of UG and GC-C transcript levels could be influenced by the number of tumors present, the severity of inflammation, and the proportion of normal tissue in the sample. A more accurate comparative analysis of UG and GC-C expression in microdissected inflamed, normal *vs* tumor tissue will be needed in the future. It is known that UG is predominantly expressed in the small intestine and proximal colon, whereas GN expression is more abundant in the colon[24]. An analysis of GN transcript levels in colon tissue would be useful to better understand the cooperative functions of UG and GN within the colon. Another limitation in this study is that expression of UG was examined only at the transcriptional level and not at the protein level. Antibodies specific for UG are being generated currently for immunohistochemical analysis of UG expression in normal, inflamed and tumor tissue; studies that are anticipated to provide new insight into the molecular basis for the disruption of GC-C signaling during colon carcinogenesis.

In summary, results from the present study suggest that administering plecanatide to overcome a deficiency in endogenous GC-C ligands ameliorates inflammation/colitis and delays progression to CRC. These findings represent a new role for GC-C agonists in the prevention of inflammation-associated CRC in humans. Recent clinical studies suggest that plecanatide is a safe and orally active drug candidate, with promising potential for use in the treatment of various GI disorders and diseases[47].

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COMMENTS

Background

Patients with long-standing inflammatory bowel disease (IBD) have a 2 to 8-fold increased relative risk of developing colorectal cancer as compared to the general population. Although prophylactic intervention with 5-aminosalicylate (5-ASA) is considered to be a promising chemopreventive strategy, additional studies are needed to elucidate its utility in IBD-promoted colorectal cancer. Plecanatide is a synthetic analog of the endogenous peptide uroguanylin (UG) and, like UG, is an activator of receptor guanylate cyclase-C (GC-C) signaling cascade that regulates fluid/ion secretion and epithelial cell homeostasis in the gastrointestinal (GI) tract. Oral treatment with plecanatide ameliorates GI inflammation in animal models of experimental colitis. Conceptually, chronic prophylactic intervention with an orally safe and locally-acting agent that not only suppresses inflammation but also regulates renewal of the GI mucosa is desirable.

Research frontiers

Therapeutic intervention with locally acting, minimally absorbed analogs of UG, represents a novel and safe approach for delaying the transition from IBD to colon carcinogenesis.

Innovations and breakthroughs

This is the first report highlighting the therapeutic potential of an orally administered and mucosally active GC-C agonist for delaying the progression of ulcerative colitis to colorectal cancer in humans.

Applications

UG is an endogenous peptide hormone that regulates fluid/ion homeostasis and epithelial cell homeostasis and maintains the barrier function within the GI tract. Several studies have demonstrated that transcript levels of UG and its related peptide guanylin are markedly reduced in inflamed colonic tissues from patients with ulcerative colitis and Crohn's disease, as well as in human colonic polyps and tumors. It can be implied from these findings that the pathogenesis of these diseases might be associated with a deficiency of UG and GN. Oral therapy with plecanatide and other UG analogs could be considered as a replacement therapy to overcome the deficiency underlying the etiology of IBD and delay its progression to colorectal cancer.

Peer-review

This manuscript is well written and illustrated.

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ORIGINAL ARTICLE

Retrospective Study

Lymphocyte-to-monocyte ratio can predict mortality in pancreatic adenocarcinoma

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Abstract

AIM

To determine if the lymphocyte-to-monocyte ratio (LMR) could be helpful in predicting survival in patients with pancreatic adenocarcinoma.

METHODS

We retrospectively reviewed the medical records of all patients diagnosed with pancreatic adenocarcinoma in the VA North Texas Healthcare System from January 2005 to December 2010. The LMR was calculated from peripheral blood cell counts obtained at the time of diagnosis of pancreatic cancer by dividing the absolute lymphocyte count by the absolute monocyte count. A Univariable Cox regression analysis was performed using these data, and hazard ratios (HR) and 95%CI were calculated. The median LMR (2.05) was used to dichotomize patients into high-LMR and low-LMR groups and the log rank test was used to compare survival

between the two groups.

RESULTS

We identified 97 patients with pancreatic adenocarcinoma (all men, 66% white, 30% African-American). The mean age and weight at diagnosis were 66.0 ± 0.9 (SEM) years and 80.4 ± 1.7 kg respectively. Mean absolute lymphocyte and monocyte values were 1.50 ± 0.07 K/μL and 0.74 ± 0.03 K/μL respectively. Mean, median and range of LMR was 2.36, 2.05 and 0.4-12 respectively. In the univariable Cox regression analysis, we found that an increased LMR was a significant indicator of improved overall survival in patients with pancreatic adenocarcinoma (HR = 0.83; 95%CI: 0.70-0.98; P = 0.027). Kaplan-Meier analysis revealed an overall median survival of 128 d (95%CI: 80-162 d). The median survival of patients in the high-LMR (> 2.05) group was significantly greater than the low-LMR group (⩽ 2.05) (194 d *vs* 93 d; P = 0.03), validating a significant survival advantage in patients with a high LMR.

CONCLUSION

The LMR at diagnosis is a significant predictor for survival and can provide useful prognostic information in the management of patients with pancreatic adenocarcinoma.

Key words: Prognosis; Lymphocyte-to-monocyte ratio; Pancreatic adenocarcinoma; Mortality; Biomarker

Core tip: Pancreatic adenocarcinoma is an aggressive malignancy and many patients are presented with aggressive treatment options at diagnosis; often times they are unsure whether they should take a palliative route or a more aggressive approach to their care. Through a retrospective analysis of patients with pancreatic adenocarcinoma, we found that a higher lymphocyte-to-monocyte ratio is associated with improved survival. The lymphocyte-to-monocyte ratio was collected at diagnosis, and is readily available on routine blood work, making it a simple way to help predict and guide treatment in patients diagnosed with pancreatic adenocarcinoma.

Singh G, Nassri A, Kim D, Zhu H, Ramzan Z. Lymphocyte-to-monocyte ratio can predict mortality in pancreatic adenocarcinoma. *World J Gastrointest Pharmacol Ther* 2017; 8(1): 60-66 Available from: URL: http://www.wjgnet.com/2150-5349/full/v8/i1/60.htm DOI: http://dx.doi.org/10.4292/wjgpt.v8.i1.60

INTRODUCTION

The fourth leading cause of death related to cancer is attributed to pancreatic adenocarcinoma in the United States[1], with an overall 5-year survival of 7.2% and 2.4% in patients with metastatic disease[2]. The disease is generally silent in the early stages and is usually detected once patient develops symptoms from local or distant metastasis. Overall, half of the patients are found to have metastatic disease at diagnosis[1]. Risk factors for pancreatic adenocarcinoma include male sex, elderly age, family history, African American race, obesity, diabetes, tobacco use, and chronic pancreatitis[3,4]. The treatment of pancreatic cancer is dependent on stage of disease, and is divided into three categories: Resectable, locally advanced, and metastatic disease. Locally advanced disease can be treated with neo-adjuvant chemotherapy followed by surgical resection. The mainstay of treatment for metastatic disease is palliative chemotherapy[5,6]. There have been numerous advances in oncologic therapeutics, however improvement of survival in patients with pancreatic cancer has been particularly slow[1].

Currently, there is no effective way to predict treatment response and survival at diagnosis aside from stage of disease. For patients with resected cancer, predictors of survival include resection margins, tumor size, and response to chemo-radiation[7,8]. Given the high morbidity and mortality associated with pancreatic cancer, any prognostic information available to risk stratify patients could be beneficial in planning treatment approaches and palliative discussions.

Inflammation and the body's cellular immune response have been shown to play an important role in the pathogenesis of malignancy and its progression from primary to metastatic disease[9,10]. These concepts have led the absolute peripheral blood lymphocyte-to-monocyte ratio (LMR) to act as a surrogate biomarker of prognosis in different malignancies, with several studies showing an association between the LMR and survival in multiple myeloma, diffuse large B cell lymphoma, osteosarcoma, non-small cell lung cancer, and breast cancer[11-15].

The primary aim of this study was to determine if the peripheral blood LMR at the time of diagnosis could be used as a prognostic biomarker in patients with pancreatic adenocarcinoma regardless of treatment modality.

MATERIALS AND METHODS

We identified a cohort of patients diagnosed with pancreatic cancer from the Dallas VA tumor registry at the Veteran's Affairs North Texas Health Care system (VANTHCS) between January 2005 and December 2010. All patients were treated based on the stage of the disease and accepted standard of care treatment protocols which included surgery, chemotherapy, radiation, and palliative stent placement.

We included patients that were only diagnosed with pancreatic adenocarcinoma; other pancreatic tumors such as lymphoma or metastases of other primaries were excluded from the analysis. The study protocol was approved by the VANTHCS Institutional Review

Board.

Data collection

Data collected included variables such as age, sex, race, weight, tobacco use, alcohol use, and medical co-morbidities. Specific variables for pancreatic cancer included age at diagnosis, largest diameter of tumor size seen on cross-sectional imaging and survival time in days (using a cutoff date of 10/11/2014 when the date of death was not available). Lab values including CA 19-9, CEA, white blood cell count, platelets, absolute lymphocyte count, lymphocyte percentage, absolute monocyte count, and monocyte percentage were all collected at or within one week of diagnosis.

LMR

The LMR was calculated by dividing the absolute lymphocyte count by the absolute monocyte count on the same blood draw that was obtained at the initial diagnosis of pancreatic cancer and prior to the initiation of any treatment.

Statistical analysis

Univariable Cox regression statistical analysis was performed to determine if LMR was a predictor of survival in patients with pancreatic adenocarcinoma; hazard ratios (HR) and 95%CI were calculated. A $P < 0.05$ was considered statistically significant. The median LMR was used to dichotomize patients into two groups: Patients with high-LMR and low-LMR. A Kaplan-Meier analysis with log rank test was used to compare survival between the two groups. The association between variables in the subgroups was evaluated by the χ^2 test for categorical variables, the *t* test for continuous variables, or the Fisher's Exact test.

These analyses were performed using SAS (version 9.2 software, The SAS Institute, Cary, NC) and R (version 2.15.1, the R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

The overall baseline demographics, histopathologic characteristics, and stage are outlined in Table 1. There were 109 total patients in the Dallas VA Tumor registry that had any type of pancreatic cancer diagnosed between January 2005 and December 2010. Twelve patients with pancreatic neuroendocrine tumors were excluded. In the final analysis, a total of ninety seven patients with pancreatic adenocarcinoma were included (demographics were 66% white, 30% African-American; all were male subjects).

The stage at presentation was Ⅰ (1%), Ⅱ (24%), Ⅲ (14%), and Ⅳ (61%). Patients had different presenting symptoms including weight loss (53%), jaundice (44%), poor appetite (21%) and abdominal pain (58%). Treatment included surgery (22%), neo-adjuvant therapy (3%) and palliative chemotherapy (37%) (Table 1). Kaplan-Meier survival analysis revealed an overall median survival for patients with pancreatic adenocarcinoma of 128 d (95%CI: 80-162 d).

Table 1 Baseline demographics pancreatic adenocarcinoma (*n* = 97) *n* (%)

Characteristic	Mean ± SEM
Age	66 ± 0.9
Weight at dx (lbs)	80. 4 ± 1.7
LMR	2.36 ± 0.16
CA 19-9	17030.2 ± 8861
CEA	960 ± 657
Tumor size (cm)	4.13 ± 0.2
WBC	9.1 ± 0.5
Platelets	276.8 ± 12.2
ALC K/μL	1.5 ± 0.07
AMC K/μL	0.74 ± 0.03
Race	
White	64 (66)
Black	29 (30)
Other	4 (4)
Location	
Head	64 (71)
Body	10 (11)
Tail	16 (18)
Stage	
I	1 (1)
II	23 (24)
III	14 (14)
IV	59 (61)
Treatment	
Surgery	22 (23)
Stent	38 (39)
Any chemo/rad	44 (45)
Palliative chemotherapy	36 (37)
Neoadjuvant chemotherapy	3 (3)
Adjuvant chemotherapy	10 (10)
Risk factors	
Alcohol	53 (55)
Tobacco	76 (78)

Ninety-three of the 97 patients with pancreatic adenocarcinoma (96%) had absolute peripheral blood lymphocyte and monocyte values available at diagnosis to calculate the LMR. Mean absolute lymphocyte and mean absolute monocyte values were 1.50 ± 0.07 K/uL and 0.74 ± 0.03 K/μL respectively. Mean, median and range of LMR was 2.36, 2.05 and 0.4-12 respectively.

Univariable Cox regression analysis showed that an increased LMR was a significant indicator of improved overall survival in patients with pancreatic adenocarcinoma (HR = 0.83; 95%CI: 0.70-0.98; $P = 0.027$). Moreover, a high LMR in this group was significantly associated with a lower risk of early mortality, *i.e.,* survival < 6 mo (OR = 0.66; 95%CI: 0.46-0.95; $P = 0.025$). The median survival of patients in the high-LMR group (> 2.05) was significantly greater than the low-LMR group (≤ 2.05) (194 d *vs* 93 d; $P = 0.03$) (Figure 1).

To investigate the value of LMR in metastatic disease (stage Ⅳ), a uni-variable logistic regression analysis was performed in this group. There was no significant association between LMR and development of metastatic disease (OR = 0.91; $P = 0.476$). The area under the ROC curve was 0.609 (Figure 2), suggesting that LMR may be a poor marker for the prediction of

Table 2 Clinical variables in patients with high and low lymphocyte-to-monocyte ratio

	LMR ≤ 2.05 (*n* = 50)	LMR > 2.05 (*n* = 43)	*P* value
Chemoradiation	20	21	0.4
Surgery	7	14	0.05
Stent	19	16	1.0
Stage			0.2
Stage 1	0	1	
Stage 2	8	14	
Stage 3	8	5	
Stage 4	34	23	
Location			0.4
Head	35	27	
Body	3	6	
Tail	9	6	
Race			0.05
White	36	25	
Black	11	18	
Other	3	0	
CEA (± SEM)	1075 ± 1041	884 ± 868	0.9
CA 19-9 (± SEM)	24957 ± 17470	10162 ± 3448	0.4
Age (± SEM)	66.6 ± 1.2	65.3 ± 1.4	0.5
Weight (± SEM)	79.3 ± 2.2	82.2 ± 2.5	0.4
Alcohol	27	24	1.0
Tobacco	38	34	0.8

LMR: Lymphocyte-to-monocyte ratio.

metastatic disease.

A uni-variable analysis of demographic and clinical variables between the high-LMR and low-LMR was performed to further characterize factors that could affect survival between the two groups. There was a marginally significant difference in the percentage receiving surgery in the high-LMR groups *vs* low-LMR group ($P = 0.05$) as well as in race between both groups ($P = 0.05$). There was no statistical significant difference between patients receiving chemo-radiation ($P = 0.4$) or stenting ($P = 1$). Furthermore, there was no difference in demographic variables such as age ($P = 0.5$), weight ($P = 0.4$), or risk factors such as tobacco ($P = 0.8$) or alcohol ($P = 1.0$) usage between the two groups. Analysis of clinical variables such as stage at presentation, location of tumor, mean CEA levels, and CA 19-9 levels between both groups did not reveal any significant difference (Table 2).

DISCUSSION

In this study we show that a higher LMR obtained from a peripheral blood count at the time of diagnosis is a predictor of improved survival in patients with pancreatic adenocarcinoma.

The LMR represents the balance between anti-tumorigenic lymphocytes and pro-tumorigenic monocytes, and may reflect the body's immune response to cancer and host-specific cancer aggressiveness. The T-lymphocytes of the native immune system play a vital role in suppressing anti-tumor immune responses and inducing apoptosis in tumor cells; low levels of T-lymphocytes have been implicated in a poor immune response to cancers[9,16,17]. Monocytes have been implicated in tumorigenesis, including differentiation into tumor-associated macrophages that support tumor invasion, angiogenesis and suppression of the body's own immune response against the tumor cells[10,18,19]. Various studies have shown that the lymphocytes have an anti-inflammatory function and their role in impeding progression of tumor may be vital in the immune surveillance of different types of malignancies. Lymphocytes have different roles in identifying and eliminating tumor cells. This phenomenon is sometimes referred to as "immunoediting", and includes a complex interplay of various cells such as the NK cells, the NKT cells, macrophages, CD4 T cells and CD8 T cells. Several studies have shown that high numbers of CD8 T cells within the tumor portend a better prognosis[20]. Further testing of these cells in a study including patients with pancreatic adenocarcinoma revealed FOXP3+ protein on immunohistochemical staining[21]. This was further evaluated in a study, which looked at the lymphocyte density and the correlation with lymph node metastasis. They found out that the presence of FOXP3+ lymphocyte was higher in patients who had a higher histological grade of tumor, lymph node metastasis, and advanced stage tumors (stage Ⅲ and Ⅳ *vs* stage Ⅰ and Ⅱ)[22]. While the studies were not prospective in nature, they do validate the importance of these inflammatory cells in dictating the prognosis of these cancers.

Moreover, cytokines released by lymphocytes have roles in both promoting and suppressing a cancer. Haabeth *et al*[23] conducted a study measuring the cytokine response in mice against cancers (myeloma and B-cell lymphoma). They found that inflammation driven by tumor specific Th1, allowed release of IFN-gamma which stimulated macrophages that were cytotoxic to the cancer cells. The $CD4^+$ Th1 cells also help cytotoxic T cells in tumor rejection. On the other hand, the $CD4^+$ Th2 cells are implicated in production of cytokines leading to B-cell activation. Similarly, Ling et al. showed that high numbers of Th1 lymphocytes in tumor tissue was associated with improved prognosis in patients with colorectal cancer[24].

The prognostic ability of the LMR has been demonstrated in various malignancies[11-15]. However, the exact utility of the LMR for primary pancreatic adenocarcinoma is unclear given the limited data available to date. Li *et al*[25] evaluated the prognostic utility of the LMR in patients with pancreatic adenocarcinoma in the People's Republic of China but only included patients who underwent pancreatic resection and excluded patients who received adjuvant treatment, had significant co-morbid conditions or a life expectancy of < 6 mo. In addition, the preoperative LMR was used and not the LMR at diagnosis. They found that an elevated preoperative LMR was associated with longer survival. Fujiwara *et al*[26] evaluated the postoperative LMR exclusively in patients who received pancreatic

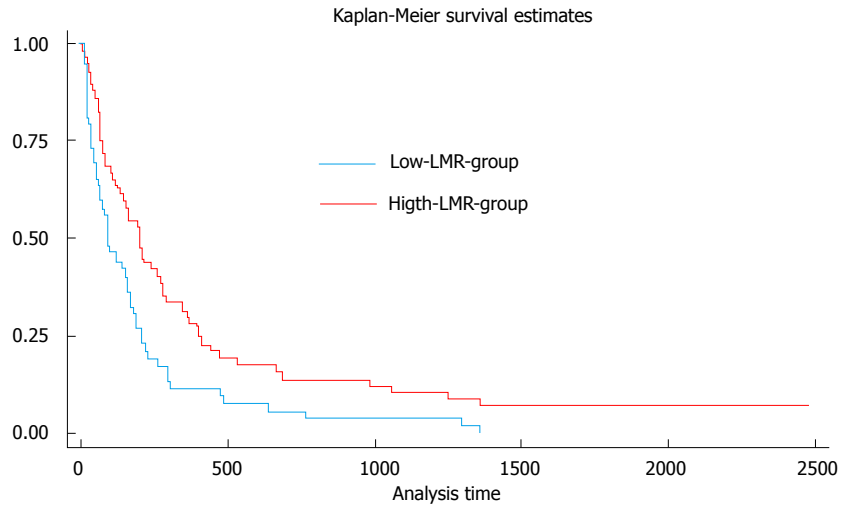

Figure 1 Kaplan meier survival curves of patients with low and high lymphocyte-to-monocyte ratio.

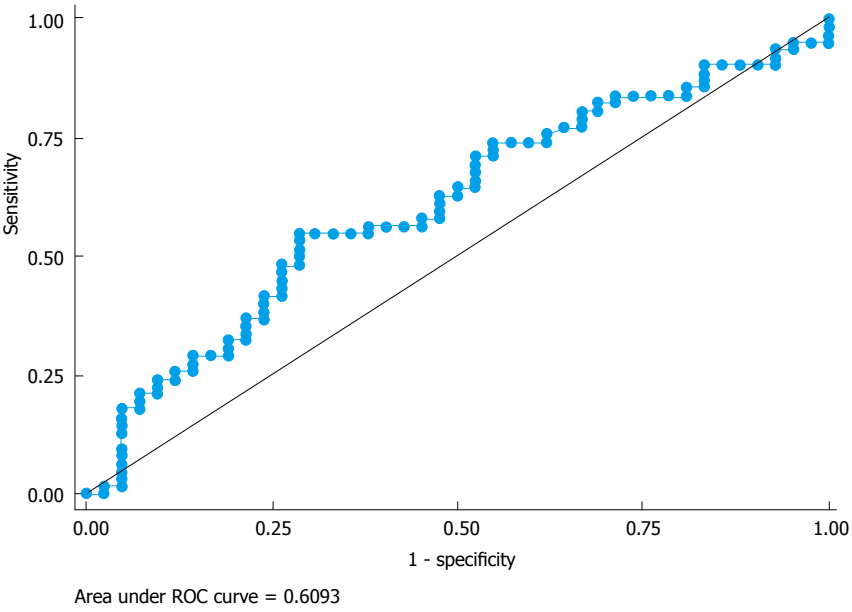

Figure 2 Price rate of change curve of the accuracy of lymphocyte-to-monocyte ratio in prediction of metastatic disease.

resections in Japan. They also reported an association between higher LMR and disease free survival.

In our study we included all adult patients diagnosed with pancreatic adenocarcinoma regardless of co-morbidities, life expectancy, functional status or intervention modality. Our study is the first study evaluating the utility of the LMR in an American cohort and shows that a higher LMR obtained at diagnosis of pancreatic cancer, regardless of the patient's functional status, various clinical factors or patient demographics validates a significant survival advantage. This information may be used in conjunction with other clinical factors to help in discussing prognosis and/or palliative options with patients as well as aid in scenarios where pateints and providers decide on surgery *vs* neoadjuvant chemotherapy for borderline resectable tumors.

There are several limitations of this study. These include problems inherent to a retrospective study design such as treatment bias, a limited number of patients, as well as a unique patient population comprising exclusively of veteran male patients. The strengths of the study include a comprehensive multidisciplinary evaluation of all patients in a tertiary care center with follow-up data available for each patient included in the analysis.

There is a role of immune-targeted therapies in the future, as it is clear that specific inflammatory cells have an impact on immune surveillance of tumors. Immunotherapies including chemicals that resemble cytokines can be used to up-regulate the cancer fighting cells. Clarifying the specific type of immune cells and chemical cytokines that attract tumor suppressing cells requires further research and understanding of the tumor biology, specifically for the different types of malignancies. Pancreatic adenocarcinoma, despite being one of the more aggressive cancers, still is in the nascent stages of research and investigators are continuing to learn the tumor biology and immunologic effects.

In the future, a large multicenter prospective trial would be beneficial to confirm our findings and validate it for routine use in the prognostication of patients with pancreatic cancer. The cutoff level of the LMR has varied in different studies, with each study using

a level specific to their cohort. In the future a single cutoff value for the LMR would need to be validated for further research purposes and clinical use. Moreover, the significance of LMR within various disease stages or a specific treatment modality (such as surgery, chemotherapy and radiation) could be further explored in adequately powered research studies.

In conclusion, the LMR is an easily acquired, minimally invasive, and inexpensive biomarker that may reflect the body's immune response to cancer and host-specific cancer aggressiveness. Our study shows that a high LMR predicts better overall survival for patients with pancreatic adenocarcinoma and can be used by clinicians and patients as a marker for prognosis.

COMMENTS

Background

Pancreatic adenocarcinoma is an aggressive malignancy and many patients are presented with aggressive treatment options at diagnosis. Inflammation plays an important role in cancer progression and metastasis, and the authors hypothesized that the lymphocyte to monocyte ratio may be a potential surrogate marker of prognosis, helping the patients make difficult decisions regarding which treatment option to pursue. Lymphocytes can be cytotoxic to tumor cells and can induce apoptosis in them, whereas monocytes have properties that promote tumorigenesis. These features might explain why a high lymphocyte-to-monocyte ratio (LMR) in peripheral blood has been found to be a favorable prognostic marker for a number of malignancies.

Research frontiers

Several malignancies have shown to have a favorable prognosis with a high peripheral blood LMR. Further research in determining the actual cellular mechanisms regarding the cytotoxic and apoptotic effects of inflammatory cells in different malignancies is where the basic science aspect would be beneficial. Also, validating a certain cutoff point for the LMR will allow clinicians to use the LMR in practice as a prognostic marker.

Innovations and breakthroughs

The LMR has been shown to be of prognostic significance in different malignanices such as breast cancer, multiple myeloma, lymphomas, osteosarcomas, and lung cancers. There are also other inflammatory markers in investigation such as the neutrophil to lymphocyte ratio, which has also been shown to have some prognostic significance.

Applications

They can use the LMR as a surrogate marker of prognosis in patients with pancreatic adenocarcinoma. It is available on routine blood work as they can calculate the LMR from a peripheral blood draw. The value of the LMR, if high, suggests a better prognosis, and may help guide treatment decisions.

Terminology

LMR is the absolute lymphocyte count divided by the absolute monocyte count.

Peer-review

The paper is well-written.

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ORIGINAL ARTICLE

Observational Study

Current practice and clinicians' perception of medication non-adherence in patients with inflammatory bowel disease: A survey of 98 clinicians

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Author contributions: Soobraty A and Boughdady S collected the data, performed the analysis and wrote the draft manuscript; Selinger CP designed the study, supervised data collection and analysis and critically reviewed the manuscript.

Institutional review board statement: The study was exempt from the requirement of research ethics committee approval as no patient data were elicited.

Informed consent statement: All study participants provided informed consent by return of the online questionnaire.

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Abstract

AIM

The survey ascertains perceptions and describes current practice of clinicians regarding medication non-adherence in patients with Inflammatory Bowel Disease.

METHODS

Gastroenterologists, trainees and inflammatory bowel disease (IBD) specialist nurses from the United Kingdom were invited to a web based survey collecting data on clinician demographics, patient volume and level of interest in IBD. Respondents were asked to estimate non-adherence levels and report use of screening tools and interventions to improve adherence.

RESULTS

Non-adherence was seen as an infrequent problem by 57% of 98 respondents. Levels of non-adherence were estimated lower than evidence suggests by 29% for mesalazine (5ASA), 26% for immunomodulators (IMM) and 21% for biologics (BIOL). Respondents reporting non-adherence as a frequent problem were more likely to report adherence levels in line with evidence (5ASA $P < 0.001$; IMM $P = 0.012$; BIOL $P = 0.015$). While 80% regarded screening as important only 25% screen

regularly (40% of these with validated assessment tools). Respondents stated forgetfulness, beliefs about necessity of medication and not immediately apparent benefits as the main reasons for non-adherence. Patient counselling on benefits and risks of medication was a commonly used intervention.

CONCLUSION

Clinicians treating IBD patients frequently underestimate non-adherence and use of validated screening tools is infrequent. Most respondents identified the main factors associated with non-adherence in line with evidence and often counselled patients accordingly. Professional education should focus more on non-adherence practice to avoid adverse treatment outcomes associated with non-adherence.

Key words: Non-adherence; Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Clinical practice

Core tip: Non-adherence to maintenance medication is a very common phenomenon occurring in up to 50% of patients with inflammatory bowel disease. This survey demonstrates that many clinicians underestimate the extent of non-adherence and screening for non-adherence is infrequent and not systematic. The lack of evidence for any intervention to improve adherence is reflected by the participants divergent practice to improve adherence. There is an urgent need for further clinician education on non-adherence and robustly tested interventions that are capable of improving adherence.

Soobraty A, Boughdady S, Selinger CP. Current practice and clinicians' perception of medication non-adherence in patients with inflammatory bowel disease: A survey of 98 clinicians. *World J Gastrointest Pharmacol Ther* 2017; 8(1): 67-73 Available from: URL: http://www.wjgnet.com/2150-5349/full/v8/i1/67.htm DOI: http://dx.doi.org/10.4292/wjgpt.v8.i1.67

INTRODUCTION

Inflammatory bowel disease (IBD) refers to a group of conditions that mainly affect the gastrointestinal tract. The two primary conditions Crohn's disease and ulcerative colitis are chronic and debilitating diseases that can have very serious consequences including hospitalisation, surgery and an increased risk of developing colorectal cancer (CRC)[1]. However, evidence has shown that if IBD is treated appropriately and patients are adherent to IBD maintenance medications, disease morbidity is improved by reducing the frequency and severity of relapses[1,2]. Adherence can lessen the risk of developing CRC and can improve other treatment outcomes for patients, for example, by providing a better quality of life[3,4].

However, despite this, there is evidence that approximately 30%-40% of patients do not adhere to their prescribed medication[4-11]. The literature also suggests that the level of non-adherence varies according to the type of medication. IBD can be treated using a wide range of medications, including mesalazine (5ASA), immunomodulators (IMMs) and biological agents (BIOL) such as infliximab, and adalimumab. Non-adherence occurs in 30%-45% with mesalazine[8-10], 15%-20% with IMMs[10,12], 5%-10% with biologics[13].

Low adherence to medication leads to poor disease control, which not only has an impact on the patient, but also the economy incurred through the cost of absenteeism, medical care and hospitalisation. Non-adherence is therefore a cause of financial burden to health services[14-16].

While forgetfulness is the main reason for non-intentional non-adherence, several reasons for intentional non-adherence have been identified[4]. Apart from psychological comorbidities and quiescent disease activity the most constant findings relate to the patient perception of their medication. Non-adherent patients were more likely to express doubts over the necessity for maintenance medication and had greater concerns over potential adverse effects[5,10].

Though there are numerous methods to determine adherence, studies have found that clinicians find this difficult to gauge amongst their patients[17,18]. Methods used to screen for adherence as identified by the literature include measuring drug metabolic levels, using scales such as the Morisky Scale[18] or Medication Adherence Report Scale (MARS)[5] and the use of simple questioning. Currently, little is known about screening behaviour of health professionals in the United Kingdom or how they manage non-adherence. In addition, there is not much information on how clinicians perceive the problem of non-adherence amongst those they are treating for IBD[17,18].

The aim of this study was to assess clinicians' awareness of the extent of non-adherence in IBD. We also aimed to explore clinicians' perception of factors associated with non-adherence and identify potential differences in perception by profession, experience or level of interest in IBD. Finally, we aimed to investigate the use of screening tools and the management of non-adherence in patients with IBD amongst health professionals.

MATERIALS AND METHODS

We developed an online survey assessing clinicians' perceptions and practice based on a literature search. The survey was piloted with 8 IBD specialists and some clarifying minor amendments were based on their feedback. The survey containing both open and closed questions is available as a supplement (S1).

The survey questionnaire collected data on the parti-

Table 1 Respondents' demographics, self-reported expertise and scope of inflammatory bowel disease practice

		Frequency	Percentage
Sex	Male	46	47%
	Female	52	53%
Age, yr	20-29	7	7%
	30-44	45	46%
	45-60	40	41%
	> 60	6	6%
Years in practice, yr	< 5	11	11%
	5-9	21	22%
	10-14	17	17%
	15-19	10	10%
	⩾ 20	39	40%
Profession	Gastroenterology trainee	17	17%
	Gastroenterology consultant	51	52%
	IBD nurse specialist	28	29%
	Other	2	2%
Geographic region	England	92	95%
	Scotland	2	2%
	Wales	2	2%
	Northern Ireland	1	1%
Self-rated level of IBD interest in medically qualified staff	General gastroenterologist	32	46%
	Interest in IBD	18	27%
	Expert IBD physician	18	27%
IBD patients per week	Range 0-150		
	Mean 25		

IBD: Inflammatory bowel disease.

cipants' demographics, level of interest in IBD and the number of patients with IBD typically seen in a week. Participants were asked about their overall impression of non-adherence amongst their local patient population. Furthermore we asked respondents to estimate the levels of non-adherence amongst those being managed with mesalazine (5ASA), IMMs and biologics (BIOL) therapy. Perceived reasons for non-adherence were explored by asking respondents to rank the significance of 8 pre-specified reasons (derived by the authors from the literature) with 1 being the most important and 8 being the least. Analysis of ranking preferences was performed by counting the number of times respondents had stated a particular reason to be in the top 3. Moreover, the survey gathered data on participants' practice regarding the use of screening tools and any interventions used in their practice to improve adherence.

The survey was distributed by email to consultant gastroenterologists, trainees *via* the British Society Gastroenterology IBD section (775 members) and IBD specialist nurses from the United Kingdom *via* the UK IBD nurse network (approximately 200 members). We aimed to include different staff groups (consultants, trainees and nurses) in order to collect the views and opinions of those involved in all aspects of patient care. The survey and data compilation were performed through Bristol Online Surveys, an academic online survey system, over a 3 mo period.

Quantitative data were analysed with SPSS Statistics (IBM, version 22) using χ^2 tests to compare responses between the different participant groups. The qualitative data responses were collated and key themes were identified and described.

In the United Kingdom Ethical approval is not required for survey studies examining the views and opinions of clinicians only.

RESULTS

Respondents

Of the 98 study participants (response rate 10%) 52 (53%) were female, 46 (47%) male, and 47% of participants were older than 44 years. Respondents included 51 consultants, 17 trainees, 28 IBD specialist nurses, 1 IBD dietitian and 1 biologics nurse specialist (Table 1). Approximately half of respondents had 15 years' experience or more. Of the 68 medically qualified respondents 32 classed themselves as general gastroenterologists, 18 had an IBD interest and 18 stated that they were IBD experts. The number of patients seen in an average week varied greatly amongst the study's participants between less 10 to over 100 patients with a mean of 25 patients per week.

Respondents' perception of non-adherence

Non-adherence in their local patient cohort was perceived as a frequent problem by 43%, as an infrequent problem by 49%, while 7% reported few cases only and 1% reported no adherence issues. Overall 57% of respondents reported non-adherence at best to be an infrequent problem. Older respondents were more likely to report non-adherence as an at best infrequent problem (P = 0.043). No other correlations between overall perception of non-adherence and other factors displayed in Table 1 were found.

Respondents estimated level of non-adherence considerably lower than suggested by the evidence base in 31% for 5ASA, in 28% for IMM and in 23% for BIOL (Table 2). Respondents who perceived non-adherence as a frequent problem were more likely to report adherence levels in line with the evidence base (5ASA P < 0.0001, IMM P = 0.002, BIOL P = 0.006; Table 3). Self-declared level of interest in IBD did not affect whether or not participants estimated non-adherence for 5ASA and IMM in line with evidence. However, a higher level of interest in IBD was found to significantly correlate with estimating level of non-adherence for biologics therapy in line with evidence (P = 0.012; Table 4). No other correlations between perception of non-adherence for 5ASA, IMM or BIOL and other factors displayed in Table 1 were found.

Perceived reasons for non-adherence

The most commonly identified reasons for non-adherence were patient's forgetfulness (rank 1), lack of belief in the necessity for medication (rank 2), benefits of

Table 2 Estimation of non-adherence levels by respondents and percentage in line with evidence

Medication	Column A: literature-based non-adherence levels	Column B: perceived mean non-adherence levels	Proportion who estimated non-adherence levels below the levels in column A
Mesalazine	30%-45%	20%	31%
Immunomodulator therapy	15%-20%	10%	28%
Biological agents	5%-10%	1%	23%

Table 3 Association between perception of non-adherence as a frequent problem and reporting non-adherence levels in line with the evidence base

Medication	χ^2 value	Degrees of freedom	*P* value
Mesalazine	33.226	1	0.000
Immunomodulator therapy	12.592	2	0.002
Biological agents	7.459	1	0.006

Table 4 Association between level of interest in inflammatory bowel disease and estimation of non-adherence to biological therapy

Pearson χ^2 test		What percentage of your patients on biological therapy are non-adherent?
Level of interest in IBD for medical staff	χ^2 value	8.863
	Degrees of freedom	2.000
	P value	0.012

IBD: Inflammatory bowel disease.

medication not immediately apparent (rank 3) and concerns over potential side effects (rank 4; Table 5).

Screening practice

Nearly all (99%) respondents thought that improving adherence to medication would improve health-related outcomes in IBD and 80% regarded screening as important. However, only 58% reported ever using screening tools and only 25% stated that they screen their patients on a regular basis. In addition, it was found that only 40% use validated assessment tools to screen for adherence on a regular basis. Among the respondents who used screening tools, 60% used simple questioning asking their patients whether they were taking all their medications and 37% used Drug metabolic levels to assess non-adherence. No participants reported using the Morisky scale or the MARS and only 3% used the Visual Analogue Scale.

Thematic coding of qualitative data found that while some screen for non-adherence routinely, others only screen if a patient is not responding to medication or if they are treating someone who has regular flares or relapses. Reasons stated for not using screening tools included not having enough time during consultations and a lack of knowledge on the different screening tools available.

Managing non-adherence

Ninety-six present of respondents believed that non-adherence can be addressed and that determining low adherence is important because interventions can increase adherence. Participants were asked to rank the effectiveness of certain interventions as a part of our survey. Fifty-two present thought that involving patients in their treatment was the most effective intervention (rank 1). Other highly ranked interventions included "general education on the disease" (rank 2) and "less frequent dosing" (rank 3) and patient counselling was ranked 4th and most commonly included information on benefits and risks of medication.

DISCUSSION

Non-adherence is a common issue amongst cohorts of patients with chronic diseases and especially in those with IBD. In contrast to the well-established evidence on the extent of adherence[4,19,20] and factors associated with it[10], little is known on how clinicians perceive non-adherence and how they combat it[17]. Yet identification of non-adherence is the vital first step in attempting to avoid the increased health burden for the patient and financial burden for the healthcare system associated with non-adherence. This study is only the 2nd survey of clinicians views overall and the first in the United Kingdom.

Our study shows that clinicians have a tendency to underestimate the extent of non-adherence as only 43% of respondents thought that non-adherence was a frequent issue. It is interesting to note that older health professionals are more likely to underestimate the problem of non-adherence more often than other clinicians. No other respondents' characteristics (nurse *vs* doctor, scope of IBD practice, self-reported level of IBD expertise) were associated with the overall impression of non-adherence. Perception of non-adherence may therefore be a generational issue that could be influenced by different methods of training over time and associated changes in practitioner-patient relationships. Further work is required to elicit why non-adherence rates are wrongly perceived as low in general. Clinicians may feel uncomfortable with the thought of patients not following agreed treatment plans and may also feel helpless when tasked with improving non-adherence given the lack of evidence based interventions.

Table 5 Perception of reasons for non-adherence

Rank	Actual side effects of medication	Patient forgets to take medication	Benefits of medication not immediately apparent	Anxiety or depression	Poor patient knowledge of disease	Frequency of dosing	Beliefs about necessity of medication	Concerns over potential side effects
Ranked 1st	14.60%	32.30%	13.70%	5.30%	7.30%	9.60%	12.40%	11.30%
Ranked 2nd	9.40%	25%	20%	7.40%	11.50%	18.10%	23.70%	14.40%
Ranked 3rd	7.30%	11.50%	18.90%	8.40%	8.30%	21.30%	20.60%	23.70%
Ranked 4th	14.60%	7.30%	11.60%	7.40%	18.80%	11.70%	15.50%	12.40%
Ranked 5th	10.40%	12.50%	16.80%	13.70%	14.60%	9.60%	10.30%	16.50%
Ranked 6th	24%	3.10%	9.50%	16.80%	18.80%	13.80%	8.20%	9.30%
Ranked 7th	10.40%	4.20%	5.30%	16.80%	14.60%	8.50%	3.10%	9.30%
Ranked 8th	9.40%	4.20%	4.20%	24.20%	6.30%	7.40%	6.20%	3.10%
Ranked in top 3 by *n* =	30	66	50	20	26	46	55	48
Overall rank	6	1	3	8	7	5	2	4

Levels of non-adherence were underestimated for all medications enquired about in our survey (5ASA, IMM and BIOL). The authors elicited observed non-adherence rates found in the majority of published cohort studies[8-10,12,13] and compared the survey respondents' perceptions with these levels. Between 23%-31% estimated the levels of non-adherence to the different medication in their local patient population much lower than the evidence suggests. This may go some way in explaining why non-adherence to maintenance medication often goes undetected. We have demonstrated that those practitioners who perceive non-adherence as a frequent issue, however, estimate non-adherence levels closer to the levels derived from the evidence base.

Respondents ranked unintentional non-adherence (patient's forgetfulness) and three reasons associated with intentional nonadherence (lack of belief of necessity for medication, benefits not immediately apparent, concerns over potential side effects) as the most common reasons for non-adherence. This closely mirrors the evidence base as these factors are most consistently associated with non-adherence[4,10,20]. Respondents ranked factors that are only inconsistently or not associated with non-adherence (frequency of dosing, anxiety or depression, patient knowledge) as less important thereby demonstrating a good understanding of the factors associated with non-adherence.

Though the majority of respondents (80%) stated that they thought screening was an important issue and that adherence to medication would improve disease outcomes, this was not reflected in the participants' clinical practice. 76% said that they screened at least occasionally for non-adherence, but of those 52% said that they only use it "rarely" or "sometimes". A similar study to this one, carried out by Trindade *et al*[18] in the United States found that 77% of participants self-reportedly screened for adherence, however, the frequency of screening is unknown[18]. The commonest screening method used was simple questioning of the patient (as in Trindade's study), which is known to be unreliable in assessing adherence as it vastly underestimates non-adherence[18,21,22]. Evidence based adherence report tools were only used by a minority and these were largely restricted to blood tests. A strong effort should be made to encourage health professionals to use validated screening tools such as the 8-item Morisky Medication Adherence Scale (MMAS-8) and MARS, which are effective at detecting non-adherence non-invasively[18].

The conundrum presented by our findings is that while 96% of respondents believed that non-adherence can be addressed and that interventions can improve adherence, only 25% of respondents reported that they screen their patients regularly. It is perceivable that respondents believe that non-adherence can be improved yet have limited experience, resources or faith in interventions' success to actually implement regular screening for non-adherence. In view of the low screening rates, interventions targeting clinicians' knowledge, skills and practices need to be found. This should include education about non-adherence, efforts at raising general awareness, especially associated consequences in terms of morbidity, and financial cost. Clinicians should also be trained in the use of validated screening tools available such as the MMAS-8 and MARS.

The field of interventions aimed at improving non-adherence is difficult as the evidence consists of underpowered studies[23], studies with non-reproducible complex interventions[24], ongoing studies[25] and review articles having to base advice on associated factors alone due to the lack of rigorously tested interventions. This dilemma is revealed by the high ranking of interventions without any positive evidence base ("general disease education", "less frequent dosing") and the high ranking of important but insufficiently defined interventions such as "patient involvement in treatment" and "counselling". Those engaged in patient counselling reassuringly report using themes around medication information concerning the evidence based necessity and concerns framework[5,10,26]. A number of technological advances allow for frequent reminders for patients, but most of these systems fail to address intentional non-adherence.

Whether patient counselling can be effectively delivered in a remote, technology based way has not been rigorously tested so far. Arguably, personal contact with clinicians and especially IBD nurses may facilitate counselling more effectively.

There are a number of limitations to our study. First of all the response rate of 10% is low, but this in line with other surveys of health care professionals[27,28]. A degree of selection bias is inherent in survey studies but the spread of self-reported expertise among respondents in our study suggest a reasonably balanced sample. We believe that the sample is likely representative of IBD clinicians in the United Kingdom, but there are no reliable data to verify this assumption. Whether non-responders hold the same views as responders is unclear. Furthermore, subjective bias may have occurred as respondents may have given answers that they thought were expected of them or answers that they think the researchers were looking for, which in turn may explain the discrepancy between the generally positive perceptions of screening and the lack of regular screening in practice. We asked respondents to rank pre-specified reasons for non-adherence and pre-specified interventions based on our valuation of the existing literature to allow for a meaningful analysis. Naturally this list will have not been comprehensive and items such as "clinician-patient relationship" were not included.

In conclusion, we found that clinicians often underestimate the problem of non-adherence in patients with IBD. We also found that the use of validated screening tools was infrequent. This is a phenomenon, which occurs across all grades and professions. In addition, we found that the factors associated with non-adherence were correctly identified by participants. Based on our findings, it seems sensible to focus educational efforts for clinicians on the issue of non-adherence and its negative impact on patients with IBD. Further research is needed to establish simple and effective interventions to manage non-adherence.

COMMENTS

Background

Non-adherence to inflammatory bowel disease (IBD) maintenance medication occurs in up to 50% of patients. It is associated with adverse clinical outcomes and increased healthcare costs. While there are a number of methods that can detect non-adherence clinicians often struggle in routine clinical practice to detect it. There is a lack of robustly tested interventions capable of improving non-adherence to IBD medication.

Research frontiers

In the absence of clear guidelines and evidence for interventions little is known how clinicians perceive and how they address the issue of non-adherence. This survey ascertained perceptions and describes current practice to inform education, research and guidelines for clinical practice.

Innovations and breakthrough

A multitude of studies have aimed to identify factors associated with non-adherence. The most frequently found modifiable factors for intentional non-adherence are a lack of belief in the necessity for medication and concerns over potential side effects. Patient friendly and easily implementable self-report tools to detect non-adherence have been assessed and validated ready for use in routine clinical practice.

Application

Further education about non-adherence is required as clinicians treating IBD patients frequently underestimate non-adherence. The use of validated screening tools should be encouraged. The respondents clearly identified the main factors associated with non-adherence and aimed to address them by counselling. A formally tested evidence based intervention to improve non-adherence is urgently required.

Terminology

IBD comprises ulcerative colitis and Crohns's disease, which are chronic inflammatory disorders of the gastrointestinal tract. Non-adherence is defined as a patient driven deviation from an agreed treatment plan.

Peer-review

This manuscript is well written and gives a clear overview of the perception of clinicians about medication non-adherence in IBD. As non-adherence is still a major problem in chronic diseases.

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ORIGINAL ARTICLE

Randomized Controlled Trial

Itopride for gastric volume, gastric emptying and drinking capacity in functional dyspepsia

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Author contributions: Abid S and Jafri W designed the study and supervised the execution of the study; Zaman MU and Bilal R contributed to the research protocol to measure gastric accommodation, gastric emptying and conduct satiety drinking capacity test; Zaman MU and Bilal R also supervised the execution of these measurements during this study; Awan S did the statistical analysis of the data; Abbas A contributed to the design and execution of the study; all contributed to the write up of the manuscript.

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Institutional review board statement: The study is given an approval for a period of one year.

Clinical trial registration statement: The study is registered at https://clinicaltrials.gov/ct2/show/NCT01226134?term=itopride&rank=2. Registration number is NCT01226134.

Informed consent statement: All the subjects included in this study gave an informed consent before getting recruited for this study.

Conflict-of-interest statement: None.

Data sharing statement: Technical appendix and dataset available from corresponding author at shahab.abid@aku.edu.

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Abstract

AIM

To study the effect of itopride on gastric accommodation, gastric emptying and drinking capacity in functional dyspepsia (FD).

METHODS

Randomized controlled trial was conducted to check the effect of itopride on gastric accommodation, gastric emptying, capacity of tolerating nutrient liquid and symptoms of FD. We recruited a total of 31 patients having FD on the basis of ROME III criteria. After randomization, itopride was received by 15 patients while 16 patients received placebo. Gastric accommodation was determined using Gastric Scintigraphy. ^{13}C labeled octanoic breadth test was performed to assess gastric emptying. Capacity of tolerating nutrient liquid drink was checked using satiety drinking capacity test. The

intervention group comprised of 150 mg itopride. Patients in both arms were followed for 4 wk.

RESULTS

Mean age of the recruited participant 33 years (SD = 7.6) and most of the recruited individuals, *i.e.*, 21 (67.7%) were males. We found that there was no effect of itopride on gastric accommodation as measured at different in volumes in the itopride and control group with the empty stomach (P = 0.14), at 20 min (P = 0.38), 30 min (P = 0.30), 40 min (P = 0.43), 50 min (P = 0.50), 60 min (P = 0.81), 90 min (P = 0.25) and 120 min (P = 0.67). Gastric emptying done on a sub sample (n = 11) showed no significant difference (P = 0.58) between itopride and placebo group. There was no significant improvement in the capacity to tolerate liquid in the itopride group as compared to placebo (P = 0.51). Similarly there was no significant improvement of symptoms as assessed through a composite symptom score (P = 0.74). The change in QT interval in itopride group was not significantly different from placebo (0.10).

CONCLUSION

Our study found no effect of itopride on gastric accommodation, gastric emptying and maximum tolerated volume in patients with FD.

Key words: Itopride; Gastric emptying; Gastric accommodation; Functional dyspepsia; Dyspepsia

Core tip: Through this study we wanted to find the effect of itopride on gastric accommodation, gastric emptying and drinking capacity in patients with functional dyspepsia (FD) in Pakistani population. The strength of our study was that we used objective measures, *i.e.*, gastric scintigraphy and ^{13}C labeled octanoic acid breath test to measure gastric accommodation and gastric emptying. Diagnosis of FD was based on ROME III criteria and was done after using extensive investigations to rule out organic cause for the symptoms. We found no effect of itopride on gastric accommodation, gastric emptying and maximum tolerated volume in patients with FD in our study.

Abid S, Jafri W, Zaman MU, Bilal R, Awan S, Abbas A. Itopride for gastric volume, gastric emptying and drinking capacity in functional dyspepsia. *World J Gastrointest Pharmacol Ther* 2017; 8(1): 74-80 Available from: URL: http://www.wjgnet.com/2150-5349/full/v8/i1/74.htm DOI: http://dx.doi.org/10.4292/wjgpt.v8.i1.74

INTRODUCTION

Patients presenting with epigastric pain and burning, early satiation and postprandial fullness without any structural, organic or systematic pathology are labeled as having functional dyspepsia (FD)[1,2]. Globally prevalence of FD varies from 1.8% to 57% depending on the geographic location and diagnostic criteria used[3]. There is no published data on community based prevalence of FD from South Asia but experts consider it to be an important problem for our population[3,4]. FD reduces productivity and incurs a considerable cost on health system[5]. Only in 2009 the cost incurred to the health system by this morbidity was $18 billion[6].

Muti-factorial pathogenesis of FD makes it a difficult condition to intervene[7]. These patients have inability of the stomach to change its volume in response to food, decreased stomach compliance and inability of the stomach to empty[7]. Delayed gastric empting is associated with the symptoms of nausea, vomiting and postprandial fullness[8]. These symptoms result in lower productivity of the patients and a compromised quality of life[7,9]. Delayed gastric emptying is found in one third of the patients with FD[8,10]. These patients showed slower gastric emptying as compare to the normal individuals[11]. This is because of sub optimal gastric myoelectric activities[12,13].

Different drug therapies used for FD that include eradication of Helicobacter pylori (*H. pylori*), use of proton pump inhibitors (PPIs) and anti-depressants failed to demonstrate a convincing effect[5,14,15]. Guidelines recommend eradication of *H. pylori* but this treatment alone depends on the type of the FD being treated[15]. Evidence in favor of efficacy for using PPIs for all the patients with FD is not clear[16]. It's argued that PPIs may only be effective in patients having comorbid reflux symptoms[16]. Though data suggest that anti-depressants like mirtazapine might be beneficial for some sub-groups of FD but still more studies are needed to recommend its usage for all patients with FD[17]. Symptoms of FD are improved by prokinetic agents[18]. Metoclopramide can cause extra pyramidal movement disorders[19]. Use of domperidone can result in rise in prolactin level leading to gynaecomastia[20]. Cisapride can result in prolonged QT interval and arrhythmias[21]. Itopride is a dopamine (D2) antagonist with peripheral action. It doesn't cause severe elevation of prolactin and or pathological changes on electrocardiogram (ECG)[22]. A recent meta-analysis concluded that itopride improves the symptoms of early satiety and postprandial fullness[23]. Through this study we wanted to find the effect of itopride on gastric accommodation, gastric emptying and capacity of tolerating nutrient liquid drink in patients with FD in Pakistani population.

MATERIALS AND METHODS

We conducted a randomized controlled trial to see the effect of itopride on gastric emptying. This study was conducted after approval from Aga Khan University Ethical Review Committee (Clinical trial registration number: NCT01226134). Subject for this study were

enrolled after written informed consent that was made on the basis of declaration of Helsinki.

Study population

Total of 31 patients were recruited for the purpose of this study from the gastroenterology clinics of Aga Khan University Hospital. Eligibility criteria used for recruiting these patients was; age equal to or greater than 18 years, diagnosed as FD on the base of Rome Ⅲ criteria, negative for *H. pylori* on gastric biopsy and Urea Breath Test, negative duodenal biopsy for giardiasis or celiac disease or any other established organic pathology, and normal upper abdominal ultrasound. We excluded; pregnant women, patients taking other medications that alter gastric motility like macrolide and anti-emetics and antibiotics.

Randomization

Before undergoing randomization patients were assessed for symptoms that included epigastric discomfort, heart burn, acid regurgitation, upper abdominal pain, belching, nausea, early satiety, and postprandial fullness. Blood samples of these patients were taken to check for serum hemoglobin level, white blood cell count, platelet count, serum alanine aminotransferese (SGPT) and prolactin level. Electrocardiogram of the patient was performed to find out the QT interval at the baseline. Single photon emission tomography and satiety drinking test was performed to measure gastric accommodation at baseline. After completing the baseline investigations, out of all patients that were recruited 15 were randomly allocated to the intervention group while 16 were randomly allocated to the placebo group. Patients in the intervention group received 150 mg of itopride for 4 wk. Patients were instructed to take antacids as and when required.

Outcome measures

Gastric accommodation: Gastric accommodation was determined by estimating the change in gastric volumes using Gastric Scintigraphy and by the help of computer software which convert the gastric images into 3D images and calculate the estimated gastric volumes[24]. Gastric volumes were determined before giving itopride or placebo agent and after completion of intervention period. We injected 99mTc pertechnetate followed by the use of Analyze software for reconstruction of tomographic images. These images were acquired after an overnight fast among all the participants and then after giving 300 mL of nutrient drink at an interval of 20, 30, 40, 50, 60, 90 and 120 min. Analyze PC 2.5 software system was used to find out stomach volume measurements[24].

Gastric emptying: After an overnight fast, ^{13}C labeled octanoic breadth test was performed to assess gastric emptying[25,26]. A test meal containing 13C was given to patient which is supposed to be completed in 10 min. Breath sample was taken before test meal (150 mL of water with a sandwich of scrambled egg containing ^{13}C octanoic acid and 250 mL of orange juice) and at an interval of every 15 min for 4 h and then half-hourly for another two hours. During the measurement time the subject remained sedentary while reading or watching television. If necessary limited movements between the breaths collections were permitted.

Satiety drinking capacity test: Subjects after an overnight fast were told to come at 8:30 AM in the morning. They were asked to grade their satiety from 0 to 5 (5 being the maximum satiety). A drink containing 6.5 g fat/100 mL, 1.1 g carbohydrate and 5 g of protein (nutridrink kcl 150/100 mL) which tasted of vanilla was taken by the participants at room temperature. Subjects drank at the rate of 15 mL/min. Symptoms were scored at every five minutes interval. Test was ceased once a score of 5 is achieved[27].

Symptoms of FD: Dyspeptic symptoms which included epigastric pain, epigastric discomfort, heart burn, acid regurgitation, upper abdominal pain, belching, nausea, early satiety and postprandial fullness were assessed at baseline and at 4 wk with validated 7-point global overall symptom scale[28].

Sample size calculation

Change in gastric volumes between baseline and postprandial (accommodation) was the primary endpoint for this study. To detect 16% difference in the Itopride and placebo with power of 80% and 5% level of significance a sample size of 15 subjects was needed in each group. This effect size of 16% [100 × (difference in group means divided by overall mean of the two groups)] corresponds to the difference in the two groups that was relevant clinically. The Analysis of coefficient of variance (ANCOVA) was done for this analysis.

Statistical analysis

For the purpose of this study, mean and standard deviation were reported for quantitative variables. Means and standard errors adjusted for covariates were reported using ANCOVA. The difference in the change in volume between itporide and the placebo group was compared using Man Whitney *U* test.

RESULTS

A total of thirty-one individuals were recruited for the purpose of this study. Mean age of these individuals was 33 years. Most of the recruited individuals, 21 (67.7%) were males. After randomization into Itopride and placebo groups, the groups were similar on variables like age, gender, serum haemoglobin, white blood cells, platelet count, serum creatinine, SGPT, prolactin level and QT interval on ECG (Table 1). There was no lost to follow up.

Table 1 Distribution of age and gender by intervention arm *n* = 31

Variable	Itopride	Placebo	*P* value
Age mean (SD)	34.2 (6.4)	31.9 (8.5)	0.40
Gender *n* (%)			
Male	10 (66.7)	11 (68.8)	1.00[1]
Female	5 (33.3)	5 (31.3)	
Hb (g/dL) mean (SD)	13.6 (2.3)	14.1 (1.7)	0.50
WBC (× 10 Eq/L) mean (SD)	8.1 (2.0)	7.7 (1.9)	0.52
Platelet count (× 10 Eq/L) mean (SD)	257.3 (59.5)	250.5 (57.2)	0.75
Creatinine (mg/mL) median (IQR)	0.9 (0.5)	0.8 (0.3)	0.05
SGPT (IU/L) median (IQR)	20 (9.0)	25.5 (17.0)	0.09
Prolactin level (mg/mL) (IQR)	7.3 (3.6)	5.7 (2.7)	0.29
QT interval mean (SD)	394.1 (21.6)	399.2 (22.9)	0.53

[1]Fischer Exact test. WBC: White blood cell; IQR: Interquartile range.

Table 2 Mean volumes to measure gastric accommodation by scintigraphy using ANCOVA adjusted for age and gender

Mean (± SE)	Itopride	Placebo
Change in volume (post-pre) using scintigraphy		
Fasting	-22.2 (± 15.4)	7.5 (± 14.9)
20 min	-201.7 (± 102.4)	-31.5 (± 99.1)
30 min	0.14 (± 22.8)	-36.4 (± 22.1)
40 min	-27.6 (± 32.2)	-37.4 (± 31.2)
50 min	-60.0 (± 31.2)	-3.0 (± 30.2)
60 min	-3.1 (± 36.5)	15.0 (± 35.4)
90 min	3.8 (± 29.8)	-31.8 (± 28.8)
120 min	16.7 (± 28.9)	23.3 (± 28.0)
Effect on gastric emptying on ^{13}C labeled octanoic acid breath test (post-pre)	0.4 (± 0.4)	0.2 (± 0.4)
Effect on drinking capacity (post-pre)	22.5 (± 18.1)	36.0 (17.5)
Change in Symptom score (post-pre)	-5.8 (1.0)	-4.7 (0.9)

Gastric accommodation

Gastric accommodation was checked using gastric scintigraphy by computing the change in gastric volume at empty stomach, 20, 30, 40, 50, 60, 90 and 120 min. We found that there was no statistically significant difference in the itopride and placebo group on gastric accommodation as measured with the difference in volume in the itopride and control group with the empty stomach (P = 0.14), at 20 min (P = 0.38), 30 min (P = 0.30), 40 min (P = 0.43), 50 min (P =0.50), 60 min (P = 0.81), 90 min (P = 0.25) and 120 min (P = 0.67) (Figure 1). Mean volumes to measure gastric accommodation by scintigraphy using ANCOVA adjusted for age and gender were computed (Table 2).

Gastric emptying

Gastric emptying was done by doing breath tests on a sub sample (n = 11). There was no statistically significant difference (P = 0.58) between intervention and control group in gastric emptying (Figure 2).

Capacity of tolerating liquid drink

At the end of the intervention (Itopride or placebo) there was no significant improvement in the capacity to tolerate liquid in the itopride group as compared to placebo (P = 0.51) (Figure 3).

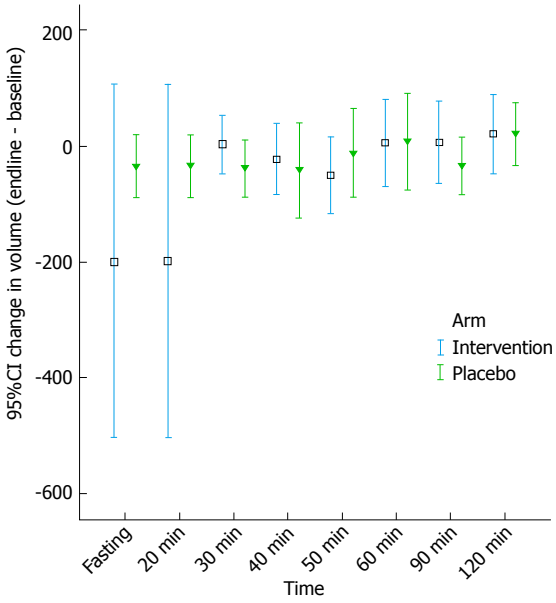

Figure 1 Schintigraphy.

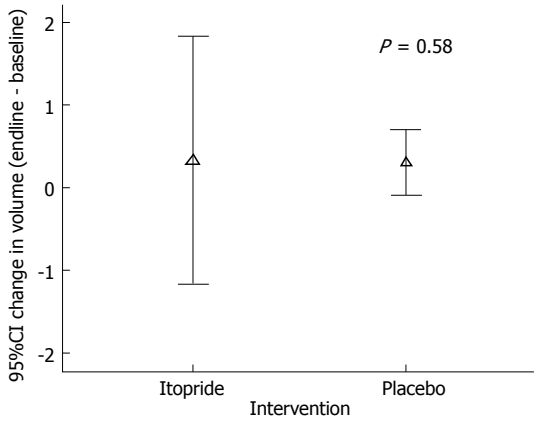

Figure 2 Breadth test.

Symptoms of FD

There was no significant improvement of symptoms as assessed through a composite symptom score (P = 0.74) in the intervention group as compared to placebo (Figure 4). Similarly we didn't find any significant improvement in the individual symptoms that included epigastric pain (P = 0.83), epigastric discomfort (P = 0.22), heart burn (P = 0.74), upper abdominal pain (P = 0.51), nausea (P = 0.08), early satiety (P = 0.34) and postprandial fullness (P = 0.25) (Table 3).

The change in QT interval as a result of itopride group was not statistically different from placebo (0.10). Similarly itopride didn't alter the serum prolactin level in the intervention group as compare to the placebo group.

Table 3 Median Symptom score along with interquartile range at baseline and end of four weeks (placebo *vs* itopride) *n* = 31

Symptoms	Baseline			End of four weeks		
	Itopride	Placebo	*P* value	Itopride	Placebo	*P* value
Epigastric pain	3.0 (1.0)	4.0 (2.0)	0.18	2.0 (1.0)	2.0 (1.0)	0.83
Epigastric discomfort	2.0 (1.0)	1.0 (1.0)	0.03	1.0 (1.0)	1.0 (1.0)	0.22
Heart burn	1.0 (3.0)	1.0 (1.8)	0.37	1.0 (1.0)	1.0 (1.0)	0.74
Upper abdominal pain	3.0 (2.0)	1.5 (2.5)	0.32	2.0 (1.0)	1.5 (1.0)	0.51
Belching	1.0 (1.0)	1.0 (0.0)	0.10	1.0 (1.0)	1.0 (0.0)	0.02
Nausea	2.0 (1.0)	1.0 (0.0)	0.04	1.0 (1.0)	1.0 (0.0)	0.08
Early satiety	2.0 (2.0)	2.0 (3.0)	0.56	1.0 (1.0)	1.0 (0.0)	0.34
Postprandial Fullness	2.0 (3.0)	2.0 (2.8)	0.82	1.0 (1.0)	1.0 (1.0)	0.25
Total Symptom score	19.0 (9.0)	16.5 (5.0)	0.41	12.0 (3.0)	11.5 (4.5)	0.71

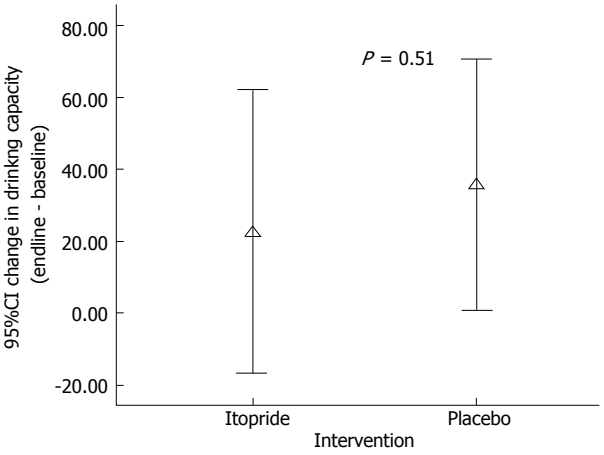

Figure 3 Drinking capacity.

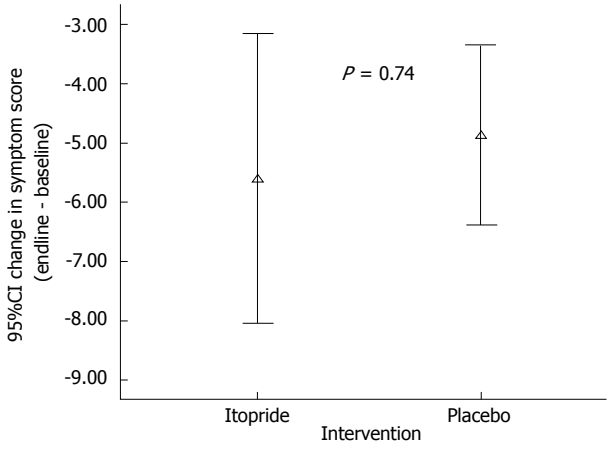

Figure 4 Change in Symptom score by intervention group.

DISCUSSION

In this study we tested the effect of itopride on some of the pathophysiological mechanisms attributed to the causation of symptoms in FD patients. Impaired accommodation is implied as one of the important factors considered to be associated with symptoms in FD patients. A primary objective of our study was to check whether itopride has any effect on gastric accommodation. We didn't find any effect of itopride on gastric accommodation when assessed through gastric scintigraphy as compared to placebo. This finding is in disagreement with a similar study which showed that itopride worsens the gastric accommodation[29].

We also found that itopride didn't effect gastric emptying as assessed through ^{13}C labeled octanoic acid breath test. A study done in Japan showed that itopride improves gastric emptying among dyspeptic patients[30]. On the other hand a cross over study that was also done in Japan showed that itopride does not improve gastric emptying[31]. Our study also found that itopride did not improve the drinking capacity as assessed through satiety drinking capacity test as compare to the placebo. This finding is in line with the similar findings in another study where it was found that itopride failed to improve the nutrient drink test induced symptoms[29].

Though in previous studies it was demonstrated that itopride improves symptoms related to FD but we found that it is not true for our set of patients. Itopride failed to show improvement in the overall symptom score as well as effect on individual symptoms including early satiety and postprandial fullness as opposed to the conclusion of a recent meta-analysis[23]. Through previous studies we know that to achieve a considerable improvement in the symptoms of one patient, we need to treat six patients of FD[32]. Itopride was efficacious in reducing the symptom score in Chinese patients having FD[33]. Small sample size might be the reason which resulted in our inability to detect an improvement in individual symptoms as a result of itopride usage as compared to placebo. Our sample included younger individuals and therefore could not study the effect of itopride in older patients with FD. Lack of variability in the age might have affected the results.

Itopride is advocated for the treatment of FD as it is safer drug as compare to other prokinetic agents. In our study we found that itopride didn't prolong the QT interval compared to placebo. Similarly itopride did not raise the prolactin level compared placebo. Therefore we can say that though itopride did not demonstrate any effect it is safer prokinetic.

Inability of itopride to effect gastric accommodation and gastric emptying might be because of the genetic variability in the dopamine-D2 receptor subtype. TaqIA

polymorphism is one example where dopamine-D2 receptor is not fully expressed resulting in compromised functionality of this receptor[34]. Mechanistic studies can identify the genetic factors like dopamine-D2 receptor variability which are anticipated to effect the efficacy of itopride among FD patients in our setting.

The strength of our study was that we used objective measures, *i.e.*, gastric scintigraphy and ^{13}C labeled octanoic acid breath test to measure gastric accommodation and gastric emptying. Diagnosis of FD was based on ROME III criteria and was done after using extensive investigations to rule out organic cause for the symptoms. The study was conducted at one center and therefore we couldn't capture a broad spectrum of patients suffering from FD. We only checked the effect of 150 mg of itopride on gastric functions and symptoms. We didn't use an objective measure to find out the absorbed amount of drug in the body.

We found no effect of itopride on gastric accommodation, gastric emptying and maximum tolerated volume in patients with FD in our study.

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COMMENTS

Background

Functional dyspepsia (FD) is defined as the presence of symptoms thought to originate in the gastro-duodenal region in the absence of any organic, systemic, or metabolic disease that is likely to explain the symptoms. Pharmacological treatments for patients with FD remain unsatisfactory. Itopride is a dopamine (D2) antagonist with acetylcholinesterase inhibitory actions. This agent is currently indicated for patients with various upper gastrointestinal (GI) symptoms. The anti-dopaminergic effects of itopride are truly "peripheral". There is a need to determine the effect of itopride on gastric function and to elaborate further the understanding on the basis of potential therapeutic benefit of this agent in FD patients. Through this study the authors wanted to find the effect of itopride on gastric accommodation, gastric emptying and drinking capacity in patients with FD in Pakistani population.

Research frontiers

Data related to the treatment of FD in Pakistani population is lacking. This study focused effect of itopride on gastric functions among patients with FD in their population.

Innovations and breakthroughs

Through this study the authors found out that there is no effect of itopride on gastric functions among patients of FD.

Applications

Itopride might not be a suitable medicine for treating patients with FD.

Peer-review

This is an original study investigating itopride in FD and showing no effect of it on physiological and clinical parameters.

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ORIGINAL ARTICLE

Randomized Clinical Trial

Role of clinical pathway in improving the quality of care for patients with faecal incontinence: A randomised trial

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Informed consent statement: No participants' identifiable information is provided. All participants have provided signed consents before participating in this study.

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Abstract

AIM

To assess the development and implementation of the Integrated Rapid Assessment and Treatment (IRAT) pathway for the management of patients with fecal incontinence and measure its impact on patients' care.

METHODS

Patients referred to the colorectal unit in our hospital for the management of faecal incontinence were randomised to either the Standard Care pathway or the newly developed IRAT pathway in this feasibility study. The IRAT pathway is designed to provide a seamless multidisciplinary care to patients with faecal incontinence in a timely fashion. On the other hand, patients in the Standard Pathway were managed in the general colorectal clinic. Percentage improvements in St. Marks Incontinence Score, Cleveland Clinic Incontinence Score and Rockwood Faecal Incontinence Quality of Life Scale after completion of treatment in both groups were the primary outcome measures. Secondary endpoints were the time required to complete the management and patients' satisfaction score. χ^2, Mann-Whitney-*U* and Kendall tau-c correlation coefficient tests were used for comparison of outcomes of the two study groups. A *P* value of 0.05 or less was considered significant.

RESULTS

Thirty-nine patients, 34 females, consented to participate. Thirty-one (79.5%) patients completed the final assessment and were included in the outcome analysis.

There was no significant difference in the quality of life scales and incontinence scores. Patients in the IRAT pathway were more satisfied with the time required to complete management (P = 0.033) and had stronger agreement that all aspects of their problem were covered (P = 0.006).

CONCLUSION

Despite of the lack of significant difference in outcome measures, the new pathway has positively influenced patient's mindset, which was reflected in a higher satisfaction score.

Key words: Pathway; Fecal incontinence; Quality improvement

Core tip: Critical pathways and process mapping methodology was used in industry since the 1950s and in medical field since the 1980s. This randomised trial describes the implementation of the Integrated Rapid Assessment and Treatment pathway, that was designed to provide a seamless multidisciplinary care to patients with faecal incontinence in a timely fashion, and compares it to the current standard of care. Although, there was no significant difference in quality-of-life and incontinence scores after completion of management, the new pathway positively influenced patient's mindset, as shown by the higher satisfaction scores. This is likely to reflect the structured support and thorough education patients in this group received.

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INTRODUCTION

Critical pathways and process mapping methodology was used in industry, particularly in the field of engineering from as early as the 1950s. In the 1980s, clinicians in the United States began to develop the pathway tools and tried to re-define the delivery of care and attempted to identify measurable outcomes. Developed and used initially for the purpose of cost containment, in the United Kingdom in the late 1980s, the emphasis has been to use clinical pathways as a quality tool[1].

The initial focus was to reduce length of stay (LOS) with an emphasis on nursing care[2]. Originally, critical pathways began with admission and ended with discharge from the hospital. Today, they are usually interdisciplinary in focus, merging the medical and nursing plans of care with those of other disciplines, such as physical therapy, nutrition, or mental health. They provide opportunities for collaborative practice and team approaches that can maximize the expertise of multiple disciplines[1].

Goals of pathways include: (1) defining standards for expected LOS and for use of specific tests and treatments; (2) giving all team members a plan and specific roles; (3) decreasing nursing and physician documentation burdens; (4) providing a framework for collecting data; and (5) educating and involving patients and families in their care; and (6) provide better care through a mechanism that is able to coordinate clinical processes and to reduce unjustified variations and, ultimately, costs[2,3].

Clinical pathways have four main components[4], these are a timeline, categories of care or activities, intermediate and long term outcome criteria and variance record to allow deviations to be documented and analysed.

Here we describe the development and implementation of the Integrated Rapid Assessment and Treatment (IRAT) Pathway in the management of patients with faecal incontinence and report the outcome of a feasibility study.

MATERIALS AND METHODS

Study design

A randomised controlled trial of patients in single centre.

Patients

Adult patients referred form primary care for management of faecal incontinence in York Teaching Hospital were prospectively recruited. Following patients' initial referral, Invitation Letter and Patient Information Sheet were sent to all potential participants. Patients were then contacted by phone by the principal investigator to discuss any query they may have and obtain initial verbal consent prior to the written informed consent that was obtained on the first clinic visit.

Objectives and end points

Primary endpoints: Percentage improvement in Faecal Incontinence Scores and Rockwood Faecal Incontinence Quality of Life Scales Faecal Incontinence Quality of Life Scale (FIQoLS).

Secondary endpoints: Time scale required to achieve full assessment and management of patients in each study group. Two periods of times were calculated; time from referral by primary care to first clinic appointment and time from initiation of management, *i.e.,* first clinic appointment to competition of management; patient satisfaction.

Randomisation: Consenting patient who chose to participate in this study were randomised to either the IRAT

pathway or the Standard Care pathway. Randomisation took place by mean of Sealed Envelope Randomisation Technique. Randomisation was performed by the Hull York Medical School Statistical Consultancy service in line with the York Hospital's Standard Operating Procedure. Patients were informed about the results of randomisation by post together with the clinic appointment letter.

Sample size: This is a feasibility study. A sample size of forty patients was arbitrarily chosen conduct the study.

Ethical consideration: This study was approved by The North and East Yorkshire Alliance Research and Development Unit and the NRES Committee of the Yorkshire and the Humber Research Ethics Office. The REC reference number is 10/H1304/27.

The pelvic floor assessment pathway form

The pelvic floor assessment pathway (PFAP) Form was developed, in cooperation with Clinical Effectiveness Team, in order to construct a data base for all participants in this study. It comprises two parts "one" and "two", consisting of four (1.a, 1.b, 1.c and 1.d) and three (2.a, 2.b and 2.c) divisions respectively. Part 1 of the PFAP is concerned with documenting demographic data, medical and obstetric history, baseline St. Marks and Cleveland Faecal Incontinence Scores, baseline Rockwood FIQoLS, quality of life Visual Analogue Scale, in addition to questionnaires specific to assessment of faecal incontinence in line with NICE Guidelines recommendations. It also documents the results of anorectal laboratory studies (anorectal manometry, endoanal ultrasound, rectal compliance and anorectal mucosal electrosensitivity) in addition to any further investigation or assessment that might be required for managing individual patients. Part 2 of the PFAP documents patients' management and monitors their progress and outcome. Patients' outcome is assessed using similar assessment tools to those used in part 1, *i.e.,* FIQoLS, St. marks incontinence score (SMIS) and cleveland clinic incontinence score (CCIS) in addition to patient satisfaction and feedback score. The later comprises 9 questions that cover patients' perception of variance aspects of their management, including waiting time from referral to first clinic appointment, time required for completion of management, adequacy of time given to the patient, protection of patient's privacy and the overall quality of care in addition to feedback about the PFAP form questionnaire itself. The patients were asked to rate these various aspects of care on a scale of 1 to 5, 1 being "strongly disagree" and 5 being "strongly agree".

CCIS

Developed in 1993, the CCIS[5] is probably still the most widely used FI severity scoring system. It gives a total score for the severity of the incontinence ranging between 0-20; where 0 represent full continence while 20 represent the worst possible incontinence. The CCIS comprises five questions accounting for incontinence to solid stool, liquid stool and flatus in addition to the use of protective pads and change in lifestyle. Each question is scored according to the frequency of occurrence of the symptom from 0 (never) - 4 (daily). This scoring system is simple and easy to understand and formed the base of almost all subsequent FI scoring systems that are currently used.

SMIS

In addition to the five questions composing CCIS, St Mark's Score[6] introduced an assessment of the ability to defer defecation, an additional score for the use of antidiarrhoeal medication and reduced the emphasis on the need to wear a pad. This scoring system comprises seven questions, each question is scored according to the frequency of occurrence of the symptom from 0 (never) - 4 (daily). The total score ranges between 0-24, where 0 indicates full continence while 24 represents the worst possible incontinence.

Rockwood faecal incontinence quality of life scale

Faecal Incontinence Quality of Life Scale[7] measures specific quality of life issues expected to affect patients with faecal incontinence. It is derived from a 29 item questionnaire comprising four domains; lifestyle, coping/behaviour, depression/self-perception and embarrassment. Each domain ranges from 1 to 4; with 1 indicating a lower functional status of quality of life.

The IRAT pathway

IRAT Pathway is designed to provide a seamless multidisciplinary care to patients with faecal incontinence in a timely fashion. Patients referred from primary care are assessed and managed by a team of surgeons, pelvic floor physiotherapist, anorectal physiology nurse practitioner and an independent researcher. Each step in patient assessment and management "event" takes place according to a preconceived timetable.

To achieve the goals of the IRAT pathway, a specialised IRAT clinic was introduced where patients are seen and assessed jointly by a colorectal surgeon with special interest in the management of faecal incontinence, pelvic floor physiotherapist and a colorectal research fellow to assess and document patient progress. This clinic takes place once every 8 wk.

Events in the IRAT pathway: Participant randomised to IRAT pathway are asked to complete part 1.a. of the PFAP before attending the first IRAT clinic; week 1: Patients are seen in IRAT clinic by surgeons and physiotherapist, completing part 1.b of PFAP; week 3: Patients undergo assessment in the Anorectal Physiology Laboratory, Part 1.c of PFAP is completed by the patients and Part 1.d. of PFAP is completed by the nurse practitioner; between week 4-week 7:

Table 1 Demographic data of patients included in analysis

Pathway	No. of patients	BMI Median (IQR)	Age Median (IQR)	Sex	
Standard care pathway	16	26.8 (23.0-31.9)	70.5 (60.0-76.0)	Female Male	14 2
IRAT	15	27.7 (22.8-35.8)	66.0 (59.0-77.0)	Female Male	12 3
P value		0.77	0.6	0.57	

IRAT: Integrated Rapid Assessment and Treatment.

All patients undergo assessment by the pelvic floor physiotherapist for suitability of biofeedback; week 8: A second IRAT clinic visit takes place for reassessment and management plan based on anorectal physiology studies and clinical and biofeedback assessments, using part 2.a of PFAP; week 16: Follow-up after completion of management.

Events in the standard care pathway: Participant randomised to Standard Care Pathway are asked to complete part 1.a. of the PFAP before attending the first clinic; patients are seen in a colorectal clinic by colorectal surgeon, completing part 1.b of PFAP; patients are assessed and treated according to the surgeon's clinical judgment. All management options available to patients in the IRAT pathway are also available to the Standard Clinic Pathway patients, including biofeedback, surgical intervention, *etc.* After completion of management, all patients, in both study arms, were asked to complete part 2.b. (final assessment) and 2.c. (patient satisfaction and feedback) of the PFAP for comparison of outcome. A reminder, by post, was sent to those who did not return the completed part 2.b. and 2.c. forms in a median of 2 mo.

Anorectal physiology laboratory assessment: Anal manometry study variables were obtained using an eight-channeled solid-state transducer catheter (Flexilog 3000, Oakfield Instruments Ltd, Evensham, Oxon, United Kingdom) using a continuous "pull through" technique. Manometric data were analysed using commercial software (Flexisoft Ⅲ, Oakfield Instruments Ltd, Evensham, Oxon, United Kingdom). This included calculation of the maximum mean resting pressure, maximum mean squeeze pressure, resting (rVV), and squeeze (sVV), vector volumes, asymmetry index, and resting and squeeze vectorgrams. In addition data from endoanal ultrasound (EAUS), rectal compliance, measured by threshold rectal volume and maximum rectal volume, and rectal mucosal electrosensitivity studies were included. EAUS was performed using a standard 2D 10 mHz probe (BandK, Denmark). Colonic imaging was also performed where indicated.

Statistical analysis

Data were assessed using Microsoft Excel Spreadsheet (Microsoft Corporation, Seattle, WA, United States) and statistical analysis was performed using SPSS v14.0 (SPSS Inc., Chicago, IL, United States). The χ^2 test was used to compare categorical variables (sex, number of deliveries, perineal tear, long labour and episiotomy, EAUS findings). The Mann-Whitney *U* test was used to compare continuous variables, including demographic data, anorectal physiology studies, time periods and the Rockwood FIQoLS. Kendall tau-c rank correlation coefficient was used to compare SMIS, CCIS and patient satisfaction score. *P* values of 0.05 or less was considered significant.

Table 2 Detailing obstetric history and concurrent urinary incontinence in patients included in analysis

Pathway	Vaginal delivery	Difficult labour	Perineal tear	Forceps delivery	Concurrent urinary incontinence	symptoms of global pelvic floor weakness
Standard care pathway	14/14	10	9	6	13	9
IRAT	12/14	9	8	4	9	6
P value	0.21	0.32	0.26	0.36	0.18	0.17

IRAT: Integrated Rapid Assessment and Treatment.

RESULTS

A total of 43 eligible patients invited to participate in this study over a period of 18 mo. Thirty-nine patients, 34 females, consented to participate. Median (IQR) age was 65 (55-75) years. Of those, 20 patients were randomised to the IRAT pathway and 19 patients were randomised to the Standard Care Pathway. Flow diagram of progress through the phases of the study is detailed in Figure 1. The median (IQR) time period from referral by primary care to first clinic appointment in our department was 5 (3-6) wk and 6 (4-8) wk for the Standard Care Pathway and the IRAT pathway respectively. The median (IQR) time period from initiation of management, *i.e.,* first clinic appointment, to competition of management, *i.e.,* discharge back to primary care was 4.5 (4-7) mo and 4 (2-6) mo for the Standard Care Pathway and the IRAT pathway respectively.

One patient withdrew from the IRAT pathway arm of this study because of resolution of her symptoms and declined further assessment. Another patient withdrew from the Standard Care Pathway without stating the reason. Of the initial 39 patients recruited in the study, 31 (79.5%) patients completed their final assessment (part 2.b) and patient satisfaction/feedback (part 2.c) components of the PFAP form. Only data from those 31 patients was included in our analysis (Figure 1).

Demographic data (age, sex, BMI) and medial and obstetric history (history of urinary incontinence, history or symptoms of pelvic floor weakness, history of vaginal delivery, difficult labour, perineal tear and forceps

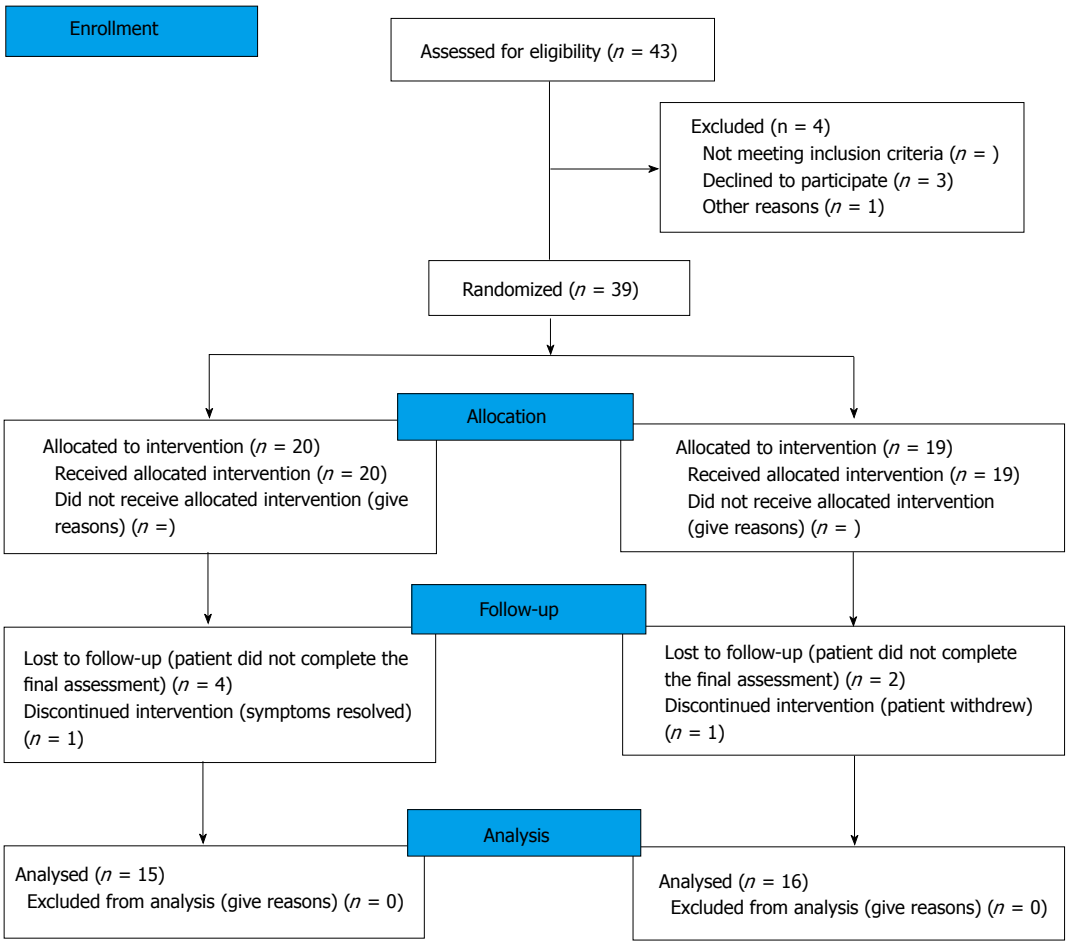

Figure 1 Flow diagram of progress through the phases of the study.

delivery) of those patients are detailed in Tables 1 and 2 respectively.

There was no significant difference in demographic data, obstetric history and anorectal laboratory test results (Table 3) between the two groups of this study. Similarly, there was no significant difference in baseline FIQoLS, SMIS and CCIS between the two study groups (Tables 4 and 5).

Three patients in Standard Care Pathway underwent perianal injection of bulking agent (Permacol®), one of them subsequently referred to SNS in a tertiary care centre due to persistence of symptoms. Another patient in the Standard Care Pathway was referred to the gynaecology team with severe uterine prolapse and subsequently underwent hysterectomy. One patient in the IRAT pathway was referred for SNS a tertiary care centre. The rest of the patients in both study groups were managed conservatively, mainly with pelvic floor exercise and biofeedback. One patient's symptoms resolved after amending his cholesterol medication.

Final follow-up with FIQoLS, SMIS, CCIS and patient satisfaction score was carried out in a median (IQR) of 1 (1-3) mo after completion of management. This shows no significant difference in any of the four scales of FIQoLS, *i.e.,* the lifestyle, coping, depression and embarrassment scales, between both study groups (Table 6). Similarly there was no difference in CCIS or SMIS at final follow-up (Table 7).

Patients' satisfaction scores in 7 of the 9 item questionnaire were not significantly different (Table 8). However patients in the IRAT pathway were more satisfied with the time required for completion of treatment (form first clinic appointment to discharge) than those in the Standard Care Pathway (P = 0.033). There was also a stronger agreement among the IRAT Pathway group that the questionnaire in the FPAP covered all aspects of their problem (P = 0.006).

The median (IQR) time period from referral by primary care to first clinic appointment was similar at 5 (3-7) wk for the both Standard Care Pathway and the IRAT pathway (P = 0.889). The median (IQR) time period for completion of management was 4.5 (4-7) mo and 4 (2-5) mo for the Standard Care Pathway and the IRAT pathway respectively. This was not significantly different (P = 0.307).

DISCUSSION

This study shows no significant difference in outcome measures such as FIQoLS, SMIS and CCIS when patients were managed in the IRAT Pathway compared to the Standard Care Pathway. The IRAT Pathway was

Table 3 detailing anorectal laboratory test results in patients included in the analysis

Anorectal physiology variables	IRAT pathway Median (IQR)	Standard care pathway Median (IQR)	*P* value
MMRP	46.0 (36.0-80.0)	55 (38.5-72)	0.96
MMSP	74.0 (57.0-89.0)	50.0 (37.0-72.0)	0.88
Resting victor volume	33308.0 (16559.2-54994.0)	51224.0 (29444.0-77663.0)	0.17
Squeeze victor volume	61168.0 (44393.0-165403.0)	81303 (51751.0-118808.5)	0.79
Squeeze asymmetry	29.7 (11.7-27.1)	14.4 (8.4-16.9)	0.07
Resting asymmetry	20.9 (13.5-31.0)	17.9 (11.2-27.1)	0.41
USS-IAS	2 abnormal	2 abnormal	1.00
USS-EAS	2 abnormal	1 abnormal	0.59
Resting vectrogram	4 abnormal	5 abnormal	0.94
Squeeze vectrogram	3 abnormal	5 abnormal	0.43
TRV	85 (50-100)	80 (50-95)	0.85
MRV	140 (100-195)	140 (100-195)	0.94
AME (high)	6.5 (5.2-10.6)	7.1 (5.5-11.3)	0.93
AME (mid)	5.3 (3.6-7.5)	5.9 (4.6-7.7)	0.89
AME (low)	4.7 (2.8-6.6)	5.1 (3.0-6.5)	0.85

MRV: Maximum rectal volume; TRV: Threshold rectal volume; MMRP: Maximum mean resting pressure.

Table 4 Comparison between baseline rockwood faecal incontinence quality of life scales of both study groups

Baseline	FIQoLS 1 Median (IQR)	FIQoLS 2 Median (IQR)	FIQoLS 3 Median (IQR)	FIQoLS 4 Median (IQR)
IRAT pathway	3.6 (2.0.2-4)	2.7 (1.4-3.4)	3.7 (2.3-4.1)	2.7 (1.3-3.8)
Standard care pathway	3.5 (2.3-3.7)	2.4 (1.6-3.0)	3.1 (2.0-3.7)	2.0 (1.3-2.7)
P value	0.44	0.94	0.11	0.22

IRAT: Integrated Rapid Assessment and Treatment; FIQoLS: Faecal Incontinence Quality of Life Scale.

Table 5 Comparison between baseline St. marks incontinence score and cleveland clinic incontinence score of both study groups

Baseline	CCIS Median (IQR)	SMIS Median (IQR)
IRAT pathway	8.0 (33.5-11.5)	13.0 (5.5-13.0)
Standard care pathway	9.5 (5.0-15.0)	12.0 (7.0-16.0)
P value	0.11	0.18

IRAT: Integrated Rapid Assessment and Treatment; CCIS: Cleveland clinic incontinence score; SMIS: St. marks incontinence score.

Table 6 Comparison between Rockwood Faecal Incontinence Quality of Life Scales of both study groups after completion of management

After completion of management	FIQoLS 1 Median (IQR)	FIQoLS 2 Median (IQR)	FIQoLS 3 Median (IQR)	FIQoLS 4 Median (IQR)
IRAT pathway	3.9 (2.2- 4.0)	2.9 (1.8 3.8)	3.9 (2.3-4.1)	3.0 (1.8-3.8)
Standard care pathway	3.6 (2.4-4.0)	3.8 (1.7-4.0)	3.5 (2.1-3.9)	2.3 (1.6-3.7)
P value	0.51	0.92	0.18	0.87

IRAT: Integrated Rapid Assessment and Treatment; FIQoLS: Faecal Incontinence Quality of Life Scale.

Table 7 Comparison between St. marks incontinence score and cleveland clinic incontinence score of both study groups after completion of management

After completion of management	CCIS Median (IQR)	SMIS Median (IQR)
IRAT pathway	6.0 (1.5 -11.5)	7.0 (30-15.5)
Standard care pathway	7.5 (3.0-12.0)	9.5 (4.0-11.0)
P value	0.37	0.85

IRAT: Integrated Rapid Assessment and Treatment; CCIS: Cleveland clinic incontinence score; SMIS: St. marks incontinence score.

designed to expedite the management of patients with FI. The IRAT clinic takes place once every 8 wk. During the time periods between first and second and second and third clinic visits, the patient would have completed their assessments and treatment respectively. However, this study shows that there was no significant difference in the waiting time for the first clinic appointment and in the time required for completion of management between the two study groups. This could well be due to the inflexibility of the preconceived timetable in the IRAT Pathway. When patients have asked to postpone or change their clinic dates for various reasons, which occurred in the case of 4 patients in the IRAT Pathway, they had to wait for another 8 wk for the next clinic appointment. The Standard Care Pathway, on the other hand, was more flexible, and since colorectal clinics take place every week, they could accommodate for patients' cancelations and appointment changes on weekly basis. By the same token, patient factors and preferences may have influenced these time scales. This is reflected in the patient satisfaction questionnaire, where patients in the IRAT pathway were more satisfied with the time required for completion of management, in spite of the lack of significant difference in the time scale itself.

Table 8 Comparison of patient satisfaction score between the integrated rapid assessment and treatment and the standard care pathways

Please rate your degree of satisfaction with each of the following aspect	Standard care pathway median (IQR)	IRAT pathway median (IQR)	*P* value
The waiting time from seeing your GP until been seen at York hospital was acceptable	4 (3-4)	4 (4-5)	0.07
The waiting time from being seen at York Hospital until completing your treatment was acceptable	4 (3-4)	4 (4-5)	0.03
The questions you were asked to complete were relevant to your problem?	4 (4-4)	4 (4-5)	0.24
The questions you were asked to complete were clear and easy to answer?	4 (4-4)	4 (4-5)	0.28
The questions you were asked to complete covered all aspect of your problem?	4 (3-4)	4 (4-5)	0.01
You were supported and given clear advices/instructions throughout management	4 (4-4)	4 (4-5)	0.08
You were given enough time to explain your problem/concerns	4 (4-4)	4 (4-5)	0.08
Your privacy and dignity were respected throughout management	4 (4-5)	4 (4-5)	0.43
The over all quality of care you received was high	4.5 (4-5)	4 (4-5)	0.85

IRAT: Integrated Rapid Assessment and Treatment.

Patients in the IRAT Pathway also had stronger agreement that all aspects of their problem were addressed. This could reflect the structured support and thorough education that patients in this group received along with interaction with pelvic floor and biofeedback therapists both in the clinic and in the laboratory.

Both study groups have rated the overall quality of care equally, which, in addition to a non-significantly different outcome measures (FIQoLS, CCIS and SMIS), means the introduction of the IRAT Pathway did not have a major impact on the quality of patient care.

In spite of the outcome measures of this study, patient satisfaction seemed to increase with the use of the IRAT pathway. This finding is compatible with outcomes of other similar studies. Lawson *et al*[8] report that patient and parent satisfaction increased because of the promptness of securing discharge prescriptions. Goode[9] discovered that patients who had a care map and a nurse case manager were more satisfied with their care.

There is evidence that pathways are more likely to be effective when applied to conditions and procedures with lower severity/complexity of illness, high volume and higher length of stay[10]. This does not apply to FI which is a multifactorial condition with complex aetiology. In addition the volume of patient referred our department for management of FI was relatively low. The risk of "contamination" of the control sample, *i.e.,* communication between experimental and control professionals, was not considered in this study, especially that some of the Standard Care Clinic were run by the same colorectal consultant conducting the IRAT Clinics. Some or all of these factors could have contributed to the final outcome of this study.

Clinical pathways applied to patients with a cardiovascular disease showed a tendency towards a decreased treatment variation, improved guideline compliance and reduced costs. However, the evidence of the effectiveness of clinical pathways in cardiovascular medicine can not be generalized because of the insufficient number of controlled studies[11]. There was a strong decline in both the average length of stay and its variation after implementation of CP in inguinal hernia repair[3]. Similar finding were observed in knee and hip arthroplasty procedures[12]. However, no significant difference in patient outcomes was seen.

On the other hand, no benefit of using clinical pathway in stoke patients was detected over conventional multidisciplinary care[10,13,14]. Functional recovery was faster and quality of life outcomes better in patients receiving conventional multidisciplinary care. Some studies reported major failures in implementation of clinical pathways for stroke and their implementation was discontinued[3].

Some studies did suggest that the use of clinical pathways had no influence on patient-care outcomes, by the same token they also stated that there was no evidence at all that they had any negative effect[15]. However, no, few, or even negative results after implementing CP hardly ever get published[15].

How health care should respond to clinical pathways that have not been shown to improve care, such as some the pathways for strokes and renal failure[3] is not clear and further research is needed to answer this question[16]. The answer depends on the risks, costs, and opportunity costs of continuing to implement critical pathways or other strategies[16].

It has been assumed that critical pathways are not associated with risk, although there are relatively few studies to support or refute that belief. However, critical pathways might be costly to develop, update, and implement. There may also be opportunity costs of not pursuing other strategies that might more effectively improve quality, reduce costs, and enhance patient safety, since these other strategies must compete for organizational resources[16].

Despite widespread enthusiasm for critical pathways, rigorous evidence to support their benefits in health care is extremely limited. However, understanding what evidence-based information is, and translating this information into practice using reminder systems or other effective implementation strategies, can potentially improve care, reduce costs, and enhance safety[16-20].

Rigorous evaluation of CP and medical management

approaches is essential in order to determine the effectiveness of CP in particular area of medical care. Pearson *et al*[21] reported significant reductions in lengths of stay after implementation of CP for surgical conditions. However, this reduction in LOS was similar to those at health care organizations at which there were no organized CP efforts in place. The CP program was responsible for very modest improvements in patient care, and was probably without a measurable "return on investment." These results occurred in an organization where the investigators are extremely knowledgeable and experienced in the field of critical pathways[22]. Only after the authors observed declining lengths of stay in organizations without critical pathways did they believe that the reductions at their organization were more likely to be a result of secular trends rather than the critical pathways[16]. In this study we randomised patients between CP and standard care which has given us the advantage to overcome this confounding factor. The findings in this study are, however, consistent with those from Pearson *et al*[21] study.

Studies should also determine the clinical and financial return on investment of these efforts. Organizations should identify which components of their current clinical quality improvement efforts are effective, and which are not. For strategies that are without measurable benefit, consideration should be given to learning from those experiences and may be redirecting resources to more effective quality improvement strategies[16].

Finally, in spite of the lack of significant difference in outcome measures, the IRAT Pathway has positively influenced patient's mindset, which was reflected in a higher satisfaction score. This has an important impact on the overall care for patients with problems such as faecal incontinence.

COMMENTS

Background

The management of faecal incontinence is widely varied, ranging from conservative management with dietary modification, medications and behavioral interventions to invasive therapy including complex surgery. No previous study has discussed the role of clinical pathway in the management of faecal incontinence.

Research frontiers

There is evidence that clinical pathways applied to patients with certain conditions, such as cardiovascular disease, showed a tendency towards a decreased treatment variation, improved guideline compliance and reduced costs. However, this evidence cannot be generalized to other conditions, such as faecal incontinence, because of the insufficient number of controlled studies

Innovations and breakthroughs

This is the first randomized controlled study to evaluate the development and implementation of clinical pathway in the management of patients with faecal incontinence and measure its impact on patients' care.

Applications

This pilot study's design and findings could be used to determine sample size for a larger randamised controlled study aiming to test the impact of clinical pathway and structured patient support and thorough education on clinical outcome and satisfaction in patients with faecal incontinence.

Terminology

Critical pathways and process mapping methodology was used in industry, particularly in the field of engineering from as early as the 1950s. In the 1980s, clinicians in the United States began to develop the pathway tools and tried to re-define the delivery of care and attempted to identify measurable outcomes. Developed and used initially for the purpose of cost containment, in the United Kingdom in the late 1980s, the emphasis has been to use clinical pathways as a quality tool.

Peer-review

The study is well designed, the manuscript is well written and new data have been provided.

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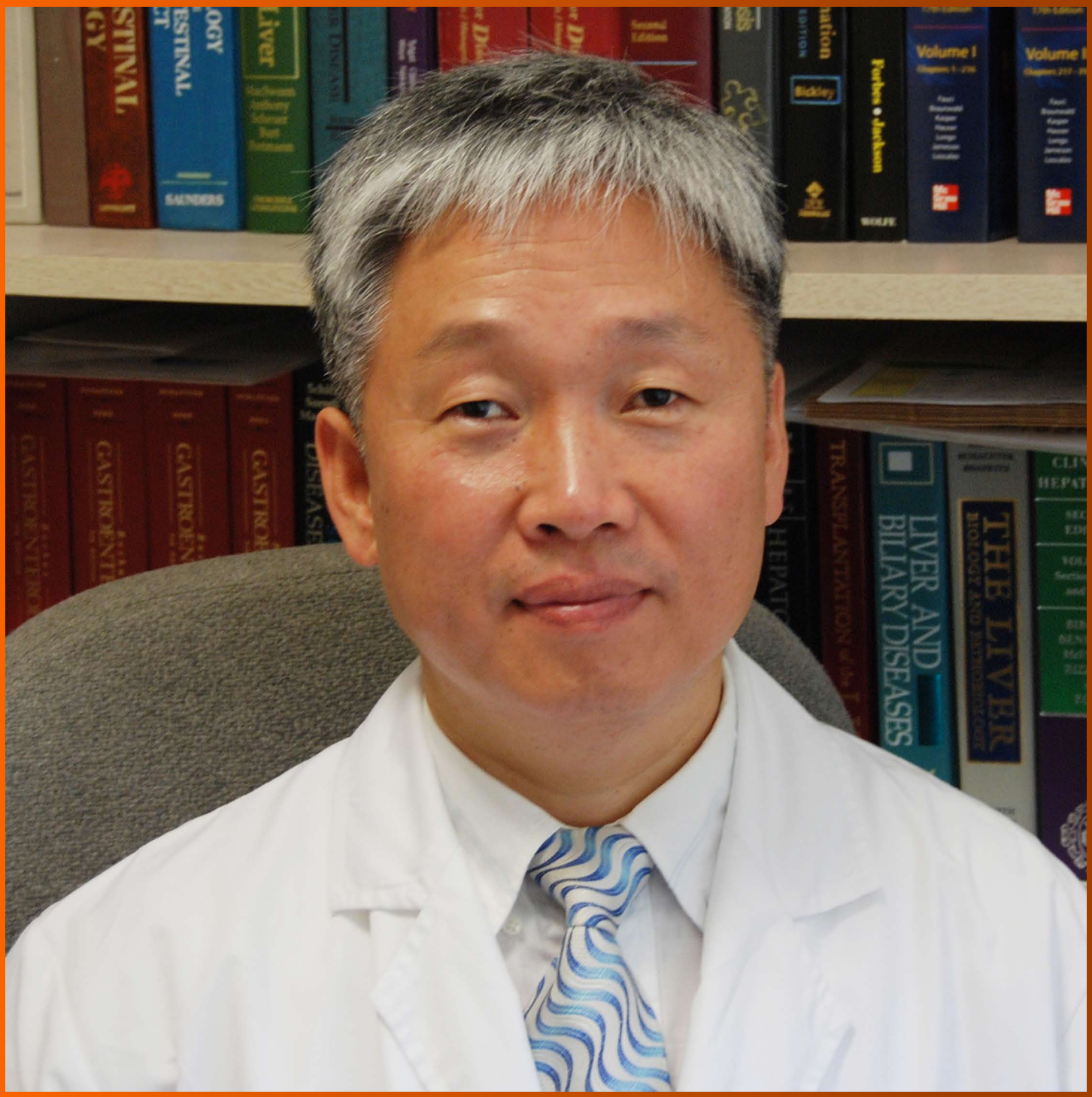

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EDITORIAL

Management of esophageal caustic injury

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Abstract

Ingestion of caustic substances and its long-term effect on the gastrointestinal system maintain its place as an important public health issue in spite of the multiple efforts to educate the public and contain its growing number. This is due to the ready availability of caustic agents and the loose regulatory control on its production. Substances with extremes of pH are very corrosive and can create severe injury in the upper gastrointestinal tract. The severity of injury depends on several aspects: Concentration of the substance, amount ingested, length of time of tissue contact, and pH of the agent. Solid materials easily adhere to the mouth and pharynx, causing greatest damage to these regions while liquids pass through the mouth and pharynx more quickly consequently producing its maximum damage in the esophagus and stomach. Esophagogastroduodenoscopy is therefore a highly recommended diagnostic tool in the evaluation of caustic injury. It is considered the cornerstone not only in the diagnosis but also in the prognostication and guide to management of caustic ingestions. The degree of esophageal injury at endoscopy is a predictor of systemic complication and death with a 9-fold increase in morbidity and mortality for every increased injury grade. Because of this high rate of complication, prompt evaluation cannot be overemphasized in order to halt development and prevent progression of complications.

Key words: Caustic ingestion; Esophageal caustic; Caustic injury; Corrosive ingestion; Esophageal injury

Core tip: Caustic ingestion maintains its place as an important public health issue in spite of the multiple efforts to educate the public. This is due to the ready availability of caustic agents and the loose regulatory control on its production. Substances with extremes of pH are very corrosive and can create severe injury in the upper gastrointestinal tract. Locations most seriously affected are in the esophagus and stomach and may lead to chronic complications like stricture formation, gastric outlet obstruction, and malignant transformation. Prompt evaluation is therefore emphasized in order to halt development and prevent progression of these

complications.

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INTRODUCTION

Ingestion of caustic substances and its long-term effect on the gastrointestinal system maintain its place as an important public health issue in spite of the multiple efforts to educate the public and contain its growing number. This is due to the ready availability of these caustic agents as items of household use and loose regulatory control on its production. According to the American Association of Poison Control (AAPCC), there were approximately two hundred thousand cases of cleaning substance exposure since 2000[1]. Data from developing countries, however, are sparse given that cases are largely underreported.

The age of occurrence presents in a bimodal fashion. The first peak is in the 1 to 5-year-old age group. Compared to adults, children are more likely to ingest caustic substances either accidentally or out of curiosity. Their higher exposure rate, however, is usually offset by a lower overall rate of complicated caustic injury because children often spit out the corrosive material immediately. The second peak is in the adolescent and young adult (21 years and older) age group. Majority of ingestions at this age group are intentional suicide attempts resulting in a greater and more extensive injury[2,3].

SUBSTANCES CAUSING CAUSTIC INJURY

Caustic agents can be acidic or alkaline in nature. Common alkali-containing caustic agents are household bleaches, drain openers, toilet bowel cleaners, dishwashing agents and detergents. Acid-containing agents implicated in caustic ingestion include toilet bowl cleaners, anti-rust compound, swimming pool cleaners, vinegar, formic acid used in the rubber tanning industry and other similar acids[3,4]. The type of caustic agent most commonly implicated in poisonings varies from country to country. In the annual report of the AAPCC in 2008, the most commonly implicated caustic agent was the alkali-sodium hypochlorite, which was found in bleaches, toilet bowl cleaners, drain cleaners and household disinfectants. Local experiences from Denmark, Israel, United Kingdom, Peru, Spain, Australia, Saudi Arabia and Turkey also showed that alkaline agents were more commonly involved in caustic injury[4]. Most caustic substances were ingested in the liquid form and events commonly occurred at home[4]. Indian data, on the other hand, showed that majority of ingestions in their country were due to acids since these were cheaper and more readily available[3,4].

PATHOPHYSIOLOGY

Substances with extremes of pH (less than 2 or greater than 12) are very corrosive and can create severe injury and burns in the upper gastrointestinal tract. Locations most seriously affected are in the esophagus and stomach since the corrosive material often remains in these areas for a longer period of time. However, injuries can also occur in any area in contact with the caustic agents such as the oral mucosa, pharyngeal area, upper airways, and duodenum[5,6].

Acids and alkali agents have contrasting characteristics and differ in how they cause tissue damage. Alkaline agents are usually colorless, relatively tasteless, more viscous, and have a less marked odor. Hence, the amount ingested tends to be more[4]. Once ingested, alkaline substances react with proteins and fats and are transformed into proteinases and soaps, resulting in liquefactive necrosis. This leads to deeper penetration into tissues with a greater likelihood of transmural injury[6]. Acids, on the other hand, have a pungent odor and an unpleasant taste. It tends to be consumed in smaller amounts and are swallowed rapidly after ingestion[4]. Once it reacts with tissue proteins, these substances are converted to acid proteins. The mode of tissue injury is coagulation necrosis[6]. The coagulum prevents the corrosive agent from spreading transmurally, hence reducing the incidence of full thickness injury[4]. This distinction, however, is not always the case. In the setting of strong acid or strong base ingestion, both these substances easily penetrate the esophageal or gastric mucosa and cause full-thickness injury[7].

The traditional opinion is that acids preferentially damage the stomach. Its lower surface tension and the formation of protective esophageal eschar allow acids to bypass the esophagus rapidly without much damage while affecting the stomach more severely. Conversely, alkalis cause more injury to the esophagus. The higher surface tension of alkalis that permit a longer contact time with esophageal tissues and the acidic contents in the stomach that act to neutralize gastric injury explain the more severe damage to the esophagus.

Mucosal injury begins within minutes of caustic ingestion. It is characterized by necrosis and hemorrhagic congestion secondary to the formation of thrombosis in the small vessels. These events continue in the next several days until approximately 4 to 7 d later when mucosal sloughing, bacterial invasion, granulation tissue and collagen deposition occur. The healing process typically begins three weeks after ingestion. It is during this time (first 3 wk) that the tensile strength of esophageal and/or gastric tissues is the lowest. If the ulcerations extend well beyond the muscularis layer, the wall becomes vulnerable to perforation[3,6].

It is for this reason that authorities advocate avoiding endoscopy between the 5th and the 15th day after caustic ingestion[3,6]. By the 3rd week, scar retraction occurs and may continue for a few more months until stricture formation occurs. The lower esophageal sphincter pressure becomes also impaired in the process causing an increased frequency and severity of acid reflux that further aggravates existing mucosal injury and accelerates the stricture formation[7].

The severity of injury depends on several aspects: concentration of the substance, amount ingested, length of time of tissue contact, and pH of the agent. Solid materials easily adhere to the mouth and pharynx, causing greatest damage to these regions. Liquids, on the other hand, pass through the mouth and pharynx more quickly consequently producing its maximum damage in the esophagus and stomach[7,8].

CLINICAL PRESENTATION

The clinical presentation of caustic ingestion is diverse and do not always correlate with the degree of injury. Symptoms mainly depend on the location of damage. Hoarseness and stridor are signs that are highly suggestive of an upper respiratory tract involvement, particularly the epiglottis and larynx. Presence of these findings may signal a potentially life-threatening respiratory event[7]. The upper gastrointestinal tract, on the other hand, may present as dysphagia or odynophagia for esophageal injury and hematemesis or epigastric pain for gastric involvement[7,8].

Short-term complications include perforation and death. Perforation of the esophagus or stomach can occur at any time during the first 2 to 3 wk of ingestion. A sudden worsening of symptoms or an acute deterioration of a previously stable condition should warrant a thorough investigation to rule out the possibility of a perforated viscus[7,8].

Chronic complications of caustic ingestion include stricture formation, gastric outlet obstruction and malignant transformation. Patients with esophageal strictures usually complain of dysphagia and substernal pressure, and may become symptomatic 3 wk or later after ingestion. Symptoms of early satiety, post-prandial nausea or vomiting, and extreme weight loss suggest gastric obstruction. The latter commonly occurs in the first 5 to 6 wk of ingestion[6].

Carcinoma of the esophagus is a well-recognized consequence of caustic ingestion - partly due to the chronic inflammation from the initial burn, the trauma induced by repeated dilation, and the continuous tissue reaction from food stasis. Patients with a history of caustic ingestion often have a 1000-3000-fold increase in the incidence of esophageal carcinoma. Conversely, up to 3% of patients with carcinoma of the esophagus may have a history of caustic ingestion[7,8]. For alkaline ingestion in particular, subsequent development of squamous cell carcinoma has been reported to occur approximately 40 years after injury. This is mainly because of the liquefactive necrosis caused by alkali agents, which causes a deeper penetration of injury compared to the less severe and often limited mucosal injury of acidic substances. Periodic endoscopic evaluation is therefore suggested starting 20 years after the caustic ingestion with an interval of 1 to 3 years.

DIAGNOSIS AND STAGING

Laboratory tests

Laboratories were not found to directly correlate with the severity or the outcome of the injury. One study showed that age, an elevated white blood cell count (> 20000 cells/mm), and the presence of gastric deep ulcer or gastric necrosis are independent predictors of death[9]. Basically, laboratory work-ups play a more important role in guiding patient management than in predicting morbidity or mortality[7,8].

Traditional radiology

Plain chest radiography may show gas shadow in the mediastinum or below the diaphragm suggesting esophageal or gastric perforation, respectively. If perforation is suspected, an upper gastrointestinal series using a water-soluble agent can be performed.

Ultrasound

Endoscopic ultrasound can also be used to evaluate the esophageal wall. Though in comparison to the conventional endoscopy, no difference was achieved in predicting early complications. Reports show that destruction of the muscularis layer on EUS could be a reliable sign of stricture formation and a marker for decreased response to balloon dilatation. However, further studies are needed to establish the role of EUS in caustic injury[7,8].

Computed tomography scan

In assessing the extent and boundary of injury, computed tomography (CT) scan has a slightly higher diagnostic contribution than upper endoscopy. It can show the depth of necrosis and even the presence of transmural damage, thereby allowing clinicians to assess threatened or established perforations[7,8]. And because of its non-invasive quality, CT scan may prove to be a promising diagnostic in the early evaluation of caustic injury[7].

Magnetic resonance imaging

Magnetic resonance imaging (MRI), in general, provides little advantage over CT scan in the assessment of caustic injury. Besides its obvious benefit of processing images without the use of ionizing radiation, it does not reliably distinguish the different layers of the esophageal wall, which is crucial for the initial assessment of the extent of mucosal involvement. In addition, some patients, particularly the acutely ill, may not be able to tolerate the slower throughput of MRI and may not be able to cooperate during the procedure resulting in movement artifacts.

Table 1 Zargar classification and its corresponding endoscopic description

Zargar classification	Description
Grade 0	Normal mucosa
Grade Ⅰ	Edema and erythema of the mucosa
Grade ⅡA	Hemorrhage, erosions, blisters, superficial ulcers
Grade ⅡB	Circumferential lesions
Grade ⅢA	Focal deep gray or brownish-black ulcers
Grade ⅢB	Extensive deep gray or brownish-black ulcers
Grade Ⅳ	Perforation

Endoscopy

Esophagogastroduodenoscopy is an important and highly recommended diagnostic tool in the evaluation of caustic injury especially during the first 12 to 48 h of caustic ingestion, though several reports indicate that it can be safely performed up to 96 h post-ingestion. Gentle and cautious insufflation during the procedure cannot be sufficiently emphasized. Endoscopy is generally not advised 5 to 15 d after caustic ingestion due to tissue softening and friability during the healing stage. With findings of extensive damage and necrosis, aborting the procedure is not mandatory[7,8]. However, endoscopy is usually contraindicated in several situations; such as hemodynamic instability, severe respiratory compromise, and suspected perforations[8].

In the absence of symptoms and in the presence of accidental ingestions (especially those of less corrosive substances), significant lesions are usually not observed on upper endoscopy. As such, it is not required in some reports to perform endoscopy for asymptomatic patients with ingestion of low potency materials. This, however, is not applicable to patients with intentional ingestions since the substances they commonly consume are more potent. Therefore, emergent endoscopy among these patients is generally recommended[7,8].

Ultimately, endoscopy is considered the cornerstone in the diagnosis, prognostication, and guide to management of caustic ingestions. Various endoscopic grading is available and Zargar's classification is one of the most commonly used (Table 1 and Figure 1). In his study, Zargar *et al*[10] found that early major complications and death were confined to patients with grade Ⅲ injuries. All patients with grade 0, Ⅰ and ⅡA burns recovered without sequelae. Majority of grade ⅡB and all survivors with grade Ⅲ injury developed eventual esophageal or gastric cicatrization[10]. In general, the degree of esophageal injury at endoscopy is a predictor of systemic complication and death with a 9-fold increase in morbidity and mortality for every increased injury grade[10].

MANAGEMENT

General measures (Figure 2)

Management of caustic injury includes immediate resuscitation and evaluation of extent of damage. In general, correlation between symptomatology and endoscopic post-corrosive severity is still unproven. The patient's initial signs and symptoms are oftentimes unreliable to gauge the extent of involvement since 20% of caustic ingestions may not present with oropharyngeal injury[11,12]. Nevertheless, for patients with a clear history of accidental ingestion of a low-volume, low-concentration caustic substance and with no signs and symptoms of oropharyngeal injury, endoscopy may be deferred. These patients may then be discharged after a 48-h observation period[11]. For those with large volume of ingestions and with significant findings on endoscopy (at least grade IIB), in-patient observation for any immediate complications in the intensive care unit is generally advised[13,14].

The cornerstone of all caustic ingestions is airway and hemodynamic stabilization. Since direct exposure of the upper respiratory tract by the corrosive substance may occur, patients should be evaluated for the need to do immediate intubation or tracheostomy. Intubation with direct visualization under fiberoptic laryngoscopy is most appropriate to avoid the risk of bleeding and further airway injury from "blind" airway access[10,15,16]. If the epiglottis and larynx are edematous, tracheostomy should be performed.

Neutralizing agents

In previous protocols, neutralizing agents (weakly acidic or basic substances) for caustic ingestion was viewed as one of the first steps for treating caustic intoxications[11]. However, it has now been emphasized that these substances should not be administered due to the additional thermal injury and chemical destruction of tissues these reactions produce[14,17].

Nasogastric tube

Routine nasogastric intubation for the purpose of evacuating any remaining caustic material is no longer warranted prior to endoscopic assessment of mucosal injury. This is due to the possibility of inducing retching or vomiting leading to further esophageal exposure by reflux of the remaining intragastric caustic material. Moreover, insertion of a foreign body in the acute setting may act as a nidus for infection, which may subsequently delay mucosal healing[16].

A preliminary survey of expert opinion from members of the world society of emergency surgery showed that 93% opted to use nasogastric tubes in patients with evidence of oropharyngeal injury while 7% avoided placement in any scenario. Among the 93%, more than two thirds opted to insert a nasogastric tube endoscopically. The theoretical advantage is said to provide a patent route for enteral feeding while serving as a stent to maintain luminal integrity and to decrease stricture formation[18].

Gastric acid suppression and mucosal protection

Upon admission, the patient should be kept fasting. Gastric acid suppression with H_2 blockers or intravenous proton pump inhibitors are often initiated to allow faster

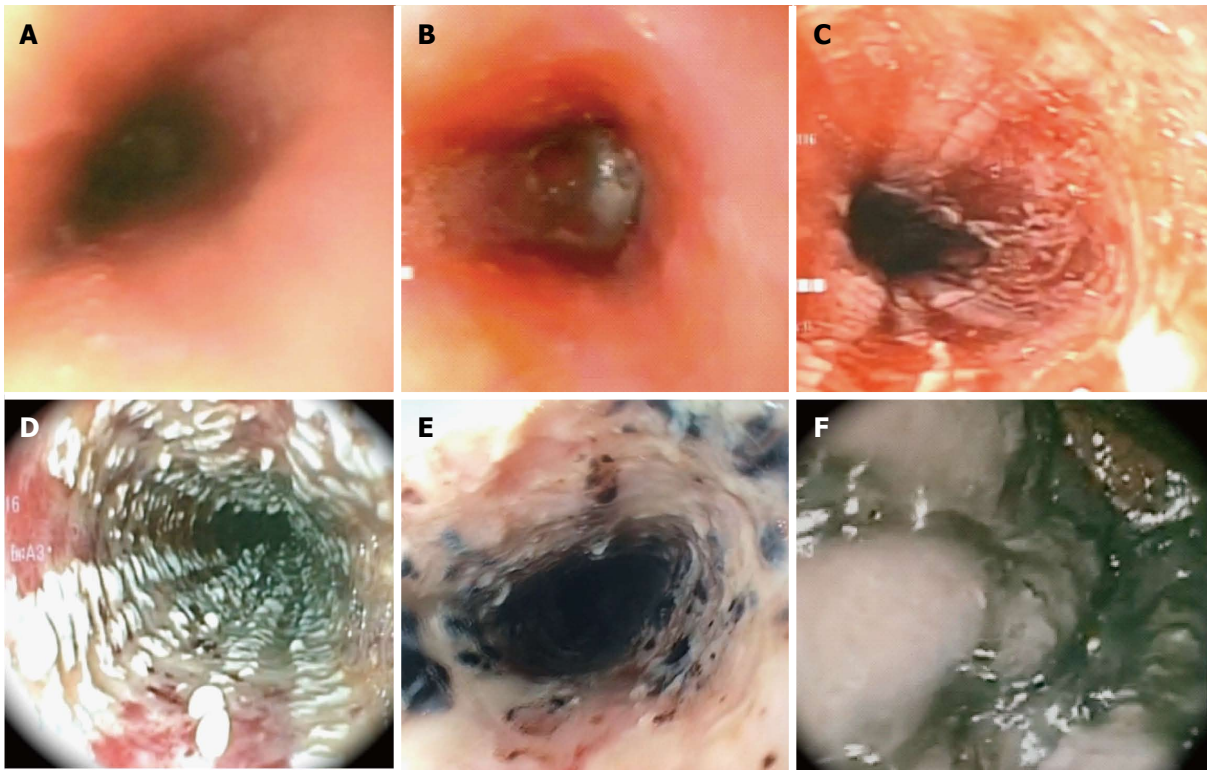

Figure 1 Endoscopic pictures of Zargar classification 0 to ⅢB. A: Zargar Grade 0: Normal mucosa; B: Zargar Grade Ⅰ: Edema and erythema of the mucosa; C: Zargar Grade ⅡA: Hemorrhage, erosions, blisters, superficial ulcers; D: Zargar Grade ⅡB: Circumferential bleeding, ulcers. Exudates; E: Zargar Grade ⅢB: Focal necrosis, deep gray or brownish black ulcers; F: Zargar Grade ⅢB: Extensive necrosis, deep gray or brownish black ulcers.

mucosal healing and to prevent stress ulcers. Efficacy of these agents for caustic ingestion has not yet been proven, although a small study done in 2013 has shown endoscopic healing after omeprazole infusion[7,16,19].

Sucralfate is now a common adjunct in the management of acute ulcers. It achieves its therapeutic effect by maintaining mucosal vascular integrity and blood flow. In the setting of caustic ingestion, sucralfate is said to hasten mucosal healing by providing a physical barrier between the harmful effects of the corrosive substance and the gastroesophageal mucosa[20-22]. Several small randomized controlled studies have assessed the efficacy of sucralfate in corrosive esophagitis. Results from these studies showed that sucralfate may decrease the frequency of stricture formation with advanced corrosive esophagitis. However, further research with a larger sample size is required to support its recommended use in this setting[20,23].

Antibiotics

To date, evidence is still conflicting with regard the use of antibiotics. A study in 1992 analyzed the utility of antibiotic together with systemic steroid administration in caustic ingestion. It was concluded that antibiotics with steroids may be useful in preventing strictures in patients with extensive burns[24]. But since it was not possible to separate the effect of the antibiotic from that of the possible effect of the steroid in this study, it may be difficult to support the use of antibiotic in preventing stricture formation with such limited data. Hence, the consensus maintains that patients treated with steroids should also be treated with antibiotics[16].

Steroids

Initial studies on corticosteroid administration to prevent stricture formation in caustic ingestion were mainly on children and results were conflicting. Methylprednisolone at a dose of 1 g/1.73 m^2 per day for 3 d showed benefit in reducing stricture development[25]. Likewise, dexamethasone (1 mg/kg per day) was shown to be better than prednisolone (2 mg/kg per day) in preventing stricture formation (38.9% *vs* 66.7%) and severe stricture development (27.8% *vs* 55.6%)[26].

However, another study showed that prednisolone at a dose of 2 mg/kg intravenous did not provide any benefit in preventing stricture development[27]. A systematic pooled analysis of caustic ingestion supported this finding as it failed to show additional benefit with the use of steroid in patients with grade II esophageal burns[28]. Based on the above evidence, it seems prudent to avoid systemic corticosteroids in caustic ingestion until further research confirms its efficacy.

Triamcinolone and mitomycin-C

Intralesional steroid such as triamcinolone (40-100 mg/session) has long been known to augment the dilatation of caustic-induced esophageal strictures although results from most studies are still conflicting[29,30].

Recently, mitomycin C has been shown to decrease

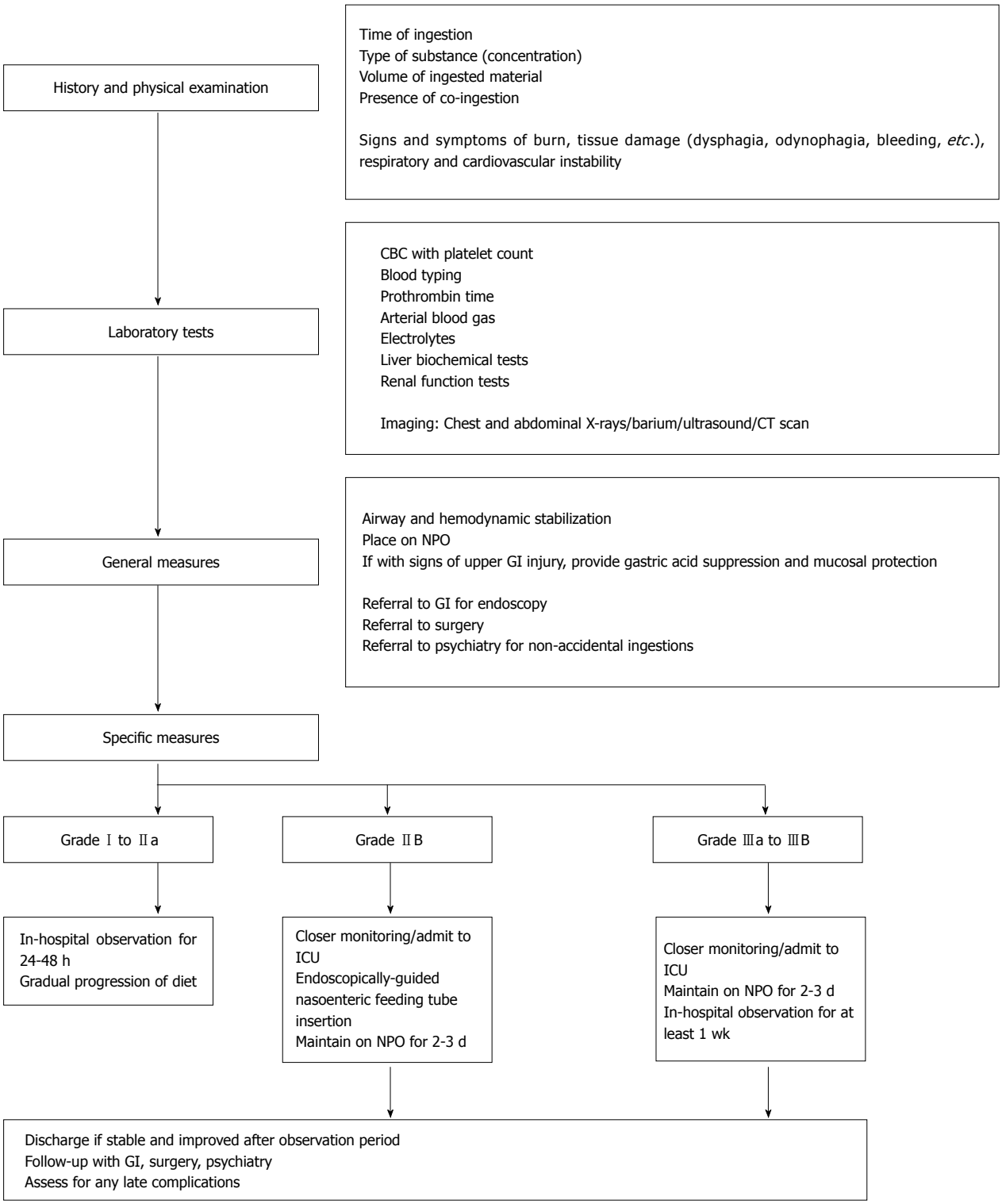

Figure 2 Management algorithm for caustic substance ingestion. CT: Computed tomography; GI: Gastroenterology; ICU: Intensive care unit.

the rate of caustic stricture formation in animals due to its antifibroblastic properties[31]. It has been used as an adjunct[32-34] after dilatation of caustic strictures in humans (including those with long strictures) by applying mitomycin-C topically at a dose of 0.4 mg/mL[34,35]. In a study of 16 patients treated with endoscopic topical application of mitomycin-C, a decrease in the number of dilatations and apparent relief of dysphagia were achieved compared to triamcinolone[35].

ENDOSCOPY

Endoscopy is important not only in the diagnosis of corrosive ingestion but also in determining subsequent management. In general, patients with normal looking mucosa or those with very mild injury may be dischar-

ged. For those with Zargar grade Ⅰ or ⅡA, in-hospital observation is advised and gradual progression of diet from liquids is done in the next 24 to 48 h. Patients with at least grade ⅡB are monitored more closely. An endoscopically-guided nasoenteric feeding tube may be placed with caution, bypassing the areas of necrosis, to facilitate feeding while initiating trial of per orem feeding. For grade Ⅲ injuries, the patient's response to treatment and feeding is usually observed for at least a week[14]. Prophylactic esophageal stenting in the acute setting is generally not recommended[36] due to a high perforation rate.

LATE COMPLICATIONS AND MANAGEMENT

Esophageal stricture is one of the most common sequelae of caustic injury. Up to 70% of patients with grade ⅡB and more than 90% of patients with grade Ⅲ injury are likely to develop esophageal stricture[37].

Peak development of strictures commonly starts on the 8th week post-ingestion, although it has been reported to occur as early as 3 wk[7,37,38]. The timing of management is crucial in achieving long-term functional effects.

Endoscopic dilatation

The primary non-surgical treatment of caustic esophageal stricture is endoscopic dilatation. This can be achieved with Bougies or balloon dilators. For tight and fibrotic strictures, bougies dilators are often more reliable than balloon dilators[37]. A prospective study published in 2015 assessed a rigorous weekly schedule of bougie dilatation (Savary-Gilliard) along with intralesional triamcinolone in patients with refractory esophageal corrosive strictures. It was noted that this intervention was safe and effective in improving dysphagia, achieving clinically significant dilatation, reducing dilatation frequency, maintaining luminal patency of $\geqslant$ 14 mm[14,39].

Using balloon dilators, a lower dilatation force should be used initially to avoid perforation[40]. This may need to be repeated and advanced slowly to achieve effective and safe dilatation. The interval between dilatations varies from 1-3 wk among different studies[16] but usually an interval of 3-4 wk is recommended.

For either technique, the goal is to achieve relief of symptoms (particularly dysphagia) and maintain efficient luminal diameter of up to to 15 mm[41].

Esophageal stents

Though endoscopic dilatation with balloon has been the standard of treatment for benign esophageal strictures, the recurrence rate still reaches 30%-40%. Approximately 10% of these patients fail to achieve clinical improvement and remain refractory to repeated dilatations. In such patients a good option is stent insertion. Recently, 3 types of stents are now available: Self expanding metal stents (SEMS), plastic sent, and biodegradable (BD) stent - each with its own advantage and disadvantage.

SEMS are often discouraged in benign esophageal stenosis due to its high rate of necrosis and ulceration, tissue hyperplasia, new stricture or fistula formation, and the tendency for the metal portion to embed within the esophageal wall. Plastic stents are said to have lesser tissue hyperplasia but with higher rate of stent migration and lower tendency to sustain significant radial force. Both of these stents require repeated endoscopic intervention for stent retrieval. Recently, BD have been introduced in the hopes of avoiding the above complications and the need for re-intervention for stent extraction[42].

A study in 2012 compared these 3 stents in patients with refractory benign esophageal stenoses. In this study, long-term resolution of dysphagia was highest in the metal stents group (40%) compared to BD stents (30%) and plastic stents (10%). Tissue migration was highest in the plastic stent group and lowest in the BD stent group[43]. To date, there is still no ideal stent recommended for universal use among patients with benign esophageal strictures, the choice for each patient should be individualized[44].

Surgery

Corrective surgery for esophageal strictures from caustic injury is done only in severe cases where endoscopic therapy fails or is deemed harmful. Surgical options include partial or total esophagectomy with gastric pull up or, preferably colonic interposition[38]. Gastric pull-up in general, is quicker and requires only one anastomosis. However, the long-term functional outcome may decrease with development of complications such as recurrence of stricture, bothersome reflux, and subsequent metaplasia over the anastomotic site[7,16,45-52]. On the other hand, colon interposition is a more complex procedure requiring 3 anastomoses, albeit with a more stable long-term functional outcome. It is often associated with a lower incidence of stricture formation than gastric pull-up hence its preferential use in the setting of a relatively spared and healthy stomach[16]. Mortality rates of late reconstructive surgery depend on local surgical expertise.

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FRONTIER

5-Aminosalicylates to maintain remission in Crohn's disease: Interpreting conflicting systematic review evidence

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Abstract

5-Aminosalicylates are a class of anti-inflammatory agents that have been used for decades in inflammatory bowel disease. Whilst they are first line for induction and an option for maintenance of remission in ulcerative colitis, the picture in Crohn's disease is variable. For maintenance of remission, key Cochrane systematic reviews have found conflicting results between the medical and surgical induced contexts. In this piece, the possible reasons for this are considered. It is proposed that clinicians should consider 5-aminosalicylates agents an option to maintain remission post-surgery. Future primary research is needed in the medical induced remission setting which considers the length of remission on enrolment and endoscopic or histological disease scores. Additionally, secondary research to rank the various treatment options in the post-surgical setting could be achieved through the use of network meta-analysis and will guide policy makers in the future.

Key words: 5-Aminosalicylate; Systematic review; Crohn's disease; Inflammatory bowel disease; Cochrane

Core tip: This paper proposes that the varying length of remission and disease activity of patients enrolled in studies for medically induced remission is different to surgical induced remission and may explain differences in findings. This guides future research proposals. Future primary research is needed in the medical induced remission setting which considers the length of remission on enrolment and endoscopic or histological disease scores. Additionally, secondary research to rank the various treatment options in the post-surgical setting could be achieved through the use of network meta-analysis.

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INTRODUCTION

There are broadly three classes of treatment that are commonly used to induce and maintain remission in inflammatory bowel disease (IBD): Antinflammatory agents, immunossupressive agents and biologic therapies. 5-aminosalicylates (5-ASAs) are a group of antiinflammatory compounds used for many years to treat IBD. The first 5-ASA used in clinical practice was to manage arthritis in the 1940s[1]. It was noted that patients who had concomitant IBD had improvements in their bowel symptoms.

COCHRANE EVIDENCE IN IBD

In the 1970s and 80s, there was growing academic and clinical concern with varying quality of primary research evidence and in particular reviews summarising evidence[2]. The concept of scientific medicine began to grow in response to this, which then became known as evidence based medicine[3]. The Cochrane Collaboration was at the forefront of evidence based medicine, leading the way in producing systematic reviews and methodological guidance for authors of reviews[4]. For 20 years, Cochrane has produced systematic reviews of primary research in human health care and health policy, and these are internationally recognized as the highest standard in evidence-based health care resources[5]. Whilst there have been criticisms of Cochrane reviews, they often inform international guidance and practice and so consideration of these reviews is vital for practising gastroenterologists.

WIDER COCHRANE EVIDENCE FOR 5-ASA AGENTS IN IBD

This class of agents have been employed in a variety of formulations within the context of IBD. In ulcerative colitis, it is well excepted in international guidance[6] and from Cochrane systematic reviews of the topic[7] that 5-ASA preparations are effective in inducing remission. Similarly, they are shown to be effective within Cochrane systematic reviews for the maintenance of remission in ulcerative colitis[8] and suggested as first line therapy for maintaining remission[6].

Interestingly despite this widespread evidence for effectiveness in ulcerative colitis, in Crohn's disease the evidence has always been more capricious. Early research demonstrated 5-ASAs are more effective for inducing remission in ileal, ileocolic, or colonic disease[9,10]. Due to this evidence, some 5-ASA agents have been frequently employed by gastroenterologists for mild Crohn's disease. However, a Cochrane review updated in 2016[11] that highlights a small benefit over placebo, but inferiority to other agents for inducing remission and mixed findings in newer studies of higher 5-ASA dosing. Until further research is performed, the authors do not suggest their use. This is also reflected in international guidance that note the variability in the evidence and do not currently suggest their use[12,13].

CONFLICTING COCHRANE EVIDENCE IN MAINTAINING REMISSION IN CROHN'S DISEASE

For maintaining remission in Crohn's disease, there is a significant difficulty in interpreting the Cochrane evidence. A recently published Cochrane review update[14] has found no evidence for the use of 5-ASA in maintaining medically induced remission. However, a review considering 5-ASA agents in post-surgical remission highlighted very different results[15]. 5-ASA was significantly more effective than placebo for averting relapses, with no statistical heterogeneity. A large number of subgroup analyses were completed to investigate length of follow up and dosage, with no change in the statistical significance of results, except when follow up was less than 12 mo. Clearly, this robust effectiveness result in the post-surgical setting[15] is at odds with the results for medically induced remission[14]. The only area of agreement between these two key reviews is related to occurrence of adverse events, with no statistical difference between 5-ASA and placebo.

The situation is further complicated in a complimentary review that investigates purine analogues for maintenance of post-surgical remission in Crohn's disease[16]. Whilst these were effective against placebo, there were only two studies in this analysis. The majority of studies compared to 5-ASA, reflecting their widespread use in this context. Meta-analysis of five studies showed no difference in preventing clinically diagnosed relapse at 12-24 mo post-surgery between 5-ASA and purine analogues. In fact, the trend in the risk ratio was towards 5-ASAs, suggesting inferiority of purine analogues. When considering adverse events that led to withdrawal of patients from treatment, these were statistically more common in the purine analogue patients compared to 5-ASA.

RECENT IMPACT ON PRACTICE GUIDANCE

As these key reviews[14-16] are reasonably contemporaneous, impact on international guidance is currently limited. However, UK guidance from the National Institute for Health and Care Excellence has recently reflected this evidence. Previous guidance clearly suggested the 5-ASA agents should not be recommended in the post-surgical settings[17], but the 2016 update now proposes 5-ASA can be offered[18] reflecting on this key Cochrane secondary evidence[15]. It remains to be seen whether other guidelines will shift advice in line with this evolving Cochrane evidence base.

UNDERSTANDING CONFLICTING RESULTS

The primary issue this spectrum of systematic review evidence raises is why 5-ASA agents have clear evidence of effectiveness in the post-surgical setting, but no

evidence in medically induced remission. There is no published research to give insight into these results, but the existing evidence base may hold the answer and allow hypotheses to be made.

Early evidence in Crohn's disease suggested that more mild disease was susceptible to 5-ASA agents[9,10], particularly in terms of the location of disease. Whilst surgery within Crohn's disease can be heterogeneous and is patient specific, it is long accepted that the most common indications relate to limited resections of particularly diseased areas with complications[19]. It is therefore possible that in the post-surgical setting, the patient has been reverted to a more disease naïve state within the remaining bowel, which due to pre-surgical medical management, is most commonly in a remission state. In many of the medical remission studies, this has been defined using clinical criteria and so at an endoscopic or histological level, there may well be disease activity. A counter view may suggest that because surgical patients had more severe disease, they do not have more mild disease. However, the author maintains that given the combination of surgical resection of these diseased areas and pre-surgical medical management, it is still likely that they represent a group with a different level of disease activity to the medical induce remission cohort of patients. This issue of clinical heterogeneity between the patient groups may explain why post-surgery evidence demonstrates efficacy of 5-ASA agents.

This hypothesis also raises a related methodological issue. Whilst studies included in the Cochrane reviews[14-16] in both medically and surgically induced remission had to define remission using accepted international rating scales, the timing of entry appears particularly capricious within the medically induced remission papers[14]. A review of the characteristics of studies suggests that patients could have been in remission for up to two years on entry within these studies. This is in stark contrast to the post-surgical remission papers reviewed that required study entry within at the most 60 d of surgery[15,16]. When this is combined with the accepted limitations of clinical disease activity scoring[20] compared to endoscopic or histological scoring methods, it is entirely possible that patients entering both sets of studies were simply not at a similar state of disease activity. In terms of 5-ASA agents and the acceptance that they are particularly efficacious in mild disease, this is a vital issue to consider.

The final issue to be considered is in the context of the post-surgical setting when comparing 5-ASA to Purine analogues. For those who have considered the individual study data within the Cochrane review[16] it will be apparent that there is clearly a contrast between primary study conclusions of purine analogue efficacy and the meta-analysis performed. This is due to the intention to treat analysis performed in the review. A per protocol analysis would suggest superiority of purine analogues, in line with the individual studies. This is not the method used in the review for several Cochrane methodological reasons related to risk of bias from incomplete outcome data. Given the clearly pervasive problem with over a quarter of patients on purine analogues not able to continue due to side effects[16] this clearly demonstrates the limitations of per protocol analysis and supports this approach from Cochrane. This was worth comment as readers may have found this discrepancy concerning. The wider relevance of this intention to treat finding is to once again suggest that 5-ASAs are not necessarily the most efficacious therapy in Crohn's disease for either induction or maintenance of remission, but there is universal agreement on their good safety profile[7,8,11,14-16].

IMPLICATIONS FOR PRACTICE

Based on the current Cochrane systematic reviews, 5-ASA agents cannot be recommended for maintenance of medically induced remission. However, in the post-surgical remission setting they are safe and effective. Given the concerning safety profile of purine analogues, it is proposed that clinicians consider this when discussing options with patients for post-surgical medication to maintain remission.

IMPLICATIONS FOR RESEARCH

There are two key areas that require further work. The first is within the medically induced remission setting. Given the volume of work suggesting the safety and potential efficacy, future large randomised controlled trials could be considered that pay particular attention to the extent and state of disease when entering the trial. Certainly, it is proposed that the use of endoscopic or histological methods to ensure induction of remission and consideration of the extent of previous disease are noted to ensure analysis can consider these factors that may be key in selecting appropriate patients for such therapy.

Secondly, given the most recent evidence now finds a role for 5-ASA agents in maintaining remission post-surgery in Crohn's disease, it is key to consider its relative efficacy to other agents, including immunosuppressive and biologic therapies. In the past, such analysis was impossible without individual primary trials investigating each comparison, but network meta-analysis offers this possibility[21]. This is a meta-analysis which allows multiple treatments to be compared directly and across trials using a common comparator, such as placebo. The end result of such analysis is to allow true conclusions to be drawn as to the relative efficacy and therefore shape future international guidance on such issues. The Cochrane Inflammatory Bowel Disease group is currently planning such a review.

CONCLUSION

It is proposed that clinicians should consider 5-ASA agents an option to maintain remission post-surgery, but evidence does not demonstrate similar efficacy in medically induced remission and so 5-ASA agents cannot be recommended in that context. Future primary research is needed in the medical induced remission setting which considers the length of remission on enrolment and endoscopic

or histological disease scores. Additionally, secondary research to rank the various treatment options in the post-surgical setting could be achieved through the use of network meta-analysis.

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MINIREVIEWS

Combination therapy for inflammatory bowel disease

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Abstract

Biologic therapies such as infliximab and adalimumab have become mainstays of treatment for inflammatory bowel disease. Early studies suggested that combination therapy (CT) with infliximab and an immunomodulator drug such as azathioprine may help optimize biologic pharmacokinetics, minimize immunogenicity, and improve outcomes. The landmark SONIC trial in Crohn's disease and the UC SUCCESS trial in ulcerative colitis demonstrated CT with infliximab and azathioprine to be superior to monotherapy with either agent alone at inducing clinical remission in treatment naïve patients with moderate to severe disease. However, many unanswered questions linger. The role of CT in non-naive patients as well as the optimal duration of CT remains unknown. The effectiveness of CT with alternate biologics and/or alternate immunomodulators is not as clear, and it is unknown whether SONIC's conclusions can be extrapolated beyond infliximab and azathioprine. Also looming are the risks of CT including opportunistic infection and malignancy; specifically, lymphoma. This review lays out the evidence as it pertains to the risks and benefits of CT as well as the areas that require further research. With this information in hand, the practitioner may develop a treatment strategy that best suits each individual patient.

Key words: Crohn's disease; Adalimumab; Vedolizumab; Ulcerative colitis; Infliximab; Inflammatory bowel disease; Methotrexate; Azathioprine

Core tip: The benefits of combination therapy (CT) with infliximab and azathioprine likely outweigh its risks in treatment naïve patients with moderate to severe Crohn's disease and ulcerative colitis. A similar benefit in patients already failing biologics or immunomodulators is not as well defined. There is a lack of strong prospective evidence demonstrating a benefit for CT with adalimumab and an immunomodulator. While expert guidelines emphasize the use of CT, its use should be preceded by a careful weighing of the risks and benefits by the physician and patient, especially in scenarios where the strongest

evidence for CT may not directly apply.

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INTRODUCTION

Traditional management of inflammatory bowel disease (IBD), both Crohn's disease (CD) and ulcerative colitis (UC), involved the stepwise use of 5-aminosalicylate compounds, followed by steroids and then an immune modulator (IMM) such as 6-mercaptopurine (6MP), azathioprine (AZA) or methotrexate (MTX) in those individuals unable to successfully taper off steroids, or those with rapid disease recurrence once steroids were withdrawn. While the IMMs are generally ineffective agents for induction of response or remission in IBD[1] the thiopurines 6MP/AZA have proven to be effective for the maintenance of response and remission in CD and UC[1,2] while the purine analogue MTX appears to offer the same benefit for CD[3]. Beginning in the 1990's, biologic therapies targeting tumor necrosis factor alpha (TNF-α) entered into this paradigm. The first in class medication infliximab (IFX) was initially shown to be effective both for induction and maintenance of remission for CD, and latter for UC. In the years that followed, IFX was followed by other TNF-α blockers including adalimumab (ADA) for CD and UC, certolizumab pegol for CD, and golimumab for UC. Even more recently we have seen the addition of biologics targeted at different points in the body's inflammatory response, such as the anti-integrins natalizumab and vedolizumab (VDZ) which inhibit the migration of white blood cells, mostly activated T cells to areas of bowel inflammation, as well ustekinumab which blocks the IL 12/23 pathway of inflammation.

Consistently, clinical trials of biologics have involved the use of these newer therapies in combination with IMMs. Initially, the use of this form of combination therapy (CT) was a natural outgrowth of the failure of IMMs to fully control disease in some of the clinical trial population, with the biologic therapy added on to continued IMM treatment. While the initial clinical trials of IFX did not show any improved response with the use of CT over monotherapy with biologic alone, there were some other findings which suggested that the use of both classes of medications together might be superior to one or the other alone. In a way, the potential benefit of CT would seem to be an answer to an obvious question: If one has access to two separate therapies with different mechanisms, each less than 100% effective, can the use of both in combination increase the rates of response over each individually?

In the following review we will address the basic questions both the clinician and patient will need to have answered before considering the use of CT; (1) Does CT work, and why does it work? (2) Is CT effective for those with either CD or UC? (3) Is CT effective for different combinations of IMM and biologic? (4) Is CT effective at all stages of IBD therapy? (5) Is CT safe? (6) Is CT being utilized? and (7) What do the experts say about CT?

DOES CT WORK/WHY DOES IT WORK?

Though the earliest clinical trials of IFX were not designed to assess the efficacy of CT, study design permitting continued IMM use provided some early data on the effect of CT. Given the few options for alternate therapy available at the time, a majority of patients in the phase 3 trials of IFX for both CD and UC had experienced prior failure of IMM therapy with either 6MP/AZA or MTX. For many, this failure to achieve remission likely involved a partial response rather than a complete lack of efficacy. In either case, large numbers of patients entering these trials continued on prior IMM therapy. This "step up" approach to CT will be discussed in more detail in the following sections. In the case of the CD trials ACCENT 1 and ACCENT 2[4,5] approximately 50% of study patients, well matched by active treatment and placebo arms, continued on IMMs. In the UC studies of IFX, ACT 1 and 2, approximately 33% of patients were on IMMs at study entry[6].

Overall, the clinical trials of IFX did not show any improved clinical efficacy associated with the use of CT. These early trials did however give the first hints of how CT might provide a benefit to the IFX patient over monotherapy in the form of decreased immunogenicity. Overall, the development of antibodies to IFX (ATI) were significantly lower in the CT patients, with 4%-20% without CT developing ATI, compared to rates of 4%-6% among those using CT[7]. This effect was noted to be greatest for those patients using the current standard 5 mg/kg dose of IFX. There was also no observed benefit in terms of higher IFX levels, but neither was there any increase in the rates of infections. Along with the lower ATI levels for those using IMM, were lower overall rates of infusion reactions at 12.5%, compared to 22.0% for those not using IMM.

Following up on these early observations, subsequent investigations began to take a closer look at the interplay between IFX, the development of anti-drug antibodies and possible impact on IFX drug levels, treatment reactions and clinical efficacy. In a prospective non-randomized trial, Baert *et al*[8] followed 123 patients on IFX, with 47% receiving concurrent IMM. In this study, as was common at that time, patients with luminal disease were treated with episodic rather than scheduled IFX therapy, while those with fistulizing disease received a week 0, 2 and 6 induction regimen followed by episodic treatment. Overall, patients received a mean number of 3.9 infusions. In total, 61% developed ATI. Higher antibody

levels > 8.0 μg/mL predicted shortened clinical response, 35 d *vs* 71 d ($P < 0.001$), with higher levels of ATI in those without IMM usage ($P < 0.001$) and lower drug levels in those without IMM usage ($P < 0.001$). Infusion reactions were found to be more common among those not using IMM [relative risk (RR) = 2.40; 95%CI: 1.65-3.66; $P < 0.001$]. Vermeire *et al*[9] performed similar work using IFX on demand for both luminal and fistulizing CD. They enrolled 174 patients who received IMM (either MTX or AZA) or no IMM in a non-randomized fashion. MTX was given subcutaneously at 25 mg weekly for 12 wk followed by 15 mg weekly, while ASA was given at a weight-based dose of 2-2.5 mg/kg. ATI levels were checked at 4 wk following IFX doses. Again, episodic treatment with IFX resulted in high ATI levels, especially for patients not receiving concomitant IMM. Overall they observed 73% of patients without IMM developing ATI, compared to 46% with IMM, $P < 0.001$. This effect was consistent across IMM types, with 44% of MTX patients developing ATI *vs* 48% of AZA patients, P = NS. There was a trend towards higher average IFX drug levels with IMM, 2.22 μg/mL *vs* 6.45 μg/mL, $P = 0.065$, and significantly less infusion reactions with IMM, 16% *vs* 40%, $P = 0.04$.

Taking into account the two main observations of IFX immunogenicity at the time, the association of lower ATIs with scheduled treatment[10,11] and concurrent use of IMM, the Study of Biologic and Immunomodulators Naïve Patients in Crohn's Disease (SONIC) trial was designed to answer the question of whether clinical response was superior with CT over monotherapy[11]. Unlike the earlier clinical trials, patients entering SONIC were entered into one of three treatment arms and followed prospectively. Additionally, given the strong association between episodic dosing, antibody formation and decreasing effectiveness of treatment, all patients in SONIC and future trials of CT were given IFX on a fixed schedule rather than episodically, as is the current practice. In total, 508 patients were randomized to either IFX monotherapy (with oral placebo), AZA monotherapy at 2.5 mg/kg (with IV placebo), and CT with IFX and AZA. All patients in the study were naïve to both IMM and biologics, had a Crohn's Disease Activity Index (CDAI) > 220, and underwent ileocolonoscopy at baseline. The primary study endpoint was steroid free clinical remission at 26 wk, defined by a CDAI < 150. This endpoint was achieved by 30.0% of those on AZA monotherapy *vs* 44.4% on IFX monotherapy, ($P = 0.006$) and 56.8% of those on CT, which was significantly greater than either AZA ($P < 0.001$) or IFX monotherapy ($P = 0.02$). Though CT achieved higher rates of mucosal healing than IFX alone, 43.9% *vs* 30.1%, this result was not found to be statistically significant, $P = 0.06$, likely due to the large number of patients without active disease found on baseline ileocolonoscopy. Additional findings again mirrored those of earlier studies, showing higher week 30 IFX trough levels with CT *vs* IFX monotherapy, 3.5 μg/mL *vs* 1.6 μg/mL ($P < 0.001$), and lower incidence of ATI, 0.9% *vs* 14.6%. Notably, serious adverse events (SAE) were actually lower with CT than IFX monotherapy (15.1% *vs* 23.9%, $P = 0.04$). Serious infections were similar across treatment groups, with 3.9% of patients on CT, 4.9% of those on IFX monotherapy, and 5.6% of those on AZA alone.

IS CT EFFECTIVE FOR UC?

Though SONIC was notable in regards to the generally short median disease duration of 2.3 years of participants, it did appear to provide an answer to the question of the superiority of CT over monotherapy with IFX, at least for the select group of treatment naïve patients with CD. Following up on these findings the UC SUCCESS trial was designed to answer the same question, and determine if CT with IFX and AZA was also more effective for UC[12]. With a similar study, 239 patients with active UC confirmed by sigmoidoscopy were enrolled to treatment arms of IFX with oral placebo, AZA with IV placebo, and AZA. Again, all patients were biologic naïve, though prior AZA exposure (discontinued at least 3 mo earlier) was permitted. The primary study endpoint of steroid free remission at week 16, defined by a MAYO score ⩽ 2, was achieved by 39.7% of CT *vs* IFX monotherapy ($P = 0.017$). Mucosal healing, defined by a subscore of 0 or 1, showed a trend towards greater effect for CT *vs* IFX monotherapy 62.8% *vs* 54.6% (P = NS), and complete mucosal healing defined by an endoscopic subscore of 0 was significantly greater for those on CT *vs* IFX monotherapy, 29.5% *vs* 11.7%, $P = 0.006$. Again, no increased incidence of SAE was observed with CT. Serious infections were similar in all three groups, (0 in the CT group, 1 in the IFX monotherapy group, and 1 in the AZA monotherapy group).

IS CT EFFECTIVE FOR OTHER IMMS?

Of course thiopurines were not the only IMMs that had shown potential benefits when used with IFX. MTX had demonstrated similar effects of decreasing ATI and increasing IFX trough levels. With that in mind, a prospective study of MTX with IFX, the COMMIT trial, was preformed comparing IFX monotherapy and subcutaneous placebo to IFX with subcutaneous MTX for patients with CD[13]. Like SONIC the study enrolled biologic naïve patients, but other inclusion criteria and study methods were notably different. There was no need for minimum baseline CDAI, and inclusion only required that patients had required steroids within 6 wk prior to enrollment. Additionally, all IFX infusions were given along with 200 mg of IV hydrocortisone as premedication. The primary study endpoint was failure to achieve steroid free remission at week 16 (defined by a CDAI < 150), or failure to maintain remission through week 50. In total 126 patients were enrolled, with an average disease duration of over 10 years in each treatment arm, as well as a relatively low CDAI of 207 for both CT and monotherapy groups. The week 14 primary endpoint of steroid free remission was not

found to be greater for CT *vs* monotherapy, 76% *vs* 78%, and neither was the week 50 endpoint, 56% *vs* 57%. Critiques of the trial have pointed at the overall low baseline levels of CDAI, suggesting that it is more difficult to detect a significant response to therapy when the disease is less severe. Also, the use of hydrocortisone along with all infusions may have offered additional clinical benefit, again obscuring any distinct MTX effect. Even so, the trial again demonstrated the ability of MTX to modify immune response to IFX, with lower ATI in the MTX arm *vs* placebo, 4% *vs* 20% (P = 0.01), and a trend towards higher IFX trough levels, 6.35 μg/mL *vs* 3.75 μg/mL (P = NS).

IS CT EFFECTIVE FOR OTHER BIOLOGICS WITH IMMS?

The next biologic, possessing a similar mechanism of action to IFX, was adalimumab (ADA). ADA differs from IFX not only by its subcutaneous route of delivery, but by its fully humanized protein structure. Given that the main benefit of IMM with IFX appeared to be linked to blunting an immune response, it could not be assumed that ADA would be as immunogenic, or that IMM with ADA would demonstrate the same benefits. In fact, early studies of ADA pharmacokinetics and clinical outcomes did demonstrate a correlation of clinical response to higher ADA trough levels and lower antibody to adalimumab, similar to prior observations with IFX. Unlike IFX however, early investigations did not find that IMM influenced these outcomes[14]. A retrospective analysis of mixed IBD patients using IFX (n = 108) again showed increased drug levels (P = 0.037) and decreased antibodies to IFX (P = 0.001) among those using IMM. This benefit to IMM was not found among the 109 ADA treated patients, with CT showing similar drug trough levels (P = 0.496) and antibody levels (P = 0.63)[15] to those on ADA monotherapy. A recent large meta-analysis of ADA pharmacokinetics of 14 studies included 1941 patients with mixed IBD diagnoses with available clinical outcome, drug trough and antibody data available. Once again, clinical response was associated with higher drug trough and low antibody to ADA, but CT did not appear to influence either antibody or drug trough levels[16]. The study suggests that antibodies to ADA do occur, they do appear to cause low levels of trough ADA and lessened clinical effect, but there is a lack of evidence suggesting that IMM have the ability to prevent the development of these antibodies.

To the present time there has been no trial of ADA matching the designs of either SOINIC or UC SUCCESS. While not a substitute for a prospectively designed trial, there is still clinical data available addressing the issue of CT with ADA and IMM. Another meta-analysis designed to look specifically at clinical outcomes with ADA monotherapy *vs* CT among CD patients included 18 studies [randomized control trials (RCT), open-label prospective, observational studies, cohort and case-control studies] with 2280 ADA monotherapy patients and 2014 CT patients[17]. Of the 6 studies analyzing induction of remission (960 ADA, 997 CT), the use of CT was associated with greater clinical response OR = 0.79 (0.65-0.96); P = 0.02, though this was not found to be the case when the analysis was limited to the RCT, OR = 1.11 (0.72-1.73); P = 0.64. There was also no evidence of clinical benefit to CT for induction of response OR = 0.68 (0.37-1.25); P = 0.22, 12 mo remission OR = 1.08 (0.79-1.48); P = 0.61, or 12 mo response OR = 1.21 (0.74-1.99); P = 0.44. At present there is even less data specifically addressing the clinical impact of IMM with ADA for UC. As was the case with the early IFX trials, almost half of the patients in the initial clinical trials were using IMM at enrollment. Though the remission rates were higher with CT, the small absolute number of patients involved and the absence of a specific prospective trial design should caution against any definitive conclusions.

Since IFX and ADA were the first biologics approved for IBD treatment, most of the current data on CT deals with IFX and ADA. Of course, biologic development has continued beyond this class of medications, most recently with the addition of the new integrin inhibitor, VDZ. While not the first in class, with that distinction going to natalizumab, the updated mechanism of action targets α4β7 on circulating white blood cells. Blockade of this gut specific integrin decreases WBC adherence to the vascular endothelial wall, and subsequent migration to areas of inflammation. As is the case for all non-IFX biologics, there is currently no prospectively designed study addressing CT of VDZ with IMM. Review of the results of the large phase 3 clinical trials offers some of the early immunologic data seen with earlier biologics. GEMINI 1, enrolling 895 patients with UC for induction and maintenance, included a third of patients with concurrent IMM use. Overall, antibodies to vedolizumab (AVA) were infrequent, found in 3.7% of patients at "any time" during testing, with a mere 1.0% testing positive on ⩾ 2 samples[18]. GEMINI 2, enrolling 1115 patients with CD for induction and maintenance also included a third with concurrent IMM use[19]. Overall AVA were again infrequent, 4.1% at "any time", and 0.4% on ⩾ 2 samples. The authors of each study commented that "concomitant immunosuppressive therapy was associated with decreased immunogenicity (data not shown)".

More recently an analysis of the phase 2 and 3 trials for both CD and UC has been preformed, addressing the issue of CT[20]. Among a total of 2830 patients, covering 4811 patient years there was no observed increased risk of adverse events. During active VDZ therapy, CT patients had a 3% risk of AVA, compared to 4% for VDZ monotherapy. As has previously been noted for TNF-α inhibitors, higher levels of anti-drug antibody were seen following completion of VDZ therapy among those patients without IMM as compared to those

Table 1 Author's summary of the evidence for combination therapy

	Crohn's disease		Ulcerative colitis	
	Clinical benefit	Pharmacokinetic/immunogenic benefit	Clinical benefit	Pharmacokinetic/immunogenic benefit
IFX + AZA/6MP (treatment naïve)	+	+	+	+
IFX + AZA/6MP (step-up from immunomodulator monotherapy)	-	NA	NA	NA
IFX + MTX	+/-	+	NA	NA
ADA + IMM	+/-	+/-	NA	NA
VDZ + IMM	NA	+	NA	NA
Ustekinumab + IMM	NA	NA	NA	NA

IFX: Infliximab; AZA: Azathioprine; 6-MP: 6-mercaptopurine; MTX: Methotrexate; ADA: Adalimumab; VDZ: Vedolizumab; IMM: immunomodulatory; +: beneficial; +/-: Possible benefit; NA: No adequate data available.

with ongoing IMM use, 18% *vs* 3%. Theoretically, this may have implications for issues such as prevention of AVA during VDZ drug holiday, and potential infusion reactions and/or drug effectiveness on resuming therapy. It does not however offer answers to the key question of risks and benefits of CT with VDZ and IMM.

Even more recently Ustekinumab, targeting the p40 subunit of IL-12/23 has obtained regulatory approval for induction and maintenance therapy for CD. In the recently published phase 3 induction and maintenance trials, approximately a third of patients received concurrent IMM with Ustekinumab or placebo[21]. The investigators have yet to publish data analyzing the effect of CT, though they did report an overall low level of antidrug antibodies at 44 wk of 2.3%. Again, while there is no prospective clinical trial data yet available on CT, a recent retrospective study from the GETAID group analyzed their experience with 122 treated patients[22]. All 122 patients were prior treatment failures with TNF-α inhibitors, with only 18 using IMM at the time of ustekinumab therapy. Of all factors analyzed, only IMM use was found to be a predictor of 3 mo clinical benefit, OR = 5.43; 95%CI: 1.14-25.77; P = 0.03 (See summary of evidence for induction CT, Table 1).

IS CT EFFECTIVE AT ALL STAGES OF IBD THERAPY?

Step up therapy: Adding biologic to failing IMM

As we have seen, most of the available evidence suggesting a benefit to CT involves the use of IFX and IMM begun simultaneously, especially in those naïve to biologic as well as to IMM. In reality, IMM is still widely used as part of a step up algorithm of care, with biologics employed as additional therapy in cases of IMM failure as in the early clinical trials. Given the frequent positioning of IMM as mono-therapy prior to biologic, a specific look is required into the role of continuing IMM as part of a combination step-up therapy.

A recent analysis by Osterman *et al*[23] retrospectively analyzed a cohort of CD patients beginning biologic therapy with either IFX or ADA, 1459 and 871 patients respectively. In total 381 CT patients using IFX and IMM were matched to 912 monotherapy IFX patients, as were 196 CT using ADA and IMM matched to 505 ADA monotherapy patients. In the IFX group, 86% of the CT patients were part of a step-up protocol, adding biologic to existing IMM, as were 89% in the ADA group. These high percentages effectively made the analysis of the effect of CT into an analysis of CT as part of a step-up treatment approach. Thiopurines accounted for 90% of IMM use. Given the retrospective design, the authors were unable to analyze for common clinical trial outcomes such as improvement in CDAI or endoscopic response and remission. Looking at alternate outcomes, they were unable to show any benefit to CT in terms of surgery (HR = 1.20, OR: 0.73-1.96), hospitalization (HR = 0.82, OR: 0.57-1.19), rates of combined biologic discontinuation and surgery (HR = 1.09, OR 0.88-1.34) or serious infections overall (HR = 0.93, OR 0.88-1.34). Rates of opportunistic infections were significantly increased (HR = 2.64, OR: 1.21-5.73), mostly due to increased rates of herpes zoster (HR = 3.16, OR: 1.25-7.97). These findings were consistent across the subgroups for both IFX and ADA. The overall conclusion: there is no apparent benefit to continuing IMM, in cases of IMM failure, once biologic therapy is begun. Similarly, a recent meta-analysis by Jones *et al*[24] reviewed the results of 11 randomized trials of anti-TNF-α therapies including IFX, ADA and certolizumab, among 1601 patients of which 40% were on CT. All patients on CT received biologic as part of a step-up approach after failing to achieve remission with IMM. Again, there was no benefit to CT for the outcomes of 6-mo remission (OR = 1.02; CI: 0.80-1.31), 6-mo response (OR = 1.08; CI: 0.79-1.48). Neither however was there any increase risks of adverse events with CT (OR = 0.71; 95%CI: 0.41-1.25).

Step up therapy: Adding IMM to failing biologic

The issue of stepping up to CT by the addition of IMM to failing biologic is less well studied. A small retrospective cohort analysis by Ben-Horin *et al*[25] examined the outcomes of 5 patients (3 with CD, 2 with UC) with a secondary loss of response to IFX associated with

high ATI levels and undetectable trough. Two patients were treated with MTX and 3 received either AZA/6MP. In all cases patients experienced a decrease in ATI, an increase in trough, and a recapturing of clinical response. Despite the questionable efficacy of CT when the anti-TNF is ADA, the same group has recently shown a similar result when adding IMM as salvage therapy to failing ADA in 23 patients (21 with CD, 2 with UC) with confirmed antibodies to ADA. Salvage therapy with IMM (14 with thiopurines, 9 with MTX) was associated with elimination of antibodies to ADA, increased ADA levels, and recapturing of response (median time to sero-reversal 5 mo) in 11 patients (48%)[26].

Optimal duration of successful CT

The final issue to address with regard to effectiveness of CT is the question of duration: For those patients in remission on CT, for how long should they continue to take the IMM? The retrospective data on de-escalation is mixed[27]. There is very limited prospective controlled data to guide therapy. Van Assche *et al*[28] from Belgium reported on a group of 80 CD patients with disease controlled on CT for a minimum of 6 mo, at IFX doses of 5 mg/kg, at intervals of every 8 wk or greater. Patients were randomized to maintenance with IFX and placebo *vs* continued CT, and followed for 104 wk. The primary outcome was the need to decrease the IFX dosing interval or discontinuation of IFX. Secondary outcomes included IFX trough levels and safety. While those patients discontinuing IMM showed significantly lower IFX trough levels at 54 wk, 1.65 μg/mL *vs* 2.87 μg/mL ($P < 0.0001$), and a trend towards higher CRP levels, there was no difference at 104 wk with regards to the need for rescue IFX, discontinuation of IFX. The authors concluded that there was no benefit to IMM beyond 6 mo in patients achieving remission with combination IFX and IMM. Another more recent prospective study however suggested a possible benefit to continued CT. Eighty-one patients on CT for at least 1 year were randomized to continuation of CT at the same dose (Cohort A), reduction of azathioprine dose by 50% (Cohort B), or complete cessation of azathioprine (Cohort C)[29]. While differences in clinical outcomes at one year were not statistically significant ($P = 0.1$), there was a trend towards higher relapse rates in Cohort C (30.7% *vs* 17.8% and 11.5% in Cohorts A and B). Only in Cohort C were infliximab trough levels significantly decreased at one year as compared to study initiation (4.2 μg/mL *vs* 2.1 μg/mL, $P = 0.02$). This data also suggests that a reduced dose of maintenance immunomodulator may provide similar benefits as full dose maintenance CT.

IS CT SAFE?

CT and lymphoma

Though most of the additional risk associated with CT relates to an increased risk of infections, particularly opportunistic infections with Candida and Herpes Zoster[30] risk of lymphoma casts a long shadow over any discussion of CT. Since CT has typically meant thiopurines with biologic, it is first important to acknowledge that the vast majority of evidence points to a 4 to 5 fold increased risk of lymphoma associated with thiopurine use. This figure has been observed both in a meta-analysis of referral center IBD patients, as well as in the recent CESAME population study from France, which noted that this risk was primarily associated with active thiopurine use[31,32]. The case for an increased risk of lymphoma with biologic monotherapy is far weaker, particularly for those with IBD[33]. Most evidence supporting this increased risk is drawn from the larger rheumatoid arthritis population, for which the disease itself is known to carry an increased risk[34].

In the absence of large population data on lymphoma risk with CT, investigators have employed mathematical modeling incorporating the observed increased risk with thiopurines to predict the risk/benefit of lymphoma with CT. Scott *et al*[35] in a recent Markov model analyzed the risk/benefit of IFX monotherapy *vs* CT at a variety of patient ages, utilizing quality of adjusted life years (QALY) as their primary outcome measure. The analysis accounted for the benefits of CT including increased response and remission rates, decreased surgery and less CD related death, balanced against the risk of death related to lymphoma and infections. They concluded that CT increased QALY for all patients, with that benefit decreasing as the patients aged. A patient 55 years or younger could expect to benefit from CT for at least 7 years. Even for those over 75 years, with the highest background risk of lymphoma, they estimated that it would take almost 5 years for QALY to suffer by continued use of CT. Another recent analysis by Siegel *et al*[36] utilized a Monte Carlo Simulation to predict the effects of one year of IFX monotherapy *vs* CT. in a theoretical population of 100000 thirty-five-year-old modeled on the SONIC trial. Here again the authors predicted that CT would result in an increased numbers of lymphomas for CT *vs* IFX monotherapy, 60 *vs* 40 cases respectively. However, since most infections observed in CD are related to the underlying disease activity rather than opportunistic infections, they also predicted that the more effective treatment of CD with CT would result in far fewer serious infections with CT *vs* IFX monotherapy, 3892 *vs* 4884, ultimately resulting in fewer deaths (399 *vs* 446). The authors concluded that the benefits of CT would continue to outweigh the risks unless serious infections occurred in over 20% of CT patients-a rate 5 fold greater than predicted, or if lymphoma occurred in over 3.9% of CT patients-a rate 65 fold higher than predicted.

No review, however, of CT can be complete without addressing the rare, but frightening complication of hepatosplenic T-cell lymphoma (HSTL), an aggressive and almost uniformly fatal disease that has been described

among IBD patients using CT. A recent systematic review of the literature by Kotlyar *et al*[37] documented 36 IBD patients who developed HSTCL. Of these, 20 received CT with a thiopurine and a TNF-α inhibitor, and 16 had thiopurine monotherapy. There were no cases reported of HSTCL with TNF-α inhibitor monotherapy. Only 2 (6.5%) were female, and the median age was 22.5 years. Notably only one patient, in the CT group, had a history of less than 2 years of thiopurine use. Overall, the authors concluded that the risk of HSTCL was highest for young men on CT, estimated at 1:3534.

Utilization of CT

Just as the literature addressing CT provides a variety of outcomes depending upon the population analyzed and the question being asked, so too does the real world data on the utilization of CT. In a recent large prospective survey study of seven high volume tertiary referral IBD practices, 1659 patients with CD, 946 with UC, and 60 indeterminate colitis, a wide variation of usage of CT was noted, particularly among those with CD[38]. While initially only including those with an IBD diagnosis of less than 4 years, the authors ultimately included patients with all disease durations in their cohort. For those with CD, the lowest site utilization rate of CT was 8%, *vs* 32% at the site with the highest frequency, adjusted OR (95%CI) 3.15 (1.79-5.56). The authors report that the results observed were similar when excluding the site with the lowest frequency from each parameter of analysis.

Among the entire CD cohort, slightly more than half of anti-TNF use was as part of CT, with 47% overall on anti-TNF and 21% on CT. For those with UC, the range of usage of CT was 6% to 13%, OR 1.14 (0.48-2.78). Among the entire UC cohort, less than a third of anti-TNF use was part of CT, with 23% overall on anti-TNF and 9% on CT. It should be noted that the authors did not provide a breakdown of the type of biologic therapy used, so we have no way of knowing if the proportion of CT usage was higher among IFX patients, where the evidence to support CT is significantly stronger. Additionally, the results do not specify rates of CT usage for induction *vs* maintenance, where we have also seen differing degrees of supporting data.

A recent population wide study from France[39] prospectively followed all IBD patients affiliated to the French national health insurance, tracking treatment and outcomes over the years 2009-2014. During that time there were 69725 new incident patients with IBD. CT was defined as the concomitant initiation of anti-TNF's and thiopurines in a period of 30 d. Among these newly diagnosed CD patients, the 5-year cumulative probability of CT and anti-TNF monotherapy was 18.3% and 33.8% respectively. For UC, the 5-year cumulative probability of CT and anti-TNF monotherapy was 7.4% and 12.9% respectively, *i.e.*, CT accounted for just slightly more than half of anti-TNF use. The authors report that CT was more frequent with IFX after one year than with ADA for both CD and UC patients (4.2% *vs* 3.1% and 1.7% *vs* 0.6%) respectively. Given that this data arises from a large/general population, it is not surprising to see lower overall rates of biologic use and CT use than in the population from the IBD referral centers. It is noteworthy however that the proportion of CT use among those using anti-TNF is fairly similar.

In a retrospective review of community trends of biologic use from the US, we analyzed referrals to our institution's infusion center which provided IFX infusion services to both the full time teaching faculty, as well as to private practice gastroenterologists[40]. Overall 247 new IFX referrals (154 CD, 93 UC) started on treatment from 2002 to 2014. Only 23.3% of patients received CT at the time of their first infusion (24% CD, 20.4% UC). These results were similar when analyzing the subgroup of 127 patients receiving IFX as part of a standard 0, 2, 6 wk induction regimen. Again, only 26% of CD and 28% of UC patients were on CT during their first induction IFX infusion. Notably, there was no trend observed of increasing use of CT over the years, despite the accumulating evidence of its benefit.

WHAT DO THE EXPERTS SAY?

Guideline recommendations

Finally, taking into account the available evidence, major GI professional societies have provided their consensus guidelines regarding CT use in the management of IBD. As with any guideline, it is important to note not only the type of recommendation provided, but also the grading of the recommendation based on the quality of supporting evidence and the year in which the guideline appeared (Table 2).

In 2009 the Practice Parameters Committee of the American College of Gastroenterology (ACG) recommended IFX monotherapy or IFX combined with AZA as more effective than AZA in the treatment of patients with moderate to severe CD failing first-line therapy with mesalamine and/or corticosteroid who were naïve to IMM and biologic[41]. Additional ACG guidelines the following year were unable to support the same recommendation for UC[42]. The 2011 guidelines from the World Congress of Gastroenterology with the European Crohn's and Colitis Organization concluded that CT of IFX and AZA was superior to induction of remission and mucosal healing over a 1 year time period. The authors further stated that it was uncertain if this was the best strategy beyond one year of treatment, and that it was unknown if this was true for other biologic/IMM combinations[43]. In 2013 the American Gastroenterological Association (AGA) published its guidelines on the use of thiopurines, MTX and anti-TNF-α drugs for the treatment of CD. The authors suggested using anti-TNF-α in combination with thiopurines over anti-TNF-α monotherapy to induce remission in cases of moderately severe CD (Weak Recommendation, moderate quality evidence), again showing the strong impact of SONIC on clinical thought.

Table 2 Summary: Major society guidelines addressing combination therapy

	CD	UC
American College of Gastroenterology (2009 CD, 2010 UC)	IFX or IFX and AZA superior to AZA	Unknown efficacy of CT
European Crohn's and Colitis Organization and World Congress of Gastroenterology (2011)	IFX and AZA superior to monotherapy (in treatment naïve)	Unknown efficacy of CT
American Gastroenterological Association (CD guidelines (2013)	Anti-TNF-α and AZA superior to monotherapy	
American Gastroenterological Association Clinical Care Pathways (2014 CD, 2015 UC)	Consider IMM with anti-TNF-α or 2nd/3rd line biologic	Consider IMM with all anti-TNF-α or VDZ use
Hong Kong IBD Society (2013)	Anti-TNF-α and AZA superior to monotherapy	CT not addressed
Indian Society of Gastroenterology (UC consensus)		CT not addressed
Asian Pacific Association of Gastroenterology (UC consensus)		CT not addressed
Japanese Society of Gastroenterology (CD guidelines)	CT Not addressed	

IFX: Infliximab; AZA: Azathioprine; IMM: Immunomodulator (includes AZA, 6-mercaptopurine, Methotrexate); VDZ: Vedolizumab; CT: Combination therapy; UC: Ulcerative colitis; CD: Crohn's disease.

The authors go on to acknowledge the uncertain benefits of CT in cases of prior IMM failure, CT with other anti-TNF-α drugs, as well as CT using MTX[44].

More recently in 2015, a panel of IBD experts in association with the AGA published pathways of care to aid clinical decision making. In the UC care pathway, at all steps where treatment with anti-TNF or VDZ is indicated, the authors recommend consideration of the addition of either a thiopurine specifically, or IMM generally. The authors support the use of MTX as an alternate IMM to thiopurine[45]. A similar AGA pathway for CD in 2014, the "Crohn's Disease Evaluation and Treatment: Clinical Decision Tool", also supports CT as an option for all patients receiving anti-TNF therapy. The pathway emphasizes that the addition of an IMM offers improved efficacy and should be considered in moderate to high risk patients receiving their 2nd or 3rd biologic[46]. Neither pathway addresses how long CT should be utilized.

Consensus statements from Asian medical societies do not emphasize CT as much as their western counterparts. The Japanese, Indian and Asia-Pacific societies for gastroenterology do not address the potential therapeutic benefits of CT in their respective IBD guidelines nor do they cite the SONIC trial[47-49]. In contrast, in a guideline issued by the Hong Kong IBD society a class A recommendation states that CT is the most effective way to induce remission in moderate to severe CD[50]. The guideline goes on to recommend an individualized weighing of risks and benefits of CT for each patient. It is likely that further patient experience and review may lead to increased attention into the role of CT in non-European/North American expert reviews and guidelines. As for now, those studies showing the greatest benefit to CT, specifically SONIC and UC SUCCESS, almost exclusively studied European/North American populations. Patient characteristics with regard to race are not addressed in UC SUCCES, but the population in SONIC is specifically identified as over 90% "white race". This raises the possibility that our strongest data on CT may not be generalizable to those in other regions.

CONCLUSION

While newer IBD therapies continue to be developed and tested in clinical trials, for the vast majority of patients and their physicians the emphasis remains on the best possible use of currently approved therapies to control disease activity. With the available choices expanding, the definition of CT may eventually broaden to include combinations of multiple biologics, but for now CT is defined by IMM use along with biologic.

The available evidence does suggest a benefit to CT, but this evidence is clearer for the use of IMM with IFX specifically, and especially in those without prior IMM or IFX use. This benefit appears to apply to both patients with CD and UC. The level of evidence for the benefit of IMM with other biologics is not a clear, nor is it certain that this combination if applied sequentially as step up therapy offers the same improved response as starting the two together. The main mechanism of benefit of IMM in the setting of biologic appears to be through the suppression of antibody formation to the biologic treatment. With less inherent immunogenicity to newer biologics, it is perhaps not surprising that the benefit of adding IMM is harder to define with other combinations. To better answer the question would require dedicated prospective studies of each CT as was the case with IFX, which are unlikely to be performed. With regards to the safety of CT, there is valid concern regarding the increased risk of opportunistic infections, though perhaps outweighed by the benefits of better disease control. As for the risks of malignancy with CT, the numbers again suggest that any increased risk is far outweighed by the potential benefits, at least over a "short term" of several years. Even though patients and physicians may understand that this risk is minor in comparison to potential benefits, the observed rates of CT use suggest that fear of this complication is still a strong motivating force away from CT. Overall, GI professional societies have advocated the use of CT when the anti-TNF is IFX, but not explicitly for other combinations. As we have seen, there is evidence to support other forms of CT, but both the physician and

the patient need to be aware of the strength of this evidence, be certain that the risks are understood, and the goals of therapy are achieved if other forms of CT are used.

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MINIREVIEWS

Inflammatory bowel disease: Efficient remission maintenance is crucial for cost containment

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Abstract

The inflammatory bowel diseases (IBD) are chronic incurable inflammatory disorders of the gut. Some 10% run a downhill course, requiring emergency medical support and often surgery; another small subset are monogenic, and, threatening pediatric patients, are the challenge of these days. The majority of the IBDs, however, are polygenic low-penetrance diseases, running a lifetime waxing-and-waning course. The prevalent trend is towards a slow worsening and steady cost increase. Each and all drugs of the available arsenal exhibit strengths and weaknesses: Mesalamines are chiefly effectively for mild-moderate colitis, and do not work in Crohn's; steroids do not control some 40% of the ulcerative colitis cases, and are not indicated for Crohn's; thiopurines are effective in the maintenance of the IBDs but do not prevent relapses on withdrawal; biologics are still being used empirically (not monitored) causing further increase of their cost over that of hospitalization. Against all these caveats, two simple rules still hold true: Strict adherence maintenance and avoidance of colitogenic drugs. This matter is expanded in this minireview.

Key words: Inflammatory bowel disease; Therapy; Cost containment; Budget; Treatment adherence; Inflammatory bowel disease managed care

Core tip: Cost-effective maintenance of remission of inflammatory bowel diseases (IBD) is a traditionally unsolved challenge for care-takers and budget supervisors. The newly released (biologic) formulations, though purported to act as disease terminators, have failed to pay back their initial cost. We have faced the issue by reappraising initial simple tenets and found the following: (1) usually, uncomplicated IBD rests in remission by using cheap traditional drugs, provided the indication is correct, and, chiefly, that adherence is tightly maintained. Non compliant IBD patients cost manifold the compliant ones, and are the main cause of budget distortion; and (2) third-party drugs (nonsteroidal anti-inflammatory drugs, *e.g.*, should be avoided. A frozen steady-state is the regime to

effectively maintain IBD.

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EPIDEMIOLOGY

The Italian health service does not cover medical expenses in full, but requires patients to partially contribute to extents that vary according to income and social positions. Patients suffering from chronic incurable disease including inflammatory bowel diseases (IBD) may apply for full coverage. Hence, the number of applications may yield an estimate of the prevalence of IBD in Italy. Such estimates, dating back to 2009[1], are showing an IBD prevalence in Italy of 177-254 cases/10^5. Regional incidence data of pooled Crohn's disease (CD) and ulcerative colitis (UC) yield figures between 2.7 and 13 cases per 10^5 per year. A recently published Survey of Italian Gastroenterology Societies[2] indicates that: (1) Despite a recent increase between 1970 and 1990, the incidence of IBD in Italy is still exceeded by the Northern Europe figures; (2) Pediatric incidence figures of both IBD phenotypes has gone up from 0.89 to 1.39 between 1996 and 2003; (3) With UC being slightly more frequent in males, general data confirm the existence of two incidence peaks, at 25/35 years and around the sixth life decade; and (4) An increased family risk, various extra-intestinal manifestations, and the association with immune-mediated disease (multiple sclerosis, psoriasis, and celiac disease just as examples) all mark the clinical IBD presentations.

MORBIDITY AND MORTALITY

Large population studies demonstrate that active IBD does significantly reduce quality of life, with young fertile women being mostly affected[3,4]. In Italy, IBD-dependent disability is quantified in classes from the lowest 15% to the maximum of 70% (fourth class) if surgery has been needed. By contrast, IBD does not seem to significantly reduce life expectancy; notably, however, mortality is estimated to be increased in the first year of diagnosis as well as in patients younger than 30 years[5].

ADMISSIONS

IBD patients do need hospitalization more frequently than the general population, with most of admissions ending in surgery. About 4.6%-7.5% of UC cases and 36% of CD patients become operated at 5 years. The figure is 17% for pediatric cases[4].

THERAPEUTIC ARSENAL

There are two main challenges in the management of IBD, and these divide the list of the available drugs into two distinct chapters: induction of a response, and maintenance of the remission.

Mesalamines and its derivatives

Pioneered by Nanna Svartz studies on salazopyrin, mesalamines and its derivatives have been a mainstay for IBD treatment for the last 60 years. The modern version of salazopyrin, 5-aminosalycilic acid (5-ASA), has been tested in a large population study employing a range of doses between 1 and 4 daily g. Such dosages were shown to induce remission in 30% of the cases (12% for placebo); the figures rose to 80% if limiting the end-point to the clinical response[6]. Looking at remission maintenance, a series of Cochrane studies have shown that each and all of the FDA-approved formulations can yield a 30% advantage over placebo[7]. Mesalamines have proven not so readily effective for the treatment of CD. Initial data suggesting that daily 4 g could strongly reduce the Crohn's Disease Activity Index (CDAI) score *vs* placebo, achieving remission in 43% of the treated patients (placebo 18%) were not duplicated[8].

Antibiotics

Some antibiotic molecules of the imidazole class have shown effectiveness in CD. A study already completed in 2005 showed that post-operative administration of ornidazole could reduce relapse rates from 37% to 7%[9]. By contrast, UC has proven unresponsive to antibiotics[10]. Ciprofloxacin and metronidazole are advantageous for CD; this issue is exhaustively illustrated in a freshly updated review[11].

Corticosteroids

Population studies have shown that 34% and 44% of UC and CD patients, respectively, need a variable number of steroid courses to achieve remission[12]; by contrast, steroids are contraindicated for remission maintenance.

Thiopurines

Experience achieved over the last 30 years has consistently indicated that thiopurines are effective in the maintenance of remission of both IBD phenotypes. A classic controlled study published in 1980, including steroid-dependent and/or fistulizing CD patients, showed that 31% experienced fistula closure, and 75% were weaned from steroids if treated with 6-mercaptopurine (6-MP); these figures were respectively 6% and 36% in the placebo-treated subgroup[13]. By contrast, evidence favoring the use of thiopurines to treat UC has lingered a little behind: A recent study from us has shown that of 127 Italian patients who had had their azathioprine withdrawn, one-third, 50%, and two-thirds did relapse

at 12 mo, at 2 years, and at 5 years respectively[14]. A significant added value of thiopurines has been documented in a recent Dutch study. This nationwide survey has shown that chronic thiopurine treatment significantly protects patients from developing colitic cancer[15]. This breakthrough finding represents an authoritative correction of previous limited work that had claimed negative results[16].

RESCUE TREATMENTS FOR REFRACTORINESS TO CONVENTIONAL DRUGS

Preliminary work published in 1990 showed that cyclosporine, a fungal derived peptide able to inhibit T-lymphocyte responses, could achieve remission in a significant proportion of patients facing colectomy for refractory acute UC[17]. Later in 1994 such initial data were then confirmed in a controlled fashion[18]. The number of refractory colitic patients that had received cyclosporine was estimated to reach the number of 700 in 2005[19]. Interestingly, an English survey showed that only 7%-8% of all hospitalized IBD patients do receive cyclosporine; however, if asked whether they would opt to be treated, their positive responses often outweigh their own doctors' intentions[20]. The literature consistently indicates that cyclosporine effectively avoids immediate colectomy in 60% to 80% of steroid refractory UC patients; subsequent ability to maintain remission may fall around 60%, and is potentiated by the concomitant administration of a thiopurine[21]. In the suggestion of a leading center[22] cyclosporine must be considered a mainstay treatment for refractory colitis. Along this line, we have recently reviewed the pharmacologic profiles of cyclosporine, mesalamine, and thiopurines on the basis of the experience of treatment of 100 consecutive patients between 1991 and 2007. We succeeded in confirming the data discussed above, and stressed the need for further efforts in the direction to improve the pharmacologic profile of these drugs[23]. As of today, official position statements[24] hold that cyclosporine is as effective as infliximab to control severe refractory colitis, whereas it is not indicated for Crohn disease.

Recent Cochrane reviews have shown that tumor necrosis factor (TNF)-inhibitors (chiefly infliximab and adalimumab) can effectively treat steroid-dependence and fistula formation in CD[25]; similar but weaker evidence (owing to patient heterogeneity) have been published for UC[26]. Such favorable evidence seems not to be reflected in real-world practice, whereby it is estimated that not more than 15% of candidate IBD patients do receive an anti-TNF molecule [27]. A recent in-depth analysis conducted in Europe[28] has found that the healthcare costs for IBD are mainly influenced by medication, chiefly anti-TNF molecules, despite the potential of these measures to restrict resort to hospitalization; notably, similar research carried out in Canada has come to the same conclusions[29]. The implications of such findings can probably be re-shaped by the evidence that in losers of response, replacement of a blind dose escalation with therapeutic drug monitoring (test-based strategy) can lead to major cost saving[30].

THE IBDS: NATURAL HISTORY

Before the release of drugs such as mesalamines and steroids, and the availability of adequate resuscitation and surgical techniques, UC turned out to be a rather ominous disease: 33% risk of death in the first year; 12% mortality rate at relapse; the cumulative death risk 20 years following diagnosis was 40%; 40 years after diagnosis the colon carcinoma risk was 40%[31]. The scenario of 1983[32], namely 30 years after Truelove and Witts demonstration of the effectiveness of steroids had begun to change, contradictory areas persisted. The survival rate of those diagnosed with mild/moderate disease matched that of controls; yet, severe disease presentation still entailed mortality rates of 31% as distributed in the first 4 years. Nowadays, severe UC is expected to present with a frequency of 10-15% at any time of disease course: According to updated evidence the expected mortality is null, but sporadic fatal cases cannot be excluded[33].

Some 50% to 80% of the patients run a waxing-and-waning course, whereas a chronic active course may be observed in 15%-30%[34]. According to a reference publication: Young age, previous relapses, and presence of residual histologic disease (plasmacytosis) are all predictors of relapse[35]. The frequencies of resort to surgery are reported to range between 9.6% after 5 years and 31% after 18 years[36].

The natural history of CD is driven by disease localization and its strength to evolve. In population studies, a non-stenosing pattern, a stenosing pattern, and a penetrating course may be described with frequencies of 70%, 17%, and 13% respectively. In the follow-up, a switch to a stenosing pattern and to a penetrating one was recorded in 27% and 28% respectively. Some 50% of the patients did exhibit an ileal localization, an ileo-colic one by contrast affected the other half[37].

The data of two population studies including some 600 patients were rewarding: 10%-30% of the cases may expect a relapse in the first year of diagnosis; 15%-25% linger in a condition of low disease activity, whereas remission might remain the outcome in 50%-65%. Resort to surgery is a likely outcome in CD: 30% probability in the first year; the patient majority undergoes an operation after 20 years of disease. Those with ileo-cecal disease are the most likely to undergo operation: Specifically, the risk of an emicolectomy is 35% after 10 years[38,39].

THE BUDGET OF UC

According to a study of 1992, the annual cost per

patient was dollars 1488[40]. The 24% of this sum included three items of 8% each: The diagnostic algorithm, the out-patient services, the drug cost; direct and indirect costs of hospitalization made the remaining 47%. Two further data are to be emphasized: In a sample observation year, 39% of the bills charged to providers had been meant to cover the needs of only 2% of the insured clients (the subset with the worst disease forms), whereas from another point of evaluation, the majority of the insured subjects were responsible for less than 7% of the costs.

This data are a spy of the heterogeneity of the sources of expense in an IBD population: As a general rule, firmly controlled disease costs dramatically less than the chronically uncontrolled presentations that often face surgery.

This trend of surgery to make the most part of the budget is further strengthened by several factors. The three-steps reconstructive proctocolectomy with pouch anal anastomosis has significantly benefited patients by ensuring digestive tract continuity, but, making the patient hospital-dependent for 6 mo, have raised the costs sky-high. One other factor is the natural tendency of the disease to worsen, to increase the need for drugs and care, and to become laden with complications, including neoplasia.

The need to aggressively maintain the disease in remission to minimize costs stems clear from the above discussion[41]. To achieve this goal one may need a costly drug armamentarium (including biologic drugs in the last years) but most American providers do retain that this cost can be paid back by the advantages of managing a disease in remission. Direct costs (hospitalization) and indirect costs (drugs, sick leaves, family disruption) are to be chiefly accounted for in an unstable disease, and can be abolished by its stabilization[42].

TWO MAIN FACTORS THREATENING REMISSION MAINTENANCE IN IBD

Lack of adherence

Among the main missions of private care systems relies the identification and control of conditions of major cost. The American health care system has long pursued this goal, eventually collecting a large wealth of data. The essential message is that disease management is mostly expensive in unstable phases[43]. Lack of compliance has been identified as one of the capital factors in the loss of IBD remission. This matter may be discussed by differentiating four main topics: (1) The facts; (2) The causes and modalities of non-compliance; (3) The costs; and (4) Possible counter measures.

Facts: One hundred UC patients had mesalamine prescribed and were then followed prospectively for two years, with checkups at 6, 12, and 24 mo; the reappearance of at least 4 bloody urgent stools a day was defined as relapse. Non-adherent patients showed a 5-fold higher relapse rate; at 24 mo, 39% of non-adherent subjects were in remission, as opposed to 89% of adherent ones[44]. IBD patients seem to be maximally keen at non-compliance, as suggested by the following data. The adherence percentages during clinical trials may attain 80%; this contrasts with data in population studies, whereby the majority of subjects in remission opt for taking the risk of relapse for non-compliance than to accept the burden of a daily drug administration[45].

Causes and modalities: Pooled results from different studies indicated that favoring factors for non-adherence were a condition of male-single, and a left-sided colitis; a colonoscopy in the preceding 2 years and being married were opposing factors, instead[46]. When requested to declare their reasons for non-adherence, the answers were: Forgetfulness (50%); two many daily administrations (30%); doubts on the indication (20%).

Costs: Non-adherence entails extra-costs: increased morbidity; rescue drugs are more expensive; risk for complications including cancer; sick leaves; stress and family disruption. In pure terms of numbers, one should bear in mind that any single failure of a mesalamine prescription costs dollars 11500 per person[41].

Countermeasures: According to the experience of American health providers, patient counseling and release of single-dose drugs are the only worthy measures to reduce non-adherence.

Improper use of third-party drugs

Nonsteroidal anti-inflammatory drugs (NSAIDs) and antibiotics have long been suspected as factors of IBD reactivation or *de-novo* IBD causation. Recently, our attention got specifically concentrated on the role of macrolide antibiotics. Capable to favor gut colonization by Candida, these antibiotics might increase intestinal permeability, a favorable ground for IBD triggering[47]. In our out-patient clinic, it has become routine to warn referring doctors and patients against the unjustified use of macrolide molecules or NSAIDs. Dental surgery and orthopedic traumatology remain the main indication source for these drugs, and merit careful surveillance by academic gastroenterology centers.

CONCLUSION

While the prevalence of IBD is roughly stable in the Western World, the figures are on the rise of its incidence in two other contexts: Pediatric IBD and migrants.

As an incurable chronic disorder capable to disable the GI tract, IBD can easily impact a country's budget. In moments of financial restriction, care providers may duly concentrate their attention on the list of "traditional therapies", seeking optimization (increasing therapeutic

effects while diminishing costs). Current evidence recommends now that this optimization path be based on the evidence that the easiest at maintenance and less costly are the quiescent phases of IBD. Thus, such phases are to be vigorously achieved and tenaciously maintained, adhering to the following rules: (1) Pursue remission by early giving steroids and/or mesalamines at full doses. Proceed to a prompt and rapid steroid tapering once clinical remission is achieved. Continue mesalamines in most of the cases; (2) Respecting safety rules, use thiopurines as the best option in maintaining remission, smartly minding the strong synergism that might develop with mesalamines, both in terms of therapeutic or toxic effects[48]; (3) As said above, patients' compliance must strictly be monitored and corrected, as faulty compliance has been shown to be a strongly negative factor in remission maintenance and cost control; (4) Instruct family physicians and patients to carefully consider prescriptions of antibiotics and NSAIDs: Though some antibiotics may be therapeutic for IBD's, some other commonly prescribed formulations (macrolides) may activate or generate de-novo IBD, as NSAIDs can do[49]; and (5) Bear in mind that average IBD can be controlled by the timely use of correctly dosed traditional molecules: Sometime the need for costly therapies is provoked by the late or inadequate use of common treatments[50].

To give the reader an idea of how important it might be to fully exploit conventional therapies before resorting to biologic strategies, we like to implement the end of this paragraph by showing the cost for one day of treatment with the molecules mentioned in this text (expressed in euro) as reported by us in 2010[51]: Mesalamine: 3.06; first-generation steroids: 1.02; thiopurines 0.87; infliximab/adalimumab: 894/1675.

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ORIGINAL ARTICLE

Case Control Study

Thiol/disulphide homeostasis in celiac disease

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Abstract

AIM

To determine dynamic thiol/disulphide homeostasis in celiac disease and to examine the associate with celiac autoantibodies and gluten-free diet.

METHODS

Seventy three patients with celiac disease and 73 healthy volunteers were enrolled in the study. In both groups, thiol/disulphide homeostasis was examined with a new colorimetric method recently developed by Erel and Neselioglu.

RESULTS

In patients with celiac disease, native thiol (P = 0.027) and total thiol (P = 0.031) levels were lower, while disulphide (P < 0.001) level, disulphide/native thiol (P < 0.001) and disulphide/total thiol (P < 0.001) ratios were higher compared to the control group. In patients who do not comply with a gluten-free diet, disulphide/native thiol ratio was found higher compared to the patients who comply with the diet (P < 0.001). In patients with

any autoantibody-positive, disulphide/native thiol ratio was observed higher compared to the patients with autoantibody-negative ($P < 0.05$). It is found that there is a negative correlation between celiac autoantibodies, and native thiol, total thiol levels and native thiol/total thiol ratio, while a positive correlation is observed between disulphide, disulphide/native thiol and disulphide/total thiol levels.

CONCLUSION

This study is first in the literature which found that the patients with celiac disease the dynamic thiol/disulphide balance shifts through disulphide form compared to the control group.

Key words: Anti-gliadin antibodies; Anti-tissue transglutaminase antibody; Gluten-free diet; Oxidative stress; Thiol oxidation

Core tip: To the best of our knowledge, for the first time in this study, total and native thiol levels in celiac patients were found lower compared to the control group while disulphide level, disulphide/total thiol and disulphide/native thiol ratios were found to be higher. Also, this study is first in which a negative correlation between celiac autoantibodies and native thiol, and total thiol levels and native thiol/total thiol ratio is observed while there is a positive correlation between disulphide level and disulphide/native thiol and disulphide/total thiol ratios.

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INTRODUCTION

Celiac disease (CD), observed in genetically predisposed individuals, is a chronic/autoimmune disease of the small intestine, characterized by symptoms such as mucosal damage induced by gliadin, malabsorption, anemia, diarrhea and growth retardation[1,2]. While environmental, genetic and immunological factors have a role in etiopathogenesis of the disease, oxidative stress has also been proved to play a critical role in development of disease[3,4].

With the development of diagnostic methods, gliadin and related toxic effects of prolamines on small intestine are understood better. Gluten peptides in enterocytes, particularly p31-43 α-gliadin peptides, induce certain signal transduction pathways by accumulating in lysosomes and increase the levels of oxidant radicals[5]. Based on increased free radicals, a deterioration occurs in the oxidation redox equilibrium[6]. At the first stage of oxidative damage in cellular level based upon free radicals, disulphide (-S-S) linkages are formed by thiol groups (-SH) of amino acids such as sulfur containing cysteine and methionine being oxidized as well as the thiol/disulphide balance collapses in favor of disulphide[7-12]. The resulting disulphide bonds are reduced to thiol groups and thiol reserves increase again. Through these reactions at the cellular level, dynamic thiol/disulphide homeostatic status is maintained[13]. This dynamic equilibrium is considered to be effective in many cellular processes such as cell death and proliferation, and especially antioxidant balance[14,15]. Due to these effects, there are certain studies showing that dynamic equilibrium collapses in cardiovascular diseases and cancer, the oxidative stress of which is particularly evident[16-18].

Dynamic thiol/disulphide homeostasis began to be measured in an easy and repeatable way in 2014 by a new method developed by Erel *et al*[19] with high accuracy and sensitivity. In the literature review, we have not found any study examining dynamic thiol/disulphide homeostatic status in celiac patients using this new method.

In this study, we aimed to measure native thiol, total thiol, disulphide, disulphide/native thiol, disulphide/total thiol and native thiol/total thiol levels in celiac patients with a new and fully automated method of analysis and determine dynamic thiol/disulphide homeostasis.

MATERIALS AND METHODS

Study population

This study was conducted in Turkey Yuksek Ihtisas Training and Research Hospital Gastroenterology Clinic and Ankara Numune Training and Research Hospital Internal Medicine Clinic between January and June 2015.

The study included a total of 146 participants including 73 celiac patients and 73 healthy volunteers. Celiac group is composed of first 73 celiac patients, over the age of 18, who admitted to the polyclinic for routine control. Patients that were diagnosed with CD *via* endoscopic biopsy and subject to regular follow-up in our clinic were included in the patient group in order of their applications. The healthy control group consisted of healthy volunteers, who have applied to our hospital for a check-up, without a chronic disease and drug use and those with similar demographic characteristics to the patient group.

Patients with known diabetes mellitus, kidney failure, malignancy, liver disease, thyroid disease, rheumatic disease, cardiovascular and cerebrovascular disease; smoking, alcohol consumption, vitamin supplements and unfollowed patients were excluded from the study.

In our clinic, the diagnosis of celiac disease in routine is made endoscopically with 2nd part duodenal biopsy and anti-gliadin antibody IgA-G or anti-tissue transglutaminase IgA-D positivity. Crypt hyperplasia, villus atrophy and submucosal lymphocytic infiltration is considered significant in the biopsy.

We created two subgroups (GCD: Patients non-

compliant with gluten free diet, GFD: Patients compliant with gluten free diet) to understand the effect of diet in oxidative stress in CD. Poor compliance to diet is defined as taking any kind of gluten containing materials. Patients' compliance to gluten diet is obtained from patient files and applied questionnaires. Patients that are compliant to gluten free diet from the beginning and for at least 5 years are included in GFD group.

Body mass index (BMI) was calculated by dividing body dry weight to the square of tall stature in meters (BMI = kg/m^2).

The study was conducted in accordance with the Declaration of Helsinki 2013 Brasil version and was approved by the Local Ethics Research Committee. All subjects provided written informed consent prior to participation in the study.

Biochemical parameters

For thiol/disulphide hemostasis tests, venous blood samples were drawn from patient and control groups after overnight fasting. Blood samples were swiftly centrifuged at 4000 rpm for 10 min, then plasma and serum samples were separated and stored at -80 ℃. Then all parameters were studied in the same session and in the same serum sample.

Laboratory parameters other than thiol/disulphide hemostasis parameters of the participants were their routine parameters at the time they were included in the study and those were recorded from patient files.

Thiol/disulphide homeostasis

Thiol/disulphide levels were measured by a newly developed, fully-automated and colorimetric method by Erel and Neselioglu[19]. When disulphide levels were divided to native thiol and total thiol levels: Disulphide/native thiol and disulphide/total thiol ratios were obtained. When native thiol level was divided to total thiol level, native thiol/total thiol ratio was obtained as a result.

Statistical analysis

Statistical Package for Social Sciences (SPSS) for Windows 20 (IBM SPSS Inc., Chicago, United States) program was employed for statistical assessments. Kolmogorov-Smirnov test was utilized to determine the distribution of data. Continuous variables with normal distribution were expressed as mean ± SD, and continuous variables without normal distribution were expressed as median (min-max). Categorical variables were presented in numbers and percentage. Continuous variables were compared to independent sample *t*-test or Mann-Whitney *U* test where necessary. The relationship between the numeric parameters was analyzed by Pearson and Spearman correlation analysis. In the examination of the relation between thiol/disulphide homeostasis parameters and celiac antibodies, the effects of demographic and clinical factors were adjusted by partial correlation. A $P < 0.05$ was considered significant for statistical analyses.

RESULTS

The demographic characteristics and laboratory findings of all groups are summarized in Table 1. The study population consisted of a total of 146 patients, including 73 celiac patients (female/male: 58/15; age: 44.1 ± 13 years, BMI: 24.5 ± 4.7 kg/m^2) and 73 controls (female/male: 55/18; age: 43.7 ± 13.6 years, BMI: 24.9 ± 4.7 kg/m^2). There were no significant difference between two groups in terms of sex, age and BMI levels ($P > 0.05$). The median disease duration of followed celiac patients was determined as 6 years (min: 1 years, max: 25 years).

The mean total protein levels in celiac patients were determined similar to the control group ($P > 0.05$). In celiac patients, mean albumin (4.4 ± 0.3 g/L *vs* 4.1 ± 0.3 g/L, respectively; $P = 0.002$), median alanine aminotransferase (23 IU/L *vs* 20 IU/L, $P = 0.035$), aspartate aminotransferase (20 IU/L, *etc.,* 17 IU/L, respectively; $P = 0.002$) and c-reactive protein (3.5 mg/L *vs* 1.2 mg/L, respectively, $P = 0.008$) levels were higher, compared to the control group.

Mean native thiol (322.7 ± 39.7 mmol/L *vs* 339.6 ± 51.3 mmol/L, respectively, $P = 0.027$) and total thiol levels (343.7 ± 41.9 mmol/L *vs* 360.4 ± 50.3 mmol/L, $P = 0.031$) were determined lower in celiac patients, compared to the control group, while native thiol/total thiol ratio did not differ significantly between the groups ($P > 0.05$). Mean disulphide level (13.0 ± 3.7 mmol/L *vs* 10.2 ± 3.9 mmol/L, respectively; $P < 0.001$), disulphide/native thiol (3.8% ± 1.2% *vs* 2.8% ± 1.1%, respectively, $P < 0.001$) and disulphide/total thiol ratio (4.1% ± 1.4% *vs* 2.9% ± 1.1%, respectively, $P < 0.001$) were determined higher in celiac patients compared to the control group.

According to dietary compliance and antibody positivity in the celiac group, the distribution of native thiol, total disulphide and disulphide/native thiol ratios were shown in detail in Table 2. According to this; in patients with dietary compliance, mean native thiol was higher (327.9 ± 35.6 mmol/L *vs* 310.6 ± 46.8 mmol/L, $P = 0.013$), mean disulphide (12.0 ± 3.3 mmol/L *vs* 13.8 ± 3.2 mmol/L, respectively; $P = 0.034$) level and disulphide/native thiol ratio (3.5% ± 1.7% *vs* 5.2% ± 1.4%, respectively, $P < 0.001$) were% much lower compared to patients without dietary compliance. As for antibody positive patients, native thiol level was determined low, disulphide level and disulphide/native thiol ratio were determined higher.

The correlation analysis of native thiol, total thiol, disulphide, disulphide/native thiol, disulphide/total thiol and native thiol/total thiol with demographic and clinical findings is shown in Table 3 in detail. Celiac autoantibodies displayed a negative correlation with native thiol, total thiol and native thiol/total thiol levels and a positive correlation with disulphide, disulphide/native thiol and disulphide/total thiol ratio. The relation

Table 1 Demographic characteristics and laboratory findings of study population

Variables	Celiac (n = 73)	Control (n = 73)	P value
Gender (male), n (%)	15 (20.5)	18 (24.7)	0.553
Age (yr)	44.1 ± 13	43.7 ± 13.6	0.866
BMI (kg/m^2)	24.5 ± 4.7	24.9 ± 4.7	0.867
Smoking, n (%)			
Non-smokers	49 (67.1)	50 (68.5)	
Smokers	18 (24.7)	17 (23.3)	0.981
Quit smoking	6 (8.2)	6 (8.2)	
Alcohol, n (%)	2 (2.7)	-	-
Duration of disease (yr)	6 (1-25)	-	-
Total protein (g/L)	7.4 ± 0.6	7.4 ± 0.5	0.964
Albumin (g/L)	4.4 ± 0.3	4.1 ± 0.3	0.002[a]
ALT (IU/L)	23 (8)	20 (7)	0.035[a]
AST (IU/L)	20 (12)	17 (9)	0.002[a]
CRP (mg/L)	3.5 (8.4)	1.2 (2)	0.008[a]
Native thiol (μmol/L)	322.7 ± 39.7	339.6 ± 51.3	0.027[a]
Total thiol (μmol/L)	343.7 ± 41.9	360.4 ± 50.3	0.031[a]
Disulphide (μmol/L)	13.0 ± 3.7	10.2 ± 3.9	< 0.001[a]
Disulphide/native thiol (%)	3.8 ± 1.2	2.8 ± 1.1	< 0.001[a]
Disulphide/total thiol (%)	4.1 ± 1.4	2.9 ± 1.1	< 0.001[a]
Native thiol/total thiol (%)	93.8 ± 2.6	94.3 ± 5.7	0.553

[a]P < 0.05. BMI: Body mass index; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CRP: C-reactive protein.

Table 2 Dietary compliance of native thiol, disulphide and disulphide/native thiol and its distribution regarding antibody positivity

Variables	Native thiol (μmol/L)	P value	Disulphide (μmol/L)	P value	Disulphide/native thiol (μmol/L)	P value
Diet						
GCD (n = 22)	310.6 ± 46.8	0.013[a]	13.8 ± 3.2	0.034[a]	5.2 ± 1.4	< 0.001[a]
GFD (n = 51)	327.9 ± 35.6		12.0 ± 3.3		3.5 ± 1.7	
AGA-IgA						
(-) (n = 33)	310.5 ± 39.5	0.015[a]	14.1 ± 3.7	0.020[a]	4.7 ± 1.9	0.005[a]
(+) (n = 40)	333.8 ± 40.1		12.0 ± 3.8		3.6 ± 1.4	
AGA-IgG						
(-) (n = 47)	308.5 ± 36.8	0.027[a]	13.9 ± 3.1	0.001[a]	4.6 ± 1.7	< 0.001[a]
(+) (n = 26)	330.5 ± 45.2		11.1 ± 3.9		3.0 ± 1.1	
Anti-t TGA						
(-) (n = 29)	315.5 ± 38.2	0.001[a]	13.9 ± 3.9	0.019[a]	4.5 ± 1.9	0.007[a]
(+) (n = 44)	346.2 ± 40.5		11.7 ± 3.8		3.4 ± 1.5	
Anti-t TGG						
(-) (n = 61)	318.1 ± 37.6	0.002[a]	13.5 ± 3.9	0.009[a]		0.035[a]
(+) (n = 12)	345.3 ± 51.5		10.3 ± 3.0			

[a]P < 0.05. GCD: Gluten-containing diet; GFD: Gluten-free diet; AGA-IgA: Anti gliadin antibodies IgA; AGA-IgG: Anti gliadin antibodies IgG; Anti-t TGA: Anti-tissue Transglutaminase IgA antibodies; Anti-t TGG: Anti-tissue Transglutaminase IgA antibodies.

between thiol/disulphide homeostasis parameters and celiac autoantibodies were observed to continue even when the effects of demographic and clinical findings were removed.

C-reactive protein level displayed a negative correlation with native thiol, total thiol and native thiol/total thiol levels, and a positive correlation between disulphide, disulphide/native thiol, disulphide/total thiol levels.

DISCUSSION

To our knowledge, for the first time in this study, total and native thiol levels in celiac patients were determined lower compared to the control group while disulphide level, disulphide/total thiol and disulphide/native thiol ratios were found to be higher. Also this is the first study to determine a negative correlation of celiac autoantibodies with native thiol and total thiol levels with native thiol/total thiol ratio, and a positive correlation of disulphide level with disulphide/native thiol and disulphide/total thiol ratios.

Celiac disease is a disease characterized by the inflammatory response created by intestinal mucosa based upon gliadin peptides taken with gluten containing food and consequently mucosal inflammation, crypt hyperplasia and villus atrophy[20]. The most important factor in etiopathogenesis of the disease is considered

Table 3 Findings related to thiol/disulphide hemostasis parameters in celiac patient group

Variables	Native thiol		Total thiol		Disulphide		Disulphide/total thiol		Disulphide/native thiol		Native thiol/total thiol	
	r	*P* value	*r*	*P* value	*r*	*P* value	*r*	*P* value	*r*	*P* value	*r*	*P* value
Age	0.261	0.026[a]	0.262	0.025[a]	-0.041	0.729	-0.132	0.264	-0.122	0.302	0.021	0.861
BMI	0.19	0.107	0.187	0.114	-0.033	0.783	-0.096	0.421	-0.113	0.34	0.045	0.702
Total protein	-0.032	0.786	0.005	0.97	0.059	0.618	0.032	0.787	0.086	0.472	-0.156	0.187
Albumin	0.009	0.937	0.025	0.835	-0.037	0.754	-0.065	0.582	-0.040	0.735	-0.071	0.548
AST	0.051	0.666	0.046	0.702	-0.03	0.801	-0.057	0.631	-0.031	0.796	0.033	0.781
ALT	-0.091	0.444	-0.116	0.329	-0.063	0.598	-0.012	0.919	0.002	0.985	0.094	0.427
CRP	-0.327	0.018[a]	-0.304	0.038[a]	0.369	0.015[a]	0.287	0.043[a]	0.332	0.029[a]	-0.244	0.038[a]
AGA-IgA	-0.325	0.009[a]	-0.266	0.035[a]	0.384	0.024[a]	0.324	0.010[a]	0.325	0.009[a]	-0.266	0.035[a]
AGA-IgG	-0.332	0.008[a]	-0.271	0.022[a]	0.298	0.010[a]	0.297	0.011[a]	0.253	0.031[a]	-0.279	0.017[a]
Anti-t TGA	-0.342	0.007[a]	-0.28	0.017[a]	0.305	0.009[a]	0.311	0.004[a]	0.280	0.035[a]	-0.294	0.028[a]
Anti-t TGG	-0.35	0.00[a]	-0.316	0.004[a]	0.315	0.007[a]	0.34	0.001[a]	0.302	0.014[a]	-0.304	0.019[a]
AGA-IgA[1]	-0.313	0.006[a]	-0.301	0.026[a]	0.334	0.004[a]	0.353	< 0.001[a]	0.315	0.011[a]	-0.319	0.013[a]
AGA-IgG[1]	-0.335	0.004[a]	-0.324	0.005[a]	0.309	0.018[a]	0.366	0.023[a]	0.349	0.033[a]	-0.288	0.045[a]
Anti-t TGA[1]	-0.261	0.043[a]	-0.243	0.035[a]	0.376	0.036[a]	0.322	0.011[a]	0.335	0.009[a]	-0.273	0.038[a]
Anti-t TGG[1]	-0.333	0.032[a]	-0.282	0.037[a]	0.294	0.031[a]	0.305	0.026[a]	0.336	0.036[a]	-0.314	0.013[a]

[a]$P < 0.05$. [1]Demographic characteristics and laboratory parameters are adjusted. BMI: Body mass index; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CRP: C-reactive protein; AGA-IgA: Anti gliadin antibodies IgA; AGA-IgG: Anti gliadin antibodies IgG; Anti-t TGA: Anti-tissue Transglutaminase IgA antibodies; Anti-t TGG: Anti-tissue Transglutaminase IgA antibodies.

to be environmental factors such as gliadin and the autoimmune response against them. Although the role of oxidative stress in the etiopathogenesis of the disease is unknown, in studies conducted with different cell models, intracellular oxidative imbalance occurs as a result of gliadin exposure and oxidant radicals are formed as a result of lipid peroxidation[21]. These oxidant radicals have been shown to form -S-S bonds by oxidizing -SH groups found in the side chain of sulfur containing amino acids and consequently increase oxidized metabolites in the cell[22]. In this case thiol/disulphide equilibrium, which is balanced under physiological conditions, is weakened and disrupted in favor of disulphide form. As a result of all these reactions, cell morphology, membrane permeability and vital cellular activities such as apoptosis and cell proliferation are disrupted[1,5].

There are certain studies in the literature investigating thiol and disulphide amounts in low molecular thiol compounds that constitute a small portion of total body thiol pool such as cysteine, glutathione (GSH) and oxidized glutathione thiol[23,24]. Until 2014, any colorimetric method that measures total thiol and disulphide amount in the body had not yet been developed. But Erel *et al*[19] developed a fully-automated method in 2014, by which total thiol, native thiol and disulphide amounts can be measured easily and repetitively with high sensitivity and specificity.

We have not found any study in the literature examining dynamic thiol/disulphide balance in celiac disease. However, there are studies conducted with low molecular weight thiol compounds. Stojiljković *et al*[25] have shown that in intestinal tissues of celiac patients in the pediatric age group, GSH level that constitutes a big part of intracellular thiol content decreases and lipid hydroperoxide level which is an oxidant substance that plays a role in cell membrane damage increases. These results have indicated that GDH redox cycle is disrupted in celiac patients. In a study conducted with asymptomatic celiac patients by Odetti *et al*[6] oxidant radicals derived from protein (carboxyl groups) and lipids (thiobarbituric acid-reactive substances) were determined high.

In our study, total and native thiol levels in celiac patients were determined lower compared to the control group; disulphide level, disulphide/total thiol and disulphide/native thiol ratios were found to be higher and eventually dynamic equilibrium was observed to shift to disulphide form. This case may be due to high levels of oxidant radicals in celiac disease. Our hypothesis is supported by the two studies mentioned above, in which the level of oxidant radicals increase in celiac patients. The increase in the level of oxidant radicals in celiac disease may be due to two cases. Firstly, the disease being a chronic inflammatory disease and secondly, being an autoimmune disease. Previously many studies have indicated that oxidant radicals increase in inflammatory/autoimmune diseases and accordingly oxidative stress level increases[26-28].

For instance, Nanda *et al*[29] have determined that in autoantibody positive hypothyroidis patients, levels of oxidant radicals are higher compared to those with autoantibody negative and observed a positive correlation between autoantibodies and oxidant radicals. Determining a positive correlation of C-reactive protein and celiac autoantibodies with disulphide/native thiol level and determining the disulphide form significantly high in autoantibody positive celiac patients strongly supports our thesis.

Another reason for determining low thiol reserve in celiac patients compared to the control group may be due to lack of thiol-containing food intake based upon deteriorated intestinal mucosa. However determining disulphide form and albumin level higher in celiac group compared to the control group indicates that this abnormal

thiol/disulphide equilibrium in celiac patients is not due to lack of oral intake but rather to oxidative stress.

In our study, disulphide/native thiol ratio was determined higher in patients who do not comply with gluten diet compared to those who comply with the diet. We think that this case could be associated with inflammation. Because previous studies have indicated that inflammation is higher in patients who do not comply with gluten diet than those who comply with diet[30,31]. Another reason may be due to the significant increase in oxidative stress based upon gliadin toxicity in patients who do not comply with gluten diet[21].

The main limitation of our study is its cross-sectional design and that repetitive measurements have not been done in the patient group. The other limitation is that the information whether participants in the control group have taken thiol containing nutrients or not, is only limited to anamnesis.

For the first time in this study, thiol/disulphide balance was shown to shift towards disulphide form in celiac patients, compared to the control group. Also this is the first study to examine the effects of celiac autoantibodies and gluten-free diet on dynamic thiol/disulphide equilibrium. According to all these results, disrupted thiol/disulphide equilibrium in celiac patients was thought to be associated with autoimmunity and inflammation. In order these results to be clarified, further studies are required to examine the association between thiol/disulphide homeostasis parameters and proinflammatory cytokines that play an active role in celiac disease.

COMMENTS

Background

Celiac disease (CD), observed in genetically predisposed individuals, is a chronic/autoimmune disease of the small intestine, characterized by symptoms such as mucosal damage induced by gliadin, malabsorption, anemia, diarrhea and growth retardation.

Research frontiers

Dynamic thiol/disulphide homeostasis began to be measured in an easy and repeatable way in 2014 by a new method developed by Erel and his colleagues with high accuracy and sensitivity.

Innovations and breakthroughs

The authors to measure native thiol, total thiol, disulphide, disulphide/native thiol, disulphide/total thiol and native thiol/total thiol levels in celiac patients with a new and fully automated method of analysis and determine dynamic thiol/disulphide homeostasis.

Applications

Disrupted thiol/disulphide equilibrium in celiac patients was thought to be associated with autoimmunity and inflammation. In order these results to be clarified, further studies are required to examine the association between thiol/disulphide homeostasis parameters and proinflammatory cytokines that play an active role in CD.

Peer-review

In the present paper, entitled "Thiol/disulphide homeostasis in celiac disease", Kaplan *et al* measured thiol/disulphide homeostasis, an indirect evaluation for oxidative stress, in patients with CD.

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ORIGINAL ARTICLE

Observational Study

Correlation of rapid point-of-care *vs* send-out fecal calprotectin monitoring in pediatric inflammatory bowel disease

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Abstract

AIM

To assess the correlation between the send-out enzyme-linked immuno sorbent assay (ELISA) and the point-of-care (POC) calprotectin test in pediatric inflammatory bowel disease (IBD) patients.

METHODS

We prospectively collected stool samples in pediatric IBD patients for concomitant send-out ELISA analysis and POC calprotectin testing using the Quantum Blue® (QB) Extended immunoassay. Continuous results between 17 to 1000 μg/g were considered for comparison. Agreement between the two tests was measured by a Bland-Altman plot and statistical significance was determined using Pitman's test.

RESULTS

Forty-nine stool samples were collected from 31 pediatric IBD patients. The overall means for the rapid and ELISA tests were 580.5 and 522.87 μg/g respectively. Among the 49 samples, 18 (37.5%) had POC calprotectin levels

of ⩽ 250 μg/g and 31 (62.5%) had levels > 250 μg/g. Calprotectin levels ⩽ 250 μg/g show good correlation between the two assays. Less correlation was observed at quantitatively higher calprotectin levels.

CONCLUSION

In pediatric IBD patients, there is better correlation of between ELISA and POC calprotectin measurements at clinically meaningful, low-range levels. Future adoption of POC calprotectin testing in the United States may have utility for guiding clinical decision making in real time.

Key words: Calprotectin; Stool biomarker; Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Point-of-care test

Core tip: Quantitative fecal calprotectin (FC) measurements, particularly in children affected by inflammatory bowel disease (IBD), is an important element of disease monitoring in a patient population vulnerable to repeated endoscopic confirmation of mucosal healing. In the United States, rapid FC assays are not yet Food and Drug Administration approved, and send-out FC assays require processing delay, preventing point-of-care usefulness. The significance of our findings in this study reiterate the clinical utility of the point-of-care FC testing in children with IBD, who are at-risk for subclinical mucosal-level inflammation. Our study confirms good correlation between the send-out and rapid point-of-care FC tests at the clinically-meaningful target range (⩽ 250 μg/g) associated with endoscopic remission.

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INTRODUCTION

Reliable mucosal-level monitoring of inflammatory bowel disease (IBD) is important for appropriate disease management response. Although endoscopy remains the current gold standard for mucosal-level evaluation, the invasive nature, anesthesia requirement, and potential for procedure-related complications including bowel perforations are valid considerations for pediatric IBD patients to be disease-monitored using non-invasive stool biomarkers[1].

As the strength of evidence for longitudinally monitoring IBD using serial calprotectin measurements is emerging, most clinical laboratories in the United States do not analyze fecal calprotectin in-house and require quantification *via* a send-out method. As a result, calprotectin measurement by the traditional enzyme-linked immunosorbent assay (ELISA) can be time intensive, potentially leading to delays in clinical decision-making - especially in children with IBD who may have discordance of biochemical markers (*e.g.*, CRP) with subjective assessments of disease activity (*e.g.*, abdominal pain).

Rapid fecal calprotectin testing, using immunochromatographic assays, could overcome this time delay and can result in point-of-care (POC) calprotectin measurements within minutes. One POC test - Quantum Blue® Extended immunoassay (Bühlmann Laboratories, Switzerland) - is approved for clinical use in Europe, Canada, and countries in Asia and South America. While there are a few studies showing good correlation of this particular assay with an ELISA test in mainly an adult, IBD and non-IBD cohort[2,3], there is only one European study to our knowledge assessing the strength of correlation for POC testing with the standard ELISA in children with IBD. In the United States, POC calprotectin testing is not yet Food and Drug Administration (FDA) approved at this time for clinical use[4]. We aimed to assess the correlation between the send-out ELISA and the POC calprotectin test in pediatric IBD patients.

MATERIALS AND METHODS

This was a Stanford University IRB approved prospective study conducted from October 2014 to May 2015. In previously diagnosed pediatric IBD patients who were being assessed for routine fecal calprotectin levels, their tested stool sample was also analyzed for calprotectin using the Quantum Blue® POC test. Informed consent by the parent or legal guardian was required for participation. During standard of care inpatient and outpatient encounters, fecal samples were collected from patients by our hospital laboratory for processing and sent to one centralized laboratory for ELISA analysis (Genova Diagnostics, NC, United States). No samples were collected from patients undergoing colonic cleanout. ELISA results were reported back within 10-14 d as μg/g within a continuous range of < 17 to 2500 μg/g. Results > 1000 were recorded as 1000 to match the range of the POC calprotectin test.

For POC calprotectin testing, stool samples (1 g) were extracted using the CALEX® cap device by unscrewing the cap and inserting it into the stool sample. The collection stick was removed with 1 g of adhering stool and inserted into the collection container that contained the antibody reagent. The device was then vigorously homogenized using a vortex mixer, and 60 μL of the mixed sample was placed in the QB test cartridge and loaded into the reader. After 12 min, the test cartridge was read and displayed the amount of FC present in the sample. The results were reported as μg/g with a continuous range of < 30 to 1000 μg/g. From stool extraction to results, the test required approximately 15 min to complete.

Previous studies and clinical experience have indicated that calprotectin ⩽ 250 μg/g correlates with lower disease activity at the mucosal-level on endoscopic evaluation[5-7].

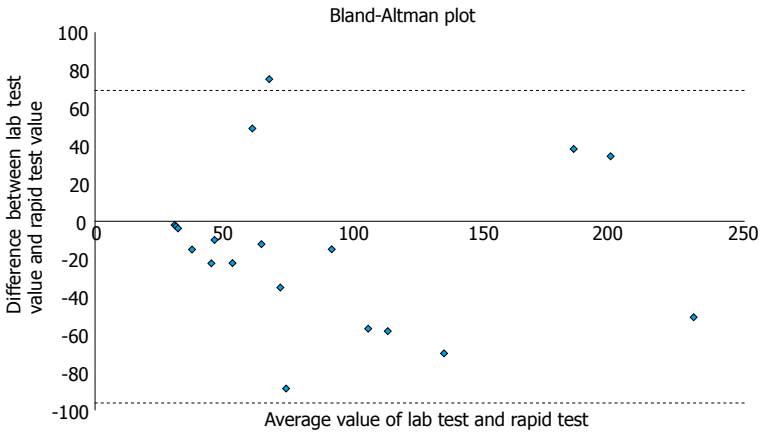

Figure 1 Bland-Altman plot for calprotectin values ≤ 250 μg/g. Pitman's test showed r = 0.072, with the value close to zero indicating good concordance between the two tests (P = 0.779).

Table 1 Patient characteristics *n* (%)

	Total (%)	FC ⩽ 250 μg/g	FC > 250 μg/g
Samples	49	21 (43)	28 (57)
Age (yr)	12.8	13.3	12.4
Diagnosis			
CD	21 (43)	9 (43)	12 (43)
UC	22 (45)	10 (48)	12 (43)
IBD-U	6 (12)	2 (9)	4 (14)
Gender			
Male	24 (49)	8 (38)	16 (57)
Female	25 (51)	13 (62)	12 (43)
CRP (mg/dL)	2.29	1.12	3.31
ESR (mm/h)	25.54	13.09	34.23

CD: Crohn's disease; UC: Ulcerative colitis; IBD: Inflammatory bowel disease; IBD-U: IBD-unclassified; FC: Fecal calprotectin; CRP: C-reactive protein.

Therefore, we were particularly interested in the strength of correlation between the ELISA and POC calprotectin test within this lower range of values. Agreement between the two tests was measured by a Bland-Altman plot and statistical significance was determined using Pitman's test in STATA 12.1 (StataCorp, College Station, TX, United States).

RESULTS

From routine inpatient or outpatient care, 49 stool samples were collected from 31 pediatric IBD patients (Table 1). The overall means for the rapid and ELISA tests were 580.5 μg/g and 522.87 μg/g respectively. Among the 49 samples, 18 (37.5%) had POC calprotectin levels of ⩽ 250 μg/g and 31 (62.5%) had levels > 250 μg/g.

Among samples resulting in ⩽ 250 μg/g, mean calprotectin levels were 74.1 μg/g from the ELISA and 86.2 μg/g from the POC calprotectin, a mean difference of 12.0 μg/g. Among samples resulting in > 250 μg/g, mean calprotectin levels were 783.5 μg/g from the ELISA and 867.6 μg/g from the POC calprotectin test, a mean difference between of 84.1 μg/g.

In order to test whether these differences were significant, we used a Bland-Altman plot, graphing the difference between the two test values against their mean value (Figure 1). Pitman's test was used to determine if there was a correlation between the two values.

For values ⩽ 250 μg/g, Pitman's test showed r = 0.072, with the value close to zero indicating good concordance between the two tests; further, P = 0.779, confirming that we cannot reject the null hypothesis of equal variances. For values > 250 μg/g (not graphed), the r = 0.109, suggesting that higher absolute values have less correlation between ELISA and POC tests, although the test of significance supported the null hypothesis P = 0.564.

DISCUSSION

Our prospective cohort study showcases the reliability of a POC calprotectin test that is currently being used in routine clinical care in Europe and Canada but not yet approved in the United States. While we acknowledge the limited sample size, the data from our study show good correlation between send-out ELISA and POC calprotectin tests. We show that agreement between the two tests appears to be stronger for lower values - a finding that is corroborated by Kolho *et al*[8] in a pediatric IBD cohort. Of note, our investigation used a classical statistical method in the Bland-Altman plot which descriptively and quantitatively showcases the strength of correlation between the two tests.

Our results also agree with previous studies that showed increased inter-test variability at higher calprotectin levels - with greater divergence from expected values above 250 μg/g[9,10]. In order to optimize the utility of our study despite our limited sample size, we focused our analysis around values ⩽ 250 μg/g since literature in IBD cohorts supports endoscopic disease quiescence at or below 300 μg/g cut-off level. Targeting low-range levels appear to be the clinical goal in calprotectin monitoring.

We also found that values of the POC test were overall higher than the values obtained from ELISA, although the Pitman's tests indicate that this difference was not statistically significant. Several previous studies from Europe and Asia demonstrate excellent correlation of a rapid assay similar to the one used in this study to ELISA[8,11], but they do not showcase the differential strength of correlation at low *vs* high calprotectin levels.

In summary, we present the first correlation study of rapid POC calprotectin testing in a pediatric IBD cohort in the United States. Unlike the conventional send-out ELISA which typically takes 10-14 d to result, the future clinical use of POC calprotectin could improve the utility in the decision-making process if levels were available at or near the time of actual care.

COMMENTS

Background

Rapid fecal calprotectin (FC) assays are useful for point-of-care decision making in inflammatory bowel disease (IBD), particularly in children. Within-patient correlation data between send-out and rapid point-of-care FC tests are incomplete in pediatric IBD.

Research frontiers

Repeated measurements of low FC in patients with IBD are associated with endoscopic remission, although more data are necessary to confirm optimal cut-off levels for different patients with various IBD subtypes. A target range of ≤ 250 μg/g is often used in clinical practice.

Innovations and breakthroughs

This study confirms good correlation between the send-out and rapid point-of-care FC tests at the clinically-meaningful target range (≤ 250 μg/g) associated with endoscopic remission.

Applications

Ensuring low levels of FC using the rapid point-of-care FC assay in children affected by IBD appear to be reliable and useful in clinical practice.

Peer-review

This is a well done prospective study about the comparison of two types of fecal calprotectin diagnostic methods as possible markers for assessment the pediatric IBD disease severity.

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ORIGINAL ARTICLE

Observational Study

Clinical and economic impact of infliximab one-hour infusion protocol in patients with inflammatory bowel diseases: A multicenter study

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Author contributions: Cappello M conceived the study and coordinated data collection; Viola A and Cappello M retrieved the data from their center, analysed the data and wrote the manuscript which has been approved by all authors; all authors contributed equally to the study design, collection of data and revision of the manuscript.

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Abstract

AIM

To assess the impact of short infliximab (IFX) infusion on hospital resource utilization and costs.

METHODS
All inflammatory bowel diseases (IBD) patients who received IFX 1 h infusion from March 2007 to September 2014 in eight centers from Southern Italy were included in the analysis. Demographic, clinical and infusion related data were collected. The potential benefits related to the short infusion protocol were assessed both in terms of time saving and increased infusion unit capacity. In addition, indirect patient-related cost savings were evaluated.

RESULTS
One hundred and twenty-five patients were recruited (64 with ulcerative colitis and 61 with Crohn's disease). Median duration of disease was of 53 mo and mean age of pts at diagnosis was of 34 years (SD: ± 13). Adverse infusion reactions were reported in less than 4% both before and after short infusion. The total number of infusions across the selected centers was of 2501 (30.5% short infusions). In the analyzed cohort, 1143 h were saved (762 in the infusion and 381 in observation phases) through the rapid IFX infusion protocol. This time saving (-15% compared to the standard protocol in infusion phase) represents, from the hospital perspective, an opportunity to optimize infusion unit capacity by allocating the saved time in alternative cost-effective treatments. This is the case of opportunity cost that represents the value of forgone benefit which could be obtained from a resource in its next-best alternative use. Hence, an extra hour of infusion in the case of standard 2-h IFX represents a loss in opportunity to provide other cost effective services. The analysis showed that the short infusion increased the infusion units capacity up to 50% on days when the IFX infusions were scheduled (infusion phase). Furthermore, the analysis showed that the short IFX infusion protocol leads to time savings also in the post-infusion phase (observation) leading to a time saving of 10% on average among the analyzed centers. Finally, the short infusion protocol has been demonstrated to lead to indirect cost savings of €138/patient (average -€17.300 on the whole cohort).

CONCLUSION
A short IFX infusion protocol can be considered time and cost saving in comparison to the standard infusion protocol both from the hospital's perspective, as it contributes to increase infusion units capacity, and the patients' perspective, as it reduces indirect costs and the impact of treatment on everyday life and work productivity.

Key words: Infliximab; One-hour infusion; Cost savings; Economic impact; Multicenter study

Core tip: Infliximab (IFX) is a monoclonal antibody anti-tumour necrosis factor used in the treatment of moderate-to-severe inflammatory bowel diseases refractory to conventional therapy. It is usually administered *i.v.* at a dose of 5 mg/kg as a 2-h infusion. Shortening the infusion protocol to 1 h is equally safe and positively affects quality of life. This paper analyzes the impact of short IFX infusion on hospital resource utilization and costs, both in terms of time saving and increased infusion unit capacity. In addition, we provide evidence of indirect patient-related cost savings.

Viola A, Costantino G, Privitera AC, Bossa F, Lauria A, Grossi L, Principi MB, Della Valle N, Cappello M. Clinical and economic impact of infliximab one-hour infusion protocol in patients with inflammatory bowel diseases: A multicenter study. *World J Gastrointest Pharmacol Ther* 2017; 8(2): 131-136 Available from: URL: http://www.wjgnet.com/2150-5349/full/v8/i2/131.htm DOI: http://dx.doi.org/10.4292/wjgpt.v8.i2.131

INTRODUCTION

Infliximab (IFX) is a chimeric monoclonal antibody anti-tumour necrosis factor (anti-TNF) effective in inducing and maintaining remission of moderate to severe luminal and fistulizing Crohn's disease (CD)[1,2] and of moderate to severe refractory ulcerative colitis (UC)[3]. It is also used to treat rheumatoid arthritis and moderate to severe psoriasis[4,5]. IFX is usually administered intravenously at a dose regimen of 5 mg/kg as a 2-h infusion followed by a monitoring time of 2 h thereafter[6-8]. This standard practice has been adopted in order to minimize infusion reactions, which are known to occur during infusion and later in the immediate post infusion period)[9] However, the standard practice has a significant impact in the setting of limited healthcare resource in terms of dedicated areas (infusion units), facilities and, mostly, time. Short infusion (1-h) protocols have been found safe in patients with rheumatoid arthritis[10]. Recently, a shorter infusion time of one hour has been used also in inflammatory bowel diseases (IBD) patients, in maintenance therapy and who tolerated a 2-h infusion without adverse events, in referral centers[11,12]. Tolerability of one hour infusion has also been reported for 10 mg/kg IFX[13]. One hour infusions are less time-consuming and might be considered in clinical practice to improve patients' quality of life and compliance to IFX therapy[14]. Moreover, infusion therapy is also costly for patient in terms of expenses related to travel to the hospital and of hours spent in the infusion clinic (work loss). At present evidence on cost savings of short infusion is scanty. We have previously confirmed in a pilot study[15] that shortening the infusion protocol to 1 h is equally effective and safe than standard protocol. The aim of the present study was to assess the impact of short IFX infusion on hospital resource utilization and costs in a multicenter study from eight referral centers in Italy.

MATERIALS AND METHODS

All patients who received 1 h infusion of IFX from

Table 1 Traditional *vs* short infusion protocols time duration

	Traditional infusion (min)	Short infusion (min)
Observation phase	90	60
Infusion phase	120	60
Total minutes	210	120
Total hours	3.5	2

March 2007 to September 2014 in eight centers from Southern Italy were included in the analysis. Written informed consent was obtained prospectively from each patient. For each patient, demographic, clinical and infusion related data were collected retrospectively on a shared dedicated database (Excel). All patients received the dose of 5 mg/kg. Optimization of therapy was achieved by shortening the interval between infusions.

On the basis of available data, the potential benefits related to the short infusion protocol were considered both in terms of potential time saving and increased infusion unit capacity. As there was no difference in terms of drug costs, nursing and specialist service costs in both protocols, it was not possible to assess the short infusion protocol impact in direct costs terms. Instead, it was possible to estimate the related productivity loss/gain of the two different protocols. Indirect costs were expressed in terms of working hours lost due to the infusion. Indirect costs were calculated on the basis of productivity lost according to the human capital approach. The value was collected through available literature[16]. In particular, the indirect costs were calculated by multiplying infusion hours by work/hour/loss in order to assess the difference between the two different protocols. Details on infusion time for both protocols are reported in Table 1. Furthermore, we assessed the impact related to the short infusion protocol on the units capacity in term of number of treated patients; a questionnaire was sent to the participating centers to collect data on the number of patients submitted to IFX infusion/day by adopting the short infusion schedule which was compared with the same data when a standard infusion time was used. This comparison was possible just in one center (University Hospital Palermo); because of different work organization, this value was not available in other centers. Continuous variables were expressed as mean ± standard deviation, and categorical variables as absolute frequency and percentage. The comparison between continuous variables was made by the Student *t*-test and categorical variables were analysed by using the chi-square test. Statistical significance was reached when P was < 0.05. Data were analyzed using the statistics software SPSS version 15.0.

RESULTS

A total of 125 patients with IBD were included in the study, 64 with UC and 61 with CD. Seventy-one (61.6%) were male and 48 (38.4%) were female. Mean age of patients at diagnosis was 34 years (SD: ± 13). Characteristics of the patients are given in Table 2. Median duration of disease was of 53 mo (IQR: 16-110.5) and median duration of follow-up was 34 mo. The mean number of total infusion/patient was 20 (range: 4-60) and the mean number of short infusions was 6.1 (range: 1-19). Patients were shifted to one-hour infusion after a median interval of 21 mo. Median follow-up of patients in short infusion was 12 mo. Indications for IFX were steroid-dependence in 61.6%, steroid-resistance in 8%, failure of thiopurines (9.6%), fistulizing disease (5.6%), rescue therapy in severe UC (2.4%). A total of 33 patients (26.4%) were taking steroids. Concomitant use of immunomodulators (azathioprine or methotrexate) was reported in 28 patients (22.4%). Seventy-five patients received mesalamine.

Table 2 Demographics and characteristics of patients

Gender	
Male	77 (61.6%)
Female	48 (38.4%)
Mean age at diagnosis	33.6 (range: 10-80)
Smoke	
No	76 (70.8%)
Yes	26 (20.8%)
Former	23 (18.4%)
Family history	
No	106 (84.8%)
Yes	19 (15.2%)
Appendicectomy	
No	116 (92.8%)
Yes	9 (7.2%)
Characteristics of disease	
Ulcerative colitis	64 (51.2%)
Crohn's disease	61 (48.8%)
Duration of disease at 1st infusion (median)	52 mo (IQR: 16-110.5)
Duration of follow-up (median)	34 mo (IQR: 19-55.5)

Fifty-seven (45.6%) patients received no premedication. A total of 68 patients (54.4%) was submitted to premedication: 51 (40.8%) with steroids, 1 with antihistaminic (0.8%) and 16 patients with both (12.8%). Details are reported in Table 3.

Adverse infusion reactions were observed in about 4% of patients both before (4 patients) and after short infusion (5 patients). Among the 9 patients who experienced an infusion reaction we recorded 7 being acute, 1 acute-severe, 1 delayed. Adverse infusion reactions occurred at a median of 3 (IQR 3-23) mo after the first infusion. In patients with mild or moderate infusion reaction the infusion was interrupted, medical therapy was administered and after resolution of symptoms, infusion was restarted slowly. The use of premedication was not significantly associated with different rates of infusion reactions. Opportunistic infections occurred in 5 patients (4%) both before and after short infusion. Opportunistic infections occurred at a median of 32 (IQR: 18-39) mo after the first infusion. No death occurred. Details are given in Table 3.

The total number of infusions across the selected centers was of 2501 (30.5% short infusions). We therefore calculated the potential related benefits both in

Table 3 Indication for biologic, concomitant therapies and premedication

Patients treated with IFX (total 125)	
Indication for IFX	
Steroid-dependent	77 (61.6%)
Steroid-resistant	16 (12.8%)
Rescue therapy severe UC	3 (2.4%)
EIM	0
Failure of thiopurine	12 (9.6%)
Fistulizing disease	7 (5.6%)
Prevention of postoperative recurrence	1 (0.8%)
Indication for IFX (dual indication)	
Steroid-dependent + EIM	3 (2.4%)
Steroid-dependent + failure of thiopurine	3 (2.4%)
Steroid-dependent + fistulzing disease	1 (0.8%)
Fistulizing disease + EIM	2 (1.6%)
Total infusions (mean)	20 (range: 4-60)
Short infusion (mean)	6.1 (range: 1-19)
Concomitant therapies	
None	12 (9.6%)
Steroids	25 (20%)
Thiopurine	10 (8%)
Methotrexate	2 (1.6%)
5ASA	56 (44.8%)
Concomitant therapies (polipharmacy)	
Steroids + thiopurine	1 (0.8%)
Steroids + 5ASA	4 (3.2%)
Steroids + thiopurine + 5ASA	3 (2.4%)
Thiopurine/methotrexate + 5ASA	12 (9.6%)
Total use of steroids	33 (26.4%)
Total COMBO therapy (Thiopurine or Mtx)	28 (22.4%)
Total use of mesalamine	75 (60%)
Premedication	
None	57 (45.6%)
Steroids	51 (40.8%)
Antihistaminic	1 (0.8%)
Steroids + antihistaminic	16 (12.8%)
Time of premedication	
None	57 (45.6%)
From first infusion	65 (52%)
From second Infusion	3 (2.4%)
Only short infusion	0

IFX: Infliximab; EIM: Excitability-inducing material; UC: Ulcerative colitis; 5ASA: 5-aminosalicylates.

terms of time saving and increased infusion unit capacity. In the analyzed cohort, 1143 h were saved (762 in the infusion and 381 in the observation phase) through the rapid IFX infusion protocol. This time saving (-15% compared to traditional protocol in infusion phase) represents, from the hospital perspective, an opportunity to optimize infusion unit capacity by allocating the saved time in alternative cost-effective treatments. This is the case of opportunity cost that represents the value of forgone benefit which could be obtained from a resource in its next-best alternative use. Hence, an extra hour of infusion in the case of standard 2-h IFX represents a loss in opportunity to provide other cost effective services. The analysis showed that the short time infusion increased the infusion units capacity up to 50% on days when the IFX infusions were scheduled (infusion phase). In the center which provided the data, by using the one-hour infusion protocol, the number of patients treated per day increased from 3 to 6 (a 50% increase), leaving enough time to schedule additional therapies such as *i.v.* iron infusions. Furthermore, our analysis showed that the short IFX infusion protocol leads to time savings also in the post-infusion phase (observation) by leading to a time saving of 10% on average among the analyzed centers. Finally, the short infusion protocol has been demonstrated to lead to indirect cost savings of €138/patient (average -€17.300 on the whole cohort). In Table 4 we report the details on the split between short and traditional infusion.

DISCUSSION

IFX therapy is effective in the management of IBD both in the induction and in maintenance of remission, in preventing the rate of postoperative recurrence in CD and in reducing the need of hospital admission and surgery. Recently, IFX therapy has been shown to promote mucosal healing, an outcome strongly related to long-term remission[17]. This treatment is widely used, since about 15%-20% of patients with IBD are currently on anti-TNFs and usually for long periods of time since most patients will be kept on maintenance therapy[18] for 12-24 mo or even longer. IFX is administered at a dose of 5 mg/kg as a 2-h infusion followed by a monitoring time of additional 2 h. Efficacy and safety of shorter IFX infusion times have been recently demonstrated both in the setting of rheumatological disorders and IBD in observational studies. A good tolerability profile of one-hour infusion (3 or 5 mg/kg) was reported first in rheumathoid arthritis, psoriasic arthritis and ankilosyng spondylitis patients[10,19] and recently for IBD patients who tolerated a 2-h infusion without adverse reactions (acute or delayed)[20]. A meta-analysis has confirmed that rapid IFX infusions of $\leqslant$ 1-h duration are safe and not associated with increased risk of infusion reaction when compared to standard infusions in patients with IBD, rheumatoid arthritis, spondylarthoparthy and psoriatic disease[20].

Short IFX infusion could also influence patients' quality of life. Principi *et al*[14] reported an improvement in social and job quality of life in patients treated with 1-h infusion of IFX. However, though some Authors[21] have suggested the possibility of reducing costs for the healthcare provider of patient daycare attendance combined with medical staffing requirements, a pharmacoeconomic evaluation of the accelerated infusion protocol has never been approached. To our knowledge, data in the literature on economic impact of one-hour infusion in IBD patients comparing standard infusion are scanty. Only one study, carried on in the United States, has been published so far, enrolling patients on accelerated infusions (both 90 min and 60 min long) at various IFX dosage[22]. This study focused on hospital cost savings, by estimating the cost required to deliver infusions over 120-min *vs* using the accelerated infusion times: 118 h of infusion time and $53632 were saved by using the accelerated protocols ($P < 0.001$).

Kuin *et al*[23] evaluated both safety and costs of home-

Table 4 Infusion time and indirect cost savings: Traditional *vs* short infusion protocol

	w/out SI (min)	w SI (min)	Delta (min)	Saving (min)	Delta %	Hours	Saving indirect costs (€)
Infusion time	300120	254400	-45.72	-45720	-15%	-762	-11.525
Post infusion time	225090	202230	-22.86	-22860	-10%	-381	-5.763
Total time	525210	456630	-68.58	-68580	-13%	-1143	-17.288
Costsaving/patient						-9	-138

based IFX infusion as an alternative to hospital-based infusions for the management of CD patients. Home-based IFX infusions were associated with a cost saving of €55 per infusion. Another study, conducted in a small pediatric population in United States, obtained similar results[24]. Home-based therapy, though fascinating, is not applicable to all health care systems. In Italy, there are also regional differences.

Our findings suggest that in terms of indirect costs a short IFX infusion protocol in the hospital can be considered time and cost saving in comparison to the traditional infusion protocol. Our analysis could not assess differences in direct costs since costs of devices and hospital staff were similar whatever protocol is used.

The strengths of our study are: Firstly, the assessment of indirect costs of the two different infusion protocols which has never been approached and that is the most relevant from the patients' perspective; secondly, the evaluation of the improvement of organizational efficiency in terms of health care utilization resources. The use of short infusions seems to increase the unit capacity up to 50%, though this evaluation was possible only in one of the participating centers.

Our study has however some limitations. Firstly, the impact on hospital resource utilization was assessed in only one center. It could be argued that this result may not be representative of all the involved centres as it depends also on hospitals' specific organizational features. Secondly, the retrospective methodology of our study could influence the accuracy of the results. However, detailed notes of infusion characteristics were made at the time of each infusion in all participating centers so that underreporting was not expected. Finally, an activity based costing approach would be recommended in order to assess the "real" direct cost impact from the hospital perspective.

In conclusion, this study can be considered an important step in the economic evaluation of the short infusion protocol within the Italian context, although it would be recommended to perform a full economic evaluation considering both costs and related outcomes in order to provide comprehensive evidence based data useful for decision makers at local level.

A short IFX infusion protocol can be considered time and cost saving in comparison to the standard 2-h infusion protocol as it contributes to increase infusion units capacity up to 50%. From the patients' perspective, reduces indirect costs and the impact of treatment on everyday life and work productivity. On the basis of our study, we believe that the one hour IFX infusion protocol in patients in stable maintenance therapy should be implemented in clinical practice.

COMMENTS

Background

Infliximab (IFX) is a chimeric monoclonal antibody anti- tumour necrosis factor effective in inducing and maintaining remission of moderate to severe luminal and fistulizing Crohn's disease and of moderate to severe refractory ulcerative colitis. It is also used to treat rheumatoid arthritis and moderate to severe psoriasis. IFX is usually administered intravenously at a dose regimen of 5 mg/kg as a 2-h infusion followed by a monitoring time of 2 h. This standard practice has been adopted in order to minimize infusion reactions. Previous reports have shown that shortening the infusion to one hour is equally safe. The key-question addressed by this manuscript is whether this accelerated infusion protocol is cost-saving both on the hospital's and on the patient's perspective.

Research frontiers

Data in the literature on economic impact of one-hour infusion in inflammatory bowel diseases patients are scanty. Only one study, carried on in the United States, focused on hospital cost savings, by estimating the cost required to deliver infusions over 120-min *vs* using the accelerated infusion times.

Innovations and breakthroughs

The methodology adopted in this research explores the potential benefits related to the short infusion protocol both in terms of potential time saving and increased infusion unit capacity. Indirect costs were expressed in terms of working hours lost due to the infusion. This approach has been recently applied in pharmacoeconomic research.

Applications

The future application of the research could be the use of the accelerated infusion protocol not only with the infliximab originator molecule, but also with biosimilars. This could significantly reduce direct and indirect costs, increase infusion units' capacities and allow access of increased number of patients to effective therapy even in low income countries.

Terminology

Standard infusion practice requires dedicated areas (infusion units), facilities and time. Saving time is an opportunity to optimize infusion unit capacity by allocating the saved time in alternative cost-effective treatments or by increasing the number of treated patients. Indirect costs reflect patients' expenses related to travel to the hospital and of hours spent in the infusion clinic (work loss).

Peer-review

Manuscript is well written and easy to follow.

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ORIGINAL ARTICLE

Observational Study

Interferon-free treatments in patients with hepatitis C genotype 1-4 infections in a real-world setting

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Abstract

AIM

To investigated the real-world effectiveness and safety of various regimens of interferon-free treatments in patients infected with hepatitis C virus (HCV).

METHODS

We performed an observational study to analyze different antiviral treatments administered to 462 HCV-infected patients, of which 56.7% had liver cirrhosis. HCV RNA after 4 wk of treatment and at 12 wk after treatment sustained virologic response (SVR) as well as serious adverse events (SAEs) was analyzed first for the whole cohort and then separately in patients who met or did not meet the inclusion criteria of a clinical trial (CT-met and CT-unmet, respectively).

RESULTS

The most frequently prescribed treatment was simeprevir/sofosbuvir (36.4%), followed by sofosbuvir/ledipasvir (24.9%) and ombitasvir/paritaprevir/ritonavir (r)/dasabuvir (19.9%). Ribavirin (RBV) was administered in 198 patients (42.9%). SVRs occurred in 437/462 patients (94.6%). The SVRs ranged between 93.3% and 100% for genotypes 1-4. SVRs were achieved in 96.2% patients in the CT-met group *vs* 91.9% patients in the CT-unmet group (P = 0.049). Undetectable HCV RNA at week 4 occurred in 72.9% of the patients. In the univariate analysis, the factors associated with SVRs were lower liver stiffness, absence of cirrhosis, higher platelet count, higher albumin levels, no RBV dose reduction, undetectable HCV RNA at week 4 and CT-met group. In the multivariate analysis, only albumin was an independent predictor of treatment failure (P = 0.04). Eleven patients (2.4%) developed SAEs; 5.2% and 0.7% of the patients in the CT-unmet and CT-met groups, respectively (P = 0.003).

CONCLUSION

A high proportion of patients with HCV infection achieved SVRs. For patients who did not meet the CT criteria, treatment regimens must be optimized.

Key words: Hepatitis C virus infection; Genotype 1-4; Real world treatment; Direct-acting antiviral agents

Core tip: Our study analyzes the hepatitis C virus (HCV) most common genotypes treatment and all the possible combinations with direct-acting antiviral agents which are nowadays available in our country. We have found sustained virological response rates up to 90%, even in genotypes 1 and 3. The current study analyzes HCV RNA after 4 wk of treatment and 12 and 24 wk after the end of the treatment, as well as the adverse events. We analyze, separately, the patients who meet or do not meet the inclusion criteria of a clinical trial, finding that in this last group the response is lower.

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INTRODUCTION

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease worldwide, and its long-term impacts range from minimal changes to extensive fibrosis and cirrhosis with or without hepatocellular carcinoma[1,2].

The objective of chronic HCV infection treatment is to achieve a sustained virological response (SVR). A SVR is stable over time, reduces morbidity and mortality, and is equivalent in most cases to curing the HCV infection[3-5].

In 2011, the association of pegylated-interferons (Peg-INFs) and ribavirin (RBV) with the first direct-acting antiviral agents (DAAs), telaprevir and boceprevir, increased the rate of SVRs in HCV genotype 1 from 30%-40% to 65%-75%[6,7]. However, all these treatments had limited efficacy and low tolerability[8-11].

Subsequently, next-generation DAAs which are produced with or without RBV, have been associated with improved efficacy (resulting in SVR rates greater than 90% in clinical trials), safety, tolerability, and shorter durations than first-generation protease inhibitor regimens[2,12,13].

However, information derived from HCV anti-viral clinical trials have limited applicability in clinical practice. Understanding the effectiveness of anti-viral regimens in real-world settings is essential to providing practical information and adopting better HCV treatment decisions[14,15].

The objective of this prospective study was to describe the clinical characteristics of real-world patients and evaluate the effectiveness and safety of different treatment regimens with different HCV genotypes according to real-world scenarios. We also aimed to investigate whether

patients who met or did not meet the usual inclusion criteria of clinical trials (CTs) have the same efficacy and safety profile when they are treated in real-world practice.

MATERIALS AND METHODS

Study design

This prospective, observational, intent-to-treat study analyzed different antiviral treatments for HCV-infected patients in routine clinical practice. The study was conducted in 9 (5 university and 4 non-university) hospitals in north-central Spain (Castilla y León).

Ethics statement

All study participants, or their legal guardian, provided informed written consent prior to study enrollment. The study protocol was performed according to the ethical guidelines of the 1975 Declaration of Helsinki and was approved in advance by the Research Ethics Committee of the Hospital Universitario de Burgos (Burgos, Spain).

Patient selection

The cohort consisted of all consecutively evaluated HCV patients of any genotype treated with INF-free treatments from December 1, 2014 to August 31, 2015. The patients were visited at baseline, at weeks 4, 12 and 24 (if necessary) during treatment, and at weeks 12 and 24 after completing treatment.

Inclusion criteria

Inclusion criteria were as follows: (1) underwent a complete clinical history and physical examination; (2) HCV documented by the presence of detectable serum RNA-HCV; (3) liver stiffness measurement was performed using transient elastography (FibroScan, Echosens, Paris France) in the six months before starting treatment and/or cirrhosis diagnosed either by liver biopsy and/or clinical plus ultrasound criteria; (4) absence of anti-HIV 1 and 2 antibodies; (5) absence of other causes of liver disease (autoimmune disorders, primary biliary cholangitis, Wilson's disease, α1-antitrypsine deficiency, and hemochromatosis); and (6) desire for and compliance with treatment.

Exclusion criteria

Exclusion criteria were as follows: (1) recipients of liver transplantation; (2) women who were pregnant or unable to adopt contraceptive measures; (3) hypersensitivity to therapy drugs; (4) previous treatment with another interferon-free combination; (5) coinfections (HBV, HDV, HIV); and (6) failure to establish the grade of fibrosis according to the criteria outlined. The presence of hepatocellular carcinoma was not considered an exclusion criterion.

Treatment

The decision to treat and the choice of treatment, including the treatment duration and the use or not of concomitant RBV, was entirely at the discretion of the treating physician in accordance, of the majority of the cases, with the product label, the European Association for the Study of the Liver clinical practice guidelines and the National Hepatitis C Plan developed by the Spanish Ministry of Health, giving priority to the treatment of patients with significant liver fibrosis (F2-F4)[2]. The availability of each DAA varied throughout the inclusion period of the patients (Supplementary material Table 1). The use of blood transfusion or erythropoietin in case of anemia was too entirely at the discretion of the treating physician.

Study variables

All data collection and analyses were performed anonymously. A range of continuous and categorical variables was tested (Supplementary material Table 2). The HCV RNA levels were determined using the COBAS AmpliPrep®/COBAS TaqMan® (Roche Molecular Systems, Pleasanton, CA, United States; lower limit of detection: 15 IU/mL). In previously treated patients, the last prescribed treatment and the type of prior response were registered. Cirrhosis (F4) was defined by a transient elastography score > 12.5 kPa, liver biopsy or data indicating clinical, analytical and ultrasound evidence of liver cirrhosis.

Virological response

The virological response, which is defined as undetectable HCV RNA, was assessed at week 4 of the treatment (undetectable HCV RNA at week 4), at week 12 after the EOT (SVR) and at week 24 after the EOT (SVR24). Virologic failure was defined as detectable HCV RNA at any time during treatment (with the exception of week 4 of treatment) or post-treatment follow-up.

Clinical trial inclusion criteria

Patients were arbitrarily divided into two groups based on the fulfillment or not of the more usual phase Ⅲ CT inclusion criteria: Age 18-70 years, HCV RNA > 10000 IU/mL, hemoglobin $\geqslant$ 11 g/dL in women and $\geqslant$ 12 g/dL in men, platelet count $\geqslant$ 50 × $10^3/\mu$L, ALT $\leqslant$ 200 UI/mL, total bilirubin $\leqslant$ 1.5 mg/dL, albumin $\geqslant$ 3.5 mg/dL, INR $\leqslant$ 1.5, Child-Pugh score A and MELD score < 12. Patients fulfilling all these criteria were classified as CT-met patients; however, if one or more criteria were unmet, they were considered CT-unmet patients.

Adverse events

Adverse events (AEs) were reported from the time of the initial drug administration to week 12 after the planned EOT. Serious adverse events (SAEs) were defined as any event that was life-threatening; an event that led to a hospital admission, prolonged an existing hospital stay or resulted in death; or an event that was considered serious based on the judgment of the treating physician. Incident hepatic decompensation was defined as the presence of variceal hemorrhage, ascites, and/or porto-

systemic (hepatic) encephalopathy. Anemia was defined as a hemoglobin levels < 10 g/dL.

End points

The primary efficacy end point was the SVR rate in all patients who received at least one dose of treatment. Secondary end points included the rate of undetectable HCV RNA at week 4, the rate of SVR in CT-met patients and CT-unmet patients and the rate of adverse events and treatment discontinuation because of adverse events.

Statistical analysis

The data analysis was performed with SPSS 19 statistical software (IBM Corp., Armonk, New York, United States) after collecting and organizing the data with Excel 2010 (Microsoft Corp., Redmond, Washington, United States). A descriptive analysis of the sample was conducted by determining the means (SD), medians (IQR), and frequencies (percentages) according to variable characteristics and distributions. Differences between variables were evaluated using the χ^2 or Fisher's tests for qualitative variables. For quantitative variables, Student's *t*-test (if normality conditions were met) or its corresponding nonparametric tests, including the Mann-Whitney *U*-test or the Kruskal-Wallis test (if data were not normally distributed), were used. Finally, a binary logistic regression was performed using the RVS as the dependent variable. The significance level was α = 0.05, and 95%CIs were calculated.

RESULTS

During the study period, 468 patients received an interferon-free treatment. Of these patients, 6 could not be reached or did not complete follow-up. Thus, 462 patients were included in the analysis.

Baseline characteristics

Of the 462 patients included in the study, 311 (67.3%) were male, and the median age was 54 years (range 15-87 years). Cirrhosis (F4) was present at baseline in 56.7% of the cohort. The majority of patients with cirrhosis (86.7%) were Child-Pugh A class (Table 1 and Supplementary material Table 1).

The most frequent treatment prescribed was SMV and SOF (36.4%), which was followed by SOF and LDV (24.9%) and OBV, PTV/r, and DSV (19.9%). A RBV occurred in 198 patients (42.9%; Table 1).

Clinical effectiveness

Overall, 437 of the 462 patients (94.6%) achieved a SVR (Figure 1A, Tables 2 and 3). The proportion of patients with HCV genotypes 1, 2, 3 and 4 who achieved a SVR was 94.5% (1a, 97.3%; 1b, 93.4), 100%, 93.3% and 95.5%, respectively. The SVR was above 91% in all genotypes and with all treatment combinations (Table 2 and Supplementary material Tables 3 and 4).

HCV RNA at week 4 data were available for 457/462 patients (98.9%), of which 333/457 (72.9%) showed an undetectable viral load at week 4 of treatment. Patients who presented an undetectable HCV RNA at week 4 achieved a SVR (96%) more frequently than patients who did not present it (90%, *P* = 0.004; Figure 1B and Supplementary Material Table 3).

Twenty-five patients (5.4%) failed to achieve a SVR. Two patients (0.4%) who had achieved a SVR experienced a relapse with RNA-HCV detectable at week 24 after EOT. Therefore, of the 437 patients with a SVR, 435 (99.6%) maintained SVR24 (positive predictive value of SVR for SVR24 of 99.5% and negative predictive value of 100%).

In the univariate analysis, the following factors were associated with a SVR: Liver stiffness (continuous, < 20 kPa *vs* ⩾ 20 kPa and < 25 kPa *vs* ⩾ 25 kPa), cirrhosis *vs* non-cirrhosis (Figure 1B), platelet count (⩾ 100000/mm³ *vs* < 100000/mm³), albumin (continuous), RBV dose reduction or not, undetectable HCV RNA at week 4 *vs* non-undetectable HCV RNA at week 4 and CT-met *vs* CT-unmet (Supplementary material Table 3 and 4). In the multivariate analysis, only baseline albumin (continuous) was an independent predictor of treatment failure (*P* = 0.04; Supplementary material Table 3 and 4).

Safety and tolerability

Four patients (0.9%) with genotype 1 discontinued treatment early, with three (0.6%) discontinuing because of a SAE and one discontinuing at the patient's request. Altogether, 321 patients (69.5%) experienced one or more AEs, and most of them (96.6%) were mild. The AEs that appeared with a frequency over 3% are described in Table 3. The most commonly reported AE was fatigue (22.5%), which was followed by headache (11.7%) and anemia (11.3%). Anemia was present in 47/198 (23.7%) of patients who received RBV, compared with 5/264 (1.9%) of patients who did not receive it (*P* = 0.000). In 21 patients (8.5%), the dose of RBV had to be modified. Two patients (0.4%) required a blood transfusion, and none required erythropoietin.

Eleven patients (2.4%) developed SAEs. Ten of these patients had liver cirrhosis (three Child-Pugh score A, 6 Child-Pugh score B and one Child-Pugh score C at baseline). Nine of the eleven patients who developed SAEs were also treated with RBV. SAEs were related to hepatic decompensation in seven patients with six of these patients experiencing ascites (one with hepatocellular carcinoma and another one with hepatic encephalopathy) and one patient developing only hepatic encephalopathy. Two patients developed severe anemia; both of these patients were cirrhotic and treated with RBV, and one patient developed suicidal ideation and the other developed hyperbilirubinemia. There were no deaths during treatment or follow up.

Subanalysis of patients with met or unmet clinical trials criteria

The predefined requirements to participate in a theoretical CT were not fulfilled by 173 patients. Regarding the

Table 1 Baseline characteristics of patients receiving direct-acting antiviral agents: Overall patients, patients subgroup clinical trial-met and clinical trial-unmet

Characteristics	Total *n* = 462	CT-met *n* = 289	CT-unmet *n* = 173	*P* value
Sex, male	311 (67.3)	196 (67.8)	115 (66.5)	0.765
Age, yr	54 (15-87)	53 (30-69)	59 (15-87)	[1]
BMI, kg/m^2, *n* = 368	26.4 (17.6-47)	26.2 (17.6-47)	26 (18.6-40.6)	0.132
IL28B genotype CC/CT/TT, *n* = 367	80/231/56	39/153/34	41/78/22	0.021
HCV genotype 1/2/3/4	78.4/2.4/9.7/9.5	76.1/2.1/10.4/11.4	82.1/2.9/8.7/6.4	0.549[2]
HCV genotype 1a/1b/1	31.2/66.6/2.2	40.1/58.6/2.7	16.2/78.9/1.4	0.000[3]
Baseline HCV RNA, log10 IU/mL	6.1 (3.0-7.8)	6.5 (4.2-7.6)	6.4 (3.0-7.8)	[1]
HCV antiviral treatment history				0.233
Naïve	186 (40.0)	112 (38.8)	74 (42.8)	
Non-responders	211 (45.7)	131 (45.6)	80 (46.2)	
Relapsers	64 (13.9)	46 (15.9)	18 (10.4)	
Fibrosis stage, *n* (%)				0
F0-1	26 (5.6)	21 (7.3)	5 (2.9)	
F2	100 (21.6)	83 (28.7)	17 (9.8)	
F3	77 (16.7)	59 (20.4)	15 (8.7)	
F4	259 (56.1)	126 (43.6)	136 (78.6)	
Transient elastography, kPa, *n* = 435	13.5 (2.8-65)	10.9 (2.8-75)	18.2 (3.5-75)	0
Cirrhosis				
No	200 (43.3)	163 (56.4)	37 (21.4)	0
Yes	262 (56.7)	126 (43.6)	136 (78.6)	
Child–Pugh Score, *n* = 209				[1]
A	180 (86.1)	116 (100)	64 (68.8)	
B	22 (10.5)	0 (0.0)	22 (23.7)	
C	7 (3.3)	0 (0.0)	7 (7.5)	
MELD score, *n* = 229	8.1 (6–29)	6.9 (6-11)	9.4 (6-29)	[1]
Hemoglobin level, g/dL,	15.3 (11-19.1)	14.3 (8-19.5)	15 (8-19.5)	[1]
Platelets, /mm^3, *n* = 446	158666 (23000-457000)	177301 (50000-457000)	124363 (23000-436000)	[1]
ALT, IU/L, *n* = 461	81 (64)	71.8 (43.9)	97.6 (79.8)	[1]
Bilirubin > 1 mg/dL, *n* = 243	94 (38.7)	19 (15.3)	75 (63.0)	[1]
Albumin < 3.5 g/dL, *n* = 239	25 (10.3)	0 (0.0)	25 (21.2)	[1]
INR	1.1 (0.7-2.9)	1.0 (0.7-1.3)	1.1 (0.9-2.9)	[1]
Treatment prescribed				0.024[4]
SMV and SOF	168 (36.4)	90 (31.1)	78 (45.1)	
SMV and DCV	7 (1.5)	1 (0.3)	6 (3.5)	
SOF and DCV	56 (12.1)	40 (13.8)	17 (9.8)	
SOF	11 (2.4)	9 (3.1)	2 (1.2)	
OMV and PTV/r	13 (2.8)	10 (3.5)	3 (1.7)	
OMV, PTV/r, and DSV	92 (19.9)	60 (20.8)	31 (17.9)	
SOF and LDV	115 (24.9)	79 (27.3)	36 (20.8)	
+ RBV	198 (42.9)	131 (45.3)	67 (38.7)	165
Treatment duration				0.973[5]
8 wk	12 (2.6)	9 (3.1)	3 (1.7)	
12 wk	407 (88.1)	253 (87.5)	154 (89.0)	
24 wk	43 (9.3)	27 (9.3)	16 (9.2)	
Treatment at University Hospital	395 (85.5)	259 (89.6)	136 (78.6)	0.001

[1]The *P* value was not calculated because the variable was part of inclusion criteria in the C-met group; [2]Genotype 3 *vs* the rest; [3]1a *vs* 1b; [4]To calculate the *P* value the SMV and DCV, SOF and OMV and PTV/r groups were excluded because of a low *n*; [5]8 plus 12 wk *vs* 24 wk. Continuous variables reported as median (range). Categorical variables reported as *n* and/or %. DDAs: Direct-acting antiviral agents; CT: Clinical trial; BMI: Body mass index; PEG: Pegylated interferon; PIs: Protease inhibitors; ALT: Alanine aminotransferase; SMV: Simeprevir; SOF: Sofosbuvir; DCV: Daclatasvir; LDV: Ledipasvir; OMV: Ombitasvir; PTV/r: Paritraprevir/ritonavir; DSV: Dasabuvir; RBV: Ribavirin.

basal characteristics and apart from the CT inclusion criteria, which were obviously different, the patients in the CT-unmet group presented the IL28B CC genotype more frequently, which is a genotype 1 subtype, and more advanced fibrosis, and they were more frequently treated in a non-university hospital (Table 1). These CT-unmet patients had a globally lower SVR than the CT-met patients (91.9% *vs* 96.2%, *P* = 0.049; Figure 1C, Supplementary material Table 3). However, the undetectable HCV RNA at week 4 was similar in both groups [75.0% in the CT-unmet group and 71.6% in the CT-met group (*P* = 0.426)] (Figure 1C). The frequency of AEs was significantly higher in the CT-unmet group (52.2% *vs* 32.9%, *P* = 0.000). However, there were no differences regarding the development of anemia and the need for RBV dose reductions between the two groups. Importantly, SAEs (including hepatic decompensation) appeared more commonly in the CT-unmet group (5.2% *vs* 0.69%, *P* = 0.003 and 3.47% *vs* 0.35%, *P* = 0.013, respectively).

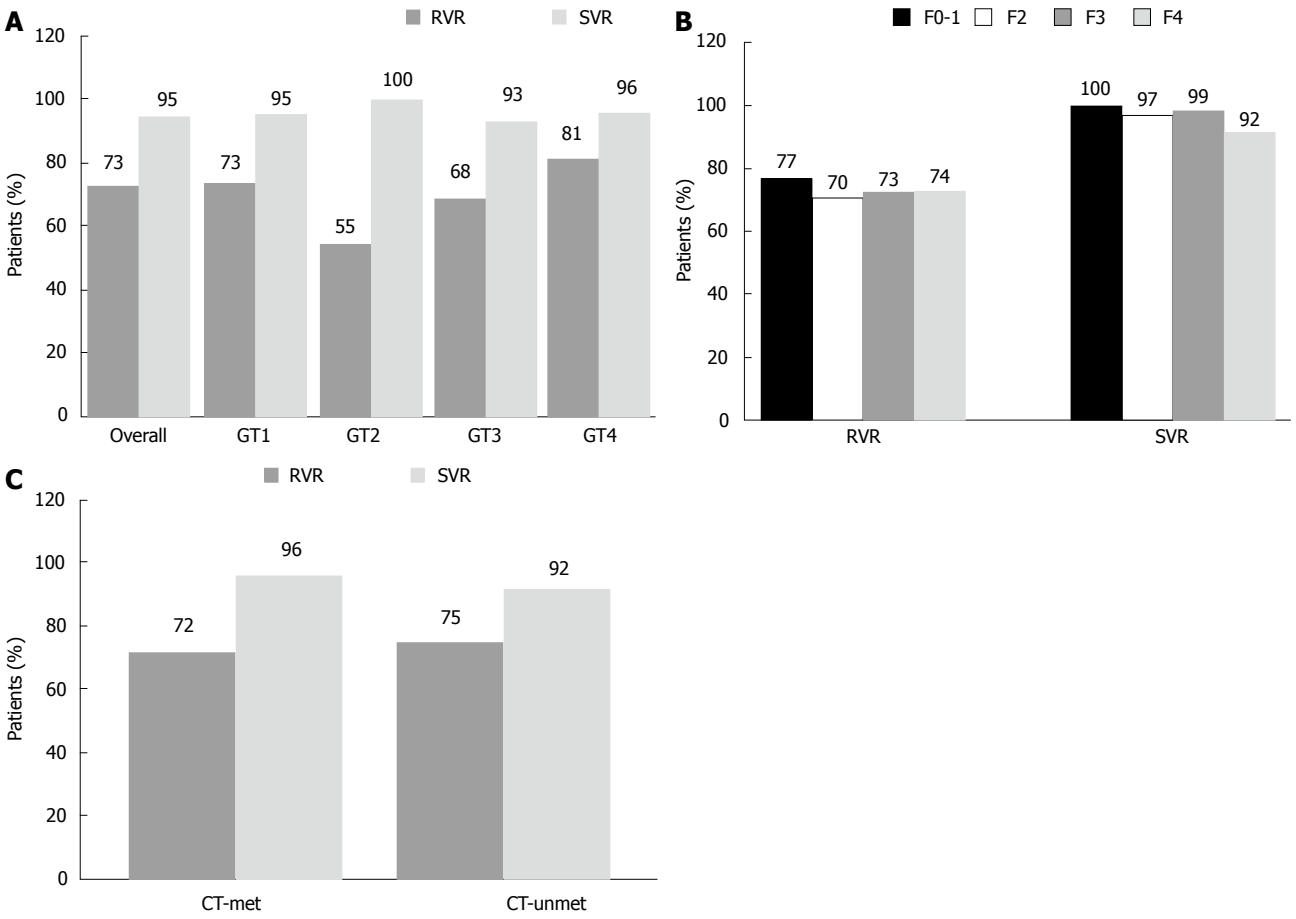

Figure 1 Rates of virological response. Patients with undetectable viral loads during and post treatment. A: At treatment week 4 and post-treatment week 12 (sustained virological response) by genotype; B: At treatment week 4 and post-treatment week 12 (sustained virological response) by fibrosis stage; C: At treatment week 4 and post-treatment week 12 (sustained virological response) by CT-met and CT-unmet. Data for 5 patients were lost: genotype 1, data from three patients were lost; genotype 3 and 4, a patient data in each genotype were lost. Data for 4 patients were lost. Data for 1 patient were lost. GT: Genotype; RVR: Undetectable HCV RNA at week 4; SVR: Sustained virological response; CT: Clinical trial.

Three of four patients who stopped treatment and 9 of 11 patients with SAEs were included in the CT-unmet group. In 6 of the 7 patients with a liver cirrhosis decompensation, a SAE was included in the CT-unmet group.

DISCUSSION

Our real-world study is representative of monoinfected, non-transplanted patients and the treatment regimens available in Spain in 2015. Because the decision to treat and the choice of treatment were entirely at the discretion of the treating physician and randomization was not possible, this study could not directly compare the effectiveness and safety of the treatment regimens.

In the general cohort, the global efficacy was high (94.6% SVR) and the results were similar to those achieved in the CTs, although almost 60% of the patients had received previous HCV antiviral treatment and more than half had liver cirrhosis.

We found that 0.4% of the subjects who achieved a SVR at week 12 subsequently relapsed at week 24 (did not achieve SVR24), and this percentage was a similar to or even lower than those found in other studies[16,17]. Therefore, this finding confirmed previous results in a real-world setting and showed good concordance between SVRs at week 12 and week 24 based on different new AAD-based regimens, including those with shorter durations and/or with drugs with lower barriers to resistance. However, in our opinion, to definitively determine a "cure" in every patient in clinical practice, a SVR must be confirmed at week 24.

Until now, few real-world setting studies have included results that consider the most frequent genotypes (1 to 4). The most significant study is the US retrospective analysis of data from 17487 patients with genotypes 1 to 4 from the Veterans Affairs (VA) National Healthcare System[18], in which a global SVR of 90.7% was found, which was lower than that in our study. This difference may be linked to early discontinuation of treatment in 4.4% of patients with available SVR data[18].

In our study, albumin was the only independent predictor of a SVR. Other studies[14,18] have also shown that albumin and other variables associated with cirrhosis or worse liver function were related to a lower SVR, thus confirming these findings in a real-world setting and with

Table 2 Sustained virological response by genotype and treatment regimen

Treatment regimen	Patients in each	SVR
Genotype 1		
SMV and SOF	149 (41.2)	139 (93.3)
SMV and DCV	7 (1.9)	7 (100)
SOF and DCV	15 (4.1)	15 (100)
OMV, PTV/r, and DSV	91 (25.1)	86 (94.5)
SOF and LDV	100 (27.6)	95 (95.0)
Total	362 (100)	342 (94.5)
Genotype 2		
SOF and DCV	5 (45.5)	5 (100)
SOF	5 (45.5)	5 (100)
SOF and LDV	1 (9.1)	1 (100)
Total	11 (100)	11 (100)
Genotype 3		
SOF and DCV	37 (82.2)	34 (91.9)
SOF	5 (11.1)	5 (100)
SOF and LDV	3 (6.7)	3 (100)
Total	45 (100)	42 (93.3)
Genotype 4		
SMV and SOF	19 (43.2)	18 (94.7)
SOF	1 (2.3)	1 (100)
OMV and PTV/r	13 (29.5)	12 (92.3)
SOF and LDV	11 (25.0)	11 (100)
Total	44 (100)	42 (95.5)

SVR: Sustained virological response; SMV: Simeprevir; SOF: Sofosbuvir; DCV: Daclatasvir; LDV: Ledipasvir; OMV: Ombitasvir; PTV/r: Paritraprevir/ritonavir; DSV: Dasabuvir.

Table 3 Safety profile *n* (%)

Patients	*n* = 462
Severe AEs	
Any AE[1]	11 (2.4)
AEs	321 (69.5)
Fatigue	104 (22.5)
Headache	55 (11.7)
Anemia	52 (11.3)
Insomnia	23 (5.0)
Infection	20 (4.3)
Arthralgia, myalgia	19 (4.1)
Dyspepsia	15 (3.2)
Rash	14 (3.0)
Deaths	0 (0.0)

[1]Adverse events (AEs) occurring during treatment or follow-up in ⩾ 3% patients.

a wide number of patients and supporting the results of CTs in which patients with a more advanced liver disease have a worse response to treatment.

Most real-world studies reported results in genotype 1 HCV patients[14,19,20]. The SVR rate in our study, which included 362 genotype 1 patients, was 94.5% of the overall genotype 1 patients, which was somewhat higher than previously reported rates (SVRs over 91%), although limited differences were observed among the different DAA combinations, treatment durations and use of RBV. SMV and SOF with or without RBV was the most used treatment in our genotype 1 patients, which was likely because it was the best combination available at the beginning of the study. This treatment was used in 149 of the total genotype 1 patients. Most of these patients had liver cirrhosis and were included in the CT-unmet group because the most severe patients were prioritized. However, these patients achieved a SVR of 93.3%. In other studies with thousands of patients with genotype 1 HCV treated with this regimen, the SVR rates were lower at between 75% and 84%[14,15,21]. The main cause of the differences between our cohort and the others was likely the lower rate of subtype 1a (31.2%) and Q80K variants in our genotype 1 patients. Although these variants were not analyzed in the current study, they appeared in only 2.7% of Spanish genotype 1 patients[22].

Other treatment combinations also showed high rates of SVR in our study; *i.e.*, 95.0% with SOF/LDV and 94.5% with OBV/PTV/r/DSV. These rates were similar to the 92.9% or 92% SVR rates derived from the first regimen presented in two US VA National Healthcare System studies[18,19] and the 94.9% or 95.1% SVR rates achieved with the second regimen in other studies in clinical practice[18,20].

In our cohort, only eleven genotype 2 patients were treated, and all of them achieved a SVR regardless of the treatment regimen used. High rates of SVR with the combination SOF + RBV were more similar to those described in Asian CTs[23] than the SVR of 79.0% or 86.2% achieved in clinical practice in the two VA studies[14,18] or the SVR of 88.2% from the recent analysis of 321 genotype 2 HCV infected HCV-TARGET participants[24]. However, the low number of genotype 2 patients in our study indicate that several of the currently recommended combinations in clinical guidelines, such as SOF and DCV[25] should be favored because they presented 100% SVR rates in all patients.

Patients with HCV genotype 3 are at a higher risk of liver disease progression and hepatocellular carcinoma development[26,27]. However, compared with other HCV genotypes, DAA combinations have lower efficacy against genotype 3 in patients with liver cirrhosis in CTs.

In the current study, the global SVR in patients with genotype 3 HCV infection was 93.3%. In our cohort, 82.2% of patients with this genotype were treated with SOF and DCV, with a global SRV rate of 90.3%-91.9% in patients with liver cirrhosis and 100% without. In others studies in real-world settings, a global SVR of 60%-70% was achieved in genotype 3 infection with SOF plus RBV[18,28]. All these studies had remarkably low rates, which was likely related to the use of combinations that are currently not recommended because of their low efficacy[25].

Patients with HCV genotype 4 infection are poorly represented in pivotal CTs of second-generation DAAs[25] and in most real-world studies. In the VA study, a SVR of 87.6% with SOF and LDV and 96.4% with OBV and PTV/r was achieved in patients with this genotype[18]. In the current study, 44 patients who were HCV genotype 4-infected were treated and the SVR rate was 95% (100% with SOF and LDV, 92.3% with OBV and PTV/r

and 94.7% with SMV and SOF).

The week 4 response data were available for almost all patients in the current study. We found that 72.9% of patients had an undetectable HCV RNA at week 4, similar to another analysis[19,29]. In this last real-world setting study, significant SVR rate reductions of 7.1% to 10.5% according to the addition of RBV or not, respectively, were observed in patients who did not have an undetectable HCV RNA at week 4 compared with those with undetectable HCV RNA at week 4, which was similar to the 6% observed in the current study[19]. The clinical implications of this finding on treatment decisions, such as potentially adding RBV or extending the treatment duration based on 4 wk of on-treatment HCV RNA, warrants further study.

Despite the real-world nature of our cohort, which included a higher proportion of elderly patients and many patients with liver cirrhosis, the safety and tolerability of all regimens were good. Discontinuation rates were low (< 1%), which is similar to that of CTs, and there were no deaths during treatment or follow up. In Backus *et al*[20] higher early discontinuation rates of 5.3% to 15.2% according to the treatment combination were found. In contrast, of the 802 patients in the genotype 1 group from the HCV-TARGET cohort treated with SMV and SOF, the rate of discontinuation for adverse events was only 2%[15].

In patients from the genotype 1 and genotype 3 groups from the HCV-TARGET cohort, the most commonly reported AEs were fatigue and headache, which is consistent with the results presented here[15,28]. However, anemia associated with RBV was less frequent in our study.

Overall, the reported rates of SAEs (2.4%) were similar to those reported in the pivotal CTs and lower than the 5.3% or the 7.3% described in other studies in "real-world"[15,28]. Again, in the three studies, the most frequent SAEs were the same decompensating events. However, in the current study, only seven of 262 cirrhotic patients experienced decompensation.

Because the real-world population is heterogeneous, it is important to investigate the treatment outcomes in patients excluded from CTs. Thus, we divided patients into two groups: Patients who met the requirements to take part in a CT and patients who did not meet these requirements. We found that the CT-unmet patients had lower rates of SVR and higher rates of SAEs, liver decompensation and treatment interruptions than the CT-met patients. Thus, in this group of patients, it might be advisable to conduct a more rigorous follow-up investigation to closely monitor tolerability and optimize treatment regimens.

This study has the usual limitations related to its observational, real-world design and electronic data collection. Resistance testing was not performed; thus, we were unable to assess the impact of this factor. The lack of randomization limited the ability to directly compare treatment groups, which is further compounded by the small number of patients in certain subgroups.

In conclusion, our study confirmed the efficacy and safety data reported in CTs in a cohort of patients with genotypes 1-4 and a wide range of basal characteristics, including a high proportion of patients with advanced fibrosis and treatment experience. Our results confirmed and occasionally improved upon the efficacy and safety results reported in other recently published real-world setting studies with a large number of patients[8,19], and these results are in sharp contrast to the lower SVR rates reported in certain early real-world studies on interferon-free therapy with second generation DAAs[14,15]. Moreover, our results indicate that treatment regimens should be optimized in patients that do not fulfill classical CT inclusion criteria because of their lower rates of SVR and higher rates of SAEs.

COMMENTS

Background

New direct-acting antiviral agents (DAAs) have shown higher efficacy (with sustained virological response, SVR, over 90%), safety, tolerability and shorter durations than previous antiviral agents used in the treatment of hepatitis C. However, information derived from hepatitis C virus (HCV) anti-viral clinical trials has limited applicability in clinical practice. Understanding the effectiveness of anti-viral regimes in real-world settings is essential to provide practical information in order to adopt better HCV treatment decisions.

Research frontiers

The research hotspot is to check whether the results of HCV anti-viral clinical trials can be extrapolated to the real world HCV population.

Innovations and breakthroughs

This study analyzes the efficacy and safety of all possible combinations of DAAs available in the authors' country in multiple HCV genotypes, in contrast to other studies where just one DAA treatment regimens and usually one genotype is analyzed. In this real world cohort, which includes a high proportion of elderly patients and patients with cirrhosis, the efficacy, safety and tolerability of all DAA regimens are good, and similar to the clinical trials results. However, patients who do not meet the requirements to participate in a theoretical clinical trial, have lower SVR rates and a higher proportion of adverse and serious adverse events, including liver disease decompensation, and more treatment interruptions.

Applications

The authors found that 0.4% of patients who achieved SVR at week 12 subsequently relapsed at week 24 so, in the authors' opinion, to definitively determine the infection cure in clinical practice, SVR should be confirmed at week 24. Moreover, as patients who do not meet clinical trial requirements have lower SVR and more adverse events, it might be advisable to conduct a more rigorous follow-up and to optimize treatment regimens in this population.

Terminology

DAAs: Direct-acting antiviral agents are molecules that target specific nonstructural proteins of the virus and result in disruption of HCV replication. There are four classes of DAAs, which are defined by their mechanism of action and therapeutic target. The four classes are nonstructural proteins 3/4A (NS3/4A), protease inhibitors (PIs), NS5B nucleoside polymerase inhibitors (NPIs), NS5B non-nucleoside polymerase inhibitors (NNPIs), and NS5A inhibitors. SVR: sustained virological response, is defined as undetectable HCV RNA at week 12 after the end of HCVtreatment. It is equivalent to the virological curie of the infection, and the goal of HCV treatment, although it does not mean the disease resolution in patients with advanced fibrosis.

Peer-review

This real-world prospective multi-center study was conducted at 9 centers in

Spain on a fair number of patients, the study is well designed and the paper is well written.

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ORIGINAL ARTICLE

Randomized Controlled Trial

Low dose oral curcumin is not effective in induction of remission in mild to moderate ulcerative colitis: Results from a randomized double blind placebo controlled trial

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Author contributions: Ahuja V planned and conducted the study, collecting and interpreting data; Kedia S and Ahuja V drafted the manuscript; Garg S analyzed data; Kedia S, Bhatia V, Thareja S, Mouli VP, Bopanna S, Tiwari V and Makharia G critically revised the manuscript for important intellectual content and final approval of the manuscript; all authors have approved the final draft submitted.

Institutional review board statement: The study was reviewed and approved institute ethics committee at All India Institute of Medical Sciences, New Delhi.

Informed consent statement: All study participants provided informed written consent prior to study enrollment.

Clinical trial registration statement: This trial was done from 2003 till 2005. Since there was no trial registry in India at that time, this trial did not have a registration number.

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Abstract

AIM

To evaluate the role of oral curcumin in inducing clinical remission in patients with mild to moderate ulcerative colitis (UC).

METHODS

A prospective randomized double-blind placebo-controlled trial comparing the remission inducing effect of oral curcumin and mesalamine 2.4 g with placebo and mesalamine 2.4 g in patients of ulcerative colitis with mild to moderate severity was conducted from January 2003 to March 2005. The included patients received 1 capsule thrice a day of placebo or curcumin (150 mg) for 8 wk. Patients were evaluated clinically and endoscopically at 0,

4 and 8 wk. The primary outcome was clinical remission at 8 wk and secondary outcomes were clinical response, mucosal healing and treatment failure at 8 wk. The primary analysis was intention to treat worst case scenario (ITT-WCS).

RESULTS

Of 300 patients with UC, 62 patients (curcumin: 29, placebo: 33) fulfilled the inclusion criteria and were randomized at baseline. Of these, 21 patients did not complete the trial, 41 patients (curcumin: 16, placebo: 25) finally completed 8 wk. There was no significant difference in rates of clinical remission (31.3% *vs* 27.3%, $P = 0.75$), clinical response (20.7% *vs* 36.4%, $P = 0.18$), mucosal healing (34.5% *vs* 30.3%, $P = 0.72$), and treatment failure (25% *vs* 18.5%, $P = 0.59$) between curcumin and placebo at 8 wk.

CONCLUSION

Low dose oral curcumin at a dose of 450 mg/d was ineffective in inducing remission in mild to moderate cases of UC.

Key words: Curcumin; Mesalamine; Ulcerative colitis; Ulcerative colitis disease activity index; Mucosal healing

Core tip: Not all patients with mild to moderate ulcerative colitis (UC) respond to available treatment options. Curcumin, an active ingredient of turmeric has anti-inflammatory properties and has been shown to play a protective role in chemically induced mouse models of inflammatory bowel disease and to reduce relapse rates in human UC. However, optimum dose ranging studies for curcumin in ulcerative colitis have not been performed. The present study shows that low dose curcumin (450 mg/d) is ineffective in inducing remission in mild to moderate ulcerative colitis. Therefore, higher doses with effective modes of delivery are required for optimal efficacy of curcumin.

Kedia S, Bhatia V, Thareja S, Garg S, Mouli VP, Bopanna S, Tiwari V, Makharia G, Ahuja V. Low dose oral curcumin is not effective in induction of remission in mild to moderate ulcerative colitis: Results from a randomized double blind placebo controlled trial. *World J Gastrointest Pharmacol Ther* 2017; 8(2): 147-154 Available from: URL: http://www.wjgnet.com/2150-5349/full/v8/i2/147.htm DOI: http://dx.doi.org/10.4292/wjgpt.v8.i2.147

INTRODUCTION

Ulcerative colitis (UC) is a chronic relapsing and remitting inflammatory condition of the intestinal tract without a known etiology[1,2]. The interaction between environmental factors, genetic susceptibility, and immune dysregulation is implicated in the pathogenesis of UC, although their precise contributions remain incompletely understood[3-6].

Oral 5-aminosalicylates (5-ASA) compounds are the first line therapy used for inducing clinical remission in mild to moderate UC. Treatment options for patients not responding to oral 5-ASA include oral corticosteroids, immunomodulators such as 6-mercaptopurine and azathioprine, topical agents like 5-ASA and steroid enemas and biologicals. However, steroids are associated with significant side effects, immunomodulators are slow to act, topical agents would only be effective in left-sided colitis and biologicals are costly and not every patient can afford them, especially in resource constraint countries like India. Surgery is an option but every patient does not want it, and one likes to defer surgery in mild to moderate cases. Therefore, there is a need for an agent which is safe, efficacious and cheap and can be added with 5-ASA to increase the remission rates, especially in developing countries like India, where the incidence of IBD is on the rise[7].

Pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, IL-12, and interferon (IFN)-γ are upregulated in patients with UC[8]. Nuclear factor (NF)κB is the main up-regulator of expression of these cytokines and is strongly activated in UC and Crohn's disease suggesting the important role in pathogenesis. Curcumin is the major constituent of turmeric powder extracted from the rhizomes of the plant *Curcuma longa Linn*. Turmeric is used as a spice to give the specific flavor and yellow color to curry. Curcumin has been identified as the most active constituent of turmeric and has been described as an anti-inflammatory, antioxidant, pro-apoptotic, and anti-proliferative compound[8,9]. As a traditional medicine, turmeric has also been widely used for centuries to treat inflammatory disorders in India[9]. The pleiotropic effects of curcumin owe to inhibition of transcriptional factor nuclear NF-κB. Curcumin blocks a signal upstream of NF-κB-inducing kinase and IκB kinase in intestinal epithelial cells[10]. The effects of curcumin on the immune response (both innate and adaptive) have been a subject of much attention in the past decade[11-15]. Curcumin has been shown to play a protective role in chemically induced mouse models of IBD[16-19] and to reduce the relapse rate in human UC[20-22].

Hence this study was carried out to determine the efficacy and safety of oral curcumin therapy in inducing remission in mild to moderate cases of UC.

MATERIALS AND METHODS

Study design

This study was a single center prospective randomized double-blind placebo-controlled trial comparing the remission inducing effect of oral curcumin and mesalamine (2.4 g/d in three divided doses) with placebo and mesalamine (2.4 g/d in three divided doses) in patients with UC with mild to moderate severity. The study was

carried out from January 2003 to March 2005. The study was approved by the institutional ethics committee.

Participants

All patients were recruited from the Inflammatory Bowel Disease clinic at All India Institute of Medical Sciences, New Delhi, India. Adult patients (≥ 18 years) who had mild-to-moderately active UC [Ulcerative Colitis Disease Activity Index (UCDAI) score[23], 3-9]; with a minimum sigmoidoscopic score of 2 with at least one previously documented attack of active disease were included in this study. Patients were excluded if they had evidence of severe disease (UCDAI, > 10), concurrent enteric infection, use of oral steroids within the past 4 wk, use of antibiotics within the past 2 wk, change in dose of oral mesalamine within the past 4 wk, and initiation of azathioprine less than 6 mo before initiation of the study. Patients requiring hospitalization and imminent need for surgery, lactating and pregnant women, and those who received any investigational medicines within 3 mo were excluded. Patients with significant hepatic, renal, endocrine, respiratory, neurologic, or cardiovascular diseases also were excluded. Demographic information was recorded on a structured proforma.

Randomization

Sequence generation: The random numbers were generated by computerized random number (The RAND corp. Inc). The randomization list and numbered packing of the intervention were prepared by a person not involved in the study. Randomization was performed using permuted blocks of 6.

Randomization-allocation concealment: Allocation concealment was ensured by the use of sequentially numbered boxes coded using alphabet containing identical curcumin or placebo capsules, according to the allocation sequence.

Randomization implementation: All the study personals were blinded to the treatment assignment (placebo or curcumin) for the duration of the study. Placebo and curcumin capsules were similar in appearance and in their method of administration. The codes were reserved with a no-interest party and were revealed only after the recruitment and data collection had been completed.

Blinding

The individual sealed box method was used to maintain blinding of the investigators and study participants.

Study intervention

Included patients were randomized to receive 1 capsule thrice a day of either placebo or curcumin for a duration of 8 wk. Each curcumin capsule contained 150 mg of purified curcumin. The placebo was supplied as an indistinguishable capsule containing starch with a yellow edible dye-caramel yellow. The curcumin and placebo capsules were supplied by Himalaya Drug Company (Bangalore, India). The patients in both the groups received mesalamine at a dose of 2.4 g/d. Other supportive treatment and standard care were provided to both the groups.

Follow-up

Patients were evaluated at the study center at weeks 0, 4, 8 (or as required) after recruitment. Clinical Assessment was done on the basis of UCDAI score. A sigmoidoscopic evaluation with endoscopic scoring was also done according to Baron score[24] at each visit. Biochemical parameters like hemoglobin, erythrocyte sedimentation rate, leukocyte count, urea, creatinine, bilirubin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and albumin were performed at each follow-up visit.

Compliance was measured by obtaining a detailed study history during a personal interview as well as compliance was judged at the 4 and 8 wk follow-up visits by a blister count of the remaining capsules. Non-compliance was defined as failure to take ≥ 80% of the medication.

Outcomes

The primary outcome measure was clinical remission (UCDAI ≤ 2) at 8 wk. Secondary outcomes were clinical response (defined as a reduction from baseline in the UCDAI of ≥ 3, sigmoidoscopic remission (Baron endoscopic score of 0/1), and treatment failure defined by an increase in UCDAI scores by ≥ 3 points or treatment intolerance by the patient.

Activity of UC

The activity of UC was assessed using the UCDAI[23]. The UCDAI was calculated by the investigator by adding the individual scores of the 4 parameters: Bowel frequency, rectal bleeding, endoscopic score, and physician's rating of severity. (Rectal bleeding and stool frequency score was assessed by asking the patient about his/her symptoms over the past 3 d. The score for these parameters was calculated individually by taking the average of the scores for the last available 3 d before the study visit. The composite score ranges from 0 (inactive disease) to 12 (severe disease activity). The Baron score[24], represents an endoscopic classification, ranges from 0 to 3, with 0 denoting normal mucosa, (1) granularity of mucosa with loss of vascular pattern; (2) bleeding to touch; and (3) spontaneous bleeding. Sigmoidoscopic remission was defined by a Baron score of 0 or 1 (normal looking mucosa or mucosal edema alone as indicated by loss of normal vascular pattern).

Sample size estimation and statistical analysis

This study was conducted in 2003 and the efficacy of curcumin in induction of remission in UC had not been

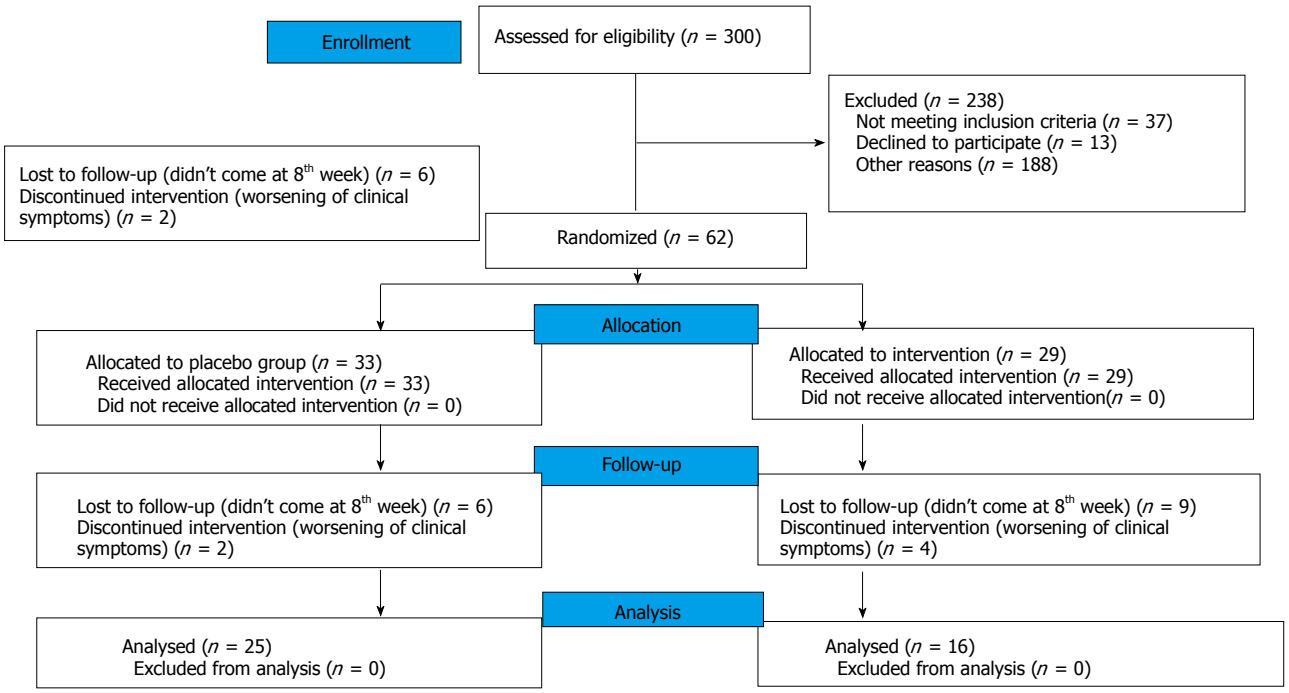

Figure 1 Flowchart demonstrating patient recruitment in curcumin and placebo group.

studied previously in any human trial. Hence, this was an exploratory pilot trial where consecutive patients of UC fulfilling the inclusion criteria were enrolled over a period of January 2003 to March 2005. The primary analysis was intention to treat worst-case scenario.

The data was entered in a Microsoft excel spreadsheet (MS Office version 2003). Descriptive statistics including measures of central tendency and dispersion were calculated for all variables. Continuous variables were compared using *t*-test for independent samples and categorical variables were compared using χ^2 test. Measures of risk were computed along with 95%CI. Changes in symptom scores and clinical sign scores from baseline to the final follow-up visit were calculated and compared between the placebo and curcumin groups. A *P*-value of < 0.05 was considered statistically significant. All calculations were done with SPSS software (v. 16).

RESULTS

A total of 300 patients presenting at the Inflammatory Bowel Disease clinic of the Department of Gastroenterology, at All India Institute of Medical Sciences (AIIMS), New Delhi, from January 2003 to March 2005 were assessed for eligibility. Of them, 62 patients fulfilled the inclusion criteria and agreed to participate (Figure 1). Thirty three patients were randomized to the placebo group and 29 to curcumin group. A total of 21 patients did not complete the trial (8 patients in the placebo group, and 13 patients in curcumin group). Thus, a total of 41 participants, 25 in the placebo group and 16 in curcumin group, completed the trial and were analyzed. The participant flow through the trial is given in Figure 1.

Demographic and clinical characteristics

Baseline characteristics were comparable between the 2 groups (Table 1). The mean baseline UCDAI score was also comparable between the two groups (5.2 ± 2 *vs* 5.5 ± 1.9, *P* = 0.63) (Table 2). All subsequent analyses are presented according to ITT-WCS.

Primary and Secondary outcome measures

Induction of clinical remission (Table 3): Clinical remission was achieved in 31.03% of patients (9 out of 29) in curcumin group and 27.27% (9 out of 33) in the placebo group at 8 wk, the difference being statistically insignificant (OR = 1.20, 95%CI: 0.40-3.60; *P* = 0.745).

Improvement in UCDAI (Table 3): The UCDAI was similar between the two randomized groups at baseline (Table 2). There was no difference in the UCDAI among the randomized patients at 4 and 8 wk. Six out of 29 (20.69%) patients in curcumin group reported an improvement in DAI score by 3 or more as compared to 12 out of 33 (36.36%) in the placebo group but the difference was not statistically significant (OR = 0.46, 95%CI: 0.14-1.43; *P* = 0.175).

Mucosal healing (Table 3): Mucosal healing was achieved in 34.48% of patients (10 out of 29) in curcumin group and 30.30% (10 out of 33) in the placebo group at 8 wk, the difference being statistically insignificant (OR = 1.21, 95%CI: 0.42-3.52; *P* = 0.725).

Treatment failure: Amongst the patients who completed the study, 1 out of 16 in curcumin group and 3 out of 25 in the placebo group were found to be the cases of treatment failure defined as increase in UCDAI

Table 1 Baseline clinical and biochemical parameters of the randomized patients *n* (%)

	Curcumin group (*n* = 29)	Placebo group (*n* = 33)
Age (yr)	36 ± 12	34 ± 7
Sex (females)	13 (44.83)	8 (24.24)
Weight (kg)	55.1 ± 10.0	55.7 ± 11.7
BMI (kg/m^2)	20.8 ± 3.1	20.5 ± 3.3
Disease duration (yr)	3.83 ± 4.00	3.64 ± 2.59
Disease extent		
E3 (pancolitis)	7 (25.9)	6 (20.7)
E2 (left sided colitis)	17 (58.6)	21 (63.6)
E1 (proctitis)	3 (11.1)	2 (6.90)
Current smoking	5 (17.24)	4 (14.81)
Current alcohol use	3 (10.34)	4 (14.81)
Hemoglobin (g/dL)	12.12 ± 2.76	13.35 ± 1.72
Total leukocyte count (per cubic millimeter)	8586 ± 2306	8221 ± 2104
Platelet count(× 1000/mm^3)	256.25 ± 131.98	257.53 ± 113.01
ESR (mm/1st h)	3 ± 2	4 ± 3
Urea (mg/dL)	22.0 ± 5.2	21.5 ± 5.2
Creatinine (mg/dL)	0.84 ± 0.26	1.19 ± 1.69
Potassium (meq/L)	4.41 ± 0.33	4.26 ± 0.78
Bilirubin (mg/dL)	0.7 ± 0.2	0.6 ± 0.1
Aspartate aminotransferase (U/L)	28 ± 6	30 ± 8
Alanine aminotransferase(U/L)	26 ± 9	28 ± 19
Total protein (g/L)	8.0 ± 0.2	7.9 ± 0.8
Albumin (g/L)	4.4 ± 0.5	4.5 ± 0.3
Current treatment		
5-ASA	29 (100)	33 (100)
Steroids	0	0
Azathioprine	2 (6.9)	2 (6.2)
Rectal steroids	0	0
Mesalamine enema	0	0

Data are given as mean ± SD. 5-ASA: 5-aminosalicylates; BMI: Body mass index; ESR: Erythrocyte sedimentation rate.

score by 3 or more. The difference between the two groups was not statistically significant (OR = 0.489, 95%CI: 0.046-5.155; *P* = 0.545).

Moreover, 4 out of 13 dropouts in curcumin group and 2 out of 8 dropouts in placebo group cited worsening of clinical symptoms as reasons for dropout were also categorized as treatment failure as per protocol. Hence, the total treatment failure rate in curcumin and placebo groups were 25% (5 out of 20) and 18.52% (5 out of 27) respectively. The difference between the treatment failure rates in the two groups was not statistically significant (OR = 1.47, 95%CI: 0.361-5.952; *P* = 0.591).

Comparison of laboratory parameters between the two randomized groups

No significant improvement in hemoglobin or albumin was reported within either group at 8th week. On comparing the two groups, no significant difference was found between any laboratory parameter at either 4 or 8 wk (Table 4).

Compliance

In the placebo group, 8 out of 33 patients did not complete the study. Two of them cited worsening of clinical symptoms, categorized as treatment failure, to be the cause of dropout. Others were lost to follow-up. In curcumin group, 13 out of 29 patients did not complete the study. Four of them cited worsening clinical symptoms, categorized as treatment failure, to be the cause of dropout. Patient drop out due to worsening of symptoms is the main reason for reporting the ITT-WCS analysis. In patients continuing in the trial, the compliance was more than 80% in all patients in both the treatment arms.

Safety and adverse drug reactions

No adverse clinical or biochemical effects were observed in either group. One patient complained of self-limited arthralgia in the placebo group.

DISCUSSION

This was the first randomized controlled trial of oral curcumin in the induction of remission in UC. This study showed that oral curcumin at a dose of 450 mg a day was ineffective in inducing remission or attaining clinical response. Curcumin has been shown to play a protective role in chemically induced mouse models of IBD[16-19]. Mechanisms by which curcumin exerts its pharmacological effects are thought to involve antioxidation[4], inhibition of kinases, interference with the activity of transcription factors such as NF-κB and AP-1[5]. Cyclooxygenase-2 (COX-2) and lipoxygenase (LOX) are inhibited by curcumin through NF-κB dependent or independent pathway[6,7]. NF-κB has been shown to activate, *via* transcription, the genes encoding pro-inflammatory cytokines (TNF-α, IL-1β and IL-12), cell adhesion molecules (vascular cell adhesion molecule (VCAM)-1 and intercellular cell adhesion molecule (ICAM)-1, inducible nitric oxide synthase (iNOS) and COX-2[25-27].

We recently published a randomized controlled trial using curcumin enemas in patients with mild to moderate distal colitis[28]. Per protocol analysis revealed significantly better outcomes in curcumin enema group, in terms of clinical response (92.9% *vs* 50%, *P* = 0.01), clinical remission (71.4% *vs* 31.3%, *P* = 0.03), and improvement on endoscopy (85.7% *vs* 50%, *P* = 0.04). However, in the present study, oral administration of curcumin did not induce remission after 8 wk of therapy. In a recent randomized controlled trial from Israel which enrolled 50 patients with mild to moderate UC, oral curcumin was found to be effective in inducing remission[29]. The dose of curcumin used was 3 g/d. In the intention-to-treat analysis, 14 patients (53.8%) receiving curcumin achieved clinical remission at week 4, compared with none of the patients receiving placebo (P = 0.01). Clinical response (reduction of ⩾ 3 points in SCCAI) was achieved by 17 patients (65.3%) in the curcumin group *vs* 3 patients (12.5%) in the placebo group (*P* < 0.001).

We did not find a significant effect of using curcumin on the response, remission, or mucosal healing at 4 and 8 wk as compared with placebo. The following factors

Table 2 Comparison of the Ulcerative Colitis Disease Activity Index between the two randomized groups at baseline, 4, and 8 wk

	Curcumin group		Placebo group		Mean difference (95%CI)	Significance
	n	UCDAI	*n*	UCDAI		
Week 0	29	5.2 ± 2.0	33	5.5 ± 1.9	-0.244 (-1.256 to 0.77)	0.632
Week 4	16	3.6 ± 2.4	23	4.4 ± 3.2	-0.823 (-2.678 to 1.020)	0.37
Week 8	16	3.4 ± 3.1	25	3.8 ± 2.8	-0.362 (-2.343 to 1.168)	0.711

UCDAI is expressed as mean ± SD. UCDAI: Ulcerative Colitis Disease Activity Index.

Table 3 Comparison of clinical remission, improvement in Ulcerative Colitis Disease Activity Index and Baron's score, and mucosal healing at 8th week between two randomized groups

	Curcumin group	Placebo group	OR (95%CI)	*P* value
Clinical remission				
PP[1]	9/16 (56.25%)	9/25 (36%)	2.28 (0.634-8.264)	0.202
ITT[2]	9/29 (31.03%)	9/33 (27.27%)	1.20 (0.40-3.60)	0.745
UCDAI improvement by ⩾ 3				
PP	6/16 (37.5%)	12/25 (48%)	0.65 (0.18-2.34)	0.509
ITT	6/29 (20.69%)	12/33 (36.36%)	0.46 (0.14-1.43)	0.175
Mucosal healing[1]				
PP	10/16 (62.5%)	10/25 (40%)	2.50 (0.69-9.09)	0.16
ITT	10/29 (34.5%)	10/33 (30.3%)	1.21 (0.4 – 3.5)	0.72

[1]Healing defined by either endoscopically normal mucosa or only mucosal granularity. Any ulceration or friability was taken as non-healed mucosa. PP: Per-protocol; ITT: Intention to treat; UCDAI: Ulcerative Colitis Disease Activity Index.

Table 4 Comparison of the biochemical parameters between the two randomized groups at 4 and 8 wk

	Week 4			Week 8		
	Curcumin group	Placebo group	*P* value	Curcumin group	Placebo group	*P* value
Hemoglobin (g/dL)	12.0 ± 2.3	13.3 ± 2.2	0.235	12.1 ± 2.7	13.2 ± 2.6	0.404
Total leukocyte count (per mm^3)	7900 ± 2449	8085 ± 2494	0.87	8957 ± 1705	7086 ± 1969	0.082
ESR (mm/1st h)	21.5 ± 13.4	22.2 ± 15.2	0.91	23.0 ± 17.1	25.9 ± 9.4	0.707
Urea (mg/dL)	23.1 ± 8.5	22.8 ± 7.0	0.922	24.7 ± 6.0	24.3 ± 6.7	0.89
Alanine aminotransferase (U/L)	26.5 ± 8.4	35.5 ± 20.6	0.178	29.5 ± 15.4	25.3 ± 5.4	0.516
Total protein (g/L)	7.9 ± 0.4	8.1 ± 0.8	0.666	8.3 ± 0.3	8.3 ± 0.9	0.963
Albumin (g/L)	4.7 ± 0.2	4.6 ± 0.3	0.869	4.8 ± 0.2	4.6 ± 0.2	0.169

were likely responsible for observed non-response. The first reason could be the use of an inadequate dose of curcumin. A daily total of 450 mg curcumin per day in three divided doses was used in this study. In another study where curcumin was used for inducing remission, a 3 g/d dose was used[29], which is much higher than the dose used in our study. In a second study, curcumin in combination with 5-ASA was shown to be effective in maintaining remission in UC patients as compared to placebo[20,22]. Again the dose of curcumin used in that study was 2 g/d, much higher than the present study. However, at the same time, none of these studies had incorporated a dose finding study design. Hence, the present study clearly adds to the knowledge that low dose oral curcumin is not effective in inducing remission in UC. The second reason could be poor bioavailability. A phase Ⅰ clinical trial conducted on 25 patients with various precancerous conditions indicated that curcumin is poorly absorbed and may have limited systemic bioavailability. Because of curcumin's rapid plasma clearance and conjugation, its therapeutic usefulness has been somewhat limited, leading researchers to investigate the benefits of complexing curcumin with other substances to increase systemic bioavailability. Other studies have also demonstrated the safety of curcumin, including a phase-1 trial in which doses of up to 8000 mg of curcumin per day were administered without toxicity[30]. We have not used any such complex formulations, which could have produced a difference. We have shown the efficacy of topical curcumin enemas in combination with oral 5-ASA in inducing remission in a similar group of patients. The dose of curcumin used in this study was just 140 mg which was lower than the dose used in this study, indicating that curcumin indeed would be effective but with proper dosage and route of administration. There have been multiple studies on this aspect that have investigated various formulations of curcumin, some of which increase systemic bioavailability

of curcumin and some have lead to increased colonic delivery[31,32]. In a mice study, Curcumin-Zn(II) complex was prepared by stirring curcumin with anhydrous zinc chloride at a molar ratio of 1:1. Kinetic stability studies showed a good stability of the metallo-complex with zinc and *in vivo* study revealed a significant reduction in severity and extent of colonic damage with this preparation[31]. Another study assessed the role of pH-triggered Eudragit-coated chitosan microspheres of curcumin in managing UC. *In vivo* organ bio-distribution study showed a negligible amount of curcumin in the stomach and small intestine and there was a significant reduction in extent and severity of colonic damage with these microspheres[32]. A trial investigating these newer formulations of curcumin could better define the role of curcumin in UC. Another limitation of this study is the small sample size as it was an exploratory pilot study. In absence of any data on the use of curcumin in UC, when this study was conducted, no sample size calculations could be done. However, the study by Lang et al which had a small sample size of 50 UC patients showed the efficacy of curcumin in inducing remission as compared to placebo[29]. Future studies are needed to prove (or disprove) the hypothesis that oral curcumin is effective in induction of remission in mild to moderate UC[33]. The dose can range from 1 to 4 g considering that doses up to 8 g are safe[30]. The sample sizes for these studies would have to be approximately 100 or 170 in each arm to detect an absolute difference of 20% and 15% respectively, assuming the baseline remission rate of 27% (from the placebo group).

In conclusion, low dose oral curcumin for 8 wk is not effective in inducing clinical remission or response in patients with mild to moderate UC. A multicenter collaborative trial using newer formulations of curcumin with higher bioavailability and a dose defining study design is required to conclusively answer this research question.

COMMENTS

Background

There is a therapeutic gap in inducing clinical remission in patients with ulcerative colitis (UC) with the available treatment options. Curcumin, an active ingredient of turmeric powder, because of it anti-inflammatory properties, can decrease the mucosal inflammation in patients with active UC. The present study was designed to evaluate the role of curcumin in inducing clinical remission in patients with mild to moderate UC.

Research frontiers

Oral 5-aminosalicylates (5-ASA) compounds are the first line therapy used for inducing clinical remission in mild to moderate UC. Treatment options for patients not responding to oral 5-ASA include oral corticosteroids, immunomodulators such as 6-mercaptopurine and azathioprine, topical agents like 5-ASA and steroid enemas, and biologicals. However, each of these agents is associated with its own side-effects and is not effective in every patient. Therefore, there is a need for an agent which is safe, efficacious and cheap and can be added with 5-ASA to increase the remission rates, especially in developing countries like India, where the incidence of inflammatory bowel disease is on the rise.

Innovations and breakthroughs

This was the first dose ranging study to evaluate the efficacy of oral curcumin in patients with mild to moderate UC. They compared oral curcumin (450 mg/d) with mesalamine (2.4 g/d) *vs* placebo with mesalamine in inducing remission in patients with mild to moderate UC and found that oral curcumin at a dose of 450 mg/d was ineffective in inducing remission in mild to moderate UC.

Applications

The results of this study indicate that low dose curcumin is ineffective in mild to moderate cases of UC. Therefore further studies with higher doses of curcumin or with better drug delivery systems are required to evaluate the efficacy of curcumin in UC.

Terminology

Low dose oral curcumin is ineffective in patients with mild to moderate UC.

Peer-review

This is a good study to point out Low dose oral curcumin at a dose of 450 mg/d, which was ineffective in inducing remission in mild to moderate cases of UC.

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EDITORIAL

Strategies for overcoming anti-tumor necrosis factor drug antibodies in inflammatory bowel disease: Case series and review of literature

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Abstract

Anti-tumor necrosis factor (TNF) biologics are currently amongst the most widely used and efficacious therapies for inflammatory bowel disease (IBD). The development of therapeutic drug monitoring for infliximab and adalimumab has allowed for measurement of drug levels and antidrug antibodies. This information can allow for manipulation of drug therapy and prediction of response. It has been shown that therapeutic anti-TNF drug levels are associated with maintenance of remission, and development of antidrug antibodies is predictive of loss of response. Studies suggest that a low level of drug antibodies, however, can at times be overcome by dose escalation of anti-TNF therapy or addition of an immunomodulator. We describe a retrospective case series of twelve IBD patients treated at the University of California-Irvine, who were on infliximab or adalimumab therapy and were found to have detectable but low-level antidrug antibodies. These patients underwent dose escalation of the drug or addition of an immunomodulator, with subsequent follow-up drug levels obtained. Eight of the twelve patients (75%) demonstrated resolution of antidrug antibodies, and were noted to have improvement in disease activity. Though data regarding overcoming low-level anti-TNF drug antibodies remains somewhat limited, cases described in the literature as well as our own experience suggest that this may be a viable strategy for preserving the use of an anti-TNF drug. Low-level anti-TNF drug antibodies may be overcome by dose escalation and/or addition of an immunomodulator, and can allow for clinical improvement in disease status. Therapeutic drug monitoring is an important tool to guide this strategy.

Key words: Inflammatory bowel disease; Adalimumab; Anti-tumor necrosis factor; Infliximab; Therapeutic drug monitoring; Drug antibody; Antidrug antibodies; Dose escalation

Core tip: One of the main challenges of anti-tumor necrosis

factor use in inflammatory bowel disease is immunogenicity, or the immune-mediated formation of drug antibodies. Therapeutic drug monitoring allows for measurement of serum drug levels and antidrug antibodies. Previously it was thought that antibody formation was indication to switch to an alternate agent; however, more recent literature, which we review in this article, suggests that a low level of antidrug antibodies can be overcome by dose escalation of the biologic drug and/or addition of an immunomodulator. We describe a small case series of patients in whom this strategy was used, in conjunction with therapeutic drug monitoring, with some success.

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INTRODUCTION

Since the initiation of their use for inflammatory bowel disease (IBD) in the late 1990s, tumor necrosis factor (TNF) inhibitors have drastically changed the face of IBD treatment. Anti-TNFs are monoclonal antibodies that inhibit the pro-inflammatory cytokine tumor necrosis factor[1]. These drugs have shown significant efficacy in the treatment of ulcerative colitis (UC) and Crohn's disease (CD)[2]. Currently approved anti-TNFs for IBD in the United States include infliximab, adalimumab, certolizumab, and golimumab.

As the use of anti-TNFs has become more widespread, we seek out tools that will help us to use these drugs more efficaciously and economically. Therapeutic drug monitoring (TDM) allows for measurement of drug levels and drug antibodies. As inadequate serum drug levels and/or development of immunogenicity are possible etiologies for treatment failure, TDM has become a helpful tool to guide therapy.

DRUG LEVELS

In the United States, there are currently commercially available assays to measure serum drug levels of infliximab, adalimumab, certolizumab and golimumab, though the therapeutic cutoffs for certolizumab and golimumab are overall less certain[3]. There are various types of assays, including enzyme-linked immunosorbent assay (ELISA), homogeneous mobility shift assay (HMSA), radioimmunoassay (RIA), reporter gene assay (RGA), and electrochemiluminescence (ECLIA)[4]. It is beyond the scope of this article to discuss details regarding the mechanism of these various assays; however, although analytic properties of the tests vary somewhat, it is thought that overall detection of drug levels and antidrug antibodies (ADAs) correlate with each other and result in similar interventions and clinical outcomes regardless of the assay used[5]. It should be noted that the newer generation assays allow for the detection of drug in the presence of drug antibodies or when bound to drug antibodies[4].

Numerous studies have clearly demonstrated that therapeutic levels of drug in the serum are associated with induction and sustaining of clinical remission, decreased inflammatory markers, endoscopic healing, and decreased risk for requiring surgery[6-9].

Anti-TNF drug levels are typically measured as a trough. There have been multiple attempts to identify target therapeutic levels; due to variable study end points (including clinical remission, decreased inflammatory markers, endoscopic and mucosal healing and lack of antibody formation), these studies have yielded mixed results[3]. A retrospective analysis in 2015 by Yanai *et al*[10] using an ELISA-based assay demonstrated trough infliximab levels of > 3.8 mcg/mL and trough adalimumab levels of > 4.5 mcg/mL to be 90% specific in identifying patients who failed to respond to dose intensification. Other studies suggest that slightly higher trough levels are necessary for the end point of mucosal healing[11,12].

ANTI-DRUG ANTIBODIES

Formation of antidrug antibodies occurs as an immune response to exposure to the TNF inhibitor, which is a foreign protein. Immunogenicity results in inability of the TNF inhibitor to bind to TNF molecules and also results in increased immune-mediated clearance of the drug from the body[13,14]. Factors thought to predict development of immunogenicity include episodic anti-TNF dosing, lack of induction dosing[15], chimeric monoclonal antibody drugs[16], route of administration, and possibly the presence of certain genetic alleles[17,18]. Commercially available assays for quantitation of anti-drug antibodies are currently available for infliximab and adalimumab only[3]. Due to lack of data on this topic with regard to other biologics, this article will focus on application of TDM for infliximab and adalimumab only.

The significance of ADAs has been evaluated in multiple studies. A relatively early study by Baert *et al*[19] in 2003 showed that antibodies to infliximab were associated with infusion reaction and decreased duration of response to the drug. Subsequent studies confirmed that ADAs are associated with decreased drug levels[20,21], and demonstrated association with flare, loss of clinical response, and discontinuation of the anti-TNF drug[20-23].

Dose escalation of the anti-TNF drug is less successful in patients with antibodies and will be discussed further below. Therefore, determining the presence or absence of drug antibodies is a useful tool to guide decision-making.

Determination of a clinically significant level of antibodies has been evaluated in multiple studies. Baert *et al*[19] determined that patients with antibodies

Table 1 Management of secondary loss of response

	No/low antibody level	High antibody level
Low drug level	Increase drug dose	Change therapy (within class or alternate class)
Normal/high drug level	Change therapy (alternate class)	(Not clinically relevant scenario)

to infliximab in a titer ⩾ 8 mcg/mL (using ELISA) had a reduced duration of drug effect. Mazor *et al*[24] showed an inverse correlation between drug levels and antibodies levels, with antibody to adalimumab ⩾ 3 mcg/mL (using ELISA) predictive of active disease. The Yanai study from 2015 determined that antibodies to infliximab > 9 mcg/mL or antibodies to adalimumab > 4 mcg/mL (using ELISA) identified patients who did not respond to dose escalation with 90% specificity[10].

Given the above, prevention of immunogenicity is regarded to be an important consideration in the approach to using a TNF inhibitor. Strategies to prevent antibody formation include maintenance, rather than episodic, dosing[25], and concomitant use of an immunomodulator[26].

However, if antidrug antibodies are identified, the subsequent management strategy is less clear. Options to salvage the current anti-TNF therapy may include dose adjustment of the TNF inhibitor and/or addition of an immunomodulator.

DOSE ESCALATION OF ANTI-TNFS

Dose intensification of the anti-TNF drug, in the form of increased dosage or frequency, can be a useful strategy for the management of secondary loss of response. The success of this strategy has been described with and without the assistance of drug monitoring, but appears to be more cost-effective when TDM is used as evidenced in multiple reviews[14,15,27]. Additionally, as recapturing of response is only demonstrated in patients who achieve measurable increase in drug levels after dose intensification[28,29], TDM may be helpful to better identify patients who will respond to this intervention.

The usefulness of dose escalation in patients with antidrug antibodies, however, is less certain. A 2010 study found that patients who underwent dose escalation of infliximab had a decreased response when antibodies to infliximab were present[30]. Similar findings were noted during a prospective study of patients on adalimumab done in 2014[31]. Vande Casteele *et al*[28] also reported in 2013 that patients with sustained levels of infliximab ADAs were more likely to fail dose adjustment, with a likelihood ratio of 3.6 to fail dose escalation when antibodies to infliximab were > 9.1 U/mL (using HMSA). Based on these data, some treatment algorithms would advocate changing drug therapy if presence of ADAs is identified.

Though studies have been able to identify with relatively reasonable consistency a cutoff beyond which dose escalation is unlikely to be successful (10, 28), the question remains whether lower levels of ADAs can be overcome by manipulation of therapy. This phenomenon has been described, albeit not in great number, in multiple studies throughout the years. Ternant *et al*[32] described as early as 2008 patients in whom infliximab antibodies resolved with dose adjustment, though disappearance seemed to occur spontaneously in other patients. In another prospective study by Bartelds *et al*[33] in 2011, 6 cases were described in which patients had loss of antibodies to adalimumab after dose escalation. Pariente *et al*[34] described a decrease in antidrug antibody titers after dose intensification. More recently, the previously cited retrospective study by Yanai noted that low level of ADAs were overall less specific for failure to respond to dose escalation[10], and a 2015 study by Steenholdt *et al*[35] demonstrated resolution of anti-infliximab antibodies in patients who underwent dose escalation.

Based on the limited information from (but not limited to) the studies listed above, and the theoretical pharmacokinetics of overcoming the presence of antibodies, more recent treatment algorithms advocate that a low level of antidrug antibodies can be overcome[14,28,36-38]. These algorithms suggest that in patients who are found to have low drug level and low level of ADA, dose intensification be performed in an attempt to avoid changing to an alternate drug (Table 1).

ADDITION OF IMMUNOMODULATOR THERAPY

An alternate strategy to mediate immunogenicity to anti-TNF biologics is to use a concurrent immunomodulator.

Concomitant therapy with an immunomodulator is a strategy already used to prevent immunogenicity. Previous data has shown that use of an immunomodulator, in the form of a thiopurine *vs* methotrexate, when used together with a biologic agent has been associated with decreased formation of antidrug antibodies[26]. Use of dual therapy is associated with lower disease activity, increased mucosal healing, increased rates of steroid-free remission, and decreased need to switch to an alternate drug. These findings are evidenced in multiple studies, including the SONIC trial[39-41]. This benefit has been postulated to be at least in part related to decreased immunogenicity of the biologic therapy when an immunomodulator is used. Additionally, studies do not show significant increase in adverse events such as malignancy, infection or death when using combination therapy *vs* monotherapy[42].

There is limited data regarding the addition of an immunomodulator drug after antidrug antibodies have already formed, but it suggests that this may be a viable strategy. In a small retrospective study of 5 patients who developed antidrug antibodies and loss of response to infliximab, Ben-Horin *et al*[43] showed

that after addition of an immunomodulator (either azathioprine/6-mercaptopurine or methotrexate), all 5 patients had gradually decreasing levels of ADAs and restored clinical response. A later study by Yarur *et al*[44] showed that 6-thioguanine (6-TG) levels > 125 pmol/8 × 10^8 RBCs correlated with more optimal levels of infliximab, and 6-TG levels lower than this threshold were associated with presence of antidrug antibodies. These studies did not include patients on adalimumab.

Though a distinct cutoff for antidrug antibody levels was not delineated in these studies, they do suggest that the addition of an immunomodulator is a viable strategy when ADAs form. This strategy has not yet been widely added to treatment algorithms, presumably pending the availability of additional supportive data.

PRACTICAL EXPERIENCE

We describe a retrospective case series of patients on anti-TNF therapy that were found to have low-level antidrug antibodies and underwent escalation of drug therapy or addition of an immunomodulator, with subsequent follow-up drug levels obtained. These patients were receiving care at the University of California-Irvine in the 36-mo period from November 2013 to October 2016.

A total of twelve patients on either infliximab or adalimumab were included in the case series - eight patients with CD and four patients with ulcerative colitis. Median patient age was 43.5 years, ranging from 21 to 71 years. Disease activity in all patients was moderate to severe. Disease extent in Crohn's patients disease included ileal, colonic, and ileocolonic disease, with some patients having stricturing or perianal disease. Disease extent in UC patients included either left-sided disease or pancolitis. The median duration of disease (from the time of diagnosis) was 8 years, ranging from 1 to 30 years. Four patients had previously been on an alternate anti-TNF drug. Four patients were being treated concurrently with immunomodulator at the time of initial evaluation. Of note, all patients had been advised to take a concurrent immunomodulator at the time of anti-TNF initiation but eight of the twelve patients had previously declined due to concern for adverse effects.

Of the twelve patients, seven were being treated with infliximab and five with adalimumab at the time of the study. Median duration of treatment on the respective anti-TNF was 18.5 mo, ranging from 4 to > 120 mo. Drug and antibody levels were checked using an ECLIA or HMSA. Reference values were defined as had been previously determined for the respective assays. Indication for checking drug and antibody levels included presence of symptoms and/or active disease noted on endoscopy. All patients had some subjective or objective evidence of active disease at the time that drug level testing was performed. In our experience, most patients either had the test covered by insurance or paid up to about $250 after subsidization from drug company assistance programs, though the list price for drug level testing is as high as $2500.

The median drug levels prior to alteration of therapy were 4.1 mcg/mL and 3.0 mcg/mL for infliximab and adalimumab, respectively. The median antidrug antibody level for infliximab was 5.5 U/mL using HMSA (negative < 3.1 U/mL). The median antidrug antibody level for adalimumab was 3.1 U/mL for patients tested using HMSA (negative < 1.7 U/mL), and was 52 ng/mL for patients tested using ECLIA (negative < 25 ng/mL). The twelve patients were determined to have a low level of antidrug antibody present, which was determined at the clinical discretion of the treating physician.

After presence of low-level antibodies was noted, eleven patients underwent dose escalation of the anti-TNF drug in the form of either increase in drug frequency or dosage, and one patient had an immunomodulator (methotrexate) added. Addition of an immunomodulator was discussed with all of the patients who were not already treated with one, but most patients declined due to concern for side effects. Those who were already on immunomodulator therapy were continued on dual therapy.

Follow-up drug level testing demonstrated resolution of anti-drug antibodies in eight patients (75%). These patients were found to have increase in drug level: Median levels drug levels increased to 20.2 mcg/mL and 7.9 mcg/mL for infliximab and adalimumab, respectively. These patients were also noted to have improvement in disease activity in the form of decreased inflammatory markers and/or symptomatic improvement. The remaining four patients did not have resolution of anti-drug antibodies after dose escalation and were therefore switched to an alternate IBD therapy. Of note, the four patients who did not have resolution of ADAs carried a diagnosis of CD, though given the small study size, it is unclear whether this is significant (Tables 2 and 3).

CONCLUSION

Though IBD treatment continues to evolve, biologic therapies still remain limited in number; therefore it may be prudent to exhaust a drug therapy before switching to an alternate drug. Therapeutic drug monitoring allows for measurement of drug levels and drug antibodies, and has become instrumental in optimizing anti-TNF drug therapy. In the case of active disease, when drug levels are low, dose escalation of the anti-TNF is recommended; when drug levels are high, therapy may need to be changed. Management of antidrug antibodies is an evolving area of interest, as immunogenicity one of the most common reasons for changing drug therapy. Antidrug antibodies have been demonstrated in multiple studies to be associated with decreased drug levels, loss of response to the drug, and active disease. Strategies to prevent antibody formation include maintenance dosing and concurrent use of an immunomodulator. A small amount of data exists about the possibility of overcoming antidrug antibodies, as reviewed above. Several studies have

Table 2 A total of twelve patients on either infliximab or adalimumab were included in the case serie

Patient	Age (yr)	Sex	Diagnosis	Montreal classification	Duration of disease (yr)	Previous anti-TNF therapy?	Immunomodulator therapy?
1	24	M	CD	A2L3B1	5	Y	Previous - MTX
2	46	M	UC	E3	10	N	Previous - 6MP
3	71	M	CD	A3L1B2	4	N	Previous - 6MP
4	31	F	CD	A2L1B2	9	Y	Current - 6MP
5	46	F	CD	A2L3B2p	5	N	None
6	41	M	UC	E2	18	N	Current - AZA
7	53	F	UC	E2	24	Y	Previous - MTX
8	21	M	UC	E3	1	N	Current - MTX
9	67	M	CD	A3L2B1	3	N	None
10	26	M	CD	A1L2B1	11	N	None
11	49	F	CD	A2L3B2	30	N	None
12	25	M	CD	A2L1B2	7	Y	Current - MTX

Montreal classification CD: Age at Diagnosis (A1: Less than 16 years; A2: Between 17 and 40 years; A3: Over 40 years). Location (L1: Ileal; L2: Colonic; L3: Ileocolonic; L4: Isolated upper digestive tract). Behavior (B1: Non-stricturing, non-penetrating; B2: Structuring; B3: Penetrating; p: Perianal). Montreal classification UC: Location (E1: Proctitis; E2: Left-sided; E3: Extensive or pancolitis). M: Male; F: Female; CD: Crohn's disease; UC: Ulcerative colitis; MTX: Methotrexate; 6-MP: 6-mercaptopurine; AZA: Azathioprine; TNF: Tumor necrosis factor.

Table 3 Adjustment therapy at the time of the study

Pt	Dx	Anti-TNF drug	On immuno-modulator?[1]	Reason for TDM	Predrug level	Pre-Ab level	Adjustment in therapy	Post-drug level	Post-Ab level	Resolved ADAs?	Did patient have improvement?
1	CD	ADA	N	Endoscopic disease	3.0 μg/mL	38 ng/mL	Frequency	10 μg/mL	< 25 ng/mL	Y	Symptom improvement; fecal calprotectin
2	UC	ADA	N	Flare symptoms	< 1.6 μg/mL	4.6 U/mL	Dose/ frequency	13.7 μg/mL	< 1.7 U/mL	Y	Symptom improvement; decreased CRP
3	CD	ADA	N	Flare symptoms	3.3 μg/mL	2.6 U/mL	Frequency	5.8 μg/mL	0	Y	Symptom improvement
4	CD	ADA	Y - 6MP	Flare symptoms, Endoscopic disease	2.6 μg/mL	66 ng/mL	Frequency	4.8 μg/mL	< 25 ng/mL	Y	Symptom improvement
5	CD	IFX	N	Flare symptoms	4.1 μg/mL	4.5 U/mL	Dose/ frequency	23.4 μg/mL	< 3.1 U/mL	Y	Symptom improvement; CRP
6	UC	IFX	Y - AZA	Flare symptoms	1.1 μg/mL	8.2 U/mL	Dose	16.9 μg/mL	< 3.1 U/mL	Y	Symptom improvement
7	UC	IFX	N	Flare symptoms; Endoscopic disease	10.4 μg/mL	5.0 U/mL	Added immuno-modulator (MTX)	11.3 μg/mL	0	Y	Symptom improvement; ESR
8	UC	IFX	Y - MTX	Flare symptoms	0	5.5 U/mL	Dose	26.8 μg/mL	0	Y	ESR/CRP
9	CD	IFX	N	Flare symptoms	23.1 μg/mL	8.6 U/mL	Dose	< 1 μg/mL	88.6 U/mL	N	-
10	CD	IFX	N	Endoscopic disease	< 1.0 μg/mL	3.7 U/mL	Frequency	< 0.4 μg/mL	34 ng/mL	N	-
11	CD	ADA	N	Flare symptoms	5.6 μg/mL	3.1 U/mL	Frequency	4.6 μg/mL	113 ng/mL	N	-
12	CD	IFX	Y - MTX	Flare symptoms	< 1 μg/mL	8.2 U/mL	Frequency	8.2 μg/mL	9.0 U/mL	N	-

[1]Refers to concurrent immunomodulator therapy. Pt: Patient; Dx: Diagnosis; TDM: Therapeutic drug monitoring; Ab: Antibody; ADA: Antidrug antibody; CD: Crohn's disease; UC: Ulcerative colitis; ADA: Adalimumab; IFX: Infliximab; AZA: Azathioprine; 6MP: 6-mercaptopurine; MTX: Methotrexate; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein.

described resolution of ADAs with therapy modification in the form of dose escalation and/or addition of an immunomodulator. Based on this strategy, recent algorithms for management of secondary loss of response now advocate that patients with low drug levels and low level ADAs undergo adjustment of therapy using these two strategies.

Our case series illustrates application of these strategies, with drug levels obtained before and after the adjustment in therapy. In 75% of cases, we were able to achieve resolution of anti-drug antibodies, confirmed with therapeutic drug monitoring, with either escalation of anti-TNF therapy or addition of an immunomodulator. These patients were noted to have improvement in their

disease activity, based on subjective and/or objective measures. With regard to the possibility of de-escalation of anti-TNF dosing or discontinuation of the anti-TNF, the authors of this study generally did not pursue this if patients are tolerating the therapy without adverse effects, due to concern for formation of recurrent drug antibodies.

Based on this literature and our own experience as described, we believe that in certain patients, low-level anti-TNF drug antibodies can be overcome by dose escalation and/or addition of an immunomodulator, and can allow for clinical improvement in disease status. As the efficacy of subsequent anti-TNF drugs generally diminishes in comparison to the initial anti-TNF drug[41], drug manipulation to overcome low-level antibodies may be a valuable strategy to preserve the use of anti-TNFs in IBD; therapeutic drug monitoring is an instrumental tool to assess success or failure of this approach.

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REVIEW

Phage therapy: An alternative to antibiotics in the age of multi-drug resistance

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Abstract

The practice of phage therapy, which uses bacterial viruses (phages) to treat bacterial infections, has been around for almost a century. The universal decline in the effectiveness of antibiotics has generated renewed interest in revisiting this practice. Conventionally, phage therapy relies on the use of naturally-occurring phages to infect and lyse bacteria at the site of infection. Biotechnological advances have further expanded the repertoire of potential phage therapeutics to include novel strategies using bioengineered phages and purified phage lytic proteins. Current research on the use of phages and their lytic proteins against multidrug-resistant bacterial infections, suggests phage therapy has the potential to be used as either an alternative or a supplement to antibiotic treatments. Antibacterial therapies, whether phage- or antibiotic-based, each have relative advantages and disadvantages; accordingly, many considerations must be taken into account when designing novel therapeutic approaches for preventing and treating bacterial infection. Although much about phages and human health is still being discovered, the time to take phage therapy serious again seems to be rapidly approaching.

Key words: Bacteriophage; Bacteriophage therapy; Phage; Phage therapy; Endolysin; Lysin; Multidrug resistance; Antibiotic resistance; Phage safety; Methicillin-resistant *S. aureus*

Core tip: Phage therapy is widely being reconsidered as an alternative to antibiotics. The use of naturally-occurring phages to treat bacterial infection has a contentious history in western medicine. However, the emergent landscape of phage-based antimicrobials has advanced well beyond traditional methods. In this rapidly evolving field, novel technologies such as bioengineered chimeras of phage-derived lytic proteins show potential as a new class of antibacterial pharmaceuticals. This review aims to

provide a topical perspective on the historical context of phage therapy, in order to highlight modern advances in phage research and innovations in the field.

Lin DM, Koskella B, Lin HC. Phage therapy: An alternative to antibiotics in the age of multi-drug resistance. *World J Gastrointest Pharmacol Ther* 2017; 8(3): 162-173 Available from: URL: http://www.wjgnet.com/2150-5349/full/v8/i3/162.htm DOI: http://dx.doi.org/10.4292/wjgpt.v8.i3.162

INTRODUCTION

Almost a decade before the discovery of penicillin, the controversial practice of phage therapy was being developed as a treatment for bacterial infections. Phages, short for bacteriophages, are bacteria-specific viruses that have been used as a treatment against pathogens such as *Shigella dysenteriae* as early as 1919[1]. With an estimated 10^{31}-10^{32} phages in the world at any given time[2], they make up the most abundant biological entity on Earth and play a crucial role in regulating bacterial populations; phages are responsible for the death of approximately 20%-40% of all marine surface bacteria every 24 h[3]. Much of the controversy surrounding phage therapy was due to poor documentation of use and variable success. The complications in implementing phage therapy stemmed from how little was known about phages at the time of their discovery. In fact, the nature of their existence was a topic of contention until they were visualized in the 1940's after the invention of electron microscopy[4]. A number of logistical and technical obstacles in developing phage therapy led to its widespread abandonment after the discovery of antibiotics.

The advent of pharmaceutical antibiotics in the mid-20th century, along with a better understanding of disease and sanitation, revolutionized healthcare and drastically improved both quality of life and life expectancy in the industrialized world. In 1900, life expectancy for men and women in the United States was 46 and 48, respectively, and the major causes of death were infectious diseases, many of which were bacterial (*e.g.*, cholera, diphtheria, typhoid fever, plague, tuberculosis, typhus, scarlet fever, pertussis, and syphilis)[5]. Antibiotics helped usher in a new era in medicine, rapidly becoming an indispensable medical tool with 262.5 million treatment courses prescribed in the United States in 2011 alone (842 prescriptions per 1000 persons) and an estimated 100000-200000 tons of antibiotics used globally between medicine, agriculture, and horticulture each year[6,7]. Antibiotic resistance genes encoding for bacterial resistance to common antibiotics, including β-lactams, aminoglycosides, chloramphenicols, and tetracycline, are posing a major threat to current medical treatment of common diseases, and these genes now appear to be abundant in the environment[8]. The spread of antibiotic resistance genes carries a unique danger in that many antibiotics have diminishing efficacy against common infections, particularly the difficult-to-treat nosocomial infections caused by the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.).

Admonitions of a return to "the pre-antibiotic era" have become increasingly common and regulatory organizations such as the Centers for Disease Control (CDC) and WHO have declared antibiotic resistance a threat to global health[9,10]. The CDC estimates antibiotic-resistant infections result in 2 million illnesses and at least 23000 deaths a year, with many more dying from conditions complicated by antibioticresistant infections, costing the United States $55 billion annually[7]. According to the United Kingdom government's 2016 Review on Antimicrobial Resistance, an estimated 700000 people die each year globally from resistant infections with a projected cost of $100 trillion and a death toll of 10 million by 2050[7]. In the United States, methicillin-resistant *S. aureus* (MRSA) infections alone account for more deaths than HIV/AIDS and tuberculosis combined[11]. Since the discovery of antibiotics, there has been a steady stream of novel antibacterial pharmaceuticals in what has been dubbed the "antibiotic pipeline". However, due to the rate at which bacteria evolve resistance to antibiotics, there has been less commercial interest in the research and development of novel compounds. In the years of 1983-1987, there were 16 new pharmaceutical antibiotics approved by the Food and Drug Administration (FDA) for use in the United States, this number has steadily trended downwards and between 2010-2016 only 6 new antibiotics were approved[12]. At the end of the antibiotic pipeline is the carbapenem class of antibiotics, often reserved as the "last resort" due to their adverse effects on health. Beginning in 2000, the incidence of carbapenem-resistant, hospital-acquired *K. pneumoniae* infections began to increase in the United States; due to the lack of treatment options these infections are associated with a 40%-50% mortality rate[13]. Reaching the end of the antibiotic pipeline could signal a shift in the global culture of infectious disease treatment and some claim is the imminent return to a pre-antibiotic era of medicine.

On September 21, 2016, the United Nations General Assembly convened to discuss the problem of antibiotic resistance and deemed it "the greatest and most urgent global risk"[14]. In the hunt for alternative strategies for prophylaxis and control of bacterial infection, one of the more popular suggestions involves revisiting the practice of phage therapy. Proponents of phage therapy tout several major advantages that phages have over antibiotics such as host-specificity, self-amplification, biofilm degradation, and low toxicity to humans[15,16]. Owing to the development of analytical tools capable of studying these small biological entities

(approximately 25-200 nm in length), such as next-generation sequencing and electron microscopy, the field of phage biology is only now reaching maturity. These technological advancements have ushered in a renaissance of phage therapy research as indicated by a wave of recent human clinical trials and animal research. The complex story of the human phageome in regards to health and disease is only beginning to unfold and will not be included in this review (for current literature review see Wahida, Ritter and Horz[17] 2016). This review aims at discussing historical use of phage therapy and current research on the feasibility of phage-based infection control with a focus on multidrug-resistant infections.

PHAGE BIOLOGY BASICS

Phages are simple, yet incredibly diverse, non-living biological entities consisting of DNA or RNA enclosed within a protein capsid. As naturally-occurring bacterial parasites, phages are incapable of reproducing independently (*i.e.*, non-living) and are ultimately dependent on a bacterial host for survival. Phages typically bind to specific receptors on the bacterial cell surface, inject their genetic material into the host cell, and then either integrate this material into the bacterial genome (so-called "temperate" phages) and reproduce vertically from mother to daughter cell, or hijack the bacterial replication machinery to produce the next generation of phage progeny and lyse the cell (so-called "lytic" phages). Upon reaching a critical mass of phage progeny, which can be anywhere from a few to over 1000 viral particles depending on environmental factors, the lytic proteins become active and hydrolyze the peptidoglycan cell wall, releasing novel phage to reinitiate the lytic cycle[18,19].

Most phages are infectious only to the bacteria that carry their complementary receptor, which, in turn, determines lytic phage host range[20]. Host specificity varies among phages, some of which are strain-specific, whereas others have demonstrated the capability of infection across a range of bacterial strains and even genera[21,22]. Bacteria have evolved numerous mechanisms to resist infection by lytic phages, and phages have an equally impressive diversity of mechanisms for breaking this resistance. For bacteria, this can include alteration or loss of receptors and integration of phage DNA into the clustered regularly interspaced palindromic repeats/CRISPR associated system (CRISPR/Cas) system[23], while for phage this can include recognition of new or altered receptors and anti-CRISPR genes[24]. The most common lytic phages associated with human pathogens and the gut microbiota are in the orders *Caudovirales*, commonly known as "tailed phages" which contain double-stranded DNA genomes, and *Microviridae*, which are tailless, single-stranded DNA viruses[25,26].

In contrast to lytic phage, lysogenic phages integrate their genetic material into the bacterial chromosome in the form of an endogenous prophage (less commonly phage DNA can remain separate as a plasmid but still be stably transmitted across bacterial generations). The bacterial lysogen then propagates the prophage with each cell division. Environmental stressors on the bacterial host are capable of inducing the lysogenic phage from the latent prophage form, triggering a transition to the lytic cycle and the release of phage progeny into the environment. When incorporating their genetic material into the bacterial genome, prophage-encoded genes become available for transcription by the host. Up to 18 prophages have been found in one bacterial genome, as in the food pathogen *Escherichia coli* (*E. coli*) O157:H7 strain Sakai[27], with prophage-encoded genes comprising up to 20% of bacterial chromosomal content[28]. These genes can be beneficial to the bacterial host and can encode for virulence factors (*e.g.*, diphtheria toxin, shiga toxin, and botulinum toxin), metabolic genes, and antibiotic resistance genes (*e.g.*, β-lactamases)[29-32]. Phage biologists now recognize that phage lifecycles fall on a spectrum between these two extremes with pseudolysogenic, chronic, and cryptic lifecycles as examples of recent classifications[19,33]. Conventional phage therapy relies on strictly lytic phages, which obligately kill their bacterial host. For treatment, lytic phages are compiled into preparations called "phage cocktails" which consist of multiple phages proven to have *in vitro* efficacy against the target pathogen.

HISTORY OF PHAGE THERAPY

Although the idea of using bacterial viruses therapeutically against bacterial infections has recently gained traction in response to the emergence of multidrug-resistant pathogens, the practice has been around for nearly a century. Since the initial observations of phage-induced bacterial lysis, the biological nature of phage, as well as their therapeutic value, has been controversial. Frederick Twort first described the characteristic zone of lysis associated with phage infection in 1915, but it was Felix d'Herelle who identified the source of this phenomenon, attributed the plaques to bacterial viruses, and coined the term "bacteriophage" (literally "bacteria-eater"). It was also d'Herelle who conceived of the idea to use phages therapeutically and is responsible for the first documented clinical use of phage in 1919 at the Hôpital des Enfants-Malades in Paris where phages were successfully used to treat 4 pediatric cases of bacterial dysentery[1]. Despite several successful trials, d'Herelle's early experiments were notorious for being poorly controlled and his research was vigorously disputed by the scientific community[3]. Nevertheless, d'Herelle continued to pioneer phage therapy with the treatment of dysentery, cholera, and the bubonic plague in the early 20th century with a series of phage therapy centers and commercial phage production plants throughout Europe and India[34]. One 1931 trial of phage therapy as a treatment for cholera in the Punjab region of India involved a cohort of 118 control

subjects and 73 experimental subjects who received phage treatment; d'Herelle observed a 90% reduction in mortality with 74 lethal outcomes in the control group and only 5 in the experimental group[1].

Along with d'Herelle, several other entrepreneurs attempted to commercialize phage production in Brazil and the United States with phage preparations for *Staphylococcus*, *Streptococcus*, *E. coli*, and other bacterial pathogens[34]. These preparations were shipped throughout the world to willing clinicians but treatment was met with mixed success; this lack of reliability, in large part, added to the preference for antibiotics in western medicine[1].

Many mistakes were made during these early trials of phage therapy and most can be attributed to a poor understanding of the biological nature of phages. Rudimentary purification and storage protocols resulted in low titers of active phage, contamination from bacterial antigens, and the inappropriate choice of a phage that lacked specificity to the target pathogen. Furthermore, delivery of phage to the site of infection was confounded by the medical limitations of the day. For example, the role of the patient's innate immune response in removing active phage and diminishing the efficacy of phage therapy was only observed recently as a potentially confounding physiological mechanism[35]. As a result, phage therapy was widely dismissed by most of western medicine after the introduction of pharmaceutical antibiotics in the 1940's. The exception to this is in the former Soviet Union and Eastern Europe where clinical phage therapy has been used extensively to treat antibiotic-resistant infections caused by a range of infectious bacteria such as *Staphylococcus*, *Pseudomonas, Klebsiella*, and *E. coli*[36,37].

PHAGE AGAINST CLINICALLY SIGNIFICANT PATHOGENS

Recent investigations using animal models have explored phage treatment against a range of clinically significant pathogens. When challenged with gut-derived sepsis due to *P. aeruginosa*, oral administration of phage saved 66.7% of mice from mortality compared to 0% in the control group[38]. In a hamster model of *Clostridium difficile* (*C. difficile*)-induced ileocecitis, a single dose of phage concurrent with *C. difficile* administration was sufficient prophylaxis against infection; phage treatments post-infection saved 11 of 12 mice whereas control animals receiving *C. difficile* and clindamycin died within 96 h[39]. Phage combinations also significantly reduced *C. difficile* growth *in vitro* and limited proliferation *in vivo* using a hamster model[40]. Intraperitoneal administration of a single phage strain was sufficient to rescue 100% of mice in bacteremia models using vancomycin-resistant *E. faecium*[41], extended spectrum β-lactamase producing *E. coli*[42], and imipenem-resistant *P. aeruginosa*[43]. Phage cocktails have also been used to treat antibiotic-resistant *P. aeruginosa* infections of the skin, lungs, and gastrointestinal tract in animal models[38,44]. Additional animal studies show similarly promising results for multidrug-resistant *E. coli* O25:H4-ST131[45], *Vibrio parahaemolyticus*[46], *S. aureus*[44,47], and *A. baumanii*[38]. There is even an indication that phage are capable of restoring antibiotic sensitivity in antibiotic-resistant bacteria, as in the case of multidrug-resistant *P. aeruginosa*[48].

Human trials for phage therapy have taken place for almost a century at several institutes in Eastern Europe, the most famous of which are the Eliava Institute of Bacteriophage and the Institute of Immunology and Experimental Therapy in Wroclaw, Poland. The Eliava Institute has extensively used phage in preclinical and clinical treatment of common bacterial pathogens such as *S. aureus*, *E. coli*, *Streptococcus* spp., *P. aeruginosa*, *Proteus* spp., *S. dysenteriae*, *Salmonella* spp., and *Enterococcus* spp.[49]. Effective applications range from surgical to gastroenterological, both therapeutic and prophylactic. In a six patient case series of antibiotic-unresponsive diabetic foot ulcers, topical application of *S. aureus*-specific phage was sufficient for recovery in all individuals[50]. In a 1938 clinical trial, 219 patients with bacterial dysentery (138 children and 81 adults) were treated solely with a phage cocktail consisting of a variety of phage targeting *Shigella flexneri*, *Shigella shiga*, *E. coli*, *Proteus* spp., *P. aeruginosa*, *Salmonella typhi*, *Salmonella paratyphi* A and B, *Staphylococcus* spp., *Streptococcus* spp. and *Enterococcus* spp.; cocktails were administered both orally and rectally. Within 24 h, 28% of patients with blood in their stools were relieved of this symptom, with a further 27% showing improvement within 2-3 d. Overall, 74% of the 219 patients showed improvement or were completely relieved of symptoms[51]. Additionally, during a 1974 typhoid epidemic, a cohort of 18577 children was enrolled in a prophylactic intervention trial using typhoid phages. Phage administration resulted in a 5-fold decrease in typhoid incidence compared to placebo[49]. The potential for phage therapy has yet to be fully realized since phages tend to be more effective against the target pathogen when used in combination with antibiotics[52], a treatment option that has not yet been investigated in humans.

Currently there are no phage therapy products approved for human use in the EU or United States. However, in the food industry, there are several commercial phage preparations used for biocontrol of bacterial pathogens that are approved by the FDA under the classification of "generally considered as safe." These preparations are used against *Salmonella* spp., *Listeria monocytogenes*, MRSA, *E. coli* O157:H7, *Mycobacterium tuberculosis*, *Campylobacter* spp., and *Pseudomonas syringae*, among others[53-56]. Phages also have potential value for pathogen detection, an example of which is using bioluminescent reporter phage to detect *Bacillus anthracis*[56]. In 2011 there was an estimated 48 million cases of food poisoning in the United States alone[55].

Table 1 Published findings on phage therapy in humans and in animal models

Causative agent	Model	Condition	Oral	Result summary[1]	Ref.
Shigella dysenteriae	Human	Dysentery	Oral	All four treated individuals recovered after 24 h	[1]
Vibrio cholerae	Human	Cholera	Oral	68 of 73 survived in treatment group and only 44 of 118 in control group	[1]
Pseudomonas aeruginosa	Murine	Sepsis	Oral	66.7% reduced mortality	[38]
Clostridium difficile	Hamster	Ileocecitis	Oral	Co-administration with *C. difficile* prevented infection	[39]
	Hamster	Ileocecitis	Oral dose every 8 h for 72 h	92% reduced mortality	[39]
Vancomycin-resistant Enterococcus faecium	Murine	Bacteremia	i.p.	100% reduced mortality	[41]
β-lactamase producing Escherichia coli	Murine	Bacteremia	i.p.	100% reduced mortality	[42]
Imipenem-resistant P. aeruginosa	Murine	Bacteremia	i.p.	100% reduced mortality	[43]
Acinetobacter baumannii, P. aeruginosa and *Staphylococcus aureus*	Murine	Sepsis	i.p.	Animals protected against fatal dose of *A. baumannii* and *P. aeruginosa* but not *S. aureus*	[44]
Escherichia coli	Murine	Meningitis and Sepsis	i.p. or s.c.	100% and 50% reduced mortality for meningitis and sepsis, respectively	[45]
MDR Vibrio parahaemolyticus	Murine	Sepsis	i.p. and oral	92% and 84% reduced mortality for *i.p.* and oral routes, respectively	[46]
S. aureus	Rabbit	Wound infection	s.c.	Co-administration with *S. aureus* prevented infection	[47]
MDR S. aureus	Human	Diabetic foot ulcer	Topical	All 6 treated patients recovered	[50]
Unclassified bacterial dysentery	Human	Dysentery	Oral	Phage cocktail improved symptoms of 74% of 219 patients	[51]
Salmonella typhi	Human	Typhoid	Oral	In cohort of 18577 children, phage treatment associated with 5-fold decrease in typhoid incidence compared to placebo	[49]
Antibiotic-resistant *P. aeruginosa*	Human	Chronic Otitis	Oral	Phage treatment safe and symptoms improved in double-blind, placebo-controlled Phase I/II trial	[61]

[1]Reduced mortality is for phage-treated groups and are relative to 100% mortality in control animals, unless otherwise specified. MDR: Multi-drug-resistant; i.p.: Intraperitoneal injection; s.c.: Subcutaneous injection.

Evidence suggests that phage biocontrol can be an effective method for improving food safety at numerous stages in meat production and processing, and also has potential to reduce bacterial contamination in fruits, vegetables, and dairy products[55]. These investigations into phage biocontrol in food production, as well as recent placebo-controlled human trials that demonstrated the safety of oral phage administration[57-60], are gradually beginning to fill the knowledge gap in phage therapy safety. The evidence on phage safety will continue to strengthen with further randomized, double-blind, and placebo-controlled phase Ⅰ/Ⅱ clinical trials of phage therapy, such as the one that established both safety and efficacy in treating chronic otitis caused by antibiotic-resistant *P. aeruginosa*[61].

Innovations in the programmable gene editing tool CRISPR/Cas have created novel opportunities for phage therapy. One example of which is the use of bioengineered phage to deliver a CRISPR/Cas programmed to disrupt antibiotic resistance genes and destroy antibiotic resistance plasmids[62]. These phages may be applied to hospital surfaces to reduce frequency and spread of antibiotic resistance genes. The field of bioengineered phages is still in its infancy but will undoubtedly yield many invaluable technologies such as this (Table 1).

DEVELOPMENT AND APPLICATION OF PHAGE-DERIVED LYTIC PROTEINS

Among the most promising of advances in phage therapy is the isolation of phage-encoded lytic enzymes, which are functionally similar to the eukaryotic enzyme lysozyme. Genes for these enzymes are expressed by the bacterial host during the lytic cycle and assist the phage by hydrolyzing the cell wall to release viral progeny. The discovery and analysis of these proteins opens the possibility for the development of novel phage-based pharmaceuticals.

Two major protein classes are employed by the majority of phage species during the lysis of the bacterial host. One of which is the transmembrane protein holin and the other is a peptidoglycan cell wall hydrolase called endolysin (lysin). These two proteins work together in triggering the lysis of the bacterial cell. The holin protein acts as a molecular "clock" in the lytic cycle. During the process of viral assembly within the cytoplasm, holin molecules accrue in the cell membrane. At the end of the lytic cycle the holin proteins trigger an opening on the cytoplasmic side of the cell membrane, allowing the lysin proteins to access and hydrolyze the cell wall[63]. Although both of these enzymes are present

across the majority of phage species, there is huge structural and biochemical variability and therefore little sequence conservation among species. Each phage can encode for several unique lysin and holin enzymes, some of which are highly specific but others can exhibit broad-spectrum activity between strains and even between species as in the case of recently discovered lysin ABgp46. ABgp46 has the ability to lyse several gram-negative and multidrug-resistant pathogens, including *A. baumannii*, *P. aeruginosa*, and *Salmonella typhimurium*[64].

Phage lysins alone are capable of bacterial cell lysis, whereas holins are not; therefore lysins have received a lot of attention as potential antimicrobial agents. These proteins are fast acting, potent, and inactive against eukaryotic cells. Lysins have successfully saved mice from bacteremia caused by multidrug-resistant *A. baumannii*[65], *Streptococcus pneumoniae*[66], and MRSA[67], among others[63]. A combination of phage lysins and antibiotics has been shown to be much more effective than antibiotics alone in eliminating *C. difficile* colonization in both an *in vitro* and an *ex vivo* colon model in the presence of intestinal contents[68]. Not all lysins show equal therapeutic potential, however, as demonstrated by Gilmer *et al*[69] who identified a uniquely potent lysin, PlySs2, which was highly effective against a range of pathogenic *Streptococcus* and *Staphylococcus* species, including MRSA, and was fully functional after 10 freeze-thaws. A single dose administered intraperitoneally to mice in a mixed *S. pyogenes* and MRSA bacteremia model provided a significantly higher survival rate than treatment with 3 previously characterized lysins[69]. A recent study exploring the isolation and application of phage proteins has revealed that lysins are even capable of crossing epithelial cell membranes to eliminate difficult to treat intracellular infections of *S. pyogenes*[70]. In addition, phage lysins can disrupt vegetative cells such as in the case of *B. anthracis* lysin PlyG which is capable of attacking endospores of bacillus, a distinct advantage over antibiotics[71]. Lysins can also be mass produced through common recombinant techniques. The gene for bacteriophage-derived cysteine, histidine-dependent amido hydrolase/peptidase (CHAPK) has been cloned and inserted into *E. coli* to be overexpressed for purification. Not only is the CHAPK lysin highly effective against MRSA, but it can disperse *S. aureus* biofilms[72].

Efforts to optimize lysins through bioengineering have yielded some promising results. Yang *et al*[73] produced a novel chimeric lysin, by combining the active site of a lysin with a cell wall binding domain, that was capable of saving mice challenged with MRSA bacteremia. Research on chimeric lysin enzymes is still in the early stages, but some of these modified lysins have also been shown to prevent death from *S. pneumoniae* bacteremia[74] and prevent development of methicillin-sensitive *S. aureus* endophthalmitis in a mouse model[75]. Since lysins act by enzymatically cleaving the bacterial cell wall, they are inherently less effective against gram-negative bacteria which have an impermeable lipopolysaccharide outer membrane. In an attempt to broaden lysin activity to target gram-negative pathogens, several researchers have begun to bioengineer artificial lysin molecules, termed Artilysins, that are capable of penetrating the outer membrane. Some of these lysins are created by combining the active site of the lysin enzyme with lipopolysaccharide-destabilizing peptides which allows the molecule to penetrate the outer membrane. So far Artilysins have been shown to decolonize *P. aeruginosa* in a nematode gut model and protect human keratinocytes when challenged with *A. baumannii*[76].

Adding to the appeal of lysins as antibacterial agents, it is widely considered unlikely that bacteria will evolve resistance to lysins due to the fact that they target sites on the peptidoglycan cell wall critical for bacterial viability[63]. Engineered recombinant phage lytic proteins would be far easier to mass produce and administer than preparations of actual phage, which can be limited by a short shelf life, removal by the reticuloendothelial system of the host, and the potential for generating neutralizing antibodies[35]. Future potential for phage lysin application includes combination therapy of lysins in conjunction with antibiotics, which has been shown to be more effective than antibiotics or lysins alone against pathogens such as MRSA and *C. difficile* in a murine model[77-79] (Table 2).

PHAGE THERAPY *VS* ANTIBIOTIC THERAPY

Both antibiotics and phages function as antibacterials that disrupt bacterial colonies through lysis or inhibition, yet several key differences make each antibacterial more or less appropriate depending on the situation.

Safety

Adverse reactions to antibiotics are well documented and include instances of anaphylaxis, nephrotoxicity, cardiotoxicity, hepatotoxicity, and neurotoxicity, as well as a number of gastrointestinal and hematological complications[80]. The majority of adverse reactions are allergic reactions and in these rare instances, the anaphylaxis is associated with specific classes of antibiotics or is the product of high tissue concentrations[81-83]. In contrast to the comprehensive literature on antibiotic safety, phage therapy has only recently gained attention by western medicine and, as a result, much of the available information on phage safety is new. Although oral phage administration is generally considered to be safe[57-60], a major consideration for phage therapy is the translocation of phage across the intestinal epithelium where they subsequently circulate within the blood[84]. Some data show that phage translocation may benefit the host by downregulating the immune response to indigenous gut microbe antigens through the inhibition of interleukin-2, tumor necrosis

Table 2 Recently published findings on phage lytic enzymes

	Lytic enzyme	Model	Target pathogens	Result summary	Ref.
Phage-derived lysins	ABgp46	*In vitro*	MDR *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*	Cross-inoculation significantly reduced bacterial density	[64]
	PlyF307	Murine	MDR *A. baumannii*	*i.p.* treatment rescued mice from lethal bacteremia	[65]
	Cpl-1	Murine	*Streptococcus pneumoniae*	*i.p.* treatment rescued mice from lethal pneumonia	[66]
	Cocktail of 6 distinct lysins	*In vitro* and murine *in vivo*	MRSA	Effective against biofilms *in vitro* and protected mice from lethal sepsis	[67]
	PlyCD	*In vitro* and *ex vivo*	*Clostridium difficile*	Reduced *C. difficile* colonization	[68]
	PlySs2	Murine	*Streptococcus pyogenes* and MRSA	i.p. treatment reduced mortality from lethal bacteremia	[69]
	PlyG	*In vitro*	*Bacillus anthracis*	Eliminated *B. anthracis* spores and vegetative cells	[71]
Bioengineered chimeric lysins	CHAPK	*In vitro*	MRSA	Eliminated MRSA and dispersed biofilms	[72]
	ClyH	Murine	MRSA	Treatment rescued mice from bacteremia	[73]
	Cpl-711	Murine	*S. pneumoniae*	Treatment rescued mice from bacteremia	[74]
	Ply187	Murine	*Staphylococcus aureus*	Prevented bacterial endophthalmitis	[75]
	Artilysins	Nematode gut	*P. aeruginosa*	Decolonized *P. aeruginosa* from gut	[76]
		Human keratinocytes	*A. baumannii*	Protected cells from bacterial challenge	[76]
Lysin and antibiotic combination therapy	CF-301	Murine	MRSA	Lysin treatment was most effective when combined with vancomycin or daptomycin	[77]
	MR-10	Murine	Burn wound infection	Lysin treatment was most effective when combined with minocycline	[78]

MDR: Multi-drug-resistant; *i.p.*: Intraperitoneal injection; MRSA: Methicillin-resistant *S. aureus*.

factor, and interferon gamma production[84]. Other studies discovered a host innate immune response aimed at removing phage after administration in mice[35,85]. While potentially beneficial in a healthy individual, the immunological response to phage may be indicative of a potential adverse immunogenicity of phage in immunocompromised patients, which could hypothetically worsen a patient's condition. On the contrary, other researchers argue it is unlikely phage therapy will elicit such an adverse response in immunocompromised patients[86].

Additional complications include the possibility that phage cocktails induce a state of intestinal barrier dysfunction, otherwise known as "leaky gut". Tetz and Tetz used a mouse model to demonstrate that oral administration of a commercial Russian phage cocktail was capable of increasing intestinal permeability and elevating serum levels of inflammatory circulating immune complexes in the blood, which are associated with a number of pathological conditions[87]. However, another study observed no significant increase in cytokine levels in response to phage treatment[88]. The potential for phage therapy to disrupt normal intestinal barrier function would have serious implications for several disorders recently linked to intestinal barrier dysfunction such as Crohn's disease, inflammatory bowel disease, and type 1 diabetes[87]. Pincus *et al*[89] found that inflammatory response to phage was dependent on site of infection. Clearly, many considerations for the safety of phage therapy still need to be addressed. It is likely that the physiological response to phages also differs between individuals and is dependent on the specific phage strains used. To determine the safety of phage treatments in regards to human health, future investigations will need to focus on human clinical trials as much of the current research on the immunological response to phage is limited to animal models.

Specificity

Relative to antibiotics, phages tend to be specific towards both species and strain. In certain situations this can be a major advantage considering the well-documented, collateral effects of broad-spectrum antibiotics on commensal gut microbes, which are notorious for secondary outcomes such as antibiotic-associated diarrhea and *C. difficile* infection[90]. Other consequences of antibiotic perturbations in the gut microbial community include risk of asthma, obesity, and diabetes[91-93]. The current understanding of collateral damage due to phage therapy is limited, but compared to antibiotics, phage therapy has been reported to result in less perturbation of the gut microbiome while still effectively reducing gut carriage of pathogens such as *Shigella sonnei* and uropathogenic *E. coli*[94,95].

Strain and species specificity of antibacterial action has many advantages, however, increased specificity also comes with several limitations. By targeting a single pathogen, phage therapy could be less effective against certain infections, such as infected burn wounds, which are often colonized by more than one strain of bacteria[96]. This can be accounted for by creating phage cocktails infective against a range of known pathogens, but the success of this approach depends on knowledge of which pathogens are being treated. With regard to logistical considerations, this

specificity significantly impacts treatment development and testing, and also limits the possibility of large-scale production and distribution, a distinct advantage of broad-spectrum antibiotics. Bourdin *et al*[15] cross-inoculated phages from 2 distinct geographic regions (Mexico and Bangladesh) against diarrhea-associated *E. coli* from the same regions and found that phage showed high strain specificity to the *E. coli* of their indigenous region. In a randomized clinical trial, Sarker *et al*[60] administered a commonly used Russian *E. coli* phage cocktail to a cohort of 120 Bangladeshi children with microbiologically-proven enterotoxogenic *E. coli* diarrhea. No improvement of clinical outcome was observed in patients receiving the phage cocktail compared to placebo[60]. These findings are in line with the *in vitro* work that suggests phage cocktails are better adapted to local bacterial populations[15], and bacterial host range can be restricted both spatially and temporally[97]. In contrast, in an *in vitro* cross-inoculation of a phage cocktail against shiga toxin-producing *E. coli* O157:H7, lysis occurred in isolates of both human and bovine origin, suggesting the possibility of regional phage cocktails for both clinical and agricultural settings[98]. Latz *et al*[99] found that phages targeting antibiotic-resistant bacteria are more likely to be found within the environment of the infected patient, which, in this case, was the hospital effluent where the antibiotic-resistant bacteria were isolated.

Regional specificity may be helpful in finding phages with the greatest infectivity towards the target pathogen, this would especially benefit regions with limited access to antibiotics. Together, the mounting evidence for the local adaptivity of phage suggests that regulatory pipelines must also be rapidly adaptable (*i.e*., allowing for the replacement or addition of phages into cocktails without requiring further clinical trials) for phage therapy to work on a global scale.

Biofilm penetration

Antibiotic therapy is highly effective with planktonic bacteria, such as *V. cholerae* and *Yersinia pestis*, yet is limited in treating biofilm-based bacterial infection[100]. Phages, however, are equipped with enzymes (*e.g*., EPS depolymerase) on the exterior of the capsid that degrade the extracellular polymeric substances (EPS) and disperse bacterial biofilms, allowing the phage to access bacteria embedded within the EPS matrix[83]. The phage progeny released upon completion of the lytic cycle propagate the dispersal of the biofilm through the removal of biofilm-embedded bacteria in subsequent layers[83,101]. In order to penetrate dense biofilms, high doses of antibiotics are typically required to observe any inhibition of bacterial growth, yet complete eradication is rare and regrowth of colonies begins after the end of antibiotic treatments[102,103]. Although low concentrations of many antibiotics are generally considered non-toxic, high concentrations of antibiotics can result in tissue toxicity[83]. Gabisoniya *et al*[104] at the Eliava Institute of Bacteriophages in Tbilisi, Georgia found that the application of phages on *in vitro* colonies of the pathogen *P. aeruginosa* not only prevented additional biofilm formation by the pathogen but also degraded existing biofilm. Phage treatments have eliminated biofilms formed by *L. monocytogenes, P. aeruginosa,* and *Staphylococcus epidermidis* on the surface of medical devices[22]. These findings are highly relevant to the problem of persistent infections caused by implanted medical devices such as catheters, lenses, and prostheses where biofilm formation is common.

Phage cocktails

Due to the massive diversity of environmental phages, designing a phage cocktail is substantially more complicated than designing a regimen for combination antibiotic therapy. Composition of the phage cocktail is critical for the success of phage therapy. Factors in the construction of a phage cocktail are beyond the scope of this review and have been thoroughly discussed elsewhere[105], but one of the major logistical challenges is whether to approach phage therapy with a standardized or a customized cocktail. Customizing phage cocktails to each infection is time consuming and costly but on the other end of the spectrum, a "one-size-fits-all" approach may not provide the strain specificity required for favorable clinical outcomes[105]. Other considerations are the collateral effects of phages on the indigenous microbiota, a topic that has not yet been fully explored[88,94,95]. In cocktail design, one must also take into account phage lifecycle. Lysogenic phages appear to be very common in the indigenous gut microbiota, with prophages comprising the majority of the gut virome[25]. Some therapeutically promising lysogenic phages effectively silence virulence genes in pathogenic bacteria or provide genes for short chain fatty acid metabolism, whereas other lysogenic phages supplement genes for virulence and antibiotic resistance[29,30,106].

Antibiotic resistance genes have been collected from the phage fraction of DNA in wastewater and have been reported to persist longer in phage when compared to bacteria[107]. Antibiotic resistance genes are also present in the phage fraction of human fecal samples and antibiotic treatment in mice enriches the abundance of phage-encoded antibiotic resistance genes, indicating a possible role for phages as a reservoir for antibiotic resistance genes[30-32]. The hypothetical potential for lysogenic phages to complicate existing infections through the horizontal transfer of antibiotic resistance genes to infectious bacteria largely excludes them from consideration for most phage cocktails. Yet, Regeimbal *et al*[106] demonstrated the possibility for an innovative application of lysogenic phages by designing an "intelligent" 5 phage cocktail that eliminated *A. baumannii* skin wound infection in a mouse model. This intelligent phage cocktail was composed of 4 phages that were incapable of lysing the *A. baumannii* host and 1 phage that only inhibited growth *in vitro*. The growth-inhibiting phage targeted capsulated *A. baumannii*, selecting for

the loss of the capsule. The removal of the capsule, a known virulence factor, decreased the virulence of the bacterium and made it susceptible to lysis from the 4 additional phages[106]. This "intelligent" cocktail represents the beginning of novel treatment options for eliminating bacterial infections that are resistant to conventional treatment. Lysogenic phages have many intriguing properties that may be useful for this type of *in situ* manipulation of individual bacterium, and potentially the human gut microbiome metagenome[108], but first much more needs to be known about the role of lysogenic phages in the human gut phageome for this to be done safely and effectively.

CONCLUSION

The available literature on the use of phages and phage-derived proteins for combating bacterial infections, specifically those of multidrug-resistant bacteria, increasingly shows promise for the prospect of phage therapy as either an alternative or a supplement to antibiotics. However, recent findings on the immunomodulatory effects of phages make it abundantly clear that we need a better understanding of the interaction between phage, microbiome, and human host before implementing phage therapy on a large scale. Phage lysins may thus be a much more practical therapeutic tool for their decreased immunological potential, among other reasons such as ease of production, purification, and storage. In spite of the promise offered by phage and phage-derived lytic proteins, it is more than likely that no panacea for antibiotic-resistant infections will arise. The increased efficacy of antibacterial agents when used in conjunction implies that therapy using some combination of phage, phage-derived lytic proteins, bioengineered phage, and/or antibiotics will be necessary for addressing the growing problem of antibiotic-resistant infections.

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MINIREVIEWS

Critically ill patients and gut motility: Are we addressing it?

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Abstract

Gastrointestinal (GI) dysmotility is a common problem in the critically ill population. It can be a reflection and an early sign of patient deterioration or it can be an independent cause of morbidity and mortality. GI dysmotility can be divided for clinical purposes on upper GI dysmotility and lower GI dysmotility. Upper GI dysmotility manifests by nausea, feeding intolerance and vomiting; its implications include aspiration into the airway of abdominal contents and underfeeding. Several strategies to prevent and treat this condition can be tried and they include prokinetics and post-pyloric feeds. It is important to note that upper GI dysmotility should be treated only when there are clinical signs of intolerance (nausea, vomiting) and not based on measurement of gastric residual volumes. Lower GI dysmotility manifests throughout the spectrum of ileus and diarrhea. Ileus can present in the small bowel and the large bowel as well. In both scenarios the initial treatment is correction of electrolyte abnormalities, avoiding drugs that can decrease motility and patient mobilization. When this fails, in the case of small bowel ileus, lactulose and polyethylene glycol solutions can be useful. In the case of colonic pseudo obstruction, neostigmine, endoscopic decompression and cecostomy can be tried when the situation reaches the risk of rupture. Diarrhea is also a common manifestation of GI dysmotility and the most important step is to differentiate between infectious sources and non-infectious sources.

Key words: Gut motility; Gut dysmotility; Intensive care unit; Gastrointestinal issues in intensive care unit; Ileus

Core tip: This manuscript presents the case for a cautious look at the gastrointestinal (GI) system during critical illness. GI dysfunction can be an early sign of decompensation, but unfortunately is often overlooked due to the natural tendency to gravitate towards the cardiovascular, respiratory and renal systems when looking for decompensation signs. It is our intention to bring attention to this system and help the clinician in using the GI tract as an early marker for decompensation and also to identify and treat potential GI complications common in

this population.

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INTRODUCTION

The gastrointestinal tract is a vast organ system with many key functions during normal state and physiology. Its functions include digestion and absorption of nutrients, immunomodulation, excretion of fluids, electrolyte balance and hormonal control[1]. These functions are integral for maintenance of homeostasis in health, adaptation in sickness and also as a source of disease.

Acute gastrointestinal injury (AGI) can occur as the result of the gastrointestinal tract been a bystander during periods of critical illness with possible grim consequences. The mechanisms responsible of this injury are diverse and include cytokines and ischemia-reperfusion injury. Observational studies have linked AGI with increased mortality and longer ICU-LOS[2].

AGI common manifestations include: Delayed gastric emptying, ileus, malabsorption, diarrhea, GI hemorrhage and GI bleed[3]. Due to this GI dysmotility in the ICU should be addressed seriously and systematically since it could be the manifestation of GI tract failure as well as manifestation of disease.

For the purpose of this review we would like to divide the problem in upper GI dysmotility and lower GI dysmotility.

UPPER GI DYSMOTILITY

Upper GI dysmotility is usually manifested as delayed gastric emptying, regurgitation and ultimately aspiration. These are signs and symptoms that should never be disregarded since they point out at AGI; the difficult questions would be how aggressive should we be monitoring and treating delayed gastric emptying? What is the optimal method of monitoring? What is the optimal treatment?

GASTRIC EMPTYING

Delayed gastric emptying is a common occurrence in the critically ill[4], multiple factors are associated to decreased gastric emptying (Table 1) and once develops there has been concern that this may be linked to aspiration pneumonia and worse outcomes[5].

The challenge for the clinician is to find a way to monitor and prevent significant dysmotility leading to reflux and aspiration.

MONITORING GASTRIC EMPTYING

Multiple direct and indirect methods of measuring gastric emptying have been studied (Table 2). Scintigraphy is the gold standard but is not practical or readily available in the ICU setting. Unfortunately all of the other indirect methods have limitations and the availability is limited and we are left with an imperfect surrogate of gastric emptying measurement: The gastric residual volume (GRV)[6], and also with a promising alternative: The ^{13}C-octanoate breath test.

Gastric residual volumes

The gastric residual volume has been used as an indirect surrogate of gastric emptying. Several limitations of using the GRV have been described. A normal patient's endogenous secretions can confuse this measurement since a patient can produce up to 4500 mL a day of saliva, gastric secretions and duodenal reflux[7].

Other limitations are technical and they include[8]: (1) a lack of standardization on the quantity of a normal GRV, 15 mL to 500 mL has been described as an upper limit; (2) location of the tip of the tube; (3) different volumes depending on the bore of the catheter; and (4) inconsistent frequency of measurements.

Several small studies have looked into the correlation of different volumes of GRV (150-250 mL), and it has been shown to be a sensitive marker for delayed gastric emptying when compared to scintigraphy and acetaminophen absorption test, but, the negative predictive value was low, thus a lot of the patients with a negative test still had abnormal gastric emptying. More importantly having an abnormal GRV did not correlate to any significant clinical outcome[9,10].

The clinical impact from checking GRV is underfeeding and early enteral nutrition has been shown to improve outcomes of critically ill patients, on the other hand checking GRV has not been shown to decrease vomiting or aspiration. In a 205 patients study, subjects were divided in two groups, one group had feedings held if a GRV were > 250 mL, the second group did not have GRV checked. Patients in the non GRV group achieved higher delivery of EN plus vomiting episodes and clinical aspiration events were not statistically different than the patient's in the GRV group[11].

Based on this data we do not recommend monitoring of GRV in the critically ill patient, but this does not mean that we should not address gastric intolerance manifested as nausea and/or vomiting.

^{13}C-octanoate breath test

The octonoate breath test has been developed as a non-invasive technique that is less cumbersome than scintigraphy since does not require patient transportation outside of the intensive care unit. It has been studied against scintigraphy in the critically ill population undergoing mechanical ventilation. In this test, carbon-13 (a non-radioactive isotope) is added to a test

Table 1 Factors associated with decreased gastric emptying

Factors associated with decreased gastric emptying
Hyperglycemia
Opiates
Elevated intracranial pressure
Electrolyte abnormalities
Ischemia
Hypoxia
Sepsis
Burns
Abdominal surgery
Hyperosmolar formulas

Adapted from Hurt RT, McClave SA. Gastric Residual Volumes in Critical Illness: What do They Really Mean? *Crit Care Clin* 2010: **26**: 481-490.

meal of 100 mL of octanoic acid. ^{13}C-Octanoic acid is not absorbed in the stomach but is rapidly absorbed by the duodenum and then metabolized in the liver to produce $^{13}CO_2$. Once the test meal is given, the $^{13}CO_2$ enrichment of the exhaled air is measured with an isotope ratio mass spectrometer at standard times for 3 to 6 h; due to the properties of the isotope this measurement is reflective of gastric emptying. The biggest study to date showed that this test had an 89% sensitivity and a 67% specificity in identifying delayed gastric emptying when compared to scintigraphy, giving it a 92% PPV and a 57% NPV. Also the authors also concluded that the wide confidence interval (45%-88%) made it a good option to test gastric emptying in the research setting but not in a real life clinical setting[12]. Other limitations include the high cost and size of spectrometer units[13].

Prevention and treatment of gastric dysmotility

Interventions to prevent and treat gastric dysmotility include: The use of continuous feeding *vs* intermittent bolus feeding, post-pyloric feeding and prokinetics.

Continuous infusions of enteral feeds have the theoretical advantage of decreasing the amount of regurgitation and aspiration compared to intermittent boluses, unfortunately the evidence is scant. Small trials[14,15] suggest a decreased incidence of elevated gastric residuals and due to this more success in meeting caloric needs with the continuous methods but there is no difference in hard clinical outcomes. The current recommendations of the American Society of Parenteral and Enteral Nutrition (ASPEN) are to choose continuous feedings on those patients that are intolerant to bolus feeding and those that are high risk for aspiration[16].

Another possible solution would be to place the enteral feeding tube past the pylorus to prevent re-gurgitation and aspiration of gastric contents. A recent meta-analysis showed that there was a decrease in the incidence of pneumonias, but there was no significant difference in nutritional outcomes, length of stay or hospital mortality[17]. But, placing a post-pyloric tube can be technically difficult and delay initiation of enteral nutrition, due to that the ASPEN guidelines suggest to use the gastric route routinely and favor the post-pyloric route to patients at high risk of aspiration or those that showed intolerance.

Table 2 Methods of measuring gastric emptying

Methods of measuring gastric emptying
Scintigraphy
Paracetamol absorption
Carbohydrate absorption
Isotope breath test
Ultrasound and MRI
Gastric residual volumes

The use of prokinetics has been associated with decreased GRV but no significant change in length of stay or mortality[18]. The most commonly studied agents include erythromycin at a dose of 3-7 mg/kg per day and metoclopramide at a dose of 10 mg every 4 h. If one chooses to use these agents, we must be aware of the side effects that include QT prolongation and diarrhea with both agents and tardive dyskinesia in the case of metoclopramide.

LOWER GI DYSMOTILITY

Lower GI dysmotility can be manifested in the ICU as ileus, acute colonic pseudo obstruction and diarrhea.

Evaluation of lower GI dysmotility

Unfortunately none of the usual tests used in the outpatient setting to evaluate motility disorders has been validated or found useful in the intensive care unit setting. The clinician is left with his clinical exam acumen and the usual routine tests performed the critically ill, this is why is important to suspect these disorders and look for them on our daily exam. We will describe the most common clinical presentations.

Ileus

Ileus is defined as the absence of physiologic motility in the bowel, leading to a lack of progression of bowel contents through the gastrointestinal tract. A more specific definition has been described and this includes: Absence of a bowel movement for $\geqslant$ 3 d, treatment for constipation, and one of the following: (1) radiologic confirmed ileus; (2) feed intolerance; (3) abdominal distention; or (4) need for gastric decompression. This has to be differentiated from acute mechanical obstruction that may be a surgical emergency. It has been reported to occur in 20%-50% of the ICU population[18]. The average duration of the episode is 6.5 d and is associated with longer ICU stays as well as underfeeding[19].

Risk factors

The critically ill patient population is specially primed to develop ileus. Inflammation, narcotic use, vasopressor use and electrolyte imbalances makes them susceptible

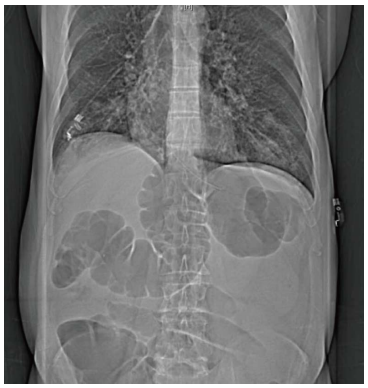

Figure 1 Abdominal plain film showing small bowel ileus and colonic distension.

to a disequilibrium between sympathetic and parasympathetic forces. Common clinical entities that predispose to ileus include: Abdominal surgery, sepsis, pancreatitis, peritonitis, narcotic use, anticholinergic use, hypokalemia, hypomagnesemia, hyperglycemia, acidosis, hypoxia, hypothermia, renal failure and mechanical ventilation[20].

Clinical manifestations

Ileus is usually manifested as inability to tolerate feeds, nausea, vomiting, abdominal distension, constipation and obstipation. The imaging studies show the presence of gas distension of bowel loops and air fluid levels within them (Figure 1). When severe enough it can develop into abdominal compartment syndrome, which is a life threatening emergency.

TREATMENT

The basic management of ileus includes the correction of electrolyte abnormalities, avoidance of opioid agonists, avoidance of anticholinergic drugs, mobilization and early enteral feedings when possible.

Other therapies may include the use of gastric decompression, osmotive laxatives, opioid antagonists and promotility agents.

A double blinded study comparing the use of placebo *vs* polyethylene glycol *vs* lactulose in ICU patients with 3 or more days without a bowel movement showed that, both lactulose and polyethylene glycol are better in promoting defecation than placebo. Patients receiving polyethylene glycol had a lower incidence of acute intestinal pseudo obstruction. Early defecation was associated to a decreased LOS. Based on these findings is reasonable to start osmotive laxatives in this patient population[21].

The use of promotility agents in ileus seems more controversial. Erythromycin has been tried due to the theoretical effect on the motilin receptor. Despite this theoretical mechanism the trials have consistently failed to show any positive effect and its use comes with risk of a prolonged QT and arrhythmias. So we recommend against its use[22]. Metoclopramide has also been tried but results have been conflicting and no clear role exists for its use.

Acute colonic pseudo obstruction (Ogilvie's syndrome)

Acute colonic pseudo obstruction is a potentially fatal condition defined as an acute dilatation of the colon without a mechanical obstruction. Clinically is characterized by abdominal distension, commonly constipation, but flatus or stools may pass as well, an abdominal exam that may be benign but also it can present with exquisite abdominal tenderness, especially at the level of the cecum. The most feared complication would be perforation that usually happens in the cecum[23].

The pathophysiology is thought to be an imbalance between the parasympathetic/sympathetic signals. Clinical factors predisposing to this condition are multiple and include medications, surgery, critical illness, neurologic factors and metabolic factors (Table 3).

Differential diagnosis

The most important alternative diagnosis to rule out is toxic megacolon and mechanical obstruction. Mechanical obstruction can be easily ruled out by the presence of gas on all colonic segments on an abdominal plain film. If there is doubt a CT of the abdomen and pelvis with oral contrast can clarify the situation. Differentiating between Ogilvie's and toxic megacolon can be more difficult. In the general population the most common cause of toxic megacolon is inflammatory bowel disease, in the critically ill the most common cause is *C. difficile* infection[24]. A thorough history and physical is warranted, other diagnostic tools include stools samples to test for *C. difficile* toxins or *C. difficile* PCR, CT abdomen pelvis and limited endoscopy with biopsies.

Treatment

The first step in management include treating underlying conditions, managing electrolyte abnormalities, avoid opiates, early mobilization when feasible and early enteral nutrition.

When this therapy fail after 24-48 h and the risk of rupture is present, defined as cecum diameter > 12 cm[25]. We must proceed with other options that include neostigmine use, endoscopic decompression, percutaneous cecal decompression or surgical management.

Neostigmine is successful in achieving decompression in more than 88% of cases[26]. The drug is used at a dose of 2 mg intravenously given slowly over 5 min with monitoring of vital signs continuously for at least 30 min. Side effects include bradycardia, hypotension, nausea, vomiting and abdominal cramping.

Endoscopic decompression is les commonly used due to the risk of perforation, when performed this should be followed by the placement of a decompression tube since this increases the success rate from 50% to 80%[27]. In patients in whom these therapies fail, the next step according to the American Society of Gastroenterology

Table 3 Factors predisposing to Ogilvie's syndrome

Factors predisposing to Ogilvie's syndrome
Medications
Opiates
Anticholinergics
Vasopressors
Calcium channel blockers
Cardiovascular factors
Shock
Heart failure
Critical illness
Severe sepsis
Pancreatitis
Mechanical ventilation
Hypoxemia
Post-operative state
Abdominal surgery
Peritonitis
Pelvic or hip fracture surgery
Metabolic factors
Hypokalemia
Renal failure
Hyperglycemia
Neurologic
Spinal cord lesions
Stroke

and Endoscopy guidelines should be either percutaneous cecostomy or surgical management[28].

DIARRHEA

Diarrhea in the ICU can be defined as > 3 loose stools a day[29]. The incidence is around 20%[30]. Diarrhea in the ICU can be divided as infectious and non-infectious. Due to its incidence and possible serious underlying conditions it should never be dismissed and proper workup should be sought.

Infectious diarrhea

Clostridium difficile infection is the most common cause of infectious diarrhea in the ICU been present in 44% of patient with either infectious or non-infectious diarrhea in the ICU[31]. Other enteric pathogens include *Salmonella*, *C. perfringens*, *S. aureus* and *P. aeruginosa*. Antibiotic use is the most widely recognized risk factor for infectious diarrhea in the ICU; other risk factors include gastric acid suppression[27], advanced age and illness severity. A review of *C. difficile* infection is beyond the scope of this review article.

Non-infectious diarrhea

The most common causes for non-infectious diarrhea in the ICU include antibiotic associated diarrhea, enteral feeding associated diarrhea and medications. Regarding antibiotic associated diarrhea, when *C. difficile* is not found the theory behind this condition is the reduction on the concentration of anaerobic organisms in the gut with subsequent reduction of carbohydrate fermentation leading to an osmotic diarrhea[31].

Enteral feeding associated diarrhea is commonly quoted as the cause of diarrhea during ICU rounds. Interestingly a recent meta-analysis comparing total parenteral nutrition *vs* enteral nutrition did not find a higher incidence of diarrhea in the enteral feeds group[32]. A common sense approach would be to avoid high caloric density formulations due to their osmotic effects when possible. Fiber use to decrease diarrhea has been proven effective in the non-icu population, but this effects have not been reproduced in the ICU population. Probiotics also did not change its incidence[33].

CONCLUSION

GI dysmotility is a common but often overlooked occurrence in the critically ill patients. By itself it may be the reflection of end organ damage and deterioration as well as a sign of a serious underlying disorder. The clinician should pay close attention to it and initiate the appropriate work up as soon as possible to prevent grim outcomes.

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ORIGINAL ARTICLE

Retrospective Study

Use of proton pump inhibitors in general practice

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Abstract

AIM

To evaluate the characteristics of the prescription of the proton pump inhibitor drugs (PPI) and the adherence to the indications of the guidelines regulating the reimbursement limitations set forth by the Italian Drug Agency.

METHODS

Thirty general practitioners (GP) participated in the study, providing data on more than 40000 patients in total. The population was divided into non occasional users of PPI drugs (PPI users) and non-users (PPI non-users) based on evidence of a prescription of at least 3 packs of PPIs in the last 90 d before analysis. The data provided allowed an assessment of compliance with the requirements of eligibility for PPI reimbursement according to the Italian Drug Agency rules, in order to obtain subpopulations which complied or not with the rules.

RESULTS

Six thousand three hundred and twenty-two patients were found to be PPI users, accounting for 14.9% of the patient population. PPI users were more frequently female, older and more frequently diagnosed with gastroesophageal reflux disease, gastric or duodenal ulcers, arthropathy, heart disease and cancer than the rest of the population. PPI users had more frequently received prescriptions for non-steroidal ant-inflammatory drugs (NSAIDS), acetylsalicylic acid (ASA), oral anticoagulant therapy (OAT) and systemic steroids. PPI reimbursement resulted applicable to 69.3% of the PPI users, but a potential for reimbursement of PPI prescriptions was identified in the non PPI users for the treatment of peptic or reflux disease (8.5%) and for the protection of gastric damage caused by NSAIDS (6.1%). Patients who are potentially eligible for reimbursement are older, diagnosed with arthropathy and heart disease more frequently and most commonly receive NSAID and ASA prescriptions compared with PPI users who do not satisfy eligibility requirements. Patients in whom it was not possible to identify conditions related to prescription suitability were more frequently associated with use of OAT.

CONCLUSION

A substantial number of patients who apparently do not meet prescription suitability conditions can be identified, but among non PPI users on the contrary, it is possible

to identify an equal number of patients for whom prescription would be suitable. Poor suitability can be identified in the population receiving OAT. Thus, there is scope for decreasing inappropriate use of PPI drugs by adhering to certain criteria and by involving all interested parties.

Key words: Proton pump inhibitors; Appropriateness; General practice; Gastroprotection; Peptic disease

Core tip: This study was carried out in a large unselected population to evaluate the characteristics of proton pump inhibitor (PPI) prescription and the adherence to the guidelines regulating the reimbursement limitations set forth by the Italian Drug Agency. A substantial number of patients who apparently do not meet prescription suitability conditions can be identified, but among non-PPI users on the contrary, it is possible to identify an equal number of patients for whom prescription would be suitable. According to our data the greatest problems in clinical decision originate in patients in antithrombotic therapy.

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INTRODUCTION

Proton pump inhibitors (PPI) are among the most prescribed drugs in the world since their indications for use are manifold, including the treatment of gastro-esophageal reflux disease (GERD), peptic ulcer disease, the prevention of gastric damage by non-steroidal ant-inflammatory drugs (NSAIDS) and acetylsalicylic acid (ASA), dyspepsia and infection by *Helicobacter pylori* (*H. pylori*)[1-4]. There are five PPIs available in Italy (omeprazole, pantoprazole, lansoprazole, rabeprazole and esomeprazole), representing between 5% and 10% of total pharmaceutical prescriptions, similar to other countries[5-7].

PPIs are generally well tolerated and have few side effects but their prolonged use has been associated with various problems due to mechanisms which are especially related to the extensive and persistent inhibition of gastric acid secretion and the competitive inhibition of hepatic cytochrome P450[8-11].

Due to the high efficacy of PPIs in controlling the symptoms of upper gastrointestinal diseases, treatment often becomes ongoing and difficult to suspend[12]. This often makes it difficult to determine the prescription suitability of PPIs[12-16].

For this reason, rules to limit the reimbursement of these drugs which are paid for by the Italian National Health Service were introduced by the Italian Drug Agency twenty years ago. These were draw up according to the conditions of proven effectiveness and following major international guidelines. Table 1 describes the eligibility requirements for reimbursement of PPI prescriptions according to the Italian Drug Agency rules.

The aim of the study was to retrospectively evaluate, using the patient files provided by a large group of General Practitioners (GPs), the characteristics of PPI prescription and their adherence to the indications of the guidelines regulating the reimbursement limitations set forth by the Italian Drug Agency.

MATERIALS AND METHODS

Forty of the 400 GPs of the Health Agency of Bologna (Northern Italy) were requested to participate in the study. GPs were asked to submit a file containing anonymous data of all adult patients at 1 June 2015. This was obtained using an automated procedure available in the software which is used to manage clinical data. Demographic variables, presence of clinical diseases and drug use were reported in the file. A single database to obtain general population data was then created. The population was divided into non occasional users of PPI drugs (PPI users) and non-users (PPI non-users) based on evidence of a prescription of at least 3 packs of PPIs in the last 90 d before analysis (1 pack = 14 tablets). The data provided allowed an assessment of compliance with the requirements of eligibility for PPI reimbursement according to the Italian Drug Agency rules, in order to obtain subpopulations which complied or not with the rules. Table 1 describes the eligibility requirements for reimbursement of PPI prescriptions according to the Italian Drug Agency rules.

Differences between populations were evaluated using analysis of variance and the chi-squared test. $P < 0.05$ values were selected as the statistical significance limit. The statistical review of the study was performed by a biomedical statistician. The study did not need to be submitted to the Ethics Committee as retrospectively conducted on anonymous database.

RESULTS

Thirty GPs participated in the project and provided anonymous data files for 42548 patients. The study population was made up of 19632 males (46.1%) and 22916 females (53.9%) with a mean age 53 years (28.4% over 64 years old). This study population did not differ from the whole population on record at Health Agency of Bologna, which comprehends about 750000 adults (44% male and 56% female), of whom about 210000 (28%) are over 64 years old.

Six thousand three hundred and twenty-two patients were found to be PPI users, accounting for 14.9% of the patient population. Table 2 summarizes the characteristics of PPI users compared to non-PPI users.

Table 1 Rules of the Italian Drug Agency for the refund of proton pump inhibitor drugs

The prescription of PPI refundable by the National Health Service is limited to
The prevention of serious complications of the upper gastrointestinal tract in patients in chronic treatment with NSAIDS or in antiaggregant therapy with low doses of ASA, provided there is one of the following conditions of risk: (1) history of past digestive hemorrhage or peptic ulcer not healed with *Helicobacter pylori* treatment; (2) concomitant therapy with anticoagulants or cortisone; and (3) advanced age
Duration of treatment 4 wk (occasionally 6 wk): Duodenal or gastric ulcer, in association with drugs eradicating the infection; GERD with or without esophagitis (first episode)
Duration of treatment extended to reevaluate after one year: Zollinger-Ellison syndrome; relapsing duodenal or gastric ulcer; GERD with and without esophagitis (relapsing)

PPI: Proton pump inhibitor; GERD: Gastroesophageal reflux disease; ASA: Acetylsalicylic acid; NSAIDS: Non-steroidal ant-inflammatory drugs.

Table 2 Characteristics of proton pump inhibitor-users (at least 3 packs in 90 d) and non-proton pump inhibitor-users *n* (%)

	All	PPI-users	Non PPI users
Patients	42548	6322	36226
Males	19632 (46.1)	2520 (39.9)	17112 (47.2)
Aged over 64 yr	12084 (28.4)	3902 (61.7)	8182 (22.6)
GERD	5769 (13.6)	2980 (47.1)	2789 (7.7)
Peptic ulcer	689 (1.6)	375 (5.9)	314 (0.9)
Arthropathy	15661 (36.8)	3786 (59.9)	11875 (32.8)
Heart disease	3932 (9.2)	1674 (26.5)	2258 (6.2)
Neoplasms	3384 (8.0)	1076 (17.0)	2308 (6.4)
Use of NSAIDS	1131 (2.7)	416 (4.6)	715 (2)
Use of ASA	4522 (10.6)	2017 (31.7)	2505 (6.9)
Use of OAT	1127 (2.6)	500 (7.9)	627 (1.7)
Use of systemic steroids	547 (1.3)	306 (4.8)	241 (0.7)
EGD scopy	5772 (13.6)	2626 (41.5)	3146 (8.7)
Test per *H. pylori*	4761 (11.2)	1641 (26.0)	3120 (8.6)
PPI refundable for prevention of gastric damage by NSAIDS	4105 (9.6)	1896 (30.0)	2209 (6.1)
PPI refundable for peptic ulcer or GERD	6340 (14.9)	3265 (51.6)	3075 (8.5)
PPI refundable for prevention of gastric damage by NSAIDS or peptic ulcer or GERD	9368 (22.0)	4383 (69.3)	4985 (13.8)

All the features differ significantly ($P < 0.01$) between the two groups. PPI: Proton pump inhibitors; GERD: Gastroesophageal reflux disease; Heart disease: Heart failure, coronary ischemic disease, major heart valves disease; NSAIDS: Non steroid inflammatory drugs; ASA: Acetylsalicylic acid; OAT: Oral anticoagulant therapy; EGDscopy: Esophageal-gastro-duodenal endoscopy; *H. pylori*: *Helicobacter pylori*.

The two groups were statistically different when all the evaluated conditions were compared. PPI users were more frequently female, older and more frequently diagnosed with gastroesophageal reflux disease, gastric or duodenal ulcers, arthropathy, heart disease and cancer than the rest of the population. PPI users had more frequently received prescriptions for NSAIDS, ASA, oral anticoagulant therapy (OAT) and systemic steroids. In addition, PPI users had been more frequently prescribed an esophagogastroduodenoscopy (EGDscopy) and tests for the diagnosis of *H. pylori* infection.

Based on the clinical characteristics of the patients, it was possible to determine the prevalence of patients in the two groups who satisfy requirements set forth by Italian Drug Agency rules and who may be eligible for PPI reimbursement. Based on the data available, PPI reimbursement for the protection of gastric damage caused by NSAIDs is applicable to 30% of PPI users, for ulcers or GERD disease it is applicable to 51.6%, for at least one of the two cases it is applicable to 69.3% of the group. One thousand nine hundred and thirty-nine out of 6322 (30.7%) patients do therefore not comply with PPI prescription suitability according to the Italian Drug Agency.

However potential conditions which are eligible for PPI reimbursement are identifiable for peptic or GERD disease in 8.5% of the non-PPI users (equal to 3075 out of 36226 patients) and for the protection of gastric damage caused by NSAIDS in 6.1% of patients (in 2209 out of 36226 patients).

Figure 1 shows, relative to the total study population, the PPI users who comply with PPI prescription suitability for the protection of gastric damage caused by NSAIDS (1896), the PPI users who are not suitable for prescription (1939) and the non-PPI users who are suitable for prescription for the protection of the gastric damage from NSAIDS (2209), relative to the total study population.

Table 3 shows the characteristics of PPI users who do or do not comply with reimbursement eligibility conditions. The two groups were statistically different in relation to some characteristics. Patients who were potentially eligible for reimbursement were older, were more frequently diagnosed with arthropathy and heart disease and more frequently received NSAID and ASA prescriptions compared with PPI users who do not satisfy eligibility requirements. Also PPI users who comply with reimbursement characteristics were most frequently associated with prescriptions of NSAIDS and ASA. Patients in whom it was not possible to identify conditions related to prescription suitability were more frequently associated with OAT prescriptions.

PPI users considered suitable for prescription were more frequently subjected to EGDscopy and tests for the diagnosis of *H. pylori* infection. No differences were found between the two groups with regard to gender, frequency of malignancies or prescription of systemic steroids.

DISCUSSION

The results of this survey describe the actual prescribing behaviour of a large group of GPs related to the use of

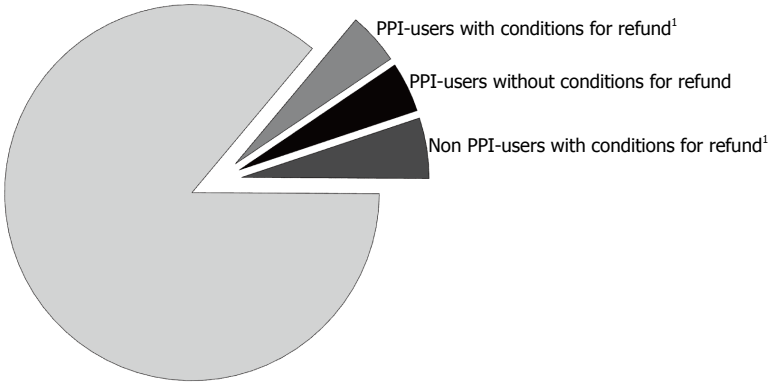

Figure 1 Subgroups of the population of the study divided according the use of proton pump inhibitors and the conditions for refund established by the Italian Drug Agency. [1]For the prevention of gastric damage of NSAIDS. Grey: PPI-users with refundable drug according the Italian Drug Agency because of protection of gastric damage by NSAIDS (4.5%); Black: PPI-users without identifiable refundable drug (4.6%); Dark Grey: Non PPI-users with identifiable need of the protection of gastric damage by NSAIDS (5.2%); White: The remaining population (85.7%); PPI: Proton pump inhibitors; NSAIDS: Non steroid inflammatory drugs.

Table 3 Characteristics of proton pump inhibitors-users with or without refundable conditions according to the Italian Drug Agency *n* (%)

	PPI refundable	PPI not refundable	*P* value
Patients	4383	1939	
Males	1762 (40.2)	758 (39.1)	ns
Aged over 64 yr	2967 (67.7)	935 (48.4)	0.01
Arthropathy	2717 (62.0)	1069 (55.1)	0.01
Heart disease	1263 (28.8)	411 (21.9)	0.01
Neoplasms	764 (17.4)	312 (16.1)	ns
Use of NSAIDS	333 (7.6)	83 (4.3)	0.01
Use of ASA	1857 (42.4)	150 (7.7)	0.01
Use of OAT	255 (5.8)	245 (12.6)	0.01
Use of systemic steroids	201 (4.6)	105 (5.4)	ns
EGDscopy	1984 (45.3)	642 (33.1)	0.01
H. pylori test	1232 (28.1)	409 (21.1)	0.01

All the features differ significantly ($P < 0.01$) between the two groups, except for gender, frequency of neoplasms and use of systemic steroids. PPI: Proton pump inhibitors; GERD: Gastroesophageal reflux disease; Heart disease: Heart failure, coronary ischemic disease, major heart valves disease; NSAIDS: Non steroid inflammatory drugs; ASA: Acetylsalicylic acid; OAR: Oral anticoagulant therapy; ns: Not significant; EGDscopy: Esophageal-gastro-duodenal endoscopy; *H. pylori*: *Helicobacter pylori*.

PPI drugs.

Unlike other analyses based purely on the assessment of administrative databases, this study allows a connection to be made between the prescription data and the records of clinical diagnoses. The analysis of a database of over 40000 patients allows us to highlight the fact that long-term prescription of PPI drugs is found in almost 15% of the population. These data are not dissimilar to those available on the whole Italian population[5], and show how PPI users present a number of clinical conditions (heart disease, cancer, arthropathy, use of ASA, OAT, systemic steroids, NSAIDS) which characterises them as a potentially fragile population. Epidemiological data showing an association between PPI and clinically dangerous conditions (*e.g.*, ischemic heart disease, renal failure, pulmonary disease) must therefore be interpreted with caution since PPIs could actually be used as markers of fragility (probably not always properly) in populations with a high prevalence of serious diseases[17-19]. It is very difficult to compare the prevalence of PPI users obtained in our work with that of other studies carried out on selected populations and in Countries with different health system, as highlighted by a recent study conducted in Sweden[20].

In any case, the main interest of this study concerns prescription suitability based on the requirements for reimbursement eligibility drawn up by the Italian Drug Agency. The methodology of the study has allowed the identification of potential reimbursement eligibility for 69% of PPI users. This is therefore a significant proportion of patients in an area of apparently poor suitability.

On the other hand over 2000 patients can be identified in the population of non-users who comply with reimbursement eligibility criteria for the prevention of damage caused by NSAIDs and over 3000 patients diagnosed with GERD or gastric/duodenal ulcers in whom the use of PPIs could be appropriate. There is therefore a need to rebalance PPI prescription patterns by reassessing patient characteristics according to overall suitability criteria[21].

The study data have made it possible to better define the characteristics of PPI users who are not suitable for PPI prescription. This population, as well as being comprised of younger patients and a lower prevalence of joint disease, heart disease and NSAIDS use, shows an extremely interesting higher prevalence of OAT use and no differences in the prevalence of cancer or use of systemic steroids.

This could indicate that the most effort to modify treatment in order to promote proper use of PPIs could be made among younger patients using OAT in the absence of further gastric hemorrhagic risk factors.

It is important to note that the current reimbursement eligibility criteria were drafted more than 10 years ago. Without an updated version it is difficult and disadvantageous to use PPIs in clinical conditions which are known to potentially cause serious gastrointestinal bleeding, such as the use of new strategies in antiplatelet and anticoagulation therapy[22] and the use of reuptake inhibitors of serotonin especially in conjunction with ASA and NSAIDS[23].

It should also be noted that these problems are widespread when used by hospital doctors[24-28], but the prescribing behaviour of GPs greatly influences PPI use since they are the main prescribers of the drug[29].

A study of the Italian College of General Practitioners showed that almost half of PPIs are suggested or encouraged by specialists, with different degrees of agreement depending on the disease and the type of specialist[30].

This study has limitations due to the retrospective method and due to the potential of poor accuracy of data logging which is typical to databases. In particular, clinical conditions related to prescription suitability such as the diagnosis of GERD or the use of ASA may not be recorded correctly, as due to their very low cost, some patients prefer to buy them without an NHS-paid prescription.

It should be noted that this study takes into account only the use of PPI and does not take account of the use of other drugs such as receptor antagonists H2.

A substantial number of patients who apparently do not meet prescription suitability conditions can be identified, but among non-PPI users on the contrary, it is possible to identify an equal number of patients for whom prescription would be suitable. It is possible that a large proportion of poor suitability can be identified in the population receiving OAT.

Even taking into account that the current rules of reimbursement eligibility in Italy have not undergone an adequate update in response to changes in the use of potentially gastrolesive medications, there is no doubt scope for decreasing inappropriate use of PPI drugs by adhering to certain criteria.

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COMMENTS

Background

Proton pump inhibitors (PPI) are among the most prescribed drugs in the world but it is often difficult to determine the prescription suitability of PPIs. The Italian National Health Service introduced rules to limit the reimbursement of these drugs that were draw up according to the conditions of proven effectiveness and following major international guidelines.

Research frontiers

Most studies showing a wide use of PPIs suspected for an inadequate compliance with the available scientific evidences are based on the analysis of administrative data. These studies cannot fully understand the relationship between clinical characteristics of the patient and the relative drug prescription.

Innovations and breakthroughs

The study clearly shows that most patients who do not meet prescription suitability conditions can be identified in the population receiving anticoagulant treatments. On the contrary, among patients not receiving PPIs, it is possible to identify an equal number of patients for whom prescription would be suitable.

Applications

The findings of this study can help the drug prescribers and the integrated units formed by specialists and general practitioners to identify specific areas of intervention to improve the suitability of the use of this class of drugs.

Terminology

There are five PPIs available in Italy (omeprazole, pantoprazole, lansoprazole, rabeprazole and esomeprazole). This study does not take account of the use of other drugs such as receptor antagonists H2. The population was divided into non occasional users of PPI drugs (PPI users) and non-users (PPI non-users) based on evidence of a prescription of at least 3 packs of PPIs in the last 90 d before analysis (1 pack = 14 tablets).

Peer-review

This is an interesting retrospective analysis of PPI use in Italy assessing adherence to the indications of the guidelines issued by the Italian Drug Agency.

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ORIGINAL ARTICLE

Observational Study

Transition care in inflammatory bowel disease: A needs assessment survey of Quebec gastroenterologists and allied nurses

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Author contributions: Strohl M initially compiled all the data from completed surveys, partook in statistical analysis and interpretation of data; additionally, wrote draft and was the first author of manuscript; Zhang X was responsible for statistical analysis of the manuscript and deriving summary descriptive data; Lévesque D conceived the idea of the survey, took part in designing the survey and assisted in draft revision and editing; Bessissow T was a major contributor to multiple revisions and edits and assisted significantly with fine-tuning the final draft.

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Abstract

AIM

To determine the tools needed and problems encountered during the transition of inflammatory bowel disease (IBD) patients from pediatric to adult gastroenterologists (GIs) in Québec, Canada.

METHODS

We conducted a needs assessment survey of Quebec health care professionals (HCPs). The survey was handed out to 136 Québec HCPs at a local conference in 2013. Additionally, it was emailed to any other HCPs in Quebec involved in caring for IBD patients. The completed surveys were compiled to derive descriptive data. Further specific subgroup analysis was then conducted.

RESULTS

Among the conference attendees and individuals emailed

77 (28.2%) completed the questionnaire. Respondents included adult GIs (61.3%), pediatric GIs (20.8%) and IBD nurses (18.3%). The majority of respondents believed that a standardized structure is important for a successful transition. Adult and pediatric GIs equally felt that patients were inadequately prepared for the transition (P = 0.6). There were significant differences between adult and pediatric GIs when it came to resource availability (55.6% *vs* 90.9%, P = 0.002) and perceived need of a formal transition clinic (21.7% *vs* 68.8%, P = 0.0006). Both transition program and medical summaries were identified as the most valuable tools to improve transition.

CONCLUSION

As described in previous studies, our survey reinforces the importance of a transition program, education for young adult IBD patients and the need to improve communication between adult and pediatric GIs.

Key words: Inflammatory bowel disease; Transition; Paediatric; Canada; Tools; Health care professionals

Core tip: Transition care and transfer of care from pediatric to adult realms is a major challenge with a paucity of published work in the inflammatory bowel disease (IBD) domain. Transition care varies across different health care systems but from other pediatric entities improved objective outcomes have been demonstrated with more effective transfer of care. This is the first published survey on health care professionals (HCPs) opinion on transition care in IBD in Canada. Barriers related to the patients from the HCPs were identified as were tools that if implemented have potential to improve the effectiveness of transition care. Differences between pediatric and adult gastroenterologists were also identified.

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INTRODUCTION

Inflammatory bowel diseases (IBD), which encompasses both ulcerative colitis and Crohn's disease are common pediatric idiopathic chronic diseases[1,2]. Estimates have shown that approximately 20%-30% of cases of IBD are diagnosed before the age of 18[2,3]. Data also indicate that the incidence and prevalence of IBD have both been increasing over time[4,5] and Canada has been shown to have one of the highest rates worldwide[5]. Hence there exists an inevitable need to manage this growing population and the chronic nature of the disease mandates to establish effective means of coordinating efficient transition care from the pediatric to the adult realm.

There are a number of significant differences between pediatric and adult systems most notably a paradigm shift from a dependent, multidisciplinary and family centered setting in pediatrics to an autonomous and self-reliant framework in the adult system[6-9]. Transition care as defined by the Society for Adolescent Medicine is the "purposeful, planned movement of adolescents and young adults with chronic physical and medical conditions from child-centered to adult-oriented health-care systems"[10]. Although lacking convincing objective evidence, there is expert consensus that a coordinated transition between the pediatric and adult realm is essential in the management of IBD[7,11-13]. Transition care provides adolescents the opportunity to acquire the set of skills required to succeed once they are integrated into the adult system. Numerous challenges, both patient and health care system related factors, currently exist making it difficult to consistently achieve successful transition care[9]. Particularly in Canada we face the challenge where patients are expected to become part of the adult system at the age of 18, as they are no longer eligible to be seen in a pediatric setting.

There remains a paucity of published literature on IBD transition. A number of publications have focused on surveying transitioning pediatric patients focusing on their knowledge of their medication, ability to identify associated adverse events, quantifying medication adherence and evaluating their capacity to demonstrate independence[14-16]. Health care providers perception has also been a focus of research to aid in identifying domains to target for transition care amelioration[11-13]. Certain tools guided at improving transition have also been studied such as The MyHealth Passport from the University of Toronto and TR$_x$ANSITION tool out North Carolina[17,18]. To date, no concrete data has been published demonstrating any improvement in a predetermined objective outcome variable with a dedicated standardized transitional care network.

In Quebec, the Transition to Adult care in patients with Crohn's and Colitis (TRACC) program has been established in collaboration between adult and pediatric gastroenterologists with the aim of facilitating and improving transition care in patients with IBD while trying to overcome some of the common obstacles encountered. Currently, in Quebec the age to initiate transition is variable with no standardized age of initiation. On the other hand, the age of transfer of care is fixed at 18 across the province. Previous studies have looked at adult and pediatric gastroenterologists (GIs) perspective of transition care, however no data exists on the perspective of adult and pediatric GIs in the Canadian health care system, more specifically from Quebec. Moreover all of the studies conducted have focused on the perception of transition and barriers to successful transition with none looking at specific tools that may be

of use to yield more successful transition care[11-13]. To our knowledge none of the published conducted studies on surveys focused specifically on needs assessment.

The primary objective of our study was to determine the necessary tools and obstacles encountered during the transition of IBD patients from pediatric to adult care from the perspective of health care professionals (HCPs) in Quebec, Canada. The information obtained *via* the survey will then serve as a basis for establishing methods to achieve more effective transition care. Secondary objectives included comparing pediatric and adult gastroenterologists and identifying any significant differences between the two groups.

MATERIALS AND METHODS

Study design

We performed a cross sectional needs assessment study that was conducted in the province of Quebec between November 2013 and August 2014. We intended to include all HCPs that care for pediatric and adult IBD patients. A total of 136 paper copies of the questionnaires were handed to all HCPs attending the annual association des gastroenterologues du Quebec (AGEQ) meeting in November 2013. In addition, through the contact list provided by the AGEQ, an email was sent to all members of the association (total of 206 individuals) of which included only board certified adult and pediatric gastroenterologists. Within this email it was specified to all members to not fill out and return the survey if they had attended the conference in November (a total of 96/206 members were at the conference). Members were all required to print out the survey and either mail it to our office or bring it by in person.

Survey/questionnaire

The questionnaire was intended to elicit the opinion of health care professionals on various aspects pertaining to transition care in pediatric patients with IBD. The questionnaire was developed by the TRACC committee, which is made up of adult and pediatric IBD specialists and expert IBD nurses. The questionnaire has not been previously validated but was developed to have a better understanding of the reality of transition care in Quebec. There is no validated questionnaire in existence geared at assessing health care provider perception of transition care in IBD. Certain components that were included in the two surveys previously published prior to our survey's inception[8-9] were also incorporated into our questionnaire. Basic demographic data was collected. With respect to age of transition care initiation and completion respondents were able to choose between different age choices (Initiation: 12, 13, 15, 16, 18; Completion: 16, 17, 19, 20). In addition respondents were asked to rank the degree of importance of different statements describing certain factors related to transition on a Linkert scale from 1 to 5, in which 1 represents "not important" and 5 represents "very important and essential". Additional questions were to investigate the current efficacy of transition through transmission of summary letters from the referring pediatricians what components should be included within the transfer note. The latter section of the questionnaire inquired about what tools, if implemented, would be perceived as beneficial in improving the quality of transition in Quebec.

Statistical analysis

All the completed surveys were compiled and entered into an Excel document, which was then used to derive summary descriptive data. Included in the summary descriptive data was subgroup breakdown. The response rate of every question varies as not all respondents answered all the questions. A respondent was included in the analysis if > 50% of the questions were answered (only 1 survey was excluded ultimately). Differences between subgroups on specific questions was explored using χ^2 analysis with the threshold for significance set at a $P < 0.05$. A two-tailed *T*-test analysis with the assumption of unequal variance was used when comparing means of two different groups for the two age-related questions.

RESULTS

Demographics of respondents

A total of 273 surveys were handed and 77 were filled out yielding a response rate of 28.2% (77/273). Respondents included adult gastroenterologists (GIs) (*n* = 47/77, 61%), pediatric GIs (*n* = 16/77, 20.8%), IBD nurses (*n* = 14/77, 18.3%). 41.6% of individuals (*n* = 32/77) worked in non-academic hospital setting while 57.1% (*n* = 44/77) work in academic centers while only 1 individual worked uniquely in an outpatient clinical setting. Looking at response rates of demographic subgroup based on total number of professionals in Québec 25.5% (*n* = 47/184) of adult GIs and 72.7% (*n* = 16/22) of pediatric GIs responded. With respect to experience 52.6% (*n* = 40/77) had greater than 10 years of experience, 23.7% (*n* = 18/77) had 5-10 years of experience and 23.7% had less than 5 years of experience (*n* = 18/77) (Table 1).

Importance and age of transition

Almost all respondents felt that a standardized structure for transitioning patients with IBD was important (97.4%, *n* = 75/77). Out of these 62.3% (*n* = 48/77) felt this was very important while 35.1% (*n* = 27/77) felt it was moderately important yet important enough to merit a standardized structure (Table S1). There was no significant difference on subgroup analysis comparing pediatric and adult GIs (*P* = 0.388). In terms of age to initiate transition and complete transfer of care from the pediatric to the adult domain the mean age was 16.2 ± 1.46 years and 18.2 ± 1.16 respectively amongst all respondents (Table S2). On subgroup analysis, pediatric GI believed transition should start earlier than adult GI

Table 1 Demographics of respondents

	# Of respondents (total *n* = 77)	Percentage
Profession		
Adult Gastroenterologist	47	61.0%
Peds Gastroenterologist	16	20.8%
Inflammatory bowel disease nurse	14	18.3%
Practice setting		
Academic center	44	57.1%
Hospital setting	32	41.6%
Outpatient	1	1.3%
Experience		
< 5 yr	18	23.7%
5-10 yr	18	23.7%
> 10 yr	40	52.6%

with mean ages of 15.4 ± 1.41 *vs* 16.7 ± 1.27 years (P = 0.003). Age of transfer completion was similar between adult and pediatric GIs with mean ages of 18.2 ± 1.25 and 18 ± 1.1 respectively (P = 0.47).

Adequate preparation for transition

The majority of respondents (58%, n = 45) felt that patients were inadequately prepared prior to being transferred from the pediatric to the adult system. This held true with stratification as pediatric (n = 11/16, 68.8%) and adult GIs (n = 25/44, 56.8%) equally felt that patients were inadequately prepared for (P = 0.4). Amongst all respondent's lack of maturity (n = 46, 60%) and independence of the patient to advocate for their needs (n = 40, 51.9%) were the 2 general domains attributed to the perceived inadequate preparation. In terms of specific factors the following were rated as the most important on the Linkert scale (mean score > 4.5): Patient's knowledge of IBD in general (mean = 4.6) and their particular disease (site affected, medication history, treatment side effects *etc.*) (mean = 4.6), patient responsibility in taking their medication (mean = 4.7), partaking in discussions during doctor visits (mean = 4.7), being able to recognize when their disease may be active and who to contact (mean = 4.8) and understanding the impact of tobacco and drugs on their condition (mean = 4.6) (Table S3)

Tools to improve transition

A significant amount of adult GIs (37%, n = 17/46) stated they do not receive enough information regarding new incoming IBD patients from the referring pediatric GIs.

The vast majority (82.6%, n = 38/46) of adults GIs prefer to obtain a chart summary prior to the first visit as opposed to at the moment of the first rendezvous.

Among a variety of tools listed which could potentially be implemented by the transition network in Québec (TRACC), a transition program (77.3%, n = 59/76) and medical summaries (76.2%, n = 58/76) were felt to be the most important. On subgroup analysis 71.7% of adult GIs and 93.8% of pediatric GIs felt that a transition program would be a useful tool (P = 0.07) with 84.7% and 62.5% respectively choosing medical summaries as important tools (P = 0.06). A structured educational day on transition care for patients and their families (47.4%, n = 36/76) was also considered useful. A checklist prior to the first adult visit was also considered important (54%, n = 41/76). Surprisingly a dedicated transition clinic, which was not clearly defined to respondents but rather listed as a response (32.9%, n = 25/76) was not perceived to be as important as some of the other tools amongst all responders (Table S4). However on subgroup analysis it became apparent that this was a more important tool amongst pediatric GIs. Only 21.7% of adult GIs compared to 68.8% of pediatric GIs selected dedicated transition clinics as being important in transition (P = 0.00006) (Table 2).

Training and resources

The majority of respondents (75.3%, n = 52/69) felt that they had adequate training to effectively deal with transitioning patients in IBD. On subgroup analysis it became apparent that IBD nurses felt less prepared compared to both pediatric and adult GIs (P = 0.005, P = 0.02 respectively). When looking at the adult GIs (78.3%, n = 36/46) compared to pediatric GIs (100%, n = 10/10) there was a trend towards significance with the adult GIs tending to feel less adequately trained (P = 0.10). Sixty percent (n = 41/68) of all respondents were interested in more training *via* either workshops (23.5%, n = 16/68) or conferences (44.1%, n = 30/68).

Amongst all respondents 64.1% (43/67) felt that they had sufficient resources to manage transitioning IBD patients. However there was a significant difference on subgroup analysis between pediatric GIs (90.1%, n = 10/11) compared to adults GIs (55.6%, n = 25/45) when it came to the opinion of adequate resources available (P = 0.0016) (Table 3). With respect to adequate resources no specifics were detailed, rather this was a general feeling amongst respondents.

DISCUSSION

Our survey of Quebec HCPs working with IBD patients reinforces the notion that a standardized structure for transition is felt to be important. It revealed specifically what HCPs felt were patient related factors that limit effective transition, emphasized the significance of having a dossier summary and identified that a transition program, medical summaries, and potentially a dedicated educational day for patients if routinely implemented might be able to improve transition care in Quebec.

Similarly to previously conducted studies, our survey revealed similar results with respect to patient related factors that are most important for successful transition, including patient's knowledge of their condition and their independence in managing their disease with

Table 2 Select subgroup analysis between adult and pediatric gastroenterologists

	Adult	Pediatrics	*P* value
Importance of transition (moderately or very important)	95.70%	100%	0.39
Age related questions			
Mean age to initiate transition	16.7	15.4	0.003[1]
Mean age to complete transfer of care	18.2	18	0.47
Are patients well prepared for transition?	56.80%	68.80%	0.4
Transition tools			
Transition programs	71.70%	93.80%	0.07
Medical summaries	84.80%	62.50%	0.06
Transition clinics	21.70%	68.80%	0.0006[1]
Pre rendezvous checklists	47.80%	56.30%	0.56
Training and resources			
Adequate training	78.30%	100%	0.1
Sufficient resources	55.60%	90.90%	0.0016[1]

[1]Variable statistically significant with *P* value < 0.05.

Table 3 Training and resources

	Respondents	Percentage
Adequate training (*n* = 69)	52	75.30
Interested in more training (*n* = 68)	41	60.00
Training *via* workshops (*n* = 68)	16	23.50
Training *via* conferences (*n* = 68)	30	44.10
Sufficient resources (*n* = 67)	43	64.10

adequate self-management skills[11-13]. Currently there is limited evidence in the literature of improved objective clinical outcomes in patients with IBD who partake in a structured transition program[9]. However, there is substantial objective evidence that has shown that transitional care can improved clinical outcomes in other pediatric chronic diseases such as diabetes mellitus type 1 and in liver transplant patients[19-22]. By comparison one can stipulate that *via* transition care in IBD their lies significant potential in improving outcomes such as decreasing rate of hospitalization, improving medical compliance, and even improving other objective outcomes. Further studies focused on IBD related transition care are warranted to demonstrate this.

In the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPHAGN) position paper one of their key recommendations was that the pediatric GIs provide a medical summary to their adult colleagues prior to the first consultation with them[6]. This study reveals that despite these recommendations a substantial proportion of adult GIs (38.2%) feel that they obtain inadequate information prior to their first encounter. No published or collected data currently exists in Québec about what percentage of adult GIs obtain summaries prior to or at the time of transfer of care. In terms of specific tools identified transition program and medical summaries were the two identified in our survey as important for successful transition care. Examples of such tools exist such as My Health Passport developed at the University of Toronto that serves as succinct summary of a multitude of chronic pediatric disease including IBD that a patient may bring with them to any encounter with health care professionals[23]. This tool was designed to be completed by the patient thereby providing the opportunity of the transitioning adolescent to educate themselves while encouraging independence[17]. The implementation of a tool such as My Health Passport has the dual benefit of acting as a dossier summary for the adult GI and as a method of improving knowledge and inspiring independence.

Respondents also identified a checklist pre 1st adult visit (52.2%) and a "readiness checklist" (39.2%) as other important tools, which provide potential in assisting transition. The TRxANSITION tool created at of the University of North Carolina serves the purpose of being a "readiness" tool with the goals of identifying whether or not a patient is adequately prepared to transition to adult care[18]. This tool also assists in identifying particular aspects that need to be addressed for transition care optimization.

As was the case in Hait *et al*[11] survey ours identified that a proportion of adult gastroenterologists (21.7%) felt they were inadequately trained to manage the population of transitioning IBD patients. Our data also showed that despite a high proportion of respondents felt they were adequately trained (60%) the majority of them were interested in more training *via* conferences or workshops (67.6%) The survey also highlighted an important difference between adult and pediatric GIs when it comes to the availability of resources (55.6% *vs* 90.9%, *P* = 0.002). This finding is consistent with what is seen in the real world practice of many adult GIs in Quebec where additional resources such as Registered Dieticians, IBD nurses, psychologists are more difficult to access in comparison to pediatrics. By implementing a standardized program for transition care there may be potential to facilitate the ability to access additional resources in the adult system.

Our study had a number of limitations. As with any survey study ours was limited by non-response bias given the response rate of 28.2%. This has the

potential of selecting out HCPs who may place less of an importance on transition care. The small sample size of the survey also limits our study's strength. In addition our study may be limited in interpretability in other geographical locations as it was intended on only assessing the current status in Quebec. Our survey was solely focused on the health care provider perception. It would be interesting to use our compiled data in conjunction with a patient based perspective which may offer the best opportunity to more clearly identify exactly where to focus our resources and which tools may be most useful in improving transition care.

As described in previous studies, our survey reinforces the importance of a transition program, education for young adult IBD patients and the need to improve communication between adult and pediatric GIs. A structured and standardized transition network offering appropriate and applicable tools is the cornerstone to optimize adherence to transition tools and ensure a genuine clinical impact for a successful transition. Further studies are warranted which will likely provide additional objective evidence of the importance of effective transition care.

COMMENTS

Background

Inflammatory bowel diseases (IBD) are showing a rising incidence an prevalence in many areas of the world. Therefore, there is an inevitable need to manage this growing population and the chronic nature of the disease mandates to establish effective means of coordinating efficient transition care from the pediatric to the adult health care domains.

Research frontiers

To date there is minimal data on the perception of transition and transfer of care of IBD patients from pediatric to adult care. Improved objective outcomes with amelioration of transition care have been demonstrated in other chronic diseases spanning pediatric life and adulthood.

Innovations and breakthroughs

As with previous survey studies looking at transition care in IBD the authors' study found that there is a lack of communication between pediatric and adult gastroenterologist with suboptimal transfer of information. This study compared pediatric and gastroenterologists and revealed significant differences between the two group's perspective of transition care. Notably it identified a significant discrepancy in terms of resource availability.

Applications

Similar to previous studies the authors' survey reinforces the importance of a transition program, education for young adult IBD patients and the need to improve communication between adult and pediatric gastroenterologists. A structured and standardized transition network offering appropriate and applicable tools has the potential to offer a genuine clinical impact for a successful transition. The idea of focusing more resources on transition of care to improve objective outcomes is applicable to many diseases and spans many different health care systems.

Terminology

Transition is defined as a process that spans before and after the transfer of care. Transfer of care is the formal process of transferring the care of a given patient from one health care professional to another.

Peer-review

The study was conducted by assessment of the survey among 136 health care professionals involved in caring for IBD patients. The findings revealed that the pediatric patients were inadequately prepared for the transition, thus indicating the importance of an educational program for young adults with IBD.

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ORIGINAL ARTICLE

Case Control Study

Association of miR-146 rs2910164, miR-196a rs11614913, miR-221 rs113054794 and miR-224 rs188519172 polymorphisms with anti-TNF treatment response in a Greek population with Crohn's disease

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Abstract

AIM

To investigate the correlation between rs2910164, rs11614913, rs113054794, and rs188519172 polymorphisms and response to anti-TNF treatment in patients with Crohn's disease (CD).

METHODS

One hundred seven patients with CD based on standard

clinical, endoscopic, radiological, and pathological criteria were included in the study. They all received infliximab or adalimumab intravenously or subcutaneously at standard induction doses as per international guidelines. Clinical and biochemical response was assessed using the Harvey-Bradshaw index and CRP levels respectively. Endoscopic response was evaluated by ileocolonoscopy at week 12-20 of therapy. The changes in endoscopic appearance compared to baseline were classified into four categories, and patients were classified as responders and non-responders. Whole peripheral blood was extracted and genotyping was performed by PCR.

RESULTS

One hundred and seven patients were included in the study. Seventy two (67.3%) patients were classified as complete responders, 22 (20.5%) as partial while 13 (12.1%) were primary non-responders. No correlation was detected between response to anti-TNF agents and patients' characteristics such as gender, age and disease duration while clinical and biochemical indexes used were associated with endoscopic response. Concerning prevalence of rs2910164, rs11614913, and rs188519172 polymorphisms of miR-146, miR-196a and miR-224 respectively no statistically important difference was found between complete, partial, and non-responders to anti-TNF treatment. Actually CC genotype of rs2910164 was not detected in any patient. Regarding rs113054794 of miR-221, normal CC genotype was the only one detected in all studied patients, suggesting this polymorphism is highly rare in the studied population.

CONCLUSION

No correlation is detected between studied polymorphisms and patients' response to anti-TNF treatment. Polymorphism rs113054794 is not detected in our population.

Key words: MicroRNA; Crohn's disease; Polymorphisms; Anti-TNF; Biomarkers

Core tip: Anti-TNF agents are the cornerstone of inflammatory bowel disease (IBD) treatment strategy so far though not effective in one third of patients in the first months of administration. Biomarkers for prediction of each patient's treatment response are vigorously sought in the era of personalized medicine. MicroRNAs have been studied as possible predictors of response to therapy in cancer and autoimmune diseases including IBD. MiRNA polymorphisms though have never been studied in IBD as markers of anti-TNF response. Our results suggest that for rs2910164, rs11614913, rs113054794, and rs188519172 no association to anti-TNF agents' response in patients with Crohn's disease can be established.

Papaconstantinou I, Kapizioni C, Legaki E, Xourgia E, Karamanolis G, Gklavas A, Gazouli M. Association of miR-146 rs2910164, miR-196a rs11614913, miR-221 rs113054794 and miR-224 rs188519172 polymorphisms with anti-TNF treatment response in a Greek population with Crohn's disease. *World J Gastrointest Pharmacol Ther* 2017; 8(4): 193-200 Available from: URL: http://www.wjgnet.com/2150-5349/full/v8/i4/193.htm DOI: http://dx.doi.org/10.4292/wjgpt.v8.i4.193

INTRODUCTION

Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), is a chronic relapsing disease of unknown etiology. It is hypothesized that it arises from a combination of genetic susceptibility and environmental factors that trigger an inappropriate mucosal inflammatory response[1]. Anti-TNF agents have revolutionized IBD therapy since their induction in the market almost 20 years ago and are nowadays considered the cornerstone of IBD treatment strategy[2]. Although their undisputable effectiveness, almost one third of patients will never respond in the first 3-6 mo of therapy (primary non response)[3]. Taking into consideration their known side-effects and the cost-effectiveness of such expensive medications it becomes clear that research and identification of novel reliable biomarkers of response is of paramount importance. Moreover, with several new therapeutic drugs lying ahead of us [anti-interleukin (IL)-12/23 monoclonal antibodies, janus kinase inhibitors, agents targeting leukocyte trafficking] options will expand rendering prediction of response to a specific drug crucial for the patient. Until now, existing clinical or serologic markers have failed to accurately predict a patient's response to anti-TNF treatment[4]. Genetic or epigenetic markers are under vigorous study in an attempt to improve our understanding of the disease and enhance the prospect of personalized medicine according to each patient's likelihood to respond to different drug classes, especially anti-TNF agents[5,6].

MicroRNAs (miRNAs) are small, single stranded, non-coding RNA molecules comprising of 19-25 nucleotides exerting post-transcriptional gene expression regulation in response to cellular or environmental changes[7]. MiRNAs have begun to attract scientists' attention as biomarkers of prognosis or response to treatment in various diseases in part due to some unique advantages they possess: They are practically noninvasive, stable in serum, and can be promptly and repeatedly detected from archived sera[8].

A series of studies have focused on miRNA expression and its impact on response to anti-TNF agents in autoimmune diseases - sharing many commons with IBD- such as rheumatoid arthritis[9,10] or psoriasis[11,12] . MiRNA expression has been investigated in differentiation of CD from UC[13,14] or in distinction of CD phenotypes[15,16] while only one study in Asian population has been done so far searching an association between miRNA expression and response to infliximab in patients with CD[17]. As far as miRNA single nucleotide polymorphisms (SNPs) in mature

or pre-miRNA are concerned no assiduous research has been done trying to unravel a possible association between autoimmune diseases and anti-TNF response. That is in contrast with cancer, where a wealth of studies exists upon miRNA variants and response to treatment[18-22], with promising results that haven't though found their role yet in clinical every day practice. Regarding autoimmune diseases and SNPs predicting treatment efficacy, only one recently published study seeks to correlate miR-146a expression and rs2910164 polymorphism to rheumatoid arthritis development and clinical outcome after anti-TNF therapy[23].

It is well known that miR-146a is implicated in regulation of immune responses through NF-κB pathway and has been extensively studied in autoimmune diseases pathogenesis[23-27] including IBD[28,29]. Concerning miR-196, apart from having been extensively studied in cancer[18-20], it has also been reported to negatively regulate IGRM, a gene associated with autophagy, thus facilitating epithelial inflammation in CD[30] while its variant rs11614913 has been recently related as possibly contributing to IBD-related colorectal cancer development[31]. Last, miR-221 and miR-224 have been detected to be up-regulated after anti-TNF treatment in patients with CD in Fujioka *et al*[17] work while both have been shown to interfere in IBD related pathways; miR-221 as a down regulator of *ICAM1* gene the protein of which has been widely studied in IBD pathogenesis[32-34] and miR-224 inducing cell proliferation in ovarian murine cells through SMAD/TGF-β pathway[35]. Assuming that SNPs in the aforementioned miRNAs would exert an alteration in their functional capacity, we chose to examine whether rs2910164 of mir-146a, rs11614913 of miR-196a, rs113054794 of miR-221, and rs188519172 of miR-224 can predict response to anti-TNF treatment in a cohort of Greek patients with CD.

MATERIALS AND METHODS

Patients

One hundred and seven patients diagnosed with CD attending the IBD Clinic at Aretaieio Hospital, Athens, Greece were enrolled in the study. The diagnosis of CD was based on standard clinical, endoscopic, radiological, and pathological criteria[36]. Patients, who were due to receive anti-TNF therapy-infliximab (IFX) or adalimumab (ADA) - and were naïve to these or any other anti-TNF agent, were eligible for the study. Patients could receive in parallel other disease related drugs as long as there was no dose change 8 wk before enrollment. Patients with the following characteristics were excluded from the study: < 18 or > 80 years old, IBD-unclasssified, and malignancy.

IFX was administered intravenously at a dose of 5 mg/kg at weeks 0, 2, 6 and every 8 wk thereafter. ADA was administered subcutaneously at a dose of 160 mg at week 0, 80 mg at week 2 and 40 mg every 2 wk thereafter. Clinical and serological response was assessed with Harvey-Bradshaw Index (HBI) and CRP, respectively at various time points: At baseline (before 1st infusion or injection), the day before each subsequent drug administration and at week 12 of treatment. Ileocolonoscopy was performed at baseline and after 12-20 wk of therapy to assess mucosal healing. Changes of endoscopic image compared to baseline were classified in four categories and patients were classified as responders or not to anti-TNF therapy as previously described[37].

Genotyping

Genomic DNA from whole peripheral blood containing EDTA was extracted using validated techniques (NucleoSpin Blood kit; Macherey-Nagel, Germany). PCR–RFLP was used to determine the rs2910164 and rs11614913 was performed using T-ARMS-PCR assay as described previously genotypes as previously described[38,39]. Regarding the rs113054794 we used PCR-RFLP method. Forward primer: 5'CAGAAACATTATAGGGGTAGCA3' and reverse: 5'GGTAGTAGGTAAGTCCCAGCA3'. Annealing was done at 62 ℃. PCR products were digested with MvaI. For rs1885191722 polymorphism we used allele-specific PCR. Two different PCR reactions are performed with one or the other allele specific primer. The primers used were a common forward 5'CCTCAAGAATCCTCCTCACT3', and a reverse for the G-allele: 5'GTGGTTCCGTTTAGTAGATGAC3' and for the A-allele: 5'GTGGTTCCGTTTAGTAGATGAT 3'.

Statistical analysis

Genotype frequencies were compared with the χ^2 test with Yate's correction using S-Plus (v.6.2 Insightful, Seattle, WA, United States). Odds ratios (OR) and 95%CI were obtained with GraphPad (v.300, GraphPad Software, San Diego, CA, United States). The *P* values are all two-sided. *P* values of < 0.05 were considered to be significant.

RESULTS

Patients' demographic and clinical characteristics are summarized in Table 1. From the 107 patients included in the study, 104 (97.19%) received infliximab while the rest received adalimumab. Seventy two (67.29%) were classified as complete responders while 22 (21.57%) as partial responders to anti-TNF induction treatment. Thirteen patients (14.74%) did not respond and were considered non-responders. No correlation was detected between complete or partial responders and non-responders to anti-TNF therapy as far as patients' characteristics, like age, gender or behavior, are concerned. Clinical and serological indexes used - HBI and CRP-were consistent with endoscopically assessed response.

The prevalence of rs2910164, rs11614913, and rs188519172 in patients with CD who responded fully, partially and those who didn't respond to anti-TNF treatment are depicted in Table 2.

Regarding the first polymorphism studied, rs2910164 C allele was not found to be significantly different between complete, partial, and non- responders (*P* =

Table 1 Patient demographic and clinical characteristic according to response to anti-TNF treatment

Characteristics	Responders	Partial responders	Primarily non-responders
n (%)	72 (67.29)	22 (21.57)	13 (14.74)
Age (yr, mean ± SD)	34.10±11.63	32.23 ± 13.31	39.09 ± 15.60
Sex (%)			
Male	30 (41.67)	14 (63.64)	10 (76.92)
Female	42 (62.69)	8 (36.36)	3 (23.08)
C-reactive protein (mg/dL, mean ± SD)			
Baseline	3.10 ± 2.03	4.13 ± 2.31	6.86 ± 2.89
After treatment	0.88 ± 1.84	2.21 ± 2.69	3.96 ± 2.81
δC-reactive protein (%)	80.44 ± 22.42	71.94 ± 45.74	58.04 ± 22.63
Duration of disease (yr)	6.38 ± 5.91	5.71 ± 3.77	4.00 ± 3.13
Infliximab dose (mg/kg)	5	5	5
Location			
L2	24 (33.34)	3 (13.64)	2 (15.38)
L3	45 (62.50)	19 (86.36)	11 (84.62)
L4	3 (4.48)	0	0
Behavior			
B1	31 (43.06)	7 (31.82)	5 (38.46)
B2	13 (18.06)	6 (27.27)	2 (15.38)
B3	28 (38.89)	9 (40.91)	6 (46.15)

0.55, OR = 1.67; 95%CI: 0.14-19.32 and *P* = 0.39, OR = 2.92; 95%CI: 0.25-34.76 respectively) while CC genotype was not found in any of the patients.

Concerning rs11614913, again neither T allele nor TT genotype was found to be statistically associated with response to anti-TNF. Specifically, T allele was not found to be different between complete, partial, and non- responders (*P* = 0.11, OR = 2.7; 95%CI: 0.86-8.39 and *P* = 1, OR = 1.03; 95%CI: 0.27-3.91 respectively); similarly for TT genotype (*P* = 0.18, OR = 3.78; 95%CI: 0.8-17.73 and *P* = 0.34, OR = 2.83; 95%CI: 0.54-4.69 respectively).

No significant difference was found for rs188519172 as well, between complete, partial, and non- responders. G allele was not statistically different between these groups (*P* = 0.44, OR = 1.56, 95%CI: 0.56-4.36; and *P* = 0.75, OR = 0.73, 95%CI: 0.19-2.78 respectively) with GG genotype not being statistically different either (*P* = 0.61, OR = 1.78, 95%CI: 0.28-11.33; and *P* = 0.58, OR = 2.86, 95%CI: 0.35-15.05). The rs113054794 SNP of miR-221 was not detected at all in our population.

DISCUSSION

Recent studies highlight the emerging role of circulating microRNAs as potential biomarkers in the pathogenesis or response to treatment of cancer and autoimmune diseases[9-22]. In the era when personalized medicine becomes the ultimate goal, vigorous research is carried out towards identification of biomarkers able to predict the exact outcome a therapy may have to a specific patient, according to his unique genetic fingerprints. In IBD, until today, no marker has achieved to fully foresee how patients will respond to anti-TNF treatment, the most popular therapy, which though will be ineffective in one out of three patients during the first months of drug administration[3].

Recently, a study from Japan investigated serum miRNA expression in CD patients receiving induction therapy with infliximab. They concluded that, among others, miR-221 and miR-224 increased during induction therapy with infliximab in patients considered as responders[17]. Castro-Villegas *et al*[9] studied serum miRNA levels as possible biomarkers of response to 6-month anti-TNFα therapy in patients with rheumatoid arthritis and concluded that, among others, miR-146a increased after anti-TNF therapy in patients who responded. In addition, Bogunia-Kubik *et al*[23] have also recently assessed miR-146 expression along with its rs2910164 polymorphism and their possible connection to rheumatoid arthritis pathogenesis and therapeutic outcome after 3 mo of anti-TNF administration. Their results showed initially reduced miR-146 levels in patients compared to controls and restoration of these levels in patients receiving a 3 mo course of anti-TNF. Moreover they concluded that although rs2910164 variant could be associated with miR-146 levels after treatment, overall this genetic variant didn't influence neither predisposition to the disease nor efficacy of anti-TNF therapy, in accordance to our results.

This is the first to our knowledge study to examine the association of polymorphisms in either pre- or mature miRNAs with response to induction therapy with anti-TNF agents in patients with CD.

Our results showed that of the SNPs genotyped, rs2910164 of mir-146, rs11614913 of miR-196a, and rs188519172 of miR-224 had no statistically significant association to anti-TNF treatment response in Greek patients with CD. Moreover, rs113054794 SNP of miR-221 was not detected in our population, indicating that this polymorphism is probably highly rare in Caucasian populations. In accordance to our results, Nguyen-Dien *et al*[40] have also demonstrated this SNP's absence in a Caucasian population studied for correlation of miRNA variants and risk of breast cancer.

Response to anti-TNF therapy was assessed by

Table 2 Genotype and allele frequencies of rs2910164, rs11614913, and rs188519172 polymorphisms in Crohn's disease patients according to response to anti-TNF treatment

Genotype	Complete responders (n = 72)	Partial responders (n = 22)	P value; OR (95%CI)	Non-responders (n = 13)	P value; OR (95%CI)
miR-146a, rs2910164					
GG	70 (97.22)	21 (95.45)	1.0 (reference)	12 (92.31)	1.0 (reference)
GC	2 (2.78)	1 (4.54)	0.55; 1.67 (0.14-19.32)	1 (7.69)	0.39; 2.92 (0.25-34.76)
CC	0	0	-	0	-
miR-196a, rs11614913					
CC	33 (45.83)	5 (22.73)	1.0 (reference)	5 (38.46)	1.0 (reference)
CT	32 (44.45)	13 (59.09)	0.11; 2.7 (0.86-8.39)	5 (38.46)	1.0; 1.03 (0.27-3.91)
TT	7 (9.73)	4 (18.18)	0.18; 3.78 (0.8-17.73)	3 (23.08)	0.34; 2.83 (0.54-14.69)
miR-224, rs188519172					
AA	38 (52.78)	9 (40.9)	1.0 (reference)	7 (53.84)	1.0 (reference)
AG	29 (40.28)	11 (50)	0.44; 1.60 (0.59-4.37)	4 (30.76)	0.75; 0.75 (0.20-2.80
GG	5 (6.94)	2 (9.09)	0.62; 1.69 (0.28-10.16)	2 (15.38)	0.59; 2.17 (0.35-13.51)

clinical, serological and endoscopic markers. Endoscopy at the end of the study was performed to measure primary response to therapy with the most objective marker of treatment efficacy, which is mucosal healing[3].

MiR-146a has been demonstrated to be an integral part of the immunological responses observed in many autoimmune diseases through NF-κB pathway. Specifically, it participates in a negative feedback system induced by microbial constituents like LPS or other pro-inflammatory elements resulting in inhibition of protein production by specific genes. These genes were shown to be interleukin-1 receptor-associated kinase (IRAK) 1 and tumor necrosis factors receptor associated factor (TRAF) 6[24]. Moreover, miR-146a was shown to be overexpressed - upon nitric oxide(NO) trigger - in the nucleotide-binding oligomerization domain (NOD2) signaling pathway thus facilitating further activation of various inflammatory genes like IL-12, TNF-α, IL-6[28]. Both abovementioned mechanisms have been implicated in IBD pathogenesis[41,42] with NF-κB pathway actually being one of the targets of anti-inflammatory effects exerted by steroids and anti-TNF agents[41,43].

MiR-196a has been demonstrated to be related to IBD pathogenesis[29] and IBD phenotype[44] with the target molecular pattern through which it exerts its effect probably being related to autophagy. Specifically it has been reported to negatively regulate Immunity Related GTPase M (IGRM)[30], a gene that has been associated to IBD susceptibility[45]. In addition, its rs11614913 SNP has only recently been implicated in IBD related colorectal cancer progression[31] while its association to other forms of cancer, mainly colorectal, has already been established[46].

MiR-221 has been shown to mediate down regulation of ICAM-1 translation in human cholangiocytes with ICAM-1 playing a major role in regulation of a balanced inflammatory response in biliary cells. MiR-221 related ICAM-1 expression has also been implicated in T cells adhesion during local inflammation[33]. Furthermore, ICAM expression in human umbilical vein endothelial cells was demonstrated to be regulated by miR-221 in response to HIV again influencing monocytes adherence[47]. Zhao *et al*[48] have connected miR-221 to TLR4 mediated production of pro-inflammatory cytokines in lung cells with simultaneous increased TNFα and IL-6 expression through NF-κB signaling, a key pathway in IBD pathogenesis.

Apart from the aforementioned cell lines, miR-221 has also been studied in colonic epithelial cells with similar results. Fang *et al*[49] have shown that down regulation of miR-221 leads to amplification of experimental colitis and increase of TNFα in histological specimens. All the above highlight a plausible role of miR-221 in inflammatory response either through ICAM regulation, a molecule suggested to interfere with IBD inflammation facilitation[34,50,51], either through still unraveled mechanisms.

Last, miR-224 expression has been shown to be up-regulated in hepatocellular cancer patients with its possible target being apoptosis inhibitor-5 (API-5), thus mediating its role by inducing apoptosis[52]. Over expression of miR-224 has also been displayed in T cells of systematic lupus erythematosus patients again through suppression of API-5 leading to T cell apoptosis[53]. Interestingly, one report has presumed API5 involvement in IBD inflammation and progression to neoplasia, as quick epithelial cell turnover, cell proliferation and finally apoptosis are present in both these situations[54]. Moreover, Olaru *et al*[55] have proved involvement of miR-224 in down-regulation of p21, a tumor suppressor gene through which miR-224 coordinates neoplasia initiation and progression through dysplasia to IBD related colorectal cancer. MiR-224 is implicated in inflammatory pathways, also linked to IBD pathogenesis. Scisciani *et al*[56] reported p65/NF-κB to be a target pathway of miR-224 in liver cells while TNFα inflammatory pathway is activated with up-regulation of miR-224. Two other reports have demonstrated that SMAD4 is the target of miR-224 with SMAD4 being a pivotal component of TGF-β pathway leading to cell proliferation[35,57]. TGF-β pathway dysregulation has long been shown to be a contributor to IBD pathogenesis[58].

Hypothesizing that miRNAs regulating IBD susceptibility genes would be a logical initial thought, we chose to study SNPs of such miRNAs, such as miR-196a, and their association to anti-TNF response in patients with CD. Among many, we presumed that SNPs of miR-146a, miR-221, and miR-224 would additionally be ideal based on previous work showing alteration of their expression after anti-TNF treatment in patients with CD and rheumatoid arthritis[9,17,23]. However, even though anti-TNF treatment interferes in pathophysiologic pathways regulated by those miRNAs, no correlation was found. This is in agreement to what Lee *et al*[59] have concluded in a very recent genome-wide association study in CD. Their data support the idea of different genetic loci contributing in susceptibility compared to prognosis - and thus potential therapeutic interventions - in adult patients with CD. Interestingly, this had been already shown in pediatric patients with CD being treated with anti-TNF agents[60]. Furthermore, absence of studied SNPs' association to treatment response may denote that other SNPs or other transciptional (ex, methylation of gene promoters) and post-transcriptional mechanisms (concerning miRNA stability or processing) interfere with alterations in miRNA expression. In addition, in the only study conducted in IBD patients assessing miRNA expression alterations and anti-TNF response, the population studied was of a different ethnic group - Japanese-compared with ours. Ethnic and geographic differences representing distinct genetic and environmental background may influence frequency or even variety of polymorphisms detected.

We chose to include in our study patients receiving both infliximab or adalimumab. It has been established that their clinical and endoscopic efficacy is similar in Crohn's disease[61,62]. Nevertheless their different structure could have interfered with our results. Notwithstanding, with only 3 patients receiving adalimumab, we believe that our findings have only minimally been influenced.

Lastly, another factor contributing in our inability to show a positive association between studied SNPs and anti-TNF response may be that due to the small effect these variants may pose, we would require a larger sample to identify a statistically significant result. IBD genetic background has not been fully elucidated but we know that a variety of risk factors contribute with a small or modest effect and not one highly penetrant, suggesting a difficulty of uncovering this effect.

Nevertheless, we believe that miRNAs constitute a small but rather attractive pawn in our effort to delineate epigenetic regulation of gene expression and its contribution to IBD susceptibility, prognosis and therapeutic possibilities. Genetic markers may need to be used as biomarkers of therapy response in combination to other clinical or serological ones to attain the maximum benefit and accurately distinguish the ideal patient for each therapeutic treatment.

In conclusion, our results demonstrate for the first time that mir-146 rs2910164, miR-196a rs11614913, miR 221 rs113054794 and miR-224 rs188519172 are not correlated with anti-TNF treatment response in Caucasian patients with CD. Hence, they cannot be used as biomarkers to predict anti-TNF drug response in candidate patients with CD. Further independent studies are required to validate our findings in a larger scale or possibly to a different ethnic population.

COMMENTS

Background

Crohn's disease (CD) is a chronic debilitating disease related to poor quality of life, increased risk of surgery and prolonged hospital admissions. Anti-TNF drugs have revolutionized therapy by reducing but not eliminating complications. Biomarkers of anti-TNF therapy response constitute essential tools for physicians considering that one third of patients will not benefit by induction therapy as well as drugs' cost and side effects.

Research frontiers

Epigenetic alterations such as those exerted by microRNAs are now studied as contributors to pathogenesis and prognosis of many diseases including inflammatory bowel disease (IBD). MiRNA polymorphism associated response to therapy has been extensively studied in cancer with promising results though our knowledge about a possible association to anti-TNF treatment response in IBD is still limited.

Innovations and breakthroughs

This is the first study trying to unravel a correlation between microRNA polymorphisms and response to anti-TNF medication in Greek patients with CD. Expression of those miRNAs has been shown to correlate to anti-TNF response in rheumatoid arthritis and in IBD in Chinese population.

Applications

Studied polymorphisms of relevant miRNAs can be used as predictive markers for anti-TNF therapy response in patients with CD thus contributing to identification of ideal drug candidates for a costly treatment with potentially serious side effects.

Terminology

MicroRNAs are small non-coding RNA sequences of a few nucleotides exerting epigenetic regulation in gene expression.

Peer-review

The result of this study demonstrates for the first time that mir-146 rs2910164, miR-196a rs11614913, miR 221 rs113054794 and miR-224 rs188519172 are not correlated with anti-TNF treatment response in Caucasian patients with CD. Hence, these markers can be used as biomarkers to predict anti-TNF drug response in candidate patients with CD.

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ORIGINAL ARTICLE

Case Control Study

Protozoan parasites in irritable bowel syndrome: A case-control study

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Abstract

AIM

To investigate the putative role of protozoan parasites in the development of irritable bowel syndrome (IBS).

METHODS

The study included 109 IBS consecutive adult patients fulfilling the Rome Ⅲ criteria and 100 healthy control subjects. All study subjects filled a structured questionnaire, which covered demographic information and clinical data. Fresh stool samples were collected from patients and control subjects and processed within less than 2 h of collection. Iodine wet mounts and Trichrome stained smears prepared from fresh stool and sediment concentrate were microscopically examined for parasites. Blastocystis DNA was detected by polymerase chain reaction, and Cryptosporidium antigens were detected by ELISA.

RESULTS

A total of 109 IBS patients (31 males, 78 females) with a mean age ± SD of 27.25 ± 11.58 years (range: 16

-60 years) were enrolled in the study. The main IBS subtype based on the symptoms of these patients was constipation-predominant (88.7% of patients). A hundred healthy subjects (30 males, 70 females) with a mean ± SD age of 25.0 ± 9.13 years (range 18-66 years) were recruited as controls. In the IBS patients, Blastocystis DNA was detected in 25.7%, Cryptosporidium oocysts were observed in 9.2%, and Giardia cysts were observed in 11%. In the control subjects, Blastocystis, Cryptosporidium and Giardia were detected in 9%, 0%, and 1%, respectively. The difference in the presence of Blastocystis (P = 0.0034), Cryptosporidium (P = 0.0003), and Giardia (P = 0.0029) between IBS patients and controls was statistically significant by all methods used in this study.

CONCLUSION

Prevalence of Blastocystis, Cryptosporidium and Giardia is higher in IBS patients than in controls. These parasites are likely to have a role in the pathogenesis of IBS.

Key words: Blastocystis; Cryptosporidium; Protozoan parasites; Giardia; Irritable bowel syndrome

Core tip: A mounting body of evidence suggests that infections with protozoan parasites may be implicated in the pathogenesis of irritable bowel syndrome (IBS). However, previous studies from different geographic regions have yielded conflicting results. The present study investigates the infection rate of protozoan parasites in Jordanian IBS patients. Our results indicate that infection with Blastocystis, Cryptosporidium, and Giardia spp. may play a role in the pathogenesis of IBS in a significant proportion of patients. Testing for these parasites in cases of presumed IBS may offer new insights into the pathogenesis of IBS and thus improve its management.

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INTRODUCTION

Irritable bowel syndrome (IBS) is a common gastrointestinal disorder with a complex etiology and pathogenesis[1,2]. The cause of IBS is not well understood and a combination of genetic, physical, mental health problems and environmental factors have been proposed[3]. Triggers vary among patients, and they may include certain foods, hormones, medications, stress, or an acute episode of gastroenteritis[4,5].

Previous infections and persistent low-grade inflammation may play an important role in the pathogenesis of IBS[6]. Numerous IBS cases develop after acute enteric infection with different pathogens causing what is called post-infectious IBS (PI-IBS), where symptoms develop and persist in patients who had not met the Rome criteria before[7,8]. The PI-IBS symptoms have been reported in a significant number of patients following infections with bacteria and viruses[9]. The duration of diarrhea during enteritis has been identified as the strongest risk factor for subsequent IBS development[10], which might reflect the severity of the initial inflammation.

Blastocystis hominis is one of the most common human intestinal protozoa in both developing and developed countries[11,12]. It is common in patients with diarrhea-predominant-IBS[1,13,14], and its possible role in the pathogenesis of IBS has been suggested. Infections with this parasite might be responsible for bowel dysfunction due to the penetration of mucosal layer[14].

Cryptosporidium parvum (C. parvum) accounts for about 20% of diarrheal episodes in children in developing countries and up to 9% of episodes in developed countries[15]. Infections are usually characterized by self-limiting diarrhea (1-2 wk) with severe abdominal pain. However, the duration of clinical symptoms is highly dependent on the person's immunological status[16], and more severe and long lasting cases may develop in children, the elderly, and immunocopmrimsed individuals[12,17]. *Cryptosporidium* has been reported in IBS patients, with the onset of gastrointestinal symptoms after an acute episode of cryptosporidiosis despite recovery and parasite clearance[18,19]. Symptoms following *C. parvum* infection are similar to those described by IBS patients, suggesting that this parasite is a potential cause of PI-IBS.

The aim of this study was to evaluate the possible association of protozoan parasites with IBS. The detection of these pathogens in IBS cases may offer new insights into the pathogenesis and management of this complex disorder.

MATERIALS AND METHODS

Study subjects

The study included 109 IBS consecutive adult patients fulfilling the Rome Ⅲ criteria[5]. The patients were recruited at the gastroenterology outpatient clinics at King Abdullah University Hospital (KAUH) between September 2013 and April 2014.

The diagnosis of IBS was ascertained by an experienced gastroenterologist (KAJ). Patients who did not agree to sign an informed consent were excluded from the study. Other exclusion criteria were: Positive stool cultures for bacteria; steatorrhea; lactose intolerance; gluten sensitivity. Healthy subjects without current or previous gastrointestinal symptoms, attending the hospital during the same time period for routine examination or accompanying other patients were enrolled in the control group. All study participants gave written informed consent prior to study enrollment.

The study protocol was reviewed and approved by the Institutional Review Board at King Abdullah

University Hospital and by the Committee of Research on Human subjects at the Jordan University of Science and Technology.

Clinical samples

Fresh stool samples were collected from patients and control subjects. All eligible subjects filled a structured questionnaire, which covered demographic information, and clinical data (*e.g.*, IBS history; IBS subtype; previous bacterial, viral, and/or parasitic infection, postinfection treatment and any antibiotic therapy received in the three months prior to enrollement).

Stool processing

Stool samples were processed within less than 2 h of collection. Each sample was divided into two portions before processing. One portion was immediately processed for protozoan parasites and the second portion was kept refrigerated in Eppendorf tube preserved in absolute ethanol (ratio 3:1) for antigen and Polymerase Chain Reaction (PCR) testing.

Microscopy

Collecting multiple stool samples per subject was not feasible. Therefore, as an alternative, a comprehensive examination of a single fresh stool sample from each subject was done using several laboratory techniques.

For each sample, normal saline wet mounts and Lugol's iodine wet mounts were microscopically examined to initially observe parasites. To maximize recovery of different parasites stages, all stool samples were concentrated using the formalin-ether acetate sedimentation technique (FEA)[20], which is the Center for Disease Control (CDC) recommended stool concentration method for clinical laboratories. Iodine wet mounts; a trichrome stained smears and modified Kinyoun acid-fast stained smears were prepared from the concentrates. Slides were thoroughly screened (200 to 300 fields) for parasites by an experienced parasitologist using 40 × and 100 × oil immersion objectives, and results were recorded.

Detection of Cryptosporidium antigen by ELISA

All stool samples were tested using an FDA-approved RIDASCREEN® *Cryptosporidium* ELISA kit (R-Biopharm AG, Darmstadt, Germany) following the manufacturer's instructions. This immunoassay is designed for the qualitative detection of *C. parvum* and *C. hominis* antigens in human stool samples.

Detection of blastocystis spp. by polymerase chain reaction

Genomic DNA was extracted from stool samples using QIAamp® Fast DNA Stool Mini Kit (Qiagen, Germany) following the manufacturer's instructions. DNA concentration and purity were measured using NanoDrop microvolume UV-Vis spectrophotometer (Thermo Scientific, United States), and DNA was stored at -20 ℃ for later testing. The primers used were F1 (5'-GGAGGTAGT GACAATAAATC-3') and BHCR seq3 (5'-TAAGACTACGAG GGT ATCTA-3') (Integrated DNA Technology, Coralville, United States) targeting the small subunit ribosomal RNA (SSU rRNA) gene of *Blastocystis* spp.[21]. Each PCR reaction contained 12.5 μL of GoTaq® Green Master Mix solution containing *Taq* DNA polymerase, dNTPs, $MgCl_2$ and reaction buffers (Promega, United States), 0.5 μL of each primer, 5 μL of sample genomic DNA extract, and 5.5 μL of nuclease free water in a total volume of 25 μL. All PCR runs included a positive control containing the same components with a *Blastocystis hominis* suspension as DNA template (Microbiologics, United States), and a negative control containing the same components, but without DNA.

PCR amplification was performed using a Perkin-Elmer GeneAmp 9600 thermal cycler. PCR amplification conditions were: Denaturation at 95 ℃ for 7 min, 35 cycles each consisting of denaturation 94 ℃ for 60 s, annealing at 56 ℃ for 45 s, followed by a final extension step at 72 ℃ for 7 min. PCR products and 100 bp DNA ladder (Promega, United States) were loaded into 2% agarose gel, in TBE buffer, and bands were visualized under UV transluminator after staining with ethidium bromide. Positive samples for *Blastocystis* spp. had the DNA bands of 550-585 bp.

Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS, version 19, Chicago, IL). Results are expressed as mean ± SD for continuous variables (*e.g.,* age), and proportions and percentages for categorical data (*e.g.,* sex). Fisher's exact test was used in the analysis of nominal variables to determine the significance of differences in parasite infection rates between the patients and the control subjects. A *P*-value $\leq$ 0.05 was considered statistically significant. All *P*-values were two sided.

RESULTS

A total of 109 patients (31 males, 78 females) with a mean age ± SD of 27.25 ± 11.58 years (range: 16 -60 years) were enrolled in the study. A hundred healthy subjects (30 males, 70 females) with a mean ± SD age of 25.0 ± 9.13 years (range 18 - 66 years) were recruited as controls.The two groups were comparable in terms of age, sex and other demographic characteristics.

Microscopy of iodine wet mounts of fresh stool

In the IBS cases, *Blastocystis* cyst-like form in moderate to many in numbers was observed in 16 (14.7%), *Cryptosporidium* oocysts were observed in 10 (9.2%) and *Giardia lamblia* cysts were observed in 9 (8.3%) (Table 1). Nonpathogenic *Entamoeba hartmanni* and *Endolimax nana* were each observed in 3 (2.8%) samples. Co-infections with 2 parasites were observed in 2 (1.8%) samples, one had *Entamoeba hartmanni* with *Endolimax nana,* and the second had *Blastocystis* spp. with *Endo-*

Table 1 Parasites observed in iodine wet mounts of cases and controls before and after concentration *n* (%)

Parasites observed	Un concentrates		*P* value	FEA concentrate		*P* value
	IBS	Controls		IBS	Controls	
Blastocystis spp.	16 (14.7)	3 (3.0)	0.0034[a]	12 (11.0)	2 (2.0)	0.0112[a]
Cryptosporidium spp.	10 (9.2)	0 (0.0)	0.0017[a]	10 (9.2)	0 (0.0)	0.0017[a]
Giardia lamblia	9 (8.3)	1 (1.0)	0.0197[a]	12 (11.0)	1 (1.0)	0.0029[a]
Endolimax nana	3 (2.8)	1 (1.0)	0.6228	3 (2.8)	1 (1.0)	0.6228
Entamoeba hartmanni	3 (2.8)	0 (0.0)	0.2477	5 (4.6)	0 (0.0)	0.2477
Entamoeba histolytica/Entamoeba dipar	0 (0.0)	0 (0.0)	1	0 (0.0)	0 (0.0)	1
Co-infections with 2 parasites	2 (1.8)	1 (1.0)	1	2 (1.8)	1 (1.0)	1

[a]*P*-value of ⩽ 0.05 is statistically significant by Fisher's test. IBS: Irritable bowel syndrome; FEA: Formalin ethyl acetate concentration technique.

Table 2 *Blastocystis* and *Cryptosporidium* spp. in irritable bowel syndrome patients and controls as detected by all methods *n* (%)

Test	*Blastocystis* spp.			*Cryptosporidium* spp.		
	IBS	Controls	*P* value	IBS	Controls	*P* value
Iodine wet mounts	14 (12.8)	3 (3.0)	0.0106[a]	10 (9.2)	0 (0.0)	0.0017[a]
FEA	12 (11.0)	2 (2.0)	0.0112[a]	12 (11.0)	0 (0.0)	0.0003[a]
Trichrome stain	14 (12.8)	2 (2.0)	0.0034[a]	12 (11.0)	0 (0.0)	0.0003[a]
PCR	28 (25.7)	9 (9.0)	0.0019[a]	-	-	-
Acid fast stain	-	-	-	14 (12.8)	0 (0.0)	0.0001[a]
ELISA	ND	ND	-	6 (5.5)	1 (1.0)	0.1211

[a]*P*-value of ⩽ 0.05 was considered statistically significant. FEA: Formalin ethyl acetate concentration technique; IBS: Irritable bowel syndrome; ELISA: Enzyme-linked immunosorbent assay; ND: Not detected.

limax nana.

In the 100 control samples, *B. hominis* was observed in 3 (3%) samples, *Cryptosporidium* in 0 (0%) samples, and *Giardia lamblia* cyst in 1 (1%) sample. Nonpathogenic *Endolimax nana* cyst was observed in 1 (1%) sample (Table 2).

FEA

In the iodine wet mount preparations of the sediment of IBS patients, *Blastocystis* was observed in 12 (11.0%) samples, *Cryptosporidium* was observed in 10 (9.2%) samples, and *Giradia* cysts in 12 (11.0%) samples. Other nonpathogenic amoebas were also observed (Table 1).

In the trichrome stained smears of the sediment of IBS patients, *Blastocystis* was observed in 14 (12.8%), *Cryptosporidium* oocysts appeared lightly stained or unstained in 12 (11.0%), and *Giardia* cysts were observed in 12 (11%) (Table 2).

In the control subjects, *Blastocystis*, *Cryptosporidium* and *Giardia* were observed in 2 (2%), 0 (0%), and 1 (1%), respectively. In the IBS patients acid fast stain of the sediment, *Cryptosporidium* was observed in 14 (12.8%) compared to none (0%) in the control samples.

Detection of Cryptosporidium antigen by ELISA

In the IBS samples, 6 (5.5%) were positive for *Cryptosporidium* compared to only 1 (1.0%) positive in the controls (Table 2).

Detection of Blastocystis DNA by PCR

The specific DNA band was observed in 28 (25.7%) of the patients, compared to 9 (9%) of the controls (Figure 1). Comparing all methods used for the detection of *Blastocystis* (Table 2), PCR was more sensitive than the other techniques used. There was a significant difference between the cases and controls in all methods used: Iodine wet mount (P = 0.0106), FEA (P = 0.0112), the trichrome stain (P = 0034), and PCR (P = 0.0019).

Four of five tests used were consistent in the detection of the *Cryptosporidium* spp. and showed significant difference between the IBS patients and the controls (P values 0.0001 and 0.0017, respectively) (Table 2). Four IBS samples were positive for *Blastocystis* by all methods tested, and one sample was also positive for *Cryptosporidium* by ELISA.

The positive results of both parasites were associated with patients having more episodes of abdominal pain and discomfort or bloating than others. The main IBS subtype based on the symptoms of these patients was constipation-predominant IBS-C (88.7% of patients). Furthermore, stool samples were tested for bacterial pathogens (unpublished data), and patients having positive results were excluded from the study.

DISCUSSION

In this study, we investigated the prevalence of protozoan parasites in stool samples of IBS patients and healthy controls. Our results suggest that the occurrence of *Blastocystis, Cryptosporidium,* and *Giardia* spp. may have a role in the pathogenesis of IBS in a significant proportion of patients.

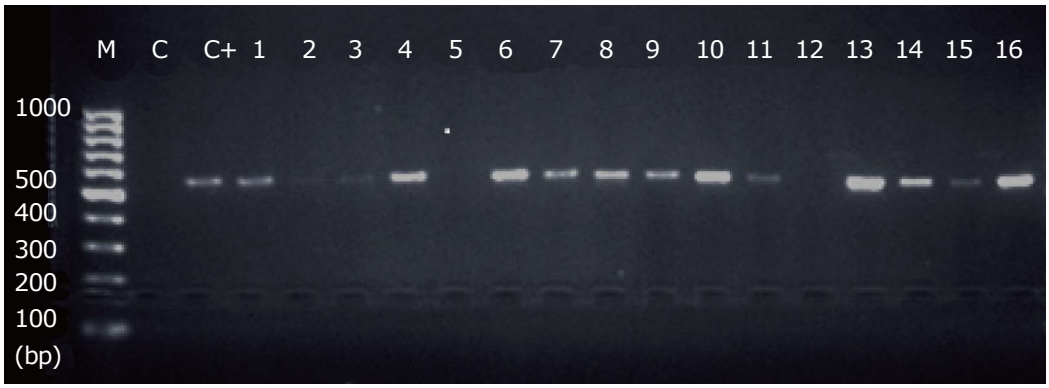

Figure 1 Ethidium bromide stained 2% agarose gel showing polymerase chain reaction products of the small subunit ribosomal RNA of *Blastocystis*.

The presence of *Blastocystis* in 25.6% IBS patients was notably different (P = 0.0019) from the controls (9%), as detected by PCR (Table 2). These results are in agreement with previous studies, which reported higher infection rates of *B. hominis* in stool samples of IBS patients compared to controls[22,23]. Of note, PCR was reported to be superior over other techniques in the detection of *Blastocystis* in clinical samples[21].

Blastocystis has been reported as the most common intestinal parasite among both healthy and immuno-compromised individuals[12], and highly prevalent in patients with acute and chronic diarrhea. In addition, a higher prevalence of *Blastocystis* spp. among IBS patients was reported as compared to patients suffering from other gastrointestinal disorders or healthy individuals[22,24]. In their epidemiological study, Giacometti *et al*[25] reported that *Blastocystis* was significantly present in IBS patients, suggesting a possible link between this parasite and IBS. Studies from the Middle East, Pakistan, Mexico and Europe[13,16,22,26,27] suggested a pathogenic role of *Blastocystis* in the etiology of IBS. However, other studies failed to demonstrate an association between *Blastocystis* and IBS[24,28]. A possible explanation for the differences in reporting could be the small number of IBS patients studied or the different diagnostic methods used. The multifactorial etiology of IBS, including microbiological factors, genetic, and environmental factors may also explain this discrepancy[23]. *Blastocystis* spp. ability to induce pathophysiological disturbances such as host cell apoptosis, the modulation of host immune response, toxic-allergic reactions leading to a nonspecific inflammation of the colonic mucosa has been linked to the pathogenesis of IBS[29,30]. It is well recognized that *B. hominis* is the single species infecting humans. However genetic analysis demonstrated antigenic heterogeneity, with nine different *Blastocystis* types that can infect humans, suggesting that virulent and avirulent species exist[24].

The detection of *Cryptosporidium* in 12.8% of IBS patients by the acid fast staining as compared to none detected in the control samples by any of the four methods used was statistically significant (Table 2). The relative prevalence of *Cryptosporidium* spp. reported in several developed countries in outbreak and non outbreak settings ranges from 0.1% to 9.1% of clinical cases[11].

Similar to bacterial or other protozoan infections in humans, *Cryptosporidium* may trigger PI-IBS-like symptoms[19]. A study of immunocompetent rats infected with *Cryptosporidium parvum* oocysts suggested that cryptosporidiosis results in increased jejunal sensitivity to distension. This infection model mimicked some features of IBS patients, who report visceral pain as a major symptom and increased sensitivity to gut distension. The results of that study allow speculation that cryptosporidiosis may be a potential etiologic factor of IBS and warrant further studies of post cryptosporidiosis bowel disturbances[30]. In addition, an association between *C. parvum* with IBS was suggested in a study of immunocompetent suckling rat similar to PI-IBS features supported by the longterm pathological changes triggered by this parasite in the intestine[31]. Infections in these rats resulted in jejunal hypersensitivity to distension that was associated with accumulation of activated mast cells at 50 d post-infection[31]. These experimental results are consistent with the observation that diarrhea-predominant IBS patients have a marked increase in mast cell numbers and higher tryptase concentrations in jejunal mucosa[32].

Additionally, our results are in agreement with a previous study[33] that reported the permanent acid fast staining being more sensitive than iodine-stained wet mounts and ELISA for the detection of *Cryptosporidium*. In fact, acid fast staining is still considered the "gold standard" method routinely used for the detection of this parasite, although it may fail to detect infections in some symptomatic patients.

The presence of *Giardia lamblia* in IBS patients (8.3%) was also statistically significant in both concentrated and unconcentrated stool sediments (P = 0.0029 and 0.0197, respectively) (Table 2). The relative prevalence of *Giardia intestinalis/lamblia* in several developed countries in outbreak and other settings ranges from 0.2% to 29.2%[12]. Although most *Giardia* infections are self-limiting, chronic infections and re-infections can occur. A previous study reported *Giardia* in 5%-10% of IBS patients and provided evidence of potential cause of chronic PI-IBS symptoms[34]. High frequency of microscopic duodenal inflammation was

found in post-giardiasis patients when the infection lasted 2-4 mo, indicating that longer duration of infection is a risk factor for PI-IBS[38]. However, the cause of the PI-IBS clinical manifestations due to *Giardia*, even after complete elimination of the parasite, remains unexplained.

Parasitic infections, and in particular *Blastocystis hominis* infection have been reported to be common in diarrhea-predominant IBS (IBS-D), suggesting a role for these parasites in the pathogenesis of IBS-D[1,13,14]. Of interest, *Blastocystis hominis, Cryptosporidium, and Giardia spp* were common also in our study patients, composed mainly of IBS-C subtype. Based on our results, we speculate that these parasites may play a role in the pathogenesis of IBS regardless of the subtype.

In conclusion, the significant presence of the protozoan parasites *Blastocystis, Cryptosporidium, and Giardia spp* in stool samples of IBS patients suggests a pathogenetic link between these parasites and IBS. Therefore, we recommend the inclusion of parasitological tests in the diagnostic work up for presumed IBS patients. Early diagnosis and treatment of these infections may shorten the duration of the infection and help reduce the risk of development of IBS. Further studies with a larger sample size and studies from epidemiologically different countries should provide more insight into the role of parasitic infections in the pathogenesis of IBS.

COMMENTS

Background

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder of uncertain etiology and multifaceted pathogenesis. Protozoa, such as *Blastocystis, Cryptosporidium, and Giardia spp*, are common enteric parasites and their carriage is believed to be linked to IBS.

Research frontiers

There were several early published studies of the possible link between parasitic infections and the development of IBS symptoms. However, many of these studies have yielded inconsistent or even contradictory results.

Innovations and breakthroughs

The present study provides new pathophysiological insights into the role of protozoan parasites in IBS. Namely, *Blastocystis, Cryptosporidium,* and *Giardia spp* have been found to play a role in the pathogenesis of IBS in a significant proportion of patients.

Applications

Investigating the role of protozoan infections in the pathophysiology of IBS is important in clinical practice, especially in light of the opportunity of developing targeted therapies. Future research should focus on the subset of IBS patients with poor response to conventional therapy, and possibly offer newer, more effective treatment options.

Terminology

Irritable bowel syndrome is a functional gastrointestinal disorder characterized by a complex pathogenesis, and various clinical manifestations. Protozoan parasites are a group of unicellular, eukaryotic microorganisms, which can infect both humans and animals; they include ciliates, amoebae, and flagellates.

Peer-review

The authors compared clinical data and test results of protozoan parasites for stool samples between in 109 patients with IBS and in 100 control individuals, demonstrated that prevalence of Blastocytosis, Cryptosporidium and Giardia is higher in OBS patients than in controls, and concluded that these parasites were likely to have a role in the pathogenesis of IBS. The paper is well-written and has interesting findings.

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LETTERS TO THE EDITOR

Fecal microbiota transplantation against irritable bowel syndrome? Rigorous randomized clinical trials are required

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Abstract

Halkjær *et al* searched systematically nine articles including 48 patients, and concluded that fecal microbiota transplantation (FMT) can be an ideal treatment option for irritable bowel syndrome (IBS) subjects. Regardless of the few successes in current traditional therapies (change in diet, herbal medicine and antibiotics) in IBS, a sharp increase in interests in the FMT option has been reported in the current century. However, there is a long list of unclear issues concerning the application of FMT for the treatment of IBS. Route of delivery and optimum dosage are the major concerns to consider before using in clinical practice.

Key words: Fecal microbiota transplantation; Irritable bowel syndrome; Microbiota; Dysbiosis

Core tip: Apart from the interesting report by Halkjær *et al*, there is a long list of tasks concerning the application of fecal microbiota transplantation for the treatment of irritable bowel syndrome. Route of delivery and optimum dosage are the major concerns to consider before using in clinical practice.

Abadi ATB. Fecal microbiota transplantation against irritable bowel syndrome? Rigorous randomized clinical trials are required. *World J Gastrointest Pharmacol Therap* 2017; 8(4): 208-209 Available from: URL: http://www.wjgnet.com/2150-5349/full/v8/i4/208.htm DOI: http://dx.doi.org/10.4292/wjgpt.v8.i4.208

TO THE EDITOR

I read with interest the article, "Can fecal microbiota transplantation cure irritable bowel syndrome?"[1]. Halkjær *et al*[1] searched systematically nine articles including 48 patients, and concluded that fecal microbiota transplantation (FMT) can be an ideal treatment option

for irritable bowel syndrome (IBS) subjects. Regardless of the few successes in current traditional therapies (change in diet, herbal medicine and antibiotics) in IBS, a sharp increase in interests in FMT option has been reported in the current century. The authors mentioned most of the important findings, but I have some concerns on their interesting paper.

First, the etiology of IBS cannot be elucidated by a unique mechanism, thus many of these unknown involved items are acting without examination by the clinicians and microbiologists. However, in order to provide a better therapeutic approach, we need to determine the actual impact on those uninvestigated factors[2,3]. Second, although Halkjær *et al*[1] found no adverse effects of all of the included studies, statistically it may be a result of weak sampling (48 patients). This lack can be compensated by further research using larger sample size to present statistically significant results. Third, the current data is not sufficient for recommending FMT as the cure of IBS, at least based on the available evidence provided by this review[1]. In the near future, more research, including controlled and randomized trials, are necessary, which can likely answer those questions.

According to the current study, FMT is able to affect therapy of IBS, at least based on the 48 subjects investigated. As a note, we are still unaware of the exact mechanistic collaboration occurring between host cells and the microbiota. Of course, new evidence describing this question can shed promising light on the better application of FMT, not only against IBS but also for other important gastric clinical disorders (*Clostridium difficile* and *Helicobacter pylori*)[4,5]. Once again, I should appreciate the paper by Halkjær *et al* since it invites the attentions to using FMT as a new approach to treating any of the gastroduodenal disorders.

Indeed, there is a long list of unclear subjects concerning the application of FMT for the treatment of IBS. Route of delivery and optimum dosage are the major concerns to consider before using in clinical practice.

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