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MINIREVIEWS

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ABOUT COVER

Editorial Board Member of *World Journal of Experimental Medicine*, Dr. James Tarbox is an a board-certified Allergist & Immunologist at Texas Tech University Health Sciences Center in Lubbock, TX (United States), where he received his medical degree. He completed an Internal Medicine internship and residency at Cleveland Clinic in Cleveland, OH, being awarded Senior Resident of the Year, and an Allergy & Immunology fellowship at Washington University in St. Louis, MO. He was promoted to Associate Professor in 2018. Dr. Tarbox's clinical and research interests include clinical immunology, primary immunodeficiency, asthma, food allergy, and urticaria/angioedema, immunological pathways, medication delivery, and diagnostic evaluation. Since joining the editorial board of the *World Journal of Experimental Medicine* in 2019, he has contributed his expert knowledge to the review of articles related to allergy and atopic disease. (L-Editor: Filipodia)

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MINIREVIEWS

Role of diet and nutrition in inflammatory bowel disease

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Abstract

Inflammatory bowel diseases (IBDs) are closely linked to nutrition. The latest research indicates that diet and nutrition are significantly involved in the etiopathogenesis of the disease, although their specific role throughout its clinical course still remains unclear. This study reviewed how diet and nutrition are associated with IBD development and management. Even though specific diets have been shown to bring about positive outcomes, there is currently no scientific consensus regarding an appropriate diet that would benefit all IBD patients. We suggest that individualized dietary recommendations are of the greatest importance and that diets should be planned to provide individual IBD patients with specific nutrient requirements while keeping all the clinical aspects of the patients in mind. Further research is clearly necessary to investigate nutritional factors involved in IBD development and, especially, to evaluate the applications of the diets during the course of the disease.

Key Words: Crohn's disease; Ulcerative colitis; Inflammatory bowel disease; Diet; Nutrition

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Core Tip: Although inflammatory bowel disease (IBD) affects the gastrointestinal tract, the role of diet in the course of disease is often underestimated. Many studies have assessed the effect of diet in the risk of developing IBD, and the importance of nutrition in the etiopathogenesis of IBD was confirmed by the fast increase in its incidence and prevalence over the last two decades. We discuss the role of diet and nutrition in the etiology and management of IBD based on the data provided in the literature and set out an agenda for future research.

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INTRODUCTION

Inflammatory bowel diseases (IBDs) are characterized by chronic and relapsing inflammation of different segments in the gastrointestinal tract. The etiology is not yet fully understood and the course of the disease is characterized by periods of exacerbation and remission. A multifactorial etiology has been confirmed. An interaction between environmental factors and gut microbiota in genetically susceptible individuals may cause a dysregulation of both the innate and adaptive immune responses[1-5]. The environmental factors include stress, pollution, breastfeeding, smoking, use of antibiotics, chemical products and diet[6]. Some of these risk factors are potentially reversible, such as smoking, use of antibiotics and diet[7].

Numerous studies point to the benefits of breastfeeding in the field of medical and public health practice, and in IBD development[8,9]. A meta-analysis performed on 17 relevant articles that examined whether breastfeeding may protect against ulcerative colitis (UC) and Crohn's disease (CD) showed heterogeneous results that nonetheless support the hypothesis that breastfeeding is associated with lower risks of IBD development[10]. In another and more recent systematic review with meta-analysis, it was established that breastfeeding duration had a dose-dependent association, with the strongest decrease in risk for CD and UC occurring when breastfeeding lasted for at least 12 mo when compared to 3 or 6 mo, which confirmed the protective effect of breastfeeding on the development of IBD[11].

These protective effects are related to the antibodies (SIgA and SIgM), cytokines, immune cells, growth factors and high concentrations of oligosaccharides provided and released by breast milk and its components. These factors seem to provide defense and promote the production of bacteria that benefit neonatal intestinal microbiota, thus improving innate mucosal immunity development[12-14]. Therefore, breastfeeding, especially in families affected by IBD, should be encouraged for all the beneficial effects already mentioned in the literature[8-10].

Although IBD affects the gastrointestinal tract, the role of diet in the course of disease is often underestimated. Several studies have evaluated the effect of diet on the risk of developing IBD. The importance of nutrition in the etiopathogenesis of IBDs was confirmed by the fast increase in the incidence and prevalence of such diseases in the last two decades in populations with a previously low incidence[7].

There was also a steady increase in the incidence and prevalence of CD in the developed world, particularly in Australia and Europe. These changes can be partially explained by the recent higher awareness and better diagnostics for IBD. However, this increase may also be related to the higher degree of Westernization, since diet is one of the key factors in the initiation, duration and treatment of the disease[15,16].

Some studies point to the association of the incidence of IBD with dietary excess or even a deficit of several nutrients. Additionally, dietary components are involved in dysbiosis on the intestinal mucosa, which can become thinner and more permeable to pathogens and antigens, leading to a low-grade, but persistent inflammation[17]. IBD is associated with intestinal dysbiosis, which is characterized by a generalized alteration in the diversity and abundance of bacterial species[18-20].

Regarding nutritional status, malnutrition was historically present mainly during periods of exacerbation of the disease, and malnourished IBD patients must be treated

properly, as they are more likely to have a worse prognosis, complication rates, mortality and quality of life[7]. However, several studies have indicated an increase in the prevalence of overweight and obesity - predominantly in remission patients[21]. We have demonstrated that in CD patients, 55% and 28% of those in remission and with active disease were overweight or obese[22], respectively, which suggests that these patients are currently receiving more effective treatments. This allows them to maintain the same behavior as the rest of the population, which typically follows a sedentary lifestyle and consumes a hypercaloric diet.

Obesity has also been associated with the risk of developing IBD. The relevant factors include epigenetic changes observed in both obesity and IBD patients, changes in the gut microbiome and high levels of intestinal inflammation[23,24].

Thus, further insight into the role of diet in the pathophysiology of IBD may help to identify preventive or therapeutic targets, and improve a patient's quality of life. Our aim is to review the literature on the role of diet and nutrition in the etiology and management of IBD and set the agenda for future research.

NUTRITIONAL FACTORS AND IBD

There is currently no consensus in the medical community regarding nutritional guidelines for adult IBD patients. The lack of randomized controlled trials testing the specific diets and dietary patterns make it impossible to make strong recommendations[25]. An exception is exclusive enteral nutrition (EEN), which is recommended as first-line therapy for children and adolescents with acute active CD in order to induce remission[7,26]. This lack of consensus is due to limited available research data. The fact that the exact action mechanism of these diets is not completely understood, in addition to the different effects caused by differences in the gastrointestinal physiology of each patient, make it impossible to formulate a guideline. The European Society for Clinical Nutrition and Metabolism (ESPEN) guideline recommends that no specific diet be followed during remission phases, as it does not seem to be effective in obtaining remission[7].

Several compounds in the diet that influence the development and maintenance of IBD have been identified, while others seem to play a protective role. In this review we will address the potential of these dietary compounds in IBD treatment based on recent literature, and will also describe the differences between UC and CD in relation to these compounds. Figure 1 shows a schematic representation of the dietetic components that influence IBD.

Macronutrients

Recently, two dose-response meta-analyses found no evidence of an association between macronutrients and IBD risk[27,28]. However, a higher intake of fiber may play a protective role in CD development, while in sugar subtypes, high sucrose intake was linked to the risk of UC and CD[27,28]. Despite the inconclusive association between the macronutrients and IBD risk demonstrated by the two meta-analyses, studies have shown that high animal fat and cholesterol intake is associated with UC risk, and that a long-term intake of fast food, which is rich in fats and sugars, is a risk factor for CD[29,30]. The high intake of saturated fats and monosaccharides and a low intake of fiber are linked with increased risk of CD development[31,32]. The EpiCom cohort study demonstrated that daily fast food and high sugar consumption were associated with earlier onset of IBD, as well as an increased risk of disease severity and surgery in UC[30]. Although diets high in fructose are associated with metabolic diseases[33], a negative association was found between fructose intake and IBD risk[29].

Diet is an important factor influencing the composition of gut microbiota and changes in bacterial species[30]. Martinez-Medina and collaborators demonstrated in 2014 that a high-fat and high-sugar diet can lead to dysbiosis, with an increase in abundance of *Bacteroides* spp. and *Ruminococcus torques* in mice. Thus, new studies have pointed out that the westernization of the diet alters the microbiota to a composition that increases the risk of developing IBD[34,35].

In addition, consumption of linoleic acid, a dietary n-6 polyunsaturated fatty acid (PUFA) has been associated with an increased risk of UC, while high intake of n-3 PUFAs is associated with a reduced incidence of UC[36,37]. Belluzzi *et al*[38] (1996) tested a new formulation of n-3 PUFAs, with the mixture of 45% eicosapentaenoic acid (EPA) and 20% docosahexaenoic acid (DHA) in enteric-coated capsules, in 78 CD patients in remission but with high risk of relapse. After 1 year of treatment, 59% of patients who were treated with the new formulation of n-3 PUFAs were still in remission, compared

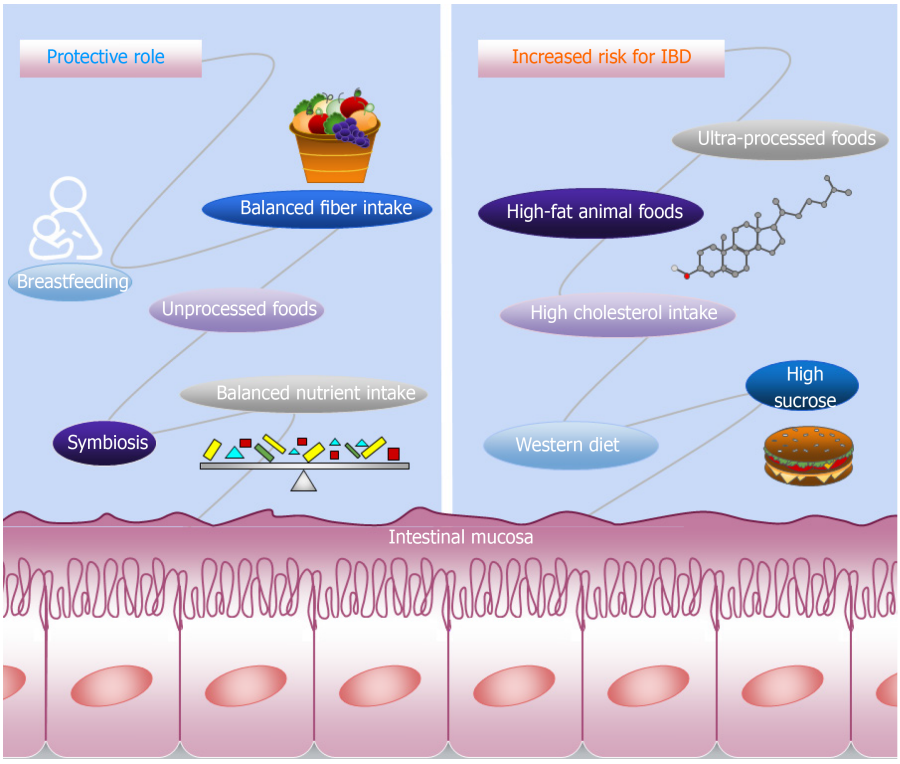


Figure 1 Influence of dietary compounds on inflammatory bowel disease. IBD: Inflammatory bowel disease.

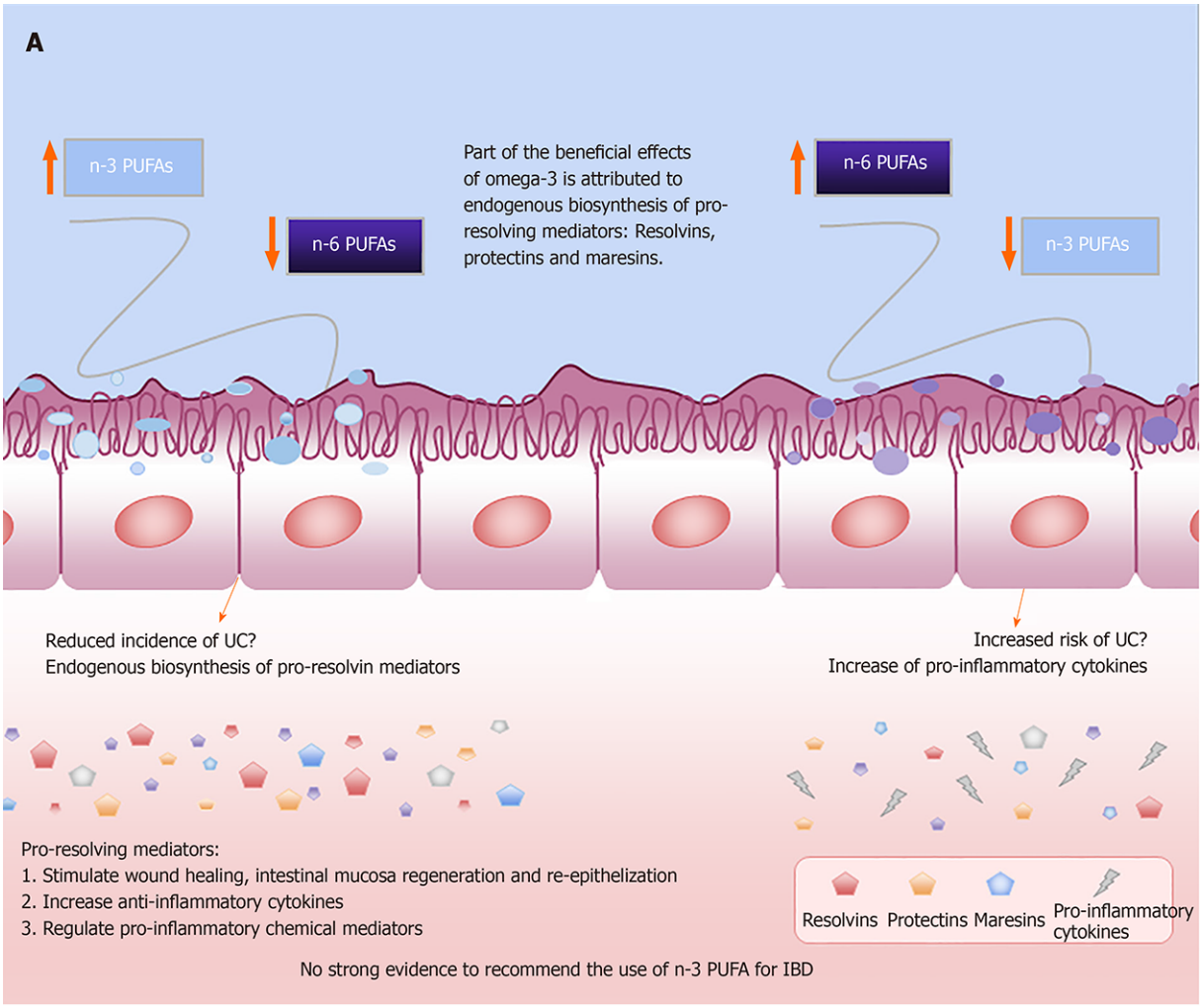
to only 26% in the placebo group and logistic regression analysis indicated that only n-3 PUFAs treatment reduced the likelihood of relapse. On the other hand, another study treated 700 CD patients with a similar preparation of n-3 PUFAs and this did not reduce the relapse rate in CD patients between groups[39]. This result can be explained by (1) the varied clinical inclusion criteria used to identify patients at high risk of relapse and (2) the inadequacy of the different therapies instituted for these patients[39,40]. A systematic review analyzing the effect of n-3 PUFAs in six studies in 1039 patients with CD did not rule out a possible beneficial effect of n-3 PUFAs in these patients[40].

Consequently, although we still have conflicting literature on whether or not to indicate omega-3 for patients with IBD, it seems that these divergent results are related to the different design of clinical trials and most likely due to the different n-6/n-3 ratio adopted in these studies. Indeed, the Western diet often results in the important disequilibrium in the n-6/n-3 PUFAs ratio, which can reach up to 20:1. Such an imbalance represents a pro-inflammatory stimulus that can affect the onset of many underlying conditions, including IBD[41-43]. The recommendation for the ratio of omega-3 to omega-6 is 5:1 according to the ESPEN guideline[44].

Furthermore, some components of n-3 PUFAs, EPA and DHA have been identified in the past decades and are related in processes of the anti-inflammatory effects of n-3 PUFAs, such as the inhibition of genes that lead to inflammatory processes and in the control of immunological and inflammatory responses. It is now known that part of the beneficial effects attributed to omega-3 in different diseases related to inflammatory disorders is attributed to endogenous biosynthesis of resolvins, protectins and maresins, as pro-resolving mediators[41,42].

These n-3 PUFAs-derived anti-inflammatory molecules can counteract and regulate pro-inflammatory chemical mediators; increase anti-inflammatory cytokines; and stimulate wound healing, intestinal mucosa regeneration and re-epithelialization. This may offer a fascinating new complementary approach to IBD treatment and its effects are currently being investigated by different research groups[40]. Despite its metabolic performance, the existing data are insufficient to justify the recommended use of n-3 PUFAs for UC and CD in clinical practice as well as for maintenance of remission in these patients. This was highlighted by the guideline for clinical nutrition in IBD[7,40,44], although it looks promising. Figure 2A describes the effects of PUFAs on IBD.

Taken together, these studies indicate that the primary factor to consider for IBD dietary recommendation is the quality of macronutrients (mainly for simple carbohydrates and animal fat) rather than the amount itself. According to the ESPEN


A
n-3 PUFAs
n-6 PUFAs
Part of the beneficial effects of omega-3 is attributed to endogenous biosynthesis of pro-resolving mediators: Resolvins, protectins and maresins.
n-6 PUFAs
n-3 PUFAs
Reduced incidence of UC?
Endogenous biosynthesis of pro-resolvin mediators
Increased risk of UC?
Increase of pro-inflammatory cytokines
Pro-resolving mediators:
1. Stimulate wound healing, intestinal mucosa regeneration and re-epithelization
2. Increase anti-inflammatory cytokines
3. Regulate pro-inflammatory chemical mediators
Resolvins
Protectins
Maresins
Pro-inflammatory cytokines
No strong evidence to recommend the use of n-3 PUFA for IBD

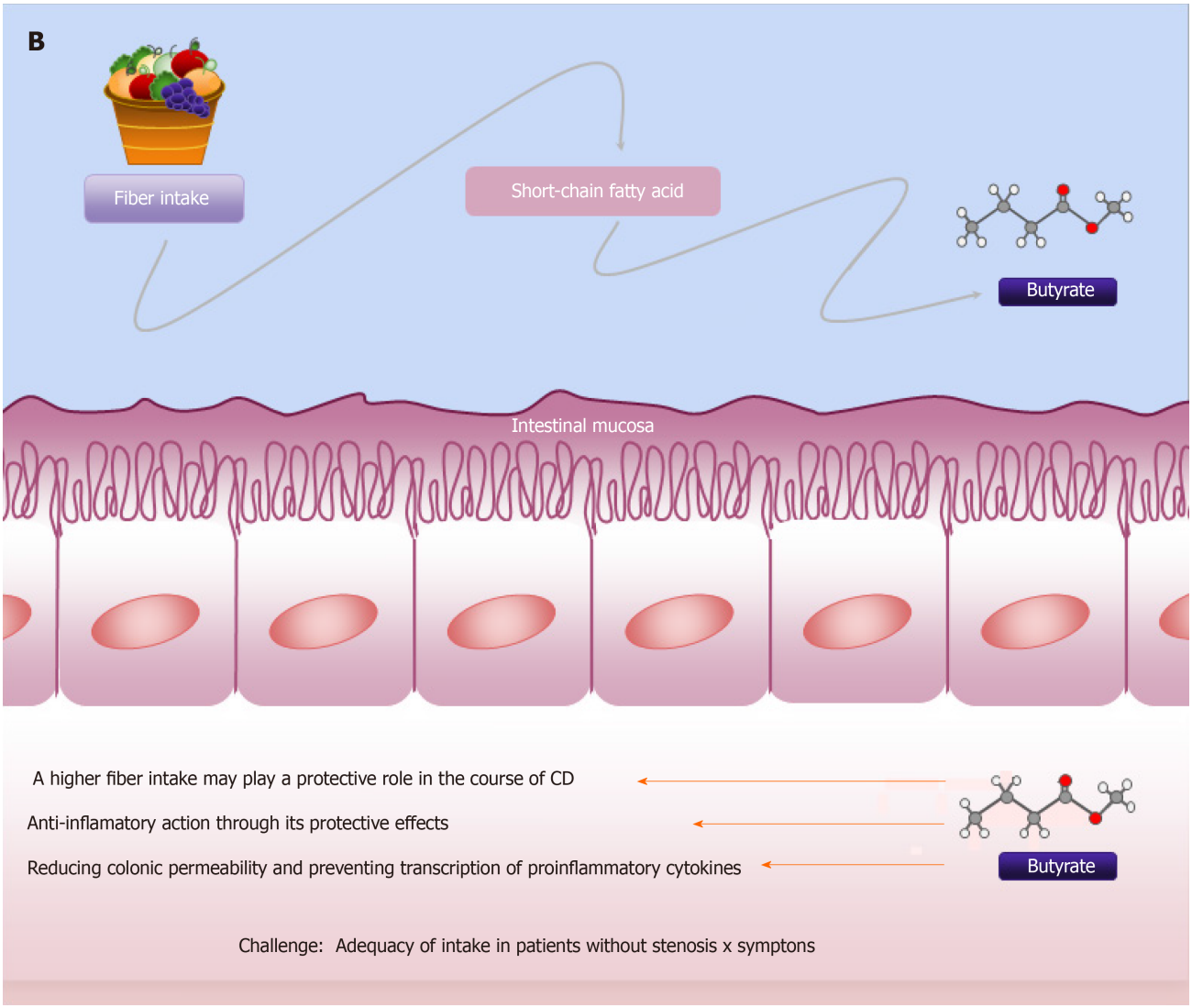


Figure 2 Description of the effects of polyunsaturated fatty acids and fiber on the intestinal mucosa in inflammatory bowel disease. A: Mechanisms related to the effects of polyunsaturated fatty acids on inflammatory bowel disease (IBD); B: Mechanisms related to the effects of fiber on IBD. PUFAs: Polyunsaturated fatty acids; UC: Ulcerative colitis; IBD: Inflammatory bowel disease; CD: Crohn's disease.

guideline, the requirements for macronutrients in IBD patients are similar to those in the healthy population[7]. However, intake of protein in active adult IBD patients should be increased to provide 1.2-1.5 g/(kg·d), due to the proteolytic and catabolic response during active inflammation[7].

Nevertheless, future large-scale prospective designed studies are necessary to evaluate the relationship between quality macronutrients with IBD risk and management, and to make strong recommendations regarding the intake of macronutrients.

Fiber

Although the literature is still unclear regarding the association between dietary components and the development of IBD, many articles demonstrate a positive effect of dietary fiber consumption[31,45,46], and its important role in the prevention of CD[7,45]. A prospective study found that patients who consumed a diet with a fiber content of 24.3 g/d resulted in a 40% decrease in the risk of CD development, although no association was observed for UC[31]. A meta-analysis of observational studies suggested that dietary fiber intake could decrease the risk of developing IBD, in addition to a reduction of 13% of CD risk for every 10 g/d increment in fiber. Among the mechanisms proposed in the literature are: (1) Anti-inflammatory action through the protective effects of butyrate; (2) Reduction in colonic permeability; and (3) Prevention of transcription of proinflammatory cytokines[47,48]. In addition, dietary fiber has been shown to have an effect on the microbiome, exerting a regulatory influence on the

immunological homeostasis[46,49].

Recently, a European prospective multi-center cohort study investigated the association between fiber intake and the development of IBD, and contrary to other studies, no association between dietary fiber intake and the odds of developing CD and UC was found. The authors indicated that the low number of cases (104 with CD and 221 with UC) could be responsible for a weak association and a lack of statistical accuracy. Another limitation is related to information that was not collected in the study, including breastfeeding and the use of antibiotics, which could influence IBD risk and present unclear associations[50].

Furthermore, dietary fermentable carbohydrates, including fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs) are a family of poorly absorbed short-chain carbohydrates that can induce gut symptoms in quiescent IBD[51]. A low FODMAP diet has been assessed for IBD patients and the results are discussed below. Patients with IBD usually report that high-fiber foods may worsen symptoms, and they also believe that foods may influence their disease course[52]. Foods rich in fiber may be hard to digest during active inflammation, so IBD patients tend to avoid fibers, despite the beneficial effect in maintaining remission that has been demonstrated by the intake of dietary fibers[47,53]. In addition, the latest dietary guidelines recommend a low insoluble fiber intake for those with intestinal stenosis. They emphasize that there is no strong evidence to support this approach, although it seems to be coherent[7,25]. Figure 2B describes the effects of fiber on IBD.

Therefore, future studies should investigate whether a protective effect by specific sources of fiber is associated with IBD development and the mechanisms involved.

SPECIFIC DIETS ON IBD

Diet is an important nutritional factor to be taken into account when assessing the impact of nutrients, as well as the existence of dietary patterns involved in both the pathogenesis and the clinical progression of IBD. Recent dietary research has shown that whole dietary patterns are more representative and more important than isolated individual nutrient evaluations[54].

For example, westernized lifestyle has been associated with changes in dietary habits. The Western diet, which is high in fat and protein, mainly from animal sources, and low in fruits and vegetables, has been shown to predispose individuals to IBD. Devkota *et al*[55] (2012) confirmed that the consumption of a high milk-fat diet altered the environments of bacterial proliferation and promoted an increase of the sulfite-reducing pathobiont, *Bilophila wadsworthia*. Furthermore, the authors observed a bloom of *Bilophila wadsworthia* in mice who were supplemented with taurocholic acid - which is a bile acid byproduct resulting from the conjugation of taurine with cholic acid – which suggests an explanation of how western high saturated fat diets may increase the prevalence of IBD[55].

Our research group has identified three dietary patterns among patients with CD. These patterns are: (1) "Traditional + FODMAP"; (2) "fitness style"; and (3) "snacks and processed foods". These patterns can be considered good indicators of patients' eating habits, and facilitate the understanding of the relationship between diets and diseases[54]. Therefore, in general, results of dietary pattern studies should be considered when providing nutritional counseling to patients.

Considering the nutritional factors mentioned above and the risk of IBD development, there are specific diets which are proposed for IBD treatment in the scientific literature. We have noted some important aspects of these dietary treatments and evaluated their effects on IBD. These are summarized in Figure 3.

EEN

EEN is based on the administration of a liquid nutrition formula for 4-12 wk (with most requiring 6-8 wk) either orally or *via* a feeding tube[26]. EEN formulas can be classified as elemental, which contain individual amino acids; semi-elemental, with peptides of varying chain length; and polymeric formulas, which contain intact proteins[56]. Information about food reintroduction after this period is still scarce and inconclusive. However, most centers follow a gradual reintroduction of the normal diet over a period of 2-3 wk[57].

The study of Connors *et al*[58] (2017) included 111 newly diagnosed pediatric CD patients. They reported that EEN as an induction therapy resulted in a reduced need for steroid treatment, with no increased need for biological therapy or surgery[58]. However, this response to EEN is not observed in UC[59,60].

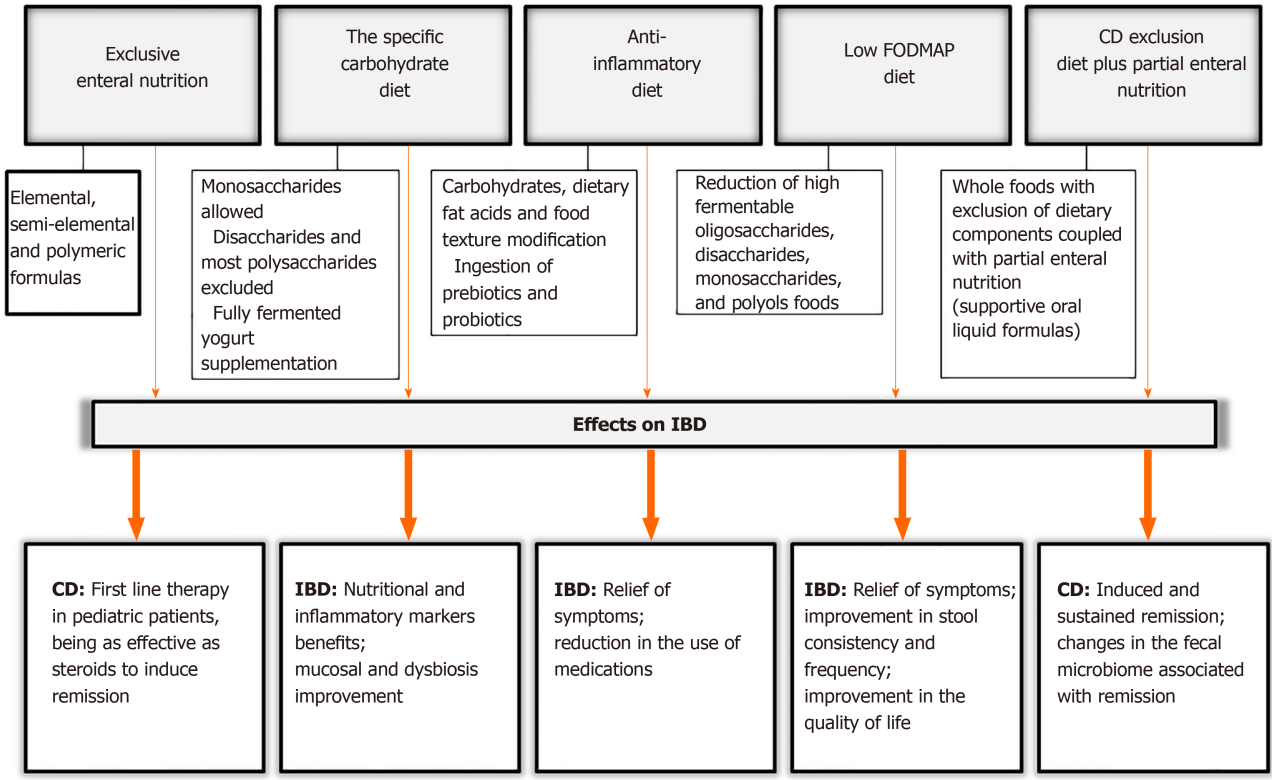


Figure 3 Effects of the specific diets on inflammatory bowel disease. FODMAP: Fermentable oligosaccharides, disaccharides, monosaccharides, and polyols; CD: Crohn's disease; IBD: Inflammatory bowel disease.

EEN has demonstrated many benefits such as mucosal healing, weight gain and linear growth, improved bone turnover and better quality of life[61-63]. The exact mode of action of EEN for CD treatment is uncertain, but may include: (1) Inhibiting the expression of tumor necrosis factor-α (TNF-α), interleukin (IL)-6 and IL-1β; (2) increased release of vascular endothelial growth factor; and (3) transforming growth factor-β. In addition, a mucosal immunity activation with maintenance of intestinal homeostasis and the formation of the intestinal mucosal barrier by essential amino acids have been observed[64].

Some studies report changes in the gut microbiome induced by EEN[64-67], which are also important in the mechanisms' understanding. The microbiome metabolic role is also relevant when considering the impact of EEN on its metabolic function[68]. Walton *et al*[68] (2016) demonstrated that there is a reduction in the concentrations of metabolites production during EEN treatment, which include potentially toxic compounds such as 1-propanol and 1-butanol, methyl and ethyl esters of short-chain fatty acids. These compounds could be involved in the immunological attack on the gut microbiota[68]. Interestingly, Mottawea *et al*[69] (2016) identified *Atopobium parvulum* as a microbe controlling the central hub of H_2S-production, which is involved in H_2S-detoxification. The decreased amount of the H_2S-detoxification protein is a hallmark of CD activity. A systematic review reporting the effects of EEN on the microbiome concluded that, despite variable methods and the sample sizes used in the studies and the microbiota compositions in different people, a reduction in microbiota diversity and richness was reported. These EEN-induced metabolomic changes may play a role in achieving remission[65].

Current guidelines recommend EEN as the first line therapy for children with CD, especially at the time of diagnosis. The rates of clinical remission in EEN-treated children are up to 80%[7,26]. However, as this dietary therapy completely excludes food intake for several weeks, the adherence by children is difficult and uncertain. This approach could cause extreme emotional and social challenges for an entire family. For this reason, an adaptation of the EEN dietary intervention has recently been proposed and is discussed below.

Specific carbohydrate diet

Specific carbohydrate diet (SCD) is a popular diet for IBD treatment, mainly in the lay literature[70,71]. The features of this diet include a modified carbohydrate diet allowing monosaccharides, excluding disaccharides and most polysaccharides. The SCD is a very restrictive diet and allows fruits and vegetables containing more amylose rather than amylopectin, nuts, nut-derived flours, dry-curd cottage cheese, meats, eggs, butter as well as oils. It eliminates sucrose, maltose, isomaltose, lactose, all true and pseudo-grains and grain-derived flours, potatoes, okra, corn, fluid milk, soy, cheeses with high amounts of lactose and most food additives and preservatives. The diet is supplemented with fully fermented yogurt, to free it from lactose. Some recommended cultures are *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, and *Streptococcus thermophilus*[71].

This diet is based on the absorption of specific selected carbohydrates that require minimal digestive processes. Although also uncertain, it is hypothesized that the mechanism of action lies in the decrease in intestinal inflammation by changes in the fecal microbiome[71].

Although studies have reported a positive impact of the SCD in IBD patients, such as improvement in anemia and albumin[72], inflammatory markers[72,73], clinical and biochemical parameters, dysbiosis, and the number of patients achieving clinical remission[74,75] and mucosal healing[75], a careful scientific evaluation is necessary, as there are limitations regarding this diet. Only a few studies with small sample sizes have assessed this diet, which limit the accuracy of estimated effects; besides, the long-term effect must be evaluated.

Additionally, a very restrictive diet requires important lifestyle changes and the patients require follow-up to provide proper nutrition, taking into consideration that specific dietary deficiencies can occur as a consequence of the restricted foods, particularly dairy products that contain vitamin D and calcium; the limited intake of grains, fruits and vegetables can result in folate, thiamine, vitamins B6, C and A deficiencies. Another important concern is reported in the pilot study by Cohen *et al*[75] (2014) in which 33% of the patients lost weight following this restrictive diet.

Anti-inflammatory diet

The anti-inflammatory diet for IBD (IBD-AID) is derived from the SCD and was developed by a group at the University of Massachusetts Medical School. This diet was proposed for patients who are refractory to pharmacological therapy. The treatment was not as beneficial as required and its goal was to obtain and maintain remission with a decreased frequency and severity of flares[76].

The IBD-AID consists of five components: The first includes the modification of carbohydrates, such as lactose and refined or processed to complex carbohydrates; the second incorporates the ingestion of prebiotics and probiotics; the third modifies dietary fat acids; the fourth detects the overall dietary pattern and missing nutrients, and identifies intolerances; and the fifth component modifies the food texture according to 4 phases, beginning with soft or pureed foods if in active flare, or based on the symptoms reported. Patients often advanced to a more whole food diet according to the improvement of their symptoms[76].

In a case-series of 11 adult patients with IBD following the IBD-AID for 4 wk or more, all of the patients were able to interrupt at least one of their prior IBD medications, in addition to experiencing a reduction of their symptoms[76]. Again, it is a very restrictive diet with risk of nutritional deficiencies as a consequence of the restriction of foods, particularly micronutrients. Due to the limited number of studies with small sample sizes, prospective studies to evaluate the application of this diet on IBD are needed with rigorous analysis and a greater number of patients. Thus, its efficacy and mechanisms have not been elucidated, which makes its recommendation very difficult, especially as the long-term effects are not known.

Low FODMAP diet

FODMAPs are poorly absorbed molecules in the small intestine which are rapidly fermented in the colon by bacteria. This process can lead to abdominal pain, bloating, flatulence, and diarrhea[77,78].

Patients with IBD usually have functional symptoms similar to those seen in irritable bowel syndrome (IBS) patients, despite having quiescent disease[79]. Most studies on the low-FODMAP diet were performed in patients with IBS, and evidence shows that this diet can lead to significant improvement in the symptoms[80-85], stool consistency[84,85] and a decrease in functional gastrointestinal symptoms[85].

Patients with IBD presenting IBS-like symptoms also had a significant reduction in

overall symptoms, as well as an increase in quality of life[86]. A meta-analysis showed that the adherence to a low-FODMAP diet compared to a normal Western diet results in the improvement of symptoms related to IBS and IBD, in addition to a significant reduction of symptom severity and improvement in the quality of life scores[87].

Additionally, patients with IBD are at risk of dysbiosis. The effects of the low-FODMAP diet on the microbiome and metabolites are important since the modification of the carbohydrate content of the diet potentially alters the gut microbiota. A study found that a variation in FODMAP intake was associated with important changes in fecal microbiota, which reflects a prebiotic effect of increasing FODMAPs in CD patients[88]. It was demonstrated in a trial of the low-FODMAP diet that IBD patients experienced relief in gut symptoms, higher health-related quality of life scores, and a reduction in the fecal abundance of microbes believed to participate in regulation of the immune response[51].

Although this diet shows positive effects for IBD patients, it should be followed with caution, as the restriction of dietary component intake may lead to nutritional deficiencies, especially in long-term use, which can have a negative impact in the course of the disease.

CD exclusion diet plus partial enteral nutrition

An alternative diet to EEN was developed based on partial enteral nutrition (PEN) that involves whole foods with exclusion of dietary components, for active CD children and young adults. The CD exclusion diet plus PEN (CDED + PEN) is a specialized diet coupled with supportive oral liquid formulas, and comprises two phases lasting 12 wk[25,89]. The first phase consists of a period of 6 wk with 50% PEN for calculated energy requirement that involves a more restrict diet: Gluten, dairy products, gluten-free baked goods and breads, animal fat, processed meats, products containing emulsifiers, canned goods, and all packaged products with an expiration date are not allowed, and a polymeric formula providing 50% of calories. The second 6-wk phase supplies 25% calories in a liquid formula, in addition to a fixed portion of whole grain bread, small amounts of nuts, fruits, legumes and vegetables are allowed. Up to 18-20 g of fiber per day is allowed.

In the Sigall-Boneh *et al*[89] (2014) study, by week 6, remission was obtained in 70.2% of patients, with similar rates in children and adults, 70.1% and 69.2%, respectively. At week 12 and after the reintroduction of some foods, 84% of patients in remission with follow-up remained in remission[89]. The same research group evaluated this diet expanding its use to anti-TNF biologics refractory patients, and showed that clinical remission was obtained in 62% of patients who were on CDED with or without PEN after 6 wk, suggesting that the effect of the decrease in inflammation may be due to the exclusion of specific dietary components rather than supplementation[90].

More recently, Levine *et al*[91] (2019) compared the efficacy of CDED + PEN with EEN in inducing and sustaining corticosteroid-free remission in children. Group 1 (CDED) received the protocol diet as documented before over 12 wk; group 2 received standard EEN for the first 6 wk and then 25% PEN + free diet during the next 6 wk with gradual reintroduction. At week 6, no statistically significant difference in inducing remission was observed between the groups[91]. However, more CDED + PEN patients achieved sustained remission (75.6%, P = 0.01) and were more likely to maintain corticosteroid-free remission compared to EEN patients at week 12 (87.5% *vs* 56%, P = 0.01). The authors also performed a microbiome analysis and demonstrated that changes within CDED + PEN patients indicated more differentiated communities with treatment than EEN patients, showing a rebound effect in which the re-exposure to food did not help to maintain the microbiological changes induced by EEN[91].

To date, of the diets proposed for CD patients, this one has some advantages, including it is not restrictive in terms of essential nutrients and it is more balanced, as it allows access to whole foods.

CONCLUSION

Diet is one of several important environmental factors associated with IBD etiopathology. The majority of studies address association but not causality. Therefore, our understanding is limited concerning how environmental factors may be involved and until now it is still unknown whether diet is a primary or secondary factor.

It has been reported that diet changes the microbiome, and thus contributes to modifying, for example, quality of life, lifestyle and clinical symptoms. For this reason, several non-lay and lay diets have been proposed and IBD patients are asking their

physicians which would be the appropriate type of diet to choose.

The data point out the importance of nutrients in the etiopathogenesis and management of IBD. In particular, components such as breastfeeding and high intake of n-3 PUFAs might have a protective effect on UC, while high consumption of sucrose, animal fat and cholesterol, as well as linoleic acid are associated with increased risk of UC. Regarding CD, breastfeeding and high intake of fiber may protect against the disease, whilst high sucrose, saturated fat and monosaccharide intake, and long-term fast food consumption are considered risk factors.

Specific diets for IBD should be considered with caution and as an adjunct to IBD therapy. Currently there is no scientific consensus regarding an appropriate diet that would benefit all IBD patients. In particular, individualized dietary recommendations are of the greatest importance and diets should be planned by a multidisciplinary team which includes a dietitian in order to provide individual IBD patients with the specific nutrient requirements while keeping in mind all of the patients' clinical aspects.

Among the diets described, CDED + PEN[91] is characterized by a reduction in the consumption of processed and industrialized foods. Interestingly, in Brazil we observe similar recommendations in 'The Dietary Guidelines for the Brazilian Population'. These guidelines recommend consuming natural or minimally processed foods, and moderating the consumption of processed foods while avoiding ultra-processed foods. These important Brazilian guidelines aim to provide information on healthy eating habits in order to reduce and prevent nutritionally related diseases[92]. We suggest that IBD patients follow a diet based on the above Guideline recommendations, because the use of processed and ultra-processed foods can result in unbalanced nutritional compositions which can lead to chronic diseases such as obesity and can also increase the risk of nutritional deficiencies.

Considering the data, there are several important questions that remain to be answered: (1) How do dietary products influence gut microbiota? (2) How does diet predispose individuals to IBD? and (3) Is IBD-associated dysbiosis a cause or a consequence of clinical outcomes?

This review aims to discuss the scientific aspects involving diet and IBD. Additional research is required to determine if diets have a role in IBD etiology and how to determine the specific diet that can benefit patients with IBD. Specific diets can affect the inflammatory pathways and the mechanisms involved. Further long-term interventional studies should explore dietary interventions for IBD. Results will support the formulation of nutritional guidelines which will assist professionals in clinical practice. Dietary therapy may be more important than it is currently believed to be.

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ORIGINAL ARTICLE

Retrospective Study

Phase I study on the safety and preliminary efficacy of allogeneic mesenchymal stem cells in hypoxic-ischemic encephalopathy

Serdar Kabataş, Erdinç Civelek, Necati Kaplan, Eyüp Can Savrunlu, Gülseli Berivan Sezen, Mourat Chasan, Halil Can, Ali Genç, Yener Akyuva, Osman Boyalı, Furkan Diren, Erdal Karaoz

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Abstract

BACKGROUND

Hypoxic-ischemic encephalopathy (HIE) is a leading cause of morbidity and mortality in the adult as well as in the neonate, with limited options for treatment and significant dysfunctionality.

AIM

To investigate the safety and preliminary efficacy of allogeneic mesenchymal stem cells (MSCs) in HIE patients.

METHODS

Patients who had HIE for at least 6 mo along with significant dysfunction and disability were included. All patients were given Wharton's jelly-derived MSCs at 1×10^6/kg intrathecally, intravenously, and intramuscularly twice a month for two months. The therapeutic effects and prognostic implications of MSCs were evaluated by multiple follow-ups. Functional independence measure (FIM), modified Ashworth, and Karnofsky scales were used to assess any side effects, neurological and cognitive functions, and overall outcomes.

RESULTS

The 8 subjects included in the study had a mean age of 33.25 ± 10.18 years. Mean HIE exposure and mean post-HIE durations were 45.63 ± 10.18 and 19.67 ± 29.04 mo, respectively. Mean FIM score was 18.38 ± 1.06, mean modified Ashworth score was 43.5 ± 4.63, and mean Karnofsky score was 20. For the first 24 h, 5 of the patients experienced a subfebrile state, accompanied by mild headaches due to intrathecally administration and muscle pain because of intramuscularly administration. Neurological and functional examinations, laboratory tests, electroencephalography, and magnetic resonance imaging were performed to assess safety of treatment. Mean FIM score increased by 20.88 ± 3.31 in the first month (P = 0.027) and by 31.38 ± 14.69 in 12 mo (P = 0.012). The rate of patients with an FIM score of 126 increased from 14.58% to 16.57% in the first month and 24.90% in 12 mo.

CONCLUSION

Multiple triple-route Wharton's jelly-derived MSC administrations were found to be safe for HIE patients, indicating neurological and functional improvement. Based on the findings obtained here, further randomized and placebo research could be performed.

Key Words: Hypoxic-ischemic encephalopathy; Stem cell; Transplantation; Wharton's jelly

Core Tip: Occurring due to the disruption of oxygen supply to the brain, hypoxic-ischemic encephalopathy is associated with substantial rates of morbidity and mortality. In addition to supportive therapy and symptomatic treatment, research on the treatment of hypoxic-ischemic encephalopathy has focused new therapautic strategies as stem cell therapy. This multi-center and open-label phase I study was performed to investigate the safety and preliminary efficacy of multiple triple-route Wharton's Jelly-Derived Mesenchymal Stem Cells administrations. The patients included in this study also had improvement in modified Ashworth scores, Functional Independence Measure scores over the course of a year, indicating long-term impact on brain functions.

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INTRODUCTION

Occurring due to the disruption of oxygen supply to the brain, hypoxic-ischemic encephalopathy (HIE) is associated with substantial rates of morbidity and mortality in both infants and adult patients[1,2]. Inflammation and neurovascular damage constitute potential warning factors for therapeutic intervention[3]. However, there are inadequate standards and specific measures available for the treatment of HIE. Recently, in addition to supportive therapy and symptomatic treatment, research on the treatment of HIE has focused on the following aspects: hypothermia therapy, neuroprotective agents, *etc*[4]. Another new therapeutic tool with promising indications for clinical practice is stem cell therapy[5]. There are ever-increasing evidences on mesenchymal stem cells (MSCs), suggesting that they promote the improvement of neurological functions, with significant immunomodulatory effects against neuroinflammatory events[6,7]. Bone marrow-derived MSCs (BM-MSCs) and umbilical cord blood-derived MSCs are now being included in efforts to prevent ischemic brain damage[8]. Although they constitute a major source of MSCs, BM-MSCs are seldom preferred due to the high incidence of viral infection and the low number of cells[9]. On the other hand, fetal-derived MSCs, which are more primitive and have less immune reactivity, have recently been suggested as better alternatives for BM-MSCs. Thus, Wharton's jelly-derived MSCs (WJ-MSCs), can easily be obtained in abundance and are readily cultured, making them good alternatives[10].

The optimal route of MSC administration remains a question of significance. While efficient and useful for avoiding negative outcomes often encountered in invasive procedures, intravenous (IV) application alone may lead to retainment in other organs, including the liver, the spleen, the kidneys, and the lungs[11]. Multiple-route administration could therefore be a better alternative, with research supporting no side effects by IV and intrathecal (IT) administration[10]. We previously reported multiple triple-route WJ-MSC administrations to be safe and applicable for HIE and cerebral palsy patients[12,13]. Based on the further research on the matter, WJ-MSCs can now be used for the clinical treatment of HIE.

This phase I study aimed to investigate the effects and preliminary estimates of multiple triple-route WJ-MSC administrations. The population of the study consisted of HIE patients with significant dysfunction. Primary outcome was considered safety of application by neurological and functional examinations, laboratory tests, electroencephalography, and magnetic resonance imaging.

MATERIALS AND METHODS

Study design

This multi-center and open-label phase I study was performed to investigate the safety and preliminary efficacy of multiple triple-route WJ-MSC administrations. Inclusion criteria were being an adult and having HIE and significant impairment and dysfunctionality (Table 1). The study made no restrictions on, and did not provide any forms of, medication or therapy (occupational, physical, or speech) during the follow-up year after WJ-MSC applications. Written informed consent forms were obtained from the legal representatives of the patients. Approval for the trial was obtained from the Turkish Ministry of Health, General Directorate of Health Services, Department of Organ/Tissue Transplantation and Dialysis Services, and the Scientific Committee (No. 56733164-203-E.2569). The findings are given in detail in Table 2.

Ethical considerations

Umbilical cords were obtained from various donors at the Good Manufacturing Practice facility of LivMedCell (Istanbul, Turkey), with informed consents approved by the institutional regulatory board. Postnatal umbilical cords were obtained from donors with full-term pregnancy[12,13].

Processing and quality control of umbilical cords

Umbilical cords were washed with phosphate-buffered saline (Invitrogen/Gibco, Paisley, United Kingdom). After removing the blood vessels, tissues were cut into explants of 5 to 10 mm^3 and cultured in humanized culture conditions (5% CO_2 at 37 °C) until cell migration occurred. The cells were harvested once they reached 70%-80% confluency and characterization tests were conducted at passage 3. Quality control was done in accordance with the standards of the Turkish Agency of Medicines and Medical Devices[12,13].

Table 1 Enrollment criteria

Inclusion criteria
Age ≥ 18
HIE ≥ 6 mo prior, radiologically confirmed at initial diagnosis and at study enrollment
The patients who does not have any chronic illness (cancer, kidney, heart/hepatic failure *etc.*) other than HIE. Adequate systemic organ function confirmed by normal ranged laboratory values
Life expectancy > 12 mo
No substiantial improvement despite of a treatment in neurological/functional status for the 3 mo before study enrollment
Severe disability defined as subject confined to a wheelchair/required to have home nursing care/needing assistance with activities of daily living
Expectation that the patient will receive standard post-treatment care and attend all visits
Signing in the written informed consent form for confirming to that know the treatment to be applied and to be willing by their parents/a surrogate
Exclusion criteria
Presence of any other clinically significant medical/psychiatric condition, or laboratory abnormality, for which study participation would pose a safety risk in the judgment of the investigator/sponsor or history within the past year of drug/alcohol abuse
Recently diagnosed severe infection (meningitis, *etc.*)/development of liver, kidney/heart failure/sepsis or skin infection at the *i.v.* infusion site or positive for Hepatitis B, C/HIV
History of uncontrolled seizure disorder
History of cerebral neoplasm, or cancer within the past 5 yr, with the exception of localized basal or squamous cell carcinoma
Having clinic symptoms that formation of white sphere number ≥ 15000/μL or platelet count ≤ 100.000/μL
Serum aspartate aminotransferase and serum alanine aminotransferase > 3 × upper limit of normal/creatinine > 1.5 × upper limit of normal
Pregnant/lactating/expectation to become pregnant during the study
Participation in an another investigational stem cell study before treatment
The patient/parents decides to abandon the treatment or the patient death

HIE: Hypoxic-ischemic encephalopathy HIV: Human immunodeficiency virus.

Characterization of WJ-MSCs by flow cytometry

According to flow cytometry results, the stem cells were positive for CD44, CD73, CD90, and CD105 and negative for CD34, CD45, and HLA-DR. Their telomerase activities were found to be stable throughout culturing, with a large and flattened morphology[12,13].

Cell differentiation and karyotyping

Expression of some markers was found, including TERT, POU5F1, SOX2, ZFP42, CD44, VCAM1, THY1, BMP2, RUNX-1, ICAM1, and NES. Differentiation tests showed that the cells had a capacity for trilineage. Although, karyotyping did not yield any structural or chromosomal abnormality[12,13].

Pre-transplantation

The final WJ-MSC preparations were harvested from cell culture passage 3 and suspended in densities of 1×10^6 in 3, 20, and 30 mL of normal saline[12,13].

Surgical procedure and WJ-MSC transplantation

All patients underwent examination in anesthesia and reanimation, neurology, physical therapy and rehabilitation, and neurosurgery departments. After ensuring that there is no contraindication for anesthesia, and no serious infectious disease, transplantation was carried out[12]. All procedures were performed by one team of doctors, including IT, IV, and intramuscular (IM) administration of allogeneic WJ-MSCs, under Sedo-anesthesia (Table 3). IT administration was done through lumbar puncture[14]. IM administration was performed under the guidance of ultrasonography. IV administration was done slowly over a period of 30 min. Then, the patients were sent to the intensive care unit for follow-up. Physical therapy and rehabilitation were initiated on the next day, avoiding exercise on the days of administration.

Table 2 Study population

		Frequency	Percent
Age	20.00	1	11.1
	25.00	1	11.1
	27.00	1	11.1
	29.00	1	11.1
	34.00	1	11.1
	37.00	1	11.1
	43.00	1	11.1
	51.00	1	11.1
Sex	M	8	100.0
	F	0	0
Cause of hypoxia	Cardiac arrest	1	12.5
	Cardiac arrest due to acute myocard infarction	3	37.5
	Cardiac arrest due to explosive devices injury	1	12.5
	Cardiac arrest due to multi-trauma	1	12.5
	Cardiac arrest, unkown ethiology	2	25.0
Duration of hypoxia	25.00	1	12.5
	30.00	1	12.5
	40.00	1	12.5
	45.00	3	37.5
	60.00	1	12.5
	75.00	1	12.5
Previous treatment	No	8	100.0
	Yes	0	0
Comorbidity	Atrial fibrilation	1	12.5
	No	7	87.5
Duration between hypoxia and first SCT	6.00	3	37.5
	10.00	1	12.5
	11.00	1	12.5
	18.00	2	25.0
	96.00	1	12.5

SCT: Stem cell therapy.

Neurological examination

Prior to treatment, all patients underwent immense neurological and functional assessment. Modified Ashworth (MA) scale was used to evaluate for spasticity and Functional independence measure (FIM) scale was used to evaluate for quality of life[15].

Criteria for safety

The criteria for safety of administration were the lack of infection, fever, headache, pain, increased C-reactive protein levels or leukocytosis, allergic reaction or shock, and complications associated with anesthesia or analgesia for 7 to 14 d. The criteria for safety of WJ-MSC were the lack of infection, neuropathic pain, cancer, and neurological deterioration for 1 year[12,13].

Table 3 Administration schedule

Rounds	Route	WJ-MSC
Round 1	IT	1×10^6/kg in 3 mL
	IV	1×10^6/kg in 30 mL
	IM	1×10^6/kg in 20 mL
Round 2 (2nd week)	IT	1×10^6/kg in 3 mL
	IV	1×10^6/kg in 30 mL
	IM	1×10^6/kg in 20 mL
Round 3 (4th week)	IT	1×10^6/kg in 3 mL
	IV	1×10^6/kg in 30 mL
	IM	1×10^6/kg in 20 mL
Round 4 (6th week)	IT	1×10^6/kg in 3 mL
	IV	1×10^6/kg in 30 mL
	IM	1×10^6/kg in 20 mL

WJ-MSC: Wharton's jelly-derived mesenchymal stem cells; IT: Intrathecal; IV: Intravenosus; IM: Intramuscular.

Evaluating treatment success

Besides MA and FIM, Karnofsky scale was used to evaluate the outcome[12,13]. Assessments also included investigation for neuropathic pain, secondary infections, urinary tract infections, or pressure ulcers of the skin.

Statistical analysis

Of nonparametric tests, Friedman and Wilcoxon Signed Rank tests were used to measure changes in FIM, MA, and Karnofsky scale scores before and after the operation. The reason for using nonparametric tests was the inadequate number of data for parametric tests.

RESULTS

Safety and negative effects

The patients showed good tolerance, with no severe side effects associated with the administration. For the first 24 h, 5 of the patients experienced a subfebrile state, accompanied by mild headaches due to IT administration and muscle pain because of IM administration (Table 4). There were no problems of safety or side effects during the 1-year follow-up.

FIM Scores: The FIM scores showed significant improvement in terms of quality of life. There was a continuous increase in the FIM Motor and Cognitive scores of patients following the operation. According to the analysis in Table 5, the difference in pre- and postoperative FIM Motor scores of the participants was statistically significant ($\chi^2 = 24.583$, $P < 0.001$). Wilcoxon Signed Rank Test was performed between the binary measurements to identify the differences between variables. The analysis yielded no difference between baseline and one-week posttest scores (z = 0.000, $P = 1.00$), nor between one-week and one-month (z = -1.00, $P = 0.32$), one-month and two-month (z = -1.342, $P = 0.18$), two-month and four-month scores (z = -1.841, $P = 0.07$). However, a statistically significant difference was noted between four-month and twelve-month scores (z = -2.226, $P = 0.026$). Concluding, increases in FIM Motor scores were not significant until the second month after the operation (Figure 1 and Table 5).

According to the analysis in Table 6, the difference in the pre- and postoperative FIM Cognitive scores of the participants was statistically significant ($\chi^2 = 37.500$, $P < 0.001$). Wilcoxon Signed Rank Test was performed between the binary measurements to identify the differences between variables. The analysis yielded no difference between baseline and one-week posttest scores (z = -1.00, $P = 0.32$), but significant differences between one-week and one-month (z = -2.207, $P = 0.027$), one-month and

Table 4 Early and late complications of the procedures

Complications		Patient No: 1				Patient No: 2				Patient No: 3				Patient No: 4				Patient No: 5				Patient No: 6				Patient No: 7				Patient No: 8			
		Administration				Administration				Administration				Administration				Administration				Administration				Administration				Administration			
		1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th
Early	Infection	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Fever	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	+	+	-	-	-	+	-	-	-	-	-	-	+	+	-	-
	Pain	-	-	-	-	-	-	-	-	+	-	+	-	+	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-	+	-	+	-
	Headache	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+	+	-	-
	Increased level of C-reactive protein	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Leukocytosis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Allergic reaction or shock	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Perioperative complications	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Late	Secondary infections	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Urinary tract infections	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Deterioration of neurological status	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Neuropathic pain	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Carcinogenesis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

–: Not present, +: Present.

two-month (z = -2.384, P = 0.017), two-month and four-month (z = - 2.552, P = 0.011), and four-month and twelve-month scores (z = -2.521, P = 0.012). Concluding, increases in FIM Cognitive scores were not significant until the first week after the operation (Figure 1 and Table 6).

MA Scores: A continuous decrease was observed in MA right and left scores of the patients after the operation. According to the analysis in Table 7, the differences in the pre-and postoperative MA right scores of the participants was statistically significant (χ^2 = 38.875, P < 0.001). Wilcoxon Signed Rank Test was performed between the binary measurements to identify the differences between variables. The analysis yielded significant differences between baseline and one-week posttest (z = -2.060, P = 0.039), one-week and one-month (z = -2.555, P = 0.011), one-month and two-month (z = -2.530, P = 0.011), two-month and four-month (z = -2.530, P = 0.011), and four-month and twelve-month scores (z = -2.533, P = 0.012). Concluding, MA right scores continued to

Table 5 Friedman test results regarding the change in the functional independence measure motor scores of the patients before and after the operation

	n	Mean	SD	Mean rank	χ^2	df	*P* value
Pre-test	8	13.00	0.00	2.69	24.583	5	0.000
Post-test 1 wk	8	13.00	0.00	2.69			
Post-test 1 mo	8	13.38	1.06	2.88			
Post-test 2 mo	8	14.00	2.45	3.25			
Post-test 4 mo	8	16.25	7.23	4.19			
Post-test 12 mo	8	17.63	9.10	5.31			

Table 6 Friedman test results regarding the change in the functional independence measure cognitive scores of the patients before and after the operation

	n	Mean	SD	Mean rank	χ^2	df	*P* value
Pre-test	8	5.38	1.06	1.63	37.500	5	0.000
Post-test 1 wk	8	5.50	1.41	1.75			
Post-test 1 mo	8	7.50	2.45	2.88			
Post-test 2 mo	8	9.13	3.83	3.88			
Post-test 4 mo	8	11.00	5.68	5.00			
Post-test 12 mo	8	13.75	6.23	5.88			

Table 7 Friedman test results regarding the change in the modified Ashworth scale right scores of the patients before and after the operation

	n	Mean	SD	Mean rank	χ^2	df	*P* value
Pre-test	8	21.88	2.17	5.81	38.875	5	0.000
Post-test 1 wk	8	19.75	3.28	5.06			
Post-test 1 mo	8	18.25	3.24	3.94			
Post-test 2 mo	8	17.00	3.59	3.19			
Post-test 4 mo	8	14.75	3.69	1.94			
Post-test 12 mo	8	13.00	4.24	1.06			

decrease significantly until the first year after the operation (Figure 2 and Table 7).

According to the analysis in Table 8, the differences in the pre- and postoperative MA left scores of the participants was statistically significant (χ^2 = 38.741, P < 0.001). Wilcoxon Signed Rank Test was performed between the binary measurements to identify the differences between variables. The analysis yielded significant differences between baseline and one-week posttest (z = -2.032, P = 0.042), one-week and one-month (z = -2.338, P = 0.017), one- month and two-month (z = -2.527, P = 0.012), two-month and four-month (z = -2.527, P = 0.012), and four-month and twelve-month scores (z = -2.539, P = 0.011). Concluding, MA left scores continued to decrease significantly until the first year after the operation (Figure 2 and Table 8).

Karnofsky Scores: An increase was observed in the Karnofsky scores of the patients after the operation. According to the analysis in Table 9, a significant difference was noted between baseline and one-year posttest scores (z = -2.546, P = 0.01) (Figure 3 and Table 9).

Table 8 Friedman test results regarding the change in the modified Ashworth scale left scores of the patients before and after the operation

	n	Mean	SD	Mean rank	χ^2	df	*P* value
Pre-test	8	21.63	2.83	5.75	38.741	5	0.000
Post-test 1 wk	8	19.50	3.21	5.066			
Post-test 1 mo	8	17.75	2.96	4.06			
Post-test 2 mo	8	16.38	3.02	3.13			
Post-test 4 mo	8	13.88	3.40	1.94			
Post-test 12 mo	8	12.13	3.64	1.06			

Table 9 Wilcoxon signed ranks test results regarding the change in the Karnofsky scale scores of the patients before and after the operation

Karnofsky scale	Ranks	*n*	Mean rank	Sum of ranks	*z*	*P* value
Pre-test-Post-test 12 mo	Negative ranks	0	0.00	0.00	-2.565	0.010
	Positive ranks	8	4.50	36.00		
	Ties	0				
	Total	8				

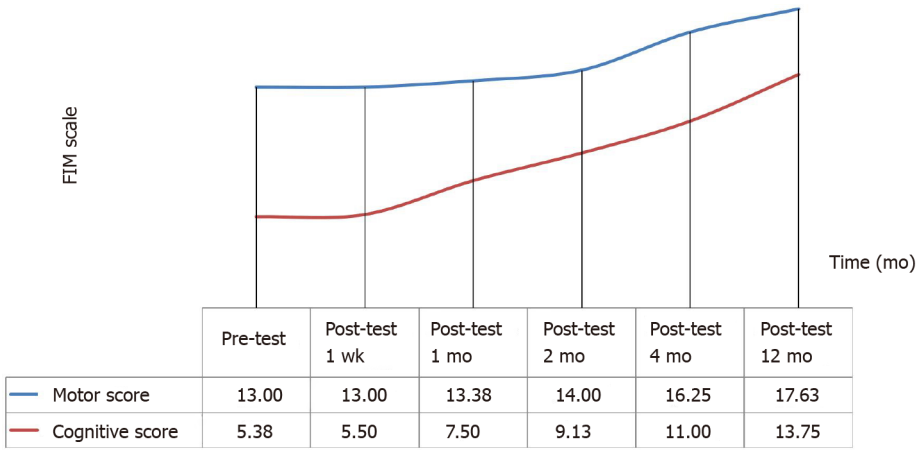
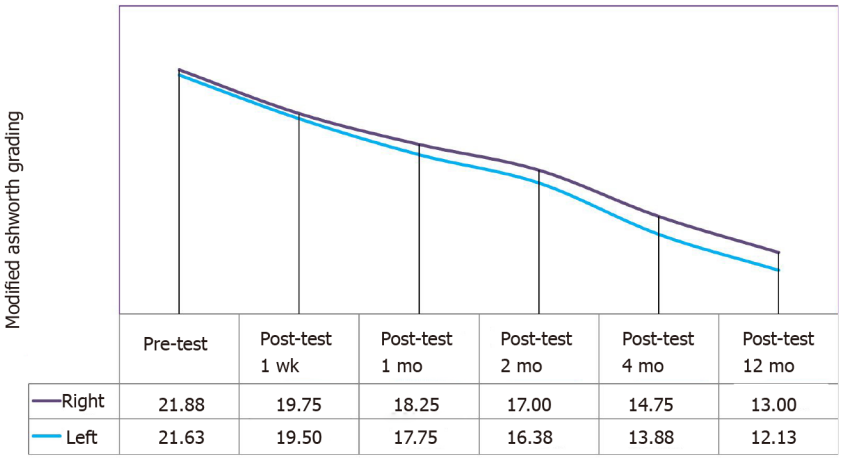


Figure 1 Change in the mean pretest and posttest functional independence measure scale motor and cognitive scores of the patients.
FIM: Functional independence measure.

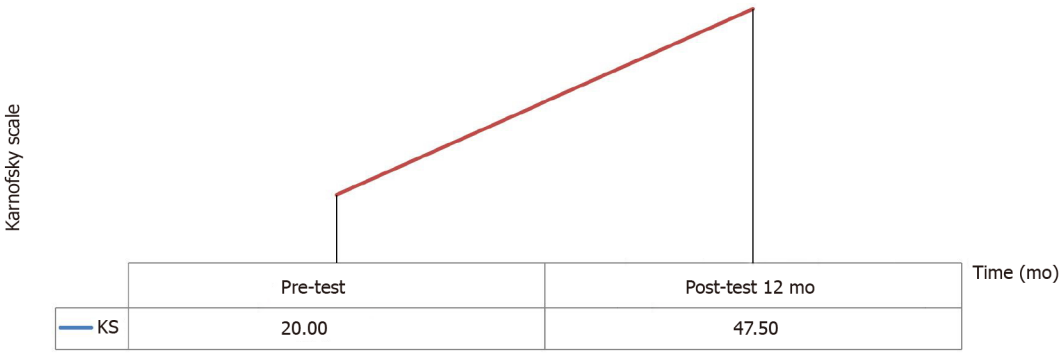
DISCUSSION

The potential disabilities to be caused by HIE can be reduced by acute therapies early after HIE and by restorative therapies during the months or years following HIE for promoting natural repair. However, there has been no specific therapy to yield particular recovery. In addition to single therapies, some options of combinations have been considered, often including moderate hypothermia, to potentially obtain better outcomes[17]. Meanwhile, the regenerative and reparative potentials of stem cells have suggested their transplantation as an alternative in the treatment of neurological disorders (*e.g.*, stroke, spinal cord injury, *etc.*)[18,19]. Among the sources for these cells are neural stem/progenitor cells derived from fetal tissue, induced pluripotent stem cells, embriyonic stem cells, and MSCs[18]. The latter are multipotent progenitor cells that have a self-renewal property and the ability to differentiate into various mesodermal tissues ranging from bone and cartilage to cardiac muscle[20]. Previously, BM was considered a good candidate as a source of MSCs. However, due to its invasive nature and decreased proliferation and differentiation capacity with advanced age, alternative sources were pursued. Fetal-derived MSCs, which are more primitive and



	Pre-test	Post-test 1 wk	Post-test 1 mo	Post-test 2 mo	Post-test 4 mo	Post-test 12 mo
Right	21.88	19.75	18.25	17.00	14.75	13.00
Left	21.63	19.50	17.75	16.38	13.88	12.13

Figure 2 Change in the mean pretest and posttest modified Ashworth scale right and left scores of the patients.



	Pre-test	Post-test 12 mo
KS	20.00	47.50

Figure 3 Change in the mean pretest and posttest Karnofsky Scale scores of the patients.

have less immune reactivity, have recently been suggested as better alternatives for BM-MSCs. WJ, which is the primitive connective tissue between the umbilical vessels and the amniotic membrane, protects these vessels from pressure and torsion. During embryogenesis, hematopoietic and mesenchymal cells migrate through the WJ, and some of them become trapped, making this tissue a good source of MSCs[21-23].

Despite the promising findings in favor of stem/progenitor cells in HIE, cellular therapy remains in the early stage for human practice[24]. Miao *et al*[14] found that UC-MSC given IT yielded functional improvements in 47 patients with various diseases (*e.g.*, spinal cord injury, cerebral palsy, *etc.*). Some recent studies have also suggested WJ-MSC therapy to be promising for patients with neurological diseases, including stroke[12]. Allogeneic WJ-MSCs are shown to demonstrate significant positive impact, even when given *via* other routes, including intracerebral[9]. The current study found that multiple (4 doses in total for 2 mo at two-week intervals) triple-route (IV, IT, and IM) implantations of allogeneic WJ-MSCs are associated with safety and potential functional recovery.

To the best of our knowledge, this study is the largest to perform multiple triple-route WJ-MSC administration on HIE patients. Also, this is the first time allogeneic WJ-MSC treatment is evaluated in this patient group[12]. When applied at a dose of 1 × 10^6/kg IV, IT, and IM, WJ-MSC yielded mild and sparse side effects, seen only in 5 of the patients and lasting only for 24 h.

Chronic stage HIE patients often show continually increasing dysfunction, contrary to the improvement noted in the patients of in this study throughout the first year after the operation. Considering the limited number of choices for functional recovery, chronic stage disease is crucial. Normally, recovering from such dysfunctions progresses in a bimodal form, with spontaneous improvements during the first couple of months, followed by a significant rise in negative conditions within the first year after onset.

Among the findings obtained here, the most significant was the 12-point increase in FIM Motor scores, which could prove substantial if confirmed by further research.

Also, at baseline, 14.28% of patients had an FIM Motor score of 91, which increased to 14.70% in the first month and 19.37% in the first year (Table 5).

The potential effects of MSCs on neurological diseases have also been marked in animal studies, and is thought to stem from some of their properties, such as restoring cellular energy, promoting neurogenesis, improving angiogenesis, and dampening inflammatory response. The 1-year sustained recovery in functional indicators demonstrated here is in line with other research on HIE[25].

The patients included in this study also had significant improvement in FIM Cognitive scores over the course of a year, indicating long-term impact on brain functions. While suggesting a promising choice for recovery in chronic stage HIE, these findings still need to be confirmed by further research, preferably of a controlled nature. In addition, such studies could measure the outcomes that are specific to certain modalities, ensuring detailed assessment regarding improvement.

This study had certain strengths in terms of its population, materials, and methods. Considering the targeted patient group, the specificity of the sample was a significant strength, in that the patients constitute a population with major dysfunction and limited options for recovery. Using allogeneic cells thanks to the nature of MSCs does not require immunosuppression and enables some treatment protocols, those that could be largely practiced on HIE patients. Using only 3 passages to harvest cell cultures was another strength, since some of the desired properties of MSCs are negatively affected by higher numbers of cell divisions, which is unavoidable when using more passages. Finally, the safety criteria used here were quite extensive in terms of both time and scope.

The study also had certain limitations, among which the most notable were the dominant focus over safety and the lack of control, as the latter complicates the interpretation of improved results. Though favorable, the findings are possibly influenced by other variables, such as growth factors and anti-inflammatory elements, perhaps even exosomes. Restorative therapies are known to aid desirable recovery outcomes, but were unfortunately lacking here.

CONCLUSION

Recently, cell therapies, particularly WJ-MSCs, have been paving the way for novel treatment protocols for preventing ischemic brain damage. However, clinical use of stem cell therapy remains conflicted and the root of its efficacy is yet to be fully clarified. Several variables still need to be elucidated, such as the mediators between cells, the optimal type and time of therapy, and the ideal patient characteristics. Thus, future multi-center studies with larger populations are needed to verify the safety and efficacy outcomes obtained here, along with an optimization for the treatment protocol in question.

ARTICLE HIGHLIGHTS

Research background

To date, hypoxic ischemic encephalopathy (HIE) is refractory, including after cardiopulmonary resuscitation, hemorrhagic shock and cerebral infarction *etc.*

Research motivation

Limited treatment options exist for patients with HIE and substantial functional deficits. Thus, further clinical research investigations on this subject could be valuable.

Research objectives

The current study examined safety and preliminary efficacy estimates of allogeneic mesenchymal stem cells (MSCs) in this population.

Research methods

Entry criteria included HIE ≥ 6 mo prior, substantial impairment and disability. Enrollees received intrathecal, intramuscular, and intravenous administrations of Wharton's jelly–derived MSCs at a target dose of 1×10^6/kg for each application route (twice a month for 2 mo).

Research results

Treatment was safe based on serial neurological and functional exams, laboratory tests, electroencephalographies, and magnetic resonance imaging.

Research conclusions

Therapeutic administration of stem cells has a theoretical role in the treatment of HIE. Although promising results from many publications have been reported, there is still no consensus on which cellular therapy should be administered to which patient at what time after HIE. There seems to be a need for a tremendous amount of work to elucidate the underlying mechanisms of how MSCs interact with damaged host tissues and how this interaction results in a cascade of events that lead to some functional neuronal recovery.

Research perspectives

These findings suggest that quality of the cells, optimization of the cell dose, standardization of the cell processing, the timing, route of administration and patient selection as well as the role of clinical experience of the physcisian are critical to the success of stem cell therapy in HIE patients.

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MINIREVIEWS

Spontaneous posterior vitreous detachment: A glance at the current literature

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Abstract

Spontaneous posterior vitreous detachment (PVD) is a common age-related condition in which prevalence tends to increase with age. Acute PVD can cause the onset of symptoms that include visual disturbances, myodesopsia and photopsia. The goal of this short review was to provide a quick glance at the important factors related to PVD based on current literature in this field, which includes incidence, symptoms, diagnosis, risk factors, and education for patients with acute symptoms, and treatments. The take home message is that an ophthalmic examination at the onset of symptoms is of utmost importance, considering that irreversible sight-threatening complications can be prevented if diagnosed and treated promptly.

Key Words: Spontaneous posterior vitreous detachment; Vitreous liquefaction; Photopsia; Vitreal adhesion; Myodesopsia; Vitreoretinal traction; Retinal detachment

Core Tip: Posterior vitreous detachment (PVD) tends to be a benign condition related to aging. Acute PVD can cause the onset of symptoms like flashes, visual disturbances, and floaters. Current literature has provided new explanations of the mechanisms underlying normal and abnormal PVD. Incidence, prevalence, and risk factors are important in assessing patients. New diagnostic tools like optical coherence tomography have assisted in providing objective evaluation of patients. Treatment with vitrectomy and laser and pharmacological vitreolysis are available, but are seldom considered because they can be invasive and can worsen symptoms. Patients must be educated to seek an ophthalmologic examination that includes a dilated fundus evaluation at the onset of important signs and symptoms, especially those with risk factors, because early diagnosis and treatment can prevent irreversible vision loss.

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INTRODUCTION

Spontaneous posterior vitreous detachment (PVD) is a common age-related condition in patients older than 45 years. Among patients aged 50-59 years, there is a prevalence of about 24%, which increases to about 87% those 80-90 years of age[1,2]. Considering that the number of studies reported in literature is relatively extensive, we wanted to provide a quick overview by concentrating mostly, but not exclusively, on studies published in the last 20 years. The aim of our review was to briefly summarize the basic concepts and important factors related to acute PVD, mainly regarding physio-pathogenesis, incidence, risk factor, symptoms, diagnosis, and treatment. We hope that this can serve to remind clinicians in all areas of specialization to send patients for an ophthalmic examination at the onset of symptoms, considering that irreversible sight-threatening complications can be prevented if diagnosed and treated on time.

The vitreous humor, the largest anatomic structure in the human eye, is located between the lens and the retina (Figure 1A). It is a transparent gel-like structure composed mostly of water (98%-99%) in addition to hyaluronic acid (0.5%) and collagen type II, a hybrid of types V/XI, and type IX (0.5%)[3]. The collagen component is organized in fibrils of 7-28 nm in diameter, with type V/XI in the core and the other types in the periphery. The vitreous body fills the posterior segment of the eye and is separated from the retina by the internal limiting lamina (ILL), which is composed mostly of type IV collagen. The embryologic origin of the ILL is the same as Bruch's membrane[4]. The most peripheral part of vitreous is known as vitreous cortex, which is formed from a concentration of collagen fibrils composed mostly of type II collagen, however, other types (IX, V/XI) are also present. Adhesion of the posterior vitreous cortex and the ILL depends on an extracellular matrix that serves as glue. With age, this adhesion weakens, most likely because of biochemical alteration of the components of the extracellular matrix.

Extensive changes of the vitreous body occur with age. The consistency of vitreous changes from a gel phase to a water phase. There is a decrease of gel volume and an increase in liquid volume of human vitreous (Figure 1B). At about 50 years of age, 25% of vitreous is in water phase, which drastically increases to about 62% at 80 years[1]. It is important to note that the adherence of the vitreous is strongest at the retina around the vitreous base (ora serrata), at the optic disc margins, at macula, and around peripheral blood vessels[4].

PVD occurs when there is a sudden separation of the from the internal limiting membrane of the retina, resulting from the liquefaction within the vitreous body (Figure 1B). For PVD to occur, two different events must happen, the liquefaction of vitreous body (synchisis) and the weakening of adhesion between vitreous posterior cortex and the ILL[5,6]. When uncomplicated PVD occurs, the liquid vitreous tends to move toward the retrocortical prepapillar and premacular space, thus causing the vitreous body to collapse (syneresis) in anterior fashion (Figure 1C and D)[7]. That event can develop in several anomalous ways when, for various causes, there is a change in the balance between the degree of gel liquefaction and the weakening of vitreoretinal adhesion. If there is insufficient weakening of the vitreoretinal adhesion in the presence of vitreous liquefaction, abnormal traction at the vitreoretinal interface can result, leading to deleterious effects on the retina (Figure 1E) and vitreous, including hemorrhages, retinal tears, retinal detachment (RD), vitreo-macular traction syndrome, macular pucker, and macular holes[3,7,8].

Women seem to be affected by a faster progression of vitreous detachment. Postmenopausal women may be more prone to PVD because of a lack of estrogen[9], which may have a protective effect against PVD[10]. Studies have shown that up to 20% of PVDs can be asymptomatic in the early stages and thus not detected clinically [8]. When the separation of vitreous from optic disc margins is complete, patients tend to present symptoms. The most common symptoms are photopsia (flashes) and myodesopsia (or floaters), which are perceived as linear dark shadows, moving cob webs or spots that move within the back of the eye (Figure 1F)[4-7]. Floaters tend to be mobile and more evident against a bright background. Symptoms are variable and can remain for months or years after uncomplicated PVD[7].

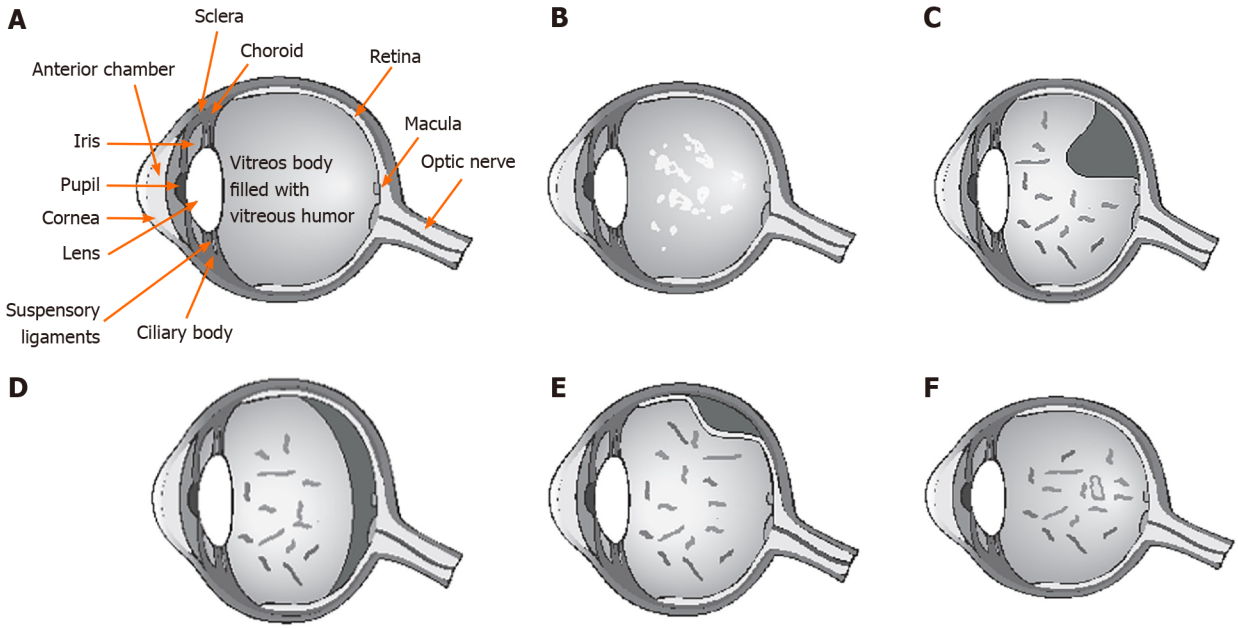

Figure 1 Posterior vitreous detachment. A: The vitreous humor in a normal young healthy eye is transparent and colorless; B: Age-related vitreous degeneration tends to starts with liquefaction, forming pockets of lacunae; C: Isolated or confluent opacities, known as floaters or myodesopsia, may appear; D: Vitreous liquefaction in conjunction with weakening of vitreoretinal adherence leads to posterior vitreous detachment (PVD). Floaters and a Weiss ring can increase symptomatic myodesopsia; E: The collapsing vitreous during PVD separates from the retina; F: PVD can be complicated by retinal detachment if the posterior cortex of the vitreous remains adherent to the retina during PVD.

It is of utmost importance that patients with acute symptoms, which include worsening of floaters and/or photopsia, undergo an ophthalmic examination. Flashes of light are rapid, often located in the temporal quadrant, and are considered to be caused by the stimulation of the retina from vitreoretinal traction and pulling. Studies have shown that the risk of damage of the retina with the onset of symptoms ranges from 8.2% to 47.65%[10,11]. Flashes and/or floaters tended to be associated with the onset or subsequent development of retinal tears[12,13]. The greatest risk factors for retinal tears include the presence of ten or more floaters, cloud-like obscuration in the visual field, and retinal or vitreous hemorrhages[12-16]. Patients may complain of acute blurred vision caused by vitreous hemorrhage, presence of a Weiss ring in the vitreous body (Figure 1F) or big floaters crowding the visual field. In symptomatic PVD, the risk of developing retinal tears and detachment can be as high as 35%, which can increase in the presence of vitreous hemorrhaging[17].

New retinal tears brought on by acute abnormal PVD can lead to RD because the vitreous fluid can enter behind the retina under traction, causing a separation of the neuroretina from retinal pigment epithelium[14-16]. RD may occur within 6 wk in untreated retinal breaks. In that period, it is possible to find new retinal breaks at different locations. Mitry *et al*[18] reported that the risk of RD was higher in men than in women, with an incidence of 13.09 and 7.41 *per* 100.000, respectively[18]. Numerous studies suggest re-examining the patient within 6 wk[14,15]. That time period may be too long, considering that retinal tears can appear within a shorter period after the onset of PVD[16-17], thus it is of utmost importance to educate patients regarding the onset of new symptoms after initial diagnosis of PVD. An earlier ophthalmic evaluation may be necessary, especially in patients at increased risk of developing RD, including those with previous retinal tears, high myopia, history of ophthalmic surgery, trauma or laser treatment, pseudophakia/afachia, *etc*.[14-17].

RD requires immediate surgical intervention to avoid the apoptosis of photoreceptors and irreversible loss of vision[19]. Patients with symptomatic PVD should be directed to an emergency ophthalmology department within 24 h of onset of symptoms. Patients should undergo a complete ophthalmic evaluation, which includes well dilated retinal examination with slit-lamp biomicroscopy, preferably with a superfield Volk lens, Goldmann three-mirror contact lens and/or indirect ophthalmoscopy with scleral indentation to examine the entire 360° of the ora serrata. During the fundus examination, it is possible to find the presence of a Weiss ring [20,21], retinal holes with or without opercula, equatorial breaks, horseshoe tears, RD or vitreous hemorrhages[14-17]. In some cases, a clinically accurate diagnosis may be

difficult if PVD is initial or partial, if there are no signs of a Weiss ring, or if the posterior hyaloid is still attached inferiorly. Fundus examination can be difficult if PVD causes a vitreous hemorrhage, which obscures proper diagnosis. B-scan ultrasonography (US) can be used to accurately assess the status of vitreous *in vivo* and to check the presence and extent of PVD[22]. US is safe, painless and noninvasive, but it is operator dependent and based on the experience and skills of the examiner, thus giving rise to variable and subjective interpretation of results.

Optical coherence tomography (OCT), is currently in widespread use in the management of retinal pathologies and glaucoma. It can also be used to noninvasively visualize the vitreoretinal interface with high-resolution images *in vivo*[23]. OCT can accurately show the separation of the posterior vitreous face and retina and can identify a shallow PVD better than slit-lamp biomicroscopy and B-scan US. Thanks to the routine clinical use of OCT, it has been shown that PVD usually starts as a vitreous RD around the fovea[21,24]. Kakehashi *et al*[25] proposed a classification of PVD into five stages based on OCT, and ranging from nonexistence of PVD in stage 0 to complete PVD with a prominent Weiss ring on slit-lamp biomicroscopy in stage 4[25]. The various stages of PVD can be seen in OCT scans (Figure 2A-E), which are normally prescribed in the management of patients with glaucoma or retinopathies, some of which derive from abnormal PVD, like macular pucker (Figure 2F and G) and macular lamellar holes (Figure 2H-J). The limit of OCT is that this technique cannot visualize the whole vitreous cavity and can give rise to false-positive interpretations of PVD[26]. The diagnostic ability of OCT for detecting PVD, especially if partial, can increase if OCT circumferential peripapillary scan images are added to the transverse OCT images[27].

Uncomplicated PVD is a benign condition with a good prognosis, typically brought on by aging, and is usually not visually threatening. Floaters and myodesopsia normally subside within about 3 mo. Over time, patients develop an adaptation to the visual symptoms and/or floaters may resolve. In many cases, however, floaters can persist for 6 mo or even years[28]. Oral supplements, vitamins and proper systemic hydration can be suggested; however, medical treatment is usually not given for vitreous floaters or for PVD. If no retinal breaks or hemorrhages are found upon initial diagnosis of PVD, it is best to repeat the dilated examination within 2 to 4 wk and then at 3 mo and 6 mo from the onset of symptoms[29]. Closer follow-up is suggested in the presence of mild vitreous hemorrhages, peripheral punctate retinal hemorrhages, and the onset of new or worsening of photopsia and myodesopsia symptoms. Vitreous hemorrhages following symptomatic PVD with photopsia have been shown to be associated with retinal breaks, and thus need to be considered in this manner even if the fundus cannot be clearly assessed in those cases[29]. Patients must be adequately educated and told to seek emergency ophthalmic evaluation in the presence of new signs and symptoms related to possible RD. If a small isolated peripheral retinal break is found, guidelines for tears or breaks, which usually recommend laser treatment, must be followed to avoid RD. Symptomatic breaks after PVD should be immediately treated with laser retinal photocoagulation, which is usually performed by applying two or three rows of confluent burns around the lesion[29]. Retinal breaks can be treated with cryoretinopexy in eyes with small pupils or a hazy cornea. Laser retinopexy is superior to cryoretinopexy, and tends to be more precise, with less risk of epiretinal membrane[29].

In rare cases, floaters can be dense and numerous, which can significantly disturb vision. If symptoms are clinically important and visual acuity levels drop, treatment can be considered. Vitrectomy is the gold-standard surgical treatment for patients with vision-degrading myodesopsia[30]. Of course, that option is used in a very limited number of cases considering the risks associated with this invasive surgery. The other options that are currently being studied involve treatment with Nd:YAG laser vitreolysis[31] and photoablation[30]. However, those treatments are still experimental and not often used in routine clinical practice. Pharmacological vitreolysis with various agents (*e.g.*, hyaluronidase, vitrase, collagenase, ocriplasmin, and others) have been proposed in the literature. However, pharmacologic options tend to be considered for abnormal PVD and treatment for symptomatic vitreomacular adhesions and pucker as opposed to reducing floaters. Studies have reported that pharmacological treatments may actually induce or increase floaters rather than dissolving them [32].

PVD is a common aging-related condition that usually happens twice in a lifetime, once in each eye. The onset of this condition tends to be asymptomatic and without complications; however, acute symptoms need to be assessed quickly. New signs and symptoms like floaters, myodesopsia and flashes can be signs of PVD. An ophthalmologic examination is important to exclude abnormal PVD and acute complications

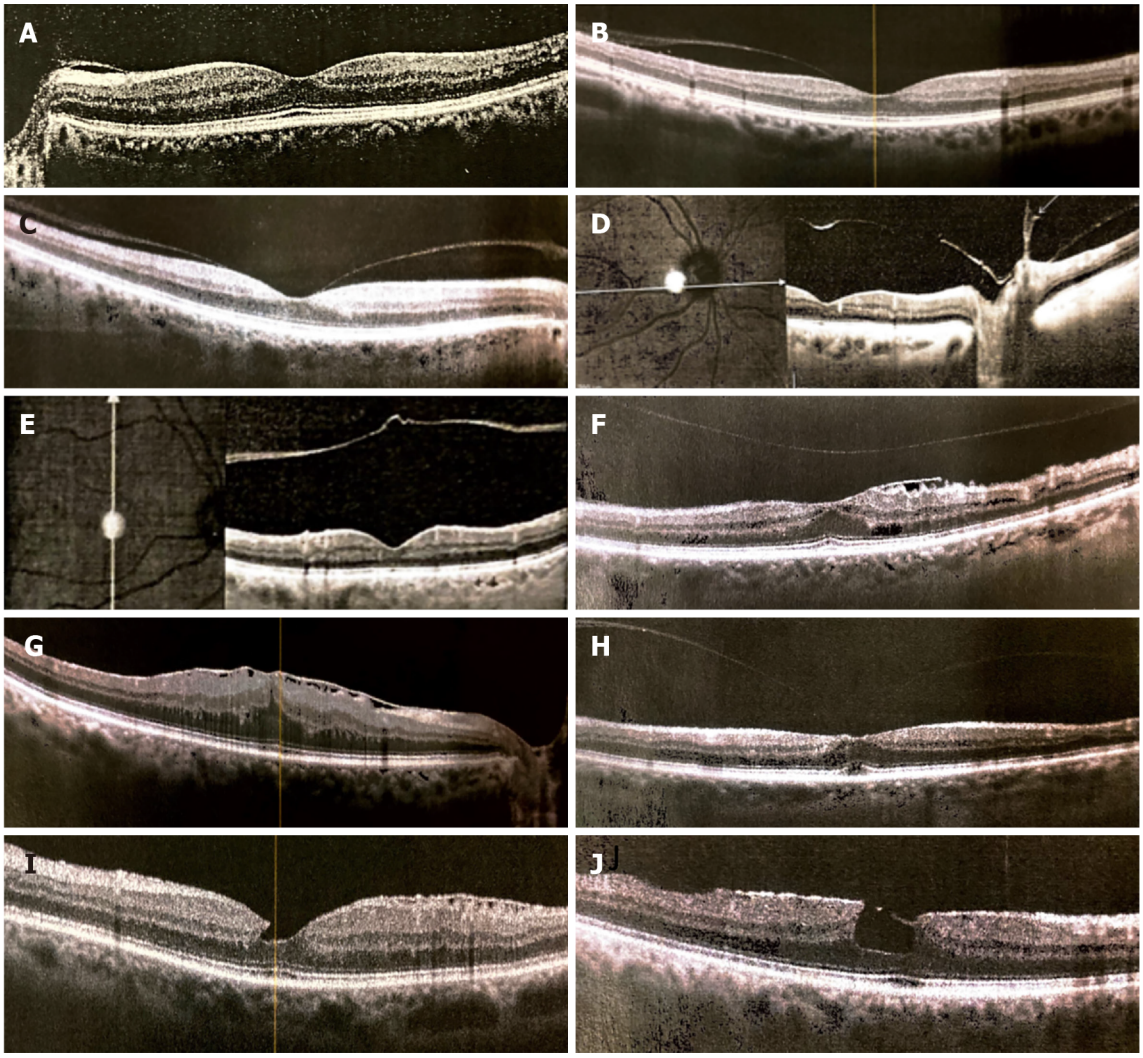

Figure 2 Different phases of posterior vitreous detachment visualized by optical coherence tomography macula scans. A-D: Various stages of posterior vitreous detachment (PVD) can be seen in focal perifoveal sectors (A-C) and around the optic nerve head (D); E: Complete PVD is seen when a crumpled translucent membrane appears floating in mid vitreous; F-J: Results of abnormal PVD caused by vitreoretinal adherence and traction can lead to macular pucker (F and G) and macular lamellar holes (H-J).

like rhegmatogenous RD, retinal breaks, and vitreal hemorrhaging. PVD may lead to other nonacute conditions that include vitreoretinal interface problems such as epiretinal membranes and macular holes. Such patients need to be assessed and managed with periodical OCT scans and ophthalmic examinations that include best corrected visual acuity and fundus examinations to determine whether or not vitrectomy surgery is needed to address visual decay caused by vitreoretinal interface problems.

CONCLUSION

PVD is part of the normal aging process. As most degenerative pathologies of aging arise in the presence of complications or abnormal acute conditions, periodic ophthalmic examinations are a must, especially after 65 years of age. Acute symptoms, such as myodesopsia and flashes need quick ophthalmic evaluation because abnormal PVD can lead to irreversible sight-threatening conditions like RD, hemorrhaging, and vitreoretinal traction, which can be avoided, prevented and treated if diagnosed early.

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MINIREVIEWS

Antenatal corticosteroids in COVID-19 perspective

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Abstract

The aim of this manuscript is to discuss the practice of antenatal corticosteroids administration for fetal maturation in severe acute respiratory syndrome coronavirus 2 positive pregnant women. Recent high-quality evidence supports the use of dexamethasone in the treatment of hospitalized patients with coronavirus disease 2019 (COVID-19). Randomized disease outcome data have identified an association between disease stage and treatment outcome. In contrast to patients with more severe forms who benefit from dexamethasone, patients with mild disease do not appear to improve and may even be harmed by this treatment. Therefore, indiscriminate usage of fluorinated corticosteroids for fetal maturation, regardless of disease trajectory, is unadvisable. Obstetrical care needs to be adjusted during the COVID-19 pandemic with careful attention paid to candidate selection and risk stratification.

Key Words: Antenatal corticosteroids; COVID-19; Dexamethasone; Pregnancy; SARS-CoV-2; Preterm delivery

Core Tip: Evidence from the randomized evaluation of coronavirus disease 2019 therapy trial supports the use of dexamethasone in the setting of maternal respiratory disease requiring either invasive mechanical ventilation or oxygen alone but not for patients receiving no respiratory support. Dexamethasone will have the added benefit of promoting fetal maturity at < 34 wk gestation in cases at risk for preterm delivery. Fetal indications for antenatal corticosteroids should be limited to obstetrical indications resulting in a high probability of preterm delivery and indiscriminate usage of fluorinated corticosteroids for fetal maturation, regardless of disease stage, is unadvisable.

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INTRODUCTION

Early in the pandemic, the use of corticosteroids as a means of immune-modulatory therapy among patients with coronavirus disease 2019 (COVID-19) was considered relatively contraindicated based on limited data suggesting adverse outcomes in the previous coronavirus outbreaks (severe acute respiratory syndrome coronavirus 1 and Middle East respiratory syndrome coronavirus)[1]. This position was supported by a 2019 meta-analysis of 6548 patients with influenza pneumonia, demonstrating that the use of corticosteroids was associated with increased mortality and duration of intensive care unit stay[2].

Notwithstanding such concerns, during the current severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, methylprednisolone, and less frequently dexamethasone (DXM), have been used globally in as great as 50% of patients with COVID-19[3].A resultant systematic review on the role of corticosteroids in the management of COVID-19 identified 5 studies (4 retrospective and 1 prospective study) with mixed findings: 3 studies have shown benefit, while 2 studies failed to demonstrate benefit with one suggesting harm from a sub-study[3].

Renewed interest in the use of corticosteroid adjunct therapy in COVID-19 followed the recent publication of the randomized evaluation of COVID-19 therapy (RECOVERY) trial, which presented preliminary compelling evidence of benefit with the use of DXM[4]. The American College of Obstetricians and Gynecologists (ACOG) and several other national and international organizations shortly thereafter reversed their initial recommendations, now prioritizing DXM as the steroid of choice in pregnant women with COVID-19. It is worth shining a light on the RECOVERY trial with a critical lens at the available data emerging from it.

BENEFICIAL EFFECT OF CORTICOSTEROIDS IS DEPENDENT ON PATIENT SELECTION

The RECOVERY trial, which is still ongoing in the United Kingdom, is an open-label, multi-center, randomized controlled study, with several arms. The study design is pragmatic, and allows for the potential differentiation between several therapeutic agents (DXM, hydroxychloroquine, lopinavir-ritonavir, azithromycin, tocilizumab, and convalescent plasma) in hospitalized patients with COVID-19. In the arm assigned to DXM treatment (6 mg daily, orally or intravenously for 10 days, or until hospital discharge), a total of 2104 patients were randomized to receive the corticosteroid and they were compared with 4324 patients randomized to the standard of care. The primary outcome (28-d mortality) was significantly reduced from 25.7% to 22.9% (rate ratio 0.83, 95%CI: 0.75-0.93; $P < 0.001$). The therapeutic effect was directly proportional to the severity of illness. In patients receiving mechanical ventilation, mortality was reduced by about one third (29.3% *vs* 41.4%; rate ratio 0.64; 95%CI: 0.51-0.81) while in those receiving oxygen without invasive mechanical ventilation, the reduction in mortality was about one fifth (23.3% *vs* 26.2%; rate ratio 0.82; 95%CI: 0.72-0.94). A striking finding occurred among patients who did not require any respiratory support to maintain adequate oxygen saturation at the time of randomization; among them, mortality was 17.8% with DXM *vs* 14.0% without DXM (non-significant difference with a rate ratio 1.19; 95%CI: 0.91-1.55). Other small observational studies have also shown a lack of benefit with corticosteroids among patients with mild COVID-19[5], and we believe that the trend towards harm with the absence of benefit warrants ongoing consideration and caution with use. Specifically, while we concur that the RECOVERY trial supports the use of DXM among hospitalized COVID-19 patients with moderate to severe respiratory disease (*i.e.*, requiring mechanical ventilation or oxygen therapy), inferring benefit in the absence of harm for patients with mild or asymptomatic disease would be premature. Our perspective is shared by the authors of the RECOVERY trial themselves, as they stated that "It is likely that the beneficial effect of glucocorticoids…is dependent on a selection of the right dose, at the right time, in the

right patient"[4].Other guiding entities have reiterated this point, including the expert consensus opinion of the Chinese Thoracic Society that stated: "Corticosteroid treatment is a double-edged sword…we oppose liberal use of corticosteroids"[6].The take-home message from the frontlines is that appropriate and judicious patient selection for potential benefit is key[4],and that corticosteroids should not be administered indiscriminately[7] nor in the outpatient setting[8].

Recognizing that every day brings better understanding of the biologic underpinnings to COVID-19, it is generally accepted that while the viral dynamics are predictable, there is marked heterogeneity among patients as to if and when they will experience clinical disease[9]. Administering DXM during early phases of disease hallmarked by viral replication may actually impair the host's functional immune response, including dampening of innate immunity, disrupting T-cell dependent initiation of humoral immunity and inhibiting requisite cognate interactions with antigen presenting cells[9,10].The net effect of disrupting initiation of functional immunity includes the potential to not only increase the circulating viral load and promote transmissibility, but also hindrance of crucial interactions within the immune system necessary for the production of lasting immunity (inclusive of the production of neutralizing antibodies, critical for immunity on re-exposure). This is not merely a theoretical consideration, as early corticosteroid administration was shown to delay viral clearance and result in higher plasma viral loads in the SARS epidemic[11].

With respect to disease severity and clinical heterogeneity, we know that COVID-19 not only presents with cardiopulmonary symptoms ranging from mild to severe but, in a subgroup of patients, is also associated with systemic autoimmune inflammation as evidenced by elevated inflammatory markers (C-reactive protein, ferritin, D-dimer, IL-1, IL-2, IL-6, IL-7, tumor necrosis factor α, granulocyte-macrophage colony-stimulating factor, macrophage inflammatory protein 1-α; the so-called "cytokine storm")[12].This dysregulated systemic inflammation is thought to be a key contributor to the COVID-19-associated fatality rate and will typically lag behind active viral replication[13].In contrast to periods with high viral replication, it is both logical and evidence-based to anticipate that corticosteroids would be of benefit amongst this subset of patients in their course of clinically evident disease. For the better part of 6 decades we have understood that corticosteroids downregulate proinflammatory cytokine transcription, consequently preventing an over-extended cytokine response and accelerating the resolution of pulmonary and systemic inflammation[14,15].

In keeping with the RECOVERY findings, DXM, a widely available and inexpensive therapeutic agent, is recommended by the World Health Organization for the treatment of patients with severe and critical COVID-19, but not in the treatment of patients with non-severe COVID-19 (www.who.int/publications/i/item/therapeutics-and-covid-19-living-guideline). Similarly, the National Institutes of Health in the US recommend against using DXM in patients with COVID-19 who do not require supplemental oxygen (www.covid19treatmentguidelines.nih.gov).

BENEFICIAL EFFECT OF CORTICOSTEROIDS IS DEPENDENT ON THE DOSE

An emerging and common pattern arising from the aggregated analysis of the experience with the use of corticosteroids in the management of COVID-19 patients is the potential for benefit with low dose corticosteroids when compared to high dose protocols[3].It is considered that a low dose of corticosteroids should not exceed 1 mg/kg per day of methylprednisolone or equivalent (Table 1). The dose of DXM used in the RECOVERY trial (6mg daily) was carefully selected to be in the low dosage range. Although high doses may exert a more rapid anti-inflammatory effect, the associated risks of secondary infections, hyperglycemia, or psychosis are also increased. High dose corticosteroids concomitantly increase the neutrophil/lymphocyte ratio and D-dimer levels. The WAYFARER Study has identified an increased risk of thromboembolism with high doses of corticosteroids, a very concerning trend since COVID-19 itself may increase the risk of coagulopathy[16].

BENEFICIAL EFFECT OF CORTICOSTEROIDS IN PREGNANCY

In the RECOVERY trial, a small number of pregnant women were enrolled, but instead

Table 1 Synthetic corticosteroids – comparative chart

Compound	Equivalent dose	Anti-inflammatory activity	Mineralocorticoid activity
Dexamethasone	0.8 mg	25	0
Betamethasone	0.8 mg	25	0
Cortisone	25 mg	0.8	0.8
Hydrocortisone	20 mg	1	1
Prednisone	5 mg	4	0.6
Prednisolone	5 mg	4	0.6
Methylprednisolone	4 mg	5	0.25

of DXM they received either prednisolone or hydrocortisone at an equivalent dosage. Prednisolone, which is inactivated by placental 17alpha-hydroxylase, as well as hydrocortisone which is rapidly inactivated by fetal enzymes, are not expected to have fetal effects and the treatment was intended exclusively for maternal benefit. Only 6 pregnant women were such treated and their number is too small to allow for valid interpretations. With the same goal, of limiting the fetal exposure, methylprednisolone, which has very limited transplacental passage, has been recommended by some to replace at least partially the DXM in the treatment of pregnant women[17].The use of methylprednisolone in COVID-19 has been studied in several small controlled trials, with a mixture of positive and negative results[18-21].Given that the sample size of many of these trials was insufficient to assess efficacy, it is reasonable to conclude that the evidence to support the use of methylprednisolone is not as robust as that demonstrated for DXM. The effectiveness of methylprednisolone or lack thereof has not been established yet and several randomized trials are currently underway or in development. Moreover, DXM may be preferable to methylprednisolone because of its higher anti-inflammatory properties and lower mineralocorticoid activity (Table 1), being therefore less likely to cause sodium and fluid retention, a concern in these critically ill patients.

The RECOVERY trial did not address the administration of antenatal corticosteroids for the purpose of fetal maturation among pregnant women with COVID-19 and it is our opinion that ACOG (www.acog.org/clinical-information/physician-faqs/covid-19-faqs-for-ob-gyns-obstetrics) and a number of other guiding bodies did not exercise sufficient caution when extrapolating the results of the RECOVERY trial to the pregnant population. Evidence from the RECOVERY trial supports the use of DXM in the setting of maternal respiratory disease, and will have the added benefit of promotion of fetal maturity at < 34 wk gestation in cases at risk for preterm delivery. Even in cases not expected to deliver prematurely, given the potential benefit of decreased maternal mortality, it is ethically acceptable to expose the fetus to a short course of low-dose DXM. In consideration here, however, is the maternal risk of morbidity and death following corticosteroid exposure in asymptomatic or mild COVID-19 cases. Indeed, the great majority of pregnant women infected with SARS-CoV-2 are not candidates for DXM by virtue of failing to meet RECOVERY criteria[22]. In a single institution study from the United States, 95% of pregnant women infected with SARS-CoV-2 remained asymptomatic or had mild disease[23].The use of antenatal corticosteroids for fetal benefit should be judiciously considered and weighed against any potential harm to the pregnant patient based on her clinical status. It has been said that in a pandemic-adjusted clinical practice, the decisions must be precisely delineated based on level of risk rather than a reflexive "one size fits all" approach[24].

CONCLUSION

Based on the above evidence and considerations, with regard to the administration of antenatal corticosteroids for fetal maturation in SARS-CoV-2 infected pregnant women, we urge consideration of the following.

The safety signal of possibly increased mortality elicited in the RECOVERY trial among patients with mild COVID-19 receiving DXM should not discourage the appropriate use of a single course of fluorinated corticosteroids (betamethasone 12 mg

daily for 2 d or dexamethasone 4 doses of 6 mg 12 h apart) for mothers with impending (within 7 d) anticipated delivery at 24 to 34 wk. The fetal indications for antenatal corticosteroids should be limited to obstetrical indications resulting in a high probability of preterm delivery. Unfortunately, the track record of antenatal corticosteroids utilization in clinical practice is inviting concern. There is a tendency to give out antenatal corticosteroids more than it is truly necessary and several studies have reported on how poorly antenatal corticosteroids are timed; 30 to 80% of women receiving them for threatened preterm birth deliver at or after 34 wk[25]. A rigorous application of the existent guidelines is necessary, promoting minimally necessary exposure and elimination of indiscriminate usage.

Contrary to the well justified, standard of care use of antenatal corticosteroids for infants delivered at 24 to 34 wk, when the anticipated benefits of antenatal corticosteroids are minimal, potential maternal adverse effects become a highly relevant concern and assuming the risk of corticosteroids administration in asymptomatic or mild COVID-19 cases is no longer warranted. Rescue corticosteroid courses are not advisable and the administration of antenatal corticosteroids after 34 wk (late preterm) may be associated with an unfavorable risk/benefit ratio. The late preterm administration of corticosteroids does not reduce neonatal mortality, overall RDS, NICU admissions or need for mechanical ventilation[26].The benefit is primarily a reduction in transient tachypnea of the newborn, a typically mild and self-limited condition. Such a modest benefit pales when weighed against maternal risks. After 34 wk, the risk of antenatal corticosteroids administered to the SARS-CoV-2 positive mothers with asymptomatic or mild disease, in our opinion, outweighs the expected modest benefit to the neonate.

The decision to use (or not use) antenatal corticosteroids is best made in consultation with a multidisciplinary team that includes maternal fetal medicine and intensive care specialists who consider the phase of the disease and the potential for maternal harm. Corticosteroids should be used prudently and withheld when maternal comorbidities pose increased risk. One such example is heart failure secondary to ischemia, where corticosteroids should be avoided since they may potentiate infarction[27].

As on so many other times before in obstetrics, our decisions have to be based on extrapolation of data from non-pregnant populations. It is hoped that in the future, pregnant and lactating women will be included in therapeutic clinical trials of COVID-19. Moreover, recognition of the further disproportionality of underserved populations and the impact of social determinants of health on both acquisition and severity of disease should prompt ardent efforts at recruiting and retaining underserved populations of reproductive age and pregnant or lactating women.

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ORIGINAL ARTICLE

Retrospective Study

Role of serological rapid antibody test in the management of possible COVID-19 cases

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Abstract

BACKGROUND

Although the detection of viral particles by reverse transcription polymerase chain reaction (RT-PCR) is the gold standard diagnostic test for coronavirus disease 2019 (COVID-19), the false-negative results constitute a big challenge.

AIM

To examine a group of patients diagnosed and treated as possible COVID-19 pneumonia whose multiple nasopharyngeal swab samples were negative for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by RT-PCR but then serological immunoglobulin M/immunoglobulin G (IgM/IgG) antibody against SARS-CoV-2 were detected by rapid antibody test.

METHODS

Eighty possible COVID-19 patients who had at least two negative consecutive COVID-19 RT-PCR test and were subjected to serological rapid antibody test were evaluated in this study.

RESULTS

The specific serological total IgM/IgG antibody against SARS-CoV-2 was detected in twenty-two patients. The mean age of this patient group was 63.2± 13.1-years-old with a male/female ratio of 11/11. Cough was the most common symptom (90.9%). The most common presenting chest computed tomography findings were bilateral ground glass opacities (77.2%) and alveolar consolidations (50.1%). The mean duration of time from appearance of first symptoms to hospital admission, to hospital admission, to treatment duration and to serological positivity were 8.6 d, 11.2 d, 7.9 d, and 24 d, respectively. Compared with reference laboratory values, serologically positive patients have shown increased levels of acute phase reactants, such as C-reactive protein, ferritin, and procalcitonin and higher inflammatory markers, such as erythrocyte sedimentation rate, lactate dehydrogenase enzyme, and fibrin end-products, such as D-dimer. A left shift on white blood cell differential was observed with increased neutrophil counts and decreased lymphocytes.

CONCLUSION

Our study demonstrated the feasibility of a COVID-19 diagnosis based on rapid antibody test in the cases of patients whose RT-PCR samples were negative. Detection of antibodies against SARS-CoV-2 with rapid antibody test should be included in the diagnostic algorithm in patients with possible COVID-19 pneumonia.

Key Words: COVID-19; Rapid antibody test; Reverse transcription polymerase chain reaction; High resolution computed tomography; Serology; Pneumonia

Core Tip: This is the first clinical retrospective study in Turkey that reports the features of the patients that were diagnosed and treated as possible coronavirus disease 2019 (COVID-19) cases whose multiple nasopharyngeal swab samples were negative by reverse transcription polymerase chain reaction (RT-PCR) but serological immunoglobulin M/immunoglobulin G antibody against severe acute respiratory syndrome coronavirus 2 was detected by a rapid antibody test. Our study demonstrated the feasibility of COVID-19 diagnosis based on rapid antibody tests in the cases of patients whose RT-PCR samples were negative. An effective diagnosis for COVID-19 is likely to require a hybrid strategy of PCR and serologic testing with the radiological

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INTRODUCTION

The coronavirus disease-2019 (COVID-19) is a unique pneumonia caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) that typically causes various degrees of respiratory disease[1]. Currently, the entire world is battling COVID-19 pneumonia, which can be lethal in high-risk patient groups. Although a COVID-19 diagnosis is generally based on clinical, laboratory, and radiological features of the patients, the gold standard test for diagnosis is the real time reverse transcription polymerase chain reaction (RT-PCR) assay from respiratory samples[2,3]. However, several studies have indicated the concerns regarding the sensitivity of RT-PCR tests[4, 5]. False negative results are thought to originate from several technical issues, including the high variability of RT-PCR tests, low nasopharyngeal viral load, manual mistakes performing the test, inappropriate collection and transportation of samples, and timing of specimen in relation to onset of symptoms, whereas false positive results are rarely seen[4].

Rapid antibody card tests can produce results in as short as fifteen minutes by detecting immunoglobulin M (IgM) and immunoglobulin G(IgG) antibodies produced against SARS-CoV-2, and they have been approved in Europe, as well as in China. Although the specificity of these tests is lower than with PCR, in some cases they can aid in the diagnosis of possible COVID-19 patients. In this retrospective study; we aimed to investigate whether these rapid antibody tests would be useful in the diagnostic challenge faced in suspected, possible COVID-19 pneumonia patients whose PCR tests were negative but has radiologically and clinically features that are consistent with COVID-19 pneumonia.

MATERIALS AND METHODS

We retrospectively evaluated the clinical characteristics, laboratory results, and radiological features of 80 possible COVID-19 patients with multiple negative RT-PCR tests and reported the characteristics of 22 serologically positive COVID-19 patients.

Patient Selection

In Turkey, rapid antibody test kits for COVID-19 were become commercially available at the beginning of April 2020. Symptomatic RT-PCR-negative patients who were suspected to be infected with SARS-CoV-2 based on epidemiological history, laboratory results, and positive radiological findings were included in the study. Until September 2020, we were able to test 80 suspected RT-PCR negative possible COVID-19 patients; 22 serologically positive cases were detected**.** All patients had a contact history and most patients had a history of a family member who tested positive with RT-PCR for COVID-19 disease. All COVID-19 antibody test positive cases had fever and at least one respiratory system symptom such as cough, dyspnea, or sputum. Herein, we introduced features of 22 serologically positive COVID-19 cases. High resolution computed tomography (HRCT) was used for the radiological assessment. In patients with possible COVID-19 pneumonia, ground-glass formation and/or consolidative opacities distributed usually bilateral, peripheral, and mostly basal, were considered as positive HRCT findings. The patients with negative RT-PCR tests were tested for specific antibodies against SARS-CoV-2 following the COVID-19 treatment, which was in average 24 d after the initiation of symptoms.

COVID-19 IgM/IgG rapid antibody test

Samples were taken from the patients with oro-nasopharyngeal and nasal swabs and analyzed by RT-PCR. The humoral responses against SARS-CoV-2 were tested with rapid card test with blood samples of patients. The blood taken from the patient was dropped on this rapid card test and the total antibody response (either IgM or IgG) was analyzed. The clinical samples were anonymized and used in accordance with local ethical guidelines. Total antibody levels against SARS-CoV-2 were noted. We used the Colloidal Gold SARS-CoV-2 IgG/IgM Rapid Test (Beijing Hotgen Biotech Co., Ltd), which is a lateral flow chromatographic immunoassay detecting total antibodies produced against the SARS-CoV-2**.** The anti-SARS-CoV-2 virus IgM, if present in the specimen, will bind to the SARS-CoV-2 conjugates. The immuno-complex is then captured by the anti-human IgM line, forming a burgundy colored M Line, indicating a SARS-CoV-2 virus IgM positive test result.

Statistical analysis

Mean and standard deviations were given for normally distributed metric variables. Frequencies and percentages were given for non-metric variables.

RESULTS

The demographic and clinical characteristics of 22 serologically positive RT-PCR negative COVID-19 patients were shown in Table 1. Each of these patients had at least two consecutive negative PCR tests, taken at a minimum of 2 d apart. The mean age was 63.2 ± 13.1-years-old and male to female ratio was 11/11. The mean duration of time from appearance of first symptoms to hospital admission, to hospital admission, to treatment duration and to serological positivity were 8.6 ± 7.2, 11.2 ± 5.4, 7.9 ± 3.2 and 24 ± 17 d, respectively.

The radiological findings and drug regimens were shown in Table 2. The radiological findings, such as bilateral reticular and ground-glass opacities were demonstrated in Figures 1-5. Also, dense consolidations were noted in Figures 3 and 5. The bilateral fibroreticular infiltrates with crazy-paving patterns are shown in Figure 6. Hydroxychloroquine and/or azithromycine and/or favipiravir therapy was initiated by the consensus of infectious disease specialists and pulmonologists according to the clinical, laboratory, and radiological findings of the patients. The selection of the drug regimen was made based on the clinical evaluation of each patient.

The laboratory results of the patients were given in Table 3. Compared with reference laboratory values, serologically positive patients have shown increased levels of acute phase reactants such as C-reactive protein, ferritin, and procalcitonin, higher inflammatory markers, such as erythrocyte sedimentation rate, lactate dehydrogenase enzyme, and fibrin end-products, such as D-dimer. A left shift on white blood cell differential observed with increased neutrophil counts and decreased lymphocytes.

DISCUSSION

In patients with possible COVID-19 pneumonia, rapid identification, isolation, and treatment of infected individuals will be a key step to prevent onward community transmission. Currently, COVID-19 diagnosis is made by the direct detection of SARS-CoV-2, supported by clinical, laboratory and radiological features of the suspected patients. According to the first COVID-19 case series by Bai *et al*[4]; the sensitivity of CT was estimated to be 97% compared to PCR tests, which had 71% sensitivity[4]. Ai *et al*[5] also reported as the sensitivity of RT-PCR assays to be in the range of 60% to 70% [5]. Here, our results supported that chest CT results were more sensitive than RT-PCR results to suspect from a possible COVID-19 diagnosis.

It was suggested that PCR-negative cases with positive CT findings and high clinical suspicion may benefit from repeated RT-PCR testing[6]. Shi *et al*[7] reported that COVID-19 pneumonia might manifest with chest CT imaging abnormalities, even in asymptomatic patients, with rapid evolution from focal unilateral to diffuse bilateral ground glass opacities that progressed to, or coexisted with, consolidations within 1–3 wk[7]. Another study with 1099 patients from China revealed that 56% of patients had ground-glass opacities, but no radiological findings were reported in 18% of COVID-

Table 1 Demographic and clinical characteristics of serologically positive reverse transcription polymerase chain reaction negative coronavirus disease 2019 patients

	***n* (%)**
Agein yr	
mean ± SD	63.2 ± 13.1
Gender	
Male/Female	11/11
Symptoms, *n* (%)	
Cough	20 (90.9)
Dyspnea	14 (63.6)
Fever	10 (45.4)
Chest pain	8 (36.3)
Duration in d, mean ± SD	
From first symptom to admission	8.6 ± 7.2
Hospital stay	11.2 ± 5.4
From symptoms to antibody test	24 ± 17
Drug treatment	7.9 ± 3.2

Table 2 Radiological findings and drug regiments of serologically positive reverse transcription polymerase chain reaction negative coronavirus disease 2019 patients

	***n* (%)**
Radiology	
GGO	17 (77.2)
Consolidation	11 (50)
Nodular infiltrates	4 (18.1)
Fibroreticular infiltrates	3 (5.9)
Drug regimens	
HCQ+Azithromycine + Favipravir	11 (50)
HCQ+Azithromycine	7 (31.8)
Favipravir	4 (18.1)

GGO: Ground-Glass Opacities; HCQ: Hydroxychloroquine.

19 cases. Although bilateral and peripheral ground-glass opacities constitute the most typical CT findings, they were not specific for the COVID-19 disease[8,9]. Since radiological evaluation of the thorax is often the key diagnostic element in patients with possible COVID-19 pneumonia, like in our present study, the patients with positive CT findings but negative RT-PCR results should be isolated and re-evaluated [9,10]. Combined assessment of imaging features with clinical and laboratory findings is key to facilitate an early diagnosis of COVID-19. Therefore, we suggest that in RT-PCR-negative cases, radiological diagnosis should be supported with specific antibody detection. Our study demonstrated that the diagnosis of COVID-19 should be confirmed by rapid antibody test at least 5 d after the treatment of RT-PCR negative patients with typical CT findings.

SARS-CoV-2 can be detected in different tissues and body fluids. In our study, the nasopharyngeal and nasal swabs samples taken from the patients were utilized and assessed by RT-PCR test. In a study on 1070 specimens collected from 205 patients with COVID-19, bronchoalveolar lavage fluid specimens showed the highest positive rates (14 of 15; 93%), followed by sputum (72 of 104; 72%), nasal swabs (5 of 8; 63%),

Table 3 The laboratory parameters of serologically positive reverse transcription polymerase chain reaction negative coronavirus disease 2019 patients

Value	mean ± SD
ESR in mm/h	68.5 ± 41.7
LDH in U/L	362 ± 152
CRP in mg/L	95 ± 101
Ferritinin µg/L	778 ± 684
WBCs	8621 ± 3549
Lymphocytes, *n* (%)	1430 ± 530
Lymphocytes, *n* (%)	22 ± 10.8
Neutrophils, *n* (%)	5390 ± 2450
Neutrophils as %	70.5 ± 12.3
D-Dimer in mg/L	1875 ± 2757
Procalcitonin in mg/L	0.15 ± 0.03

CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; LDH: Lactate dehydrogenase; WBCs:White blood cells.

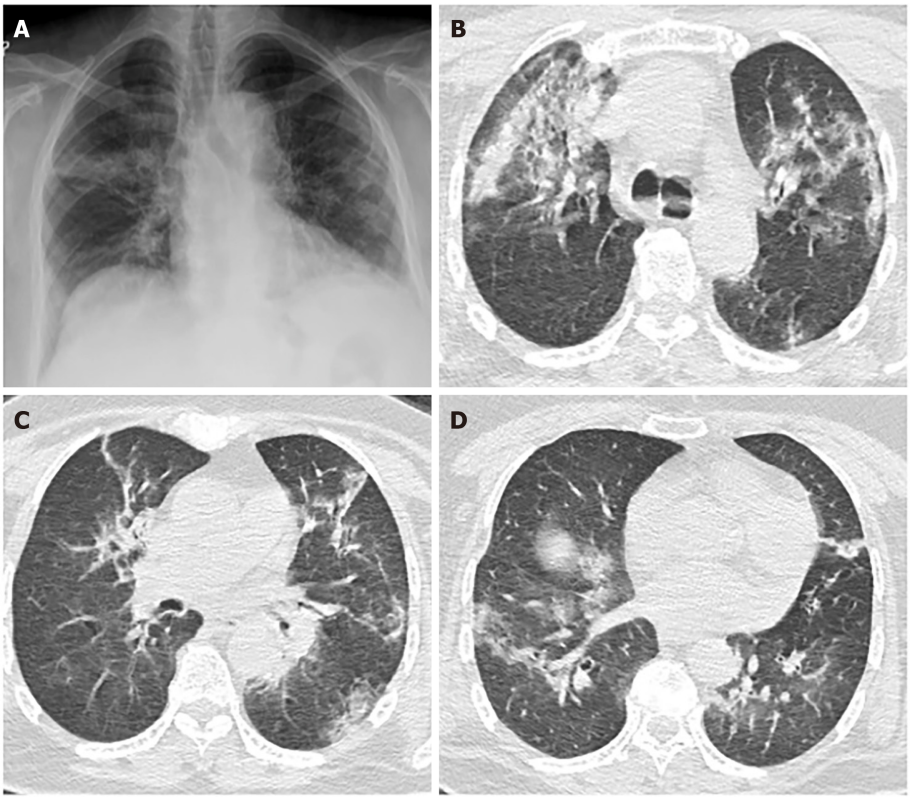

Figure 1 Example of the radiological images of a patient whose multiple reverse transcription polymerase chain reaction were negative but serological immunoglobulin M/immunoglobulin G against severe acute respiratory syndrome coronavirus 2 positive. A: Chest radiograph of coronavirus disease 2019 (COVID-19) patient showing the bilateral infiltrates; B-D: High resolution computed tomography images showing the bilateral reticular and ground-glass opacities of COVID-19 patient.

fibro-bronchoscopy brush biopsy (6 of 13; 46%), pharyngeal swabs (126 of 398; 32%), feces (44 of 153; 29%), and blood (3 of 307; 1%). None of the 72 urine specimens tested positive[9]. That study by Ding *et al*[9] supported that sensitivity of nasal and nasopharyngeal swabs for PCR tests remained questionable.

The first comprehensive study on the host humoral response against SARS-CoV-2 has shown that serological response can aid in the diagnosis of COVID-19, including

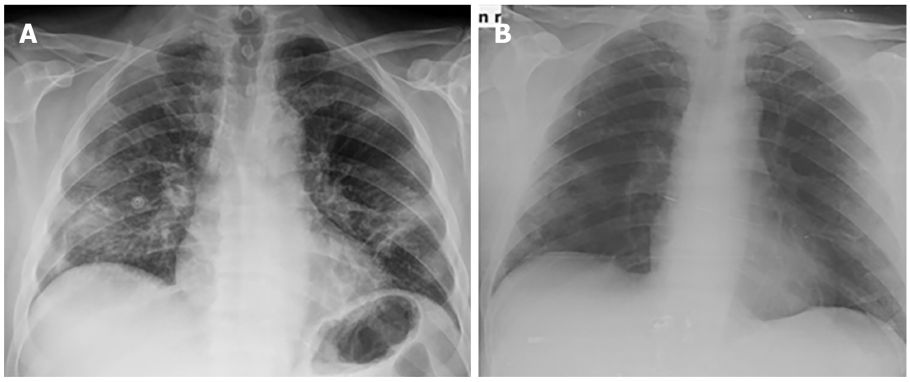

Figure 2 Example of the radiological images of a patient whose multiple reverse transcription polymerase chain reaction were negative but serological immunoglobulin M/immunoglobulin G against severe acute respiratory syndrome coronavirus 2 positive. A: Chest x-ray of the coronavirus disease 2019 (COVID-19) patient showing the bilateral infiltrates before treatment; B: Chest x-ray of the COVID-19 patient showing reduced bilateral infiltrates after treatment.

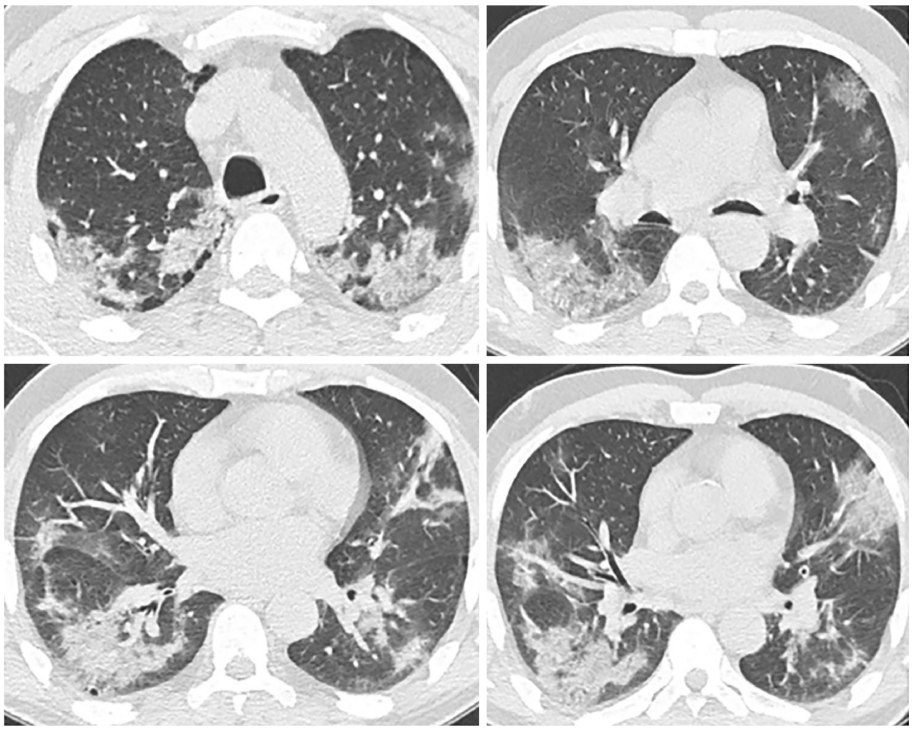

Figure 3 High resolution computed tomography images of coronavirus disease 2019 patient showing the bilateral ground-glass opacities and consolidations.

those subclinical cases. In that study, IgA, IgM, and IgG response using an ELISA-based assay on the recombinant viral nucleocapsid protein was analyzed in 208 plasma samples from 82 confirmed and 58 probable cases[11,12]. The median duration of IgM and IgA antibody detection were 5 d (IQR 3-6), while IgG was detected on day 14 (IQR 10-18) after symptom onset, with a positive rate of 85.4%, 92.7% and 77.9% respectively. It was shown that detection efficiency by IgM ELISA was higher than that of PCR after 5.5 d of onset of symptoms. In another study of 173 patients, the seroconversion rates (median time) for IgM and IgG were 82.7% (12 d) and 64.7% (14 d), respectively. Our study also reported the mean duration of time from appearance of first symptoms to hospital admission, to hospital admission, to treatment duration and to serological positivity were 8.6 ± 7.2, 11.2 ± 5.4, 7.9 ± 3.2 and 24 ± 17 d, respectively. It was also reported that a higher titter of antibody was independently associated with severe course of diseases[13]. Since our study included only RT-PCR-negative serologically positive COVID-19 patients who were diagnosed and treated based on radiological and clinical findings, we were unable to compare the severity of RT-PCR-positive and RT-PCR-negative COVID-19 patients.

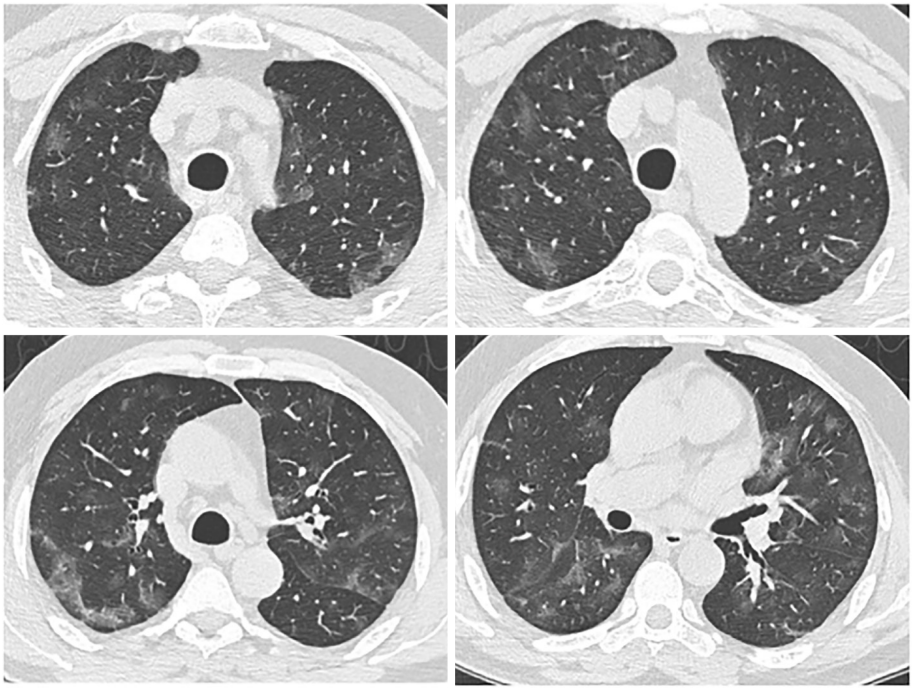

Figure 4 High resolution computed tomography images showing the bilateral patchy ground-glass opacities in a coronavirus disease 2019 patient.

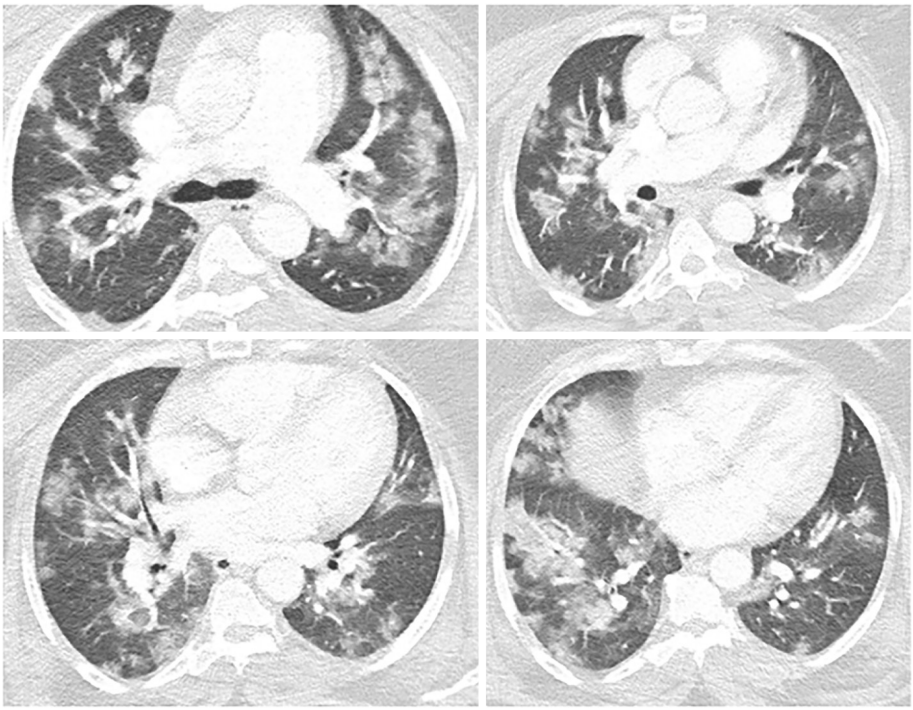

Figure 5 High resolution computed tomography images in a severe coronavirus disease 2019 patient showing the bilateral patchy ground-glass opacities with consolidations.

To date, several population-based studies demonstrated false-negative RT-PCR is a particular concern in the diagnosis of COVID-19. Baron *et al*[14] reported that among COVID-19 patients, the ratio of false-negative RT-PCR results was 18% compared to a negative serology ratio of 4%[14]. West *et al*[15] clearly stated that the variety in the test performance and diagnostic validity of different methods have not been well investigated, which raises concern for generating a false sense of security[15]. As Benoit[16] suggested, a multi-step strategy to limit the likelihood of COVID-19 patients to be labeled incorrectly as negative should be applied, which includes RT-

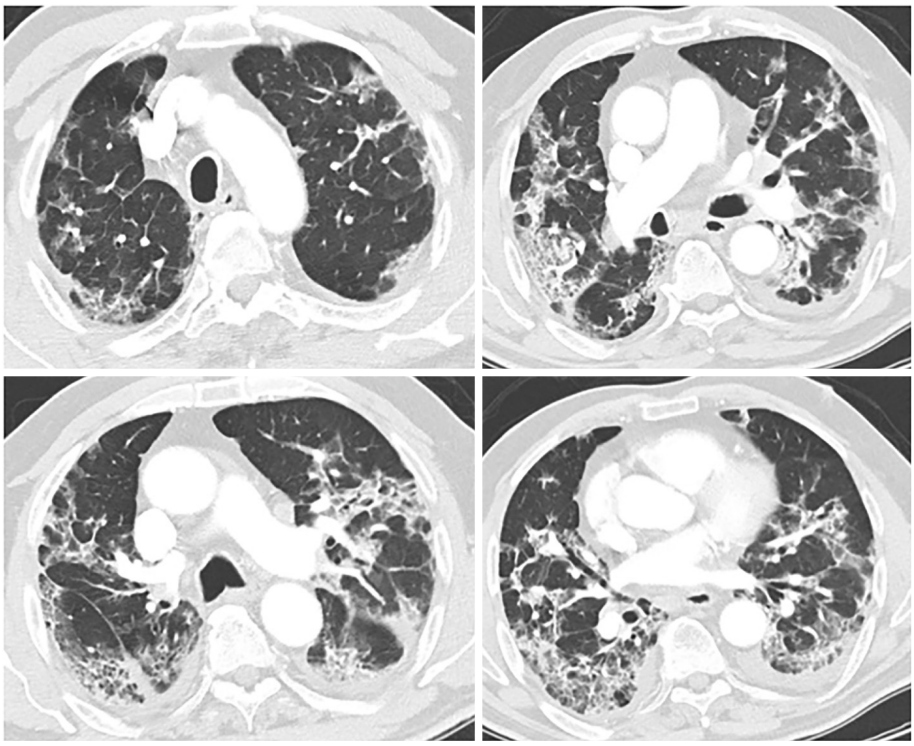

Figure 6 High resolution computed tomography images of a severe coronavirus disease 2019 patient showing the bilateral fibroreticular infiltrates with crazy-paving pattern.

PCR tests, serological testing, and clinical and radiological findings of the patients[16]. It should be noted that RT-PCR tests alone to define COVID-19 negative cohorts are not valid and likely to produce biased results based on many concerns regarding the sensitivity of RT-PCR assays.

Our study has several limitations, including low sample size and follow-up for serology results due to its retrospective nature; however, ideal research conditions are often difficult to be establish during a pandemic situation. Also, the comparison of laboratory and radiological findings between patients who demonstrated a seroconversion and those who did not could better reveal the differences and may give information about the severity of the disease course. In addition, this study did not differentiate the serological results in terms of specific IgM and IgG against SARS-CoV-2.

CONCLUSION

In conclusion, our study remarks the feasibility of total antibody testing by a rapid card test in the diagnosis of suspected PCR-negative COVID-19 patients who are likely to have false negative results or viral clearance of the upper respiratory tract. Even though there is no specific treatment for COVID-19, it is highly important to confirm the diagnosis of highly suspected cases to prevent further transmission and to prevent long-term complications. We suggest that detection of antibodies against SARS-CoV-2 with rapid-card test should be included in the diagnostic algorithm in PCR-negative patients with COVID-19 pneumonia. An effective diagnosis is likely to require a hybrid strategy of PCR and serologic testing with radiological demonstration.

ARTICLE HIGHLIGHTS

Research background

Novel coronavirus disease (COVID-19) is unique pneumonia caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that typically causes various degrees of respiratory disease. Currently, the entire world is battling COVID-19 pneumonia, which can be lethal in high-risk patient groups. Although COVID-19

diagnosis is generally made based on clinical, laboratory, and radiological features of the patients, the most common standard of care for diagnosis is the reverse transcription polymerase chain reaction (RT-PCR) assay.

Research motivation

Several studies have indicated concerns regarding the sensitivity of RT-PCR tests, and an alternative rapid test is required to confirm the diagnosis by RT-PCR test.

Research objectives

In this study; we aimed to investigate whether rapid antibody tests would be useful in the diagnostic challenge faced in suspected COVID-19 patients whose PCR tests were negative but has radiologically and clinically consistent features with COVID-19.

Research methods

Eighty suspected COVID-19 patients who had at least two negative consecutive COVID-19 PCR tests and were subjected to serological rapid antibody tests were evaluated. The clinical and laboratory characteristics of serologically positive RT-PCR negative COVID-19 patients were presented in this study.

Research results

The specific serological total immunoglobulin M/immunoglobulin G antibody against SARS-CoV-2 was detected in 22 patients. The most common presenting chest computed tomography findings were bilateral ground glass opacities (77.2%) and alveolar consolidations (50.09%). The mean duration of time from appearance of first symptoms to hospital admission, to hospital admission, to treatment duration and to serological positivity were 8.6, 11.2, 7.9, and 24 d, respectively. Compared with reference laboratory values, serologically positive patients have shown increased levels of acute phase reactants such as C-reactive protein, ferritin, and procalcitonin, higher inflammatory markers, such as erythrocyte sedimentation rate, lactate dehydrogenase enzyme, and fibrin end-products, such as D-dimer. A left shift on white blood cell differential was observed with increased neutrophil counts and decreased lymphocytes.

Research conclusions

Rapid serological card tests can be a feasible alternative in the diagnosis and treatment algorithm of suspected COVID-19 cases.

Research perspectives

An effective diagnosis for COVID-19 is likely to require a hybrid strategy of PCR and serologic testing with radiological demonstration.

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MINIREVIEWS

Emerging role of cell-free DNA in kidney transplantation

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Abstract

Monitoring kidney transplants for rejection conventionally includes serum creatinine, immunosuppressive drug levels, proteinuria, and donor-specific antibody (DSA). Serum creatinine is a late marker of allograft injury, and the predictive ability of DSA regarding risk of rejection is variable. Histological analysis of an allograft biopsy is the standard method for diagnosing rejection but is invasive, inconvenient, and carries risk of complications. There has been a long quest to find a perfect biomarker that noninvasively predicts tissue injury caused by rejection at an early stage, so that diagnosis and treatment could be pursued without delay in order to minimize irreversible damage to the allograft. In this review, we discuss relatively novel research on identifying biomarkers of tissue injury, specifically elaborating on donor-derived cell-free DNA, and its clinical utility.

Key Words: Biomarker; Donor-derived cell-free DNA; Kidney allograft outcomes; Kidney transplant; Allograft biopsy

Core Tip: Donor-derived cell-free DNA (dd-cfDNA) is now available as a noninvasive biomarker to evaluate the risk of rejection in kidney allografts and other organ transplants. The technology utilizes next generation sequencing and does not require donor genotyping. In this review we discuss the current literature on the utility of dd-cfDNA in kidney transplantation, the limitations, and future directions.

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INTRODUCTION

Kidney transplants offer the best survival to patients with end-stage kidney disease [1]. Conventional monitoring of kidney transplant recipients includes serum creatinine, proteinuria, and donor-specific antibodies (DSA), which are neither sensitive nor specific. Surveillance biopsies are performed for allograft monitoring in a few centers, but are invasive and have multiple disadvantages including bleeding risk, inconvenience, sampling error, and poor reproducibility in interpretation. They generally have low yield as the majority reveal normal histology and have not been validated to improve outcomes.

The risk of renal allograft dysfunction from acute rejection (AR) during the first year after transplant is around 10%-15%. AR can be either acute T cell-mediated rejection (TCMR) characterized by lymphocytic infiltration of tubules, interstitium, and in severe cases, vessels causing cytotoxic injury or acute antibody-mediated rejection (ABMR) caused by DSA resulting in complement activation and lysis of target cells. A rise of serum creatinine is a delayed marker of the assessment of AR. By the time the serum creatinine rises, significant histological damage has already occurred, thereby significantly lowering the chance of complete recovery from injury. There have been multiple efforts to develop a noninvasive biomarker that promptly, accurately, and inexpensively predicts immunological allograft injury at an early stage with high sensitivity, specificity, and predictive value[2]. An ideal biomarker should assess the risk of injury, diagnose and monitor the injury and the pharmacological response, have prognostic value, and assess the safety of treatment[3].

NONINVASIVE BIOMARKERS IN ORGAN TRANSPLANTATION

Over the last decade, the emphasis has been on finding the perfect biomarker, which will help to predict, diagnose, and treat rejection in order to improve short- and long-term transplant outcomes. Various biomarkers in different categories have been studied, including blood mRNA (Granzyme B, Perforin, FasL, HLA-DRA, and multigene signature); blood donor-derived cell-free DNA (dd-cfDNA); blood proteins (DSA, C1Q binding, Pleximune, Immuknow, kSORT, IFN-γ Elispot and, TCR repertoire); urinary mRNA (Perforin, Granzyme B, PI-9, CD103, FOXP3, CXCL10, NKG2D, TIM3, Granulysin, and multigene signature); and urinary proteins (CXCL9, CXCL10, and Fractalkine)[4]. The markers have varying degrees of sensitivity and specificity and are summarized in Table 1. dd-cfDNA has recently become as one of the most commonly used biomarkers. The purpose of this review is to outline the discovery and utility of dd-cfDNA and to evaluate the available data regarding its use in kidney transplantation.

DD-CFDNA

Plasma cell-free DNA has been used as a biomarker in prenatal testing, cancer diagnosis, and organ transplantation[5-8]. Multiple studies have shown that allograft-derived cell-free DNA can be detected and quantified as a fraction of total cell-free DNA in the plasma or serum of various solid organ transplant recipients[8-10] such as kidney[11], heart[12], lung[13], pancreas[14], and liver[15]. Similar studies were also done looking at cell-free DNA excretion in urine[16]. This noninvasive marker was extensively studied in heart transplant recipients by Snyder *et al*[9], where significantly increased levels of dd-cfDNA were noted with biopsy-proven AR. Severity of rejection worsened with increasing levels of dd-cfDNA. In addition to being a marker of rejection, dd-cfDNA can also be used as an individualized tool to assess the efficacy of immunosuppressive treatment. In a study of liver transplant recipients, higher tacrolimus levels were associated with lower amounts of dd-cfDNA, suggesting that the relationship could be used as a tool for optimizing immunosuppressant drug dosing[17]. The presence of dd-cfDNA in the plasma of solid organ transplant patients was first described in 1990's, and involved measurement of Y chromosome DNA

Table 1 Biomarkers studied in the field of transplantation

Ref.	Biomarker name	Biomarker assay method	Sample size	Rejection type	Sensitivity/ specificity	PPV/NPV	Comments
Patel *et al*[56]	CDC crossmatch	Micro-cytotoxicity assay	225	Hyper acute rejection/ early graft loss	0.75/0.97	0.80/0.97	FDA approved
Mahoney *et al* [57]	Flow crossmatch	Flow cytometry	90	Early graft loss	0.71/0.74	0.33/0.93	FDA approved
Pei *et al*[58]	Luminex	HLA beads; flow cytometry	10	Anti HLA Ab	-	-	FDA approved
Ashokkumar *et al* [59]	Pleximmune	T cytotoxicmemory cell	32	Acute rejection	0.88/0.94	0.93/0.88	FDA approved
He *et al*[60]	Cylex-Immuknow	Lymphocyte ATPgeneration	42	CD4 T cell function	-	-	FDA approved
Loupy *et al*[61]	C1q bindingassay	Flow cytometric C1q binding	1016	TCMR/ABMR/graft loss	-	-	FDA approved
Hricik *et al*[62]	IFN-γ ELISPOT	Donor-reactive memory T cell	21	De novo DSA/ rejection	1.0/0.67	0.67/1.0	Not FDA approved
Roedder *et al*[63]	KSORT	PBLRNA by qPCR	143	AR	0.83/0.91	0.81/0.91	Not FDA approved
Bloom *et al*[33]	dd-cfDNA	PBL single gene sequencing	102 (107 samples)	ABMR and I b or higher TCMR	0.59/0.85	0.61/0.84	FDA approved
Acquino–Dias *et al*[64]	FOXP3	PBL, urine (PCR)	65 (78 sample)	AR *vs* DGF	0.94/0.95	0.94/0.95	
Li *et al*[65]	Granzyme B, perforin	Urine mRNA (PCR)	85 (151 samples)	AR	0.79-0.83 /0.77-0.83	-	
Hricik *et al*[66]	Urine CXCL9	Urine ELISA	258	TCMR	0.85/0.81	0.68/0.92	Not FDA approved
Suthanthiran *et al* [67]	Urine 3 gene; CD3E, CXCL10, 18SrRna	Urine RNA by PCR	485 pts (4300 samples)	Diagnosis AR 20 d early	0.79/0.78	-	Not FDA approved
Renesto *et al*[68]	TIM-3	PBL, urine mRNA PCR	115 (160 samples)	Diagnose AR, values normal post treatment	0.87/0.95	0.87/0.93	
Valujskikh *et al* [69]	miRNA-210	Urine miR-210 PCR	81 (88 samples)	Diagnose AR, values normal post treatment	0.52/0.74	-	

ABMR: Antibody-mediated rejection; AR: Acute rejection; CDC: Complement dependent cell cytotoxicity; dd-cfDNA: Donor-derived cell-free DNA; ELISA: Enzyme-linked immunosorbent assay; FDA: Food and Drug Administration; HLA: Human leukocyte antigen; IFN: Interferon; PBL: Peripheral blood lymphocyte; PCR: Polymerase chain reaction; TCMR: T cell-mediated rejection.

particles in a female recipient from a male donor[18].

BIOLOGY AND KINETICS OF CELL-FREE DNA

Levels of cell-free DNA fluctuate randomly during the day[19,20] and vary with multiple factors including age[21], exercise, obesity[22], malignancy, transplant[20], acute coronary syndrome[23], stroke[24], and other pathological conditions. The concentration of cell-free DNA may vary from 3.5-100 ng/mL[20,25]. Cell-free DNA is rapidly cleared from the plasma. Its The mean clearance half-life of fetal Y chromosome DNA particles from maternal plasma was reported by Lo *et al*[26] to be 16 min. Cell-free DNA is primarily cleared by apoptosis, necrosis, and active secretion. Less than 20% is secreted in urine[27] and partly degraded by the liver[28] and endonucleases in the plasma and other tissues. Yu *et al*[29] found clearance of fetal cell-free DNA to occur in two phases, one with a half-life of about 1 h and a slower phase with a half-life of about 13 h. Nearly complete disappearance of fetal cell-free DNA occurs by 2 d postpartum.

In kidney transplant recipients, the kinetics of dd-cfDNA were described in detail by Shen *et al*[30], where the authors compared the dynamics of degradation of dd-cfDNA in the immediate post transplant period in kidney transplant recipients from

living donors (LD) compared with deceased donors (DD) where donors had cardiac death, and some experienced delayed graft function. Based on their analysis, the mean dd-cfDNA concentration was 20.69% at 3 h, 5.22% by about 16 h, and 0.85% by day 7. The concentrations were significantly higher in recipients of kidneys from DDs than LDs initially (45% *vs* 10%) and on day 7 d (1.11% *vs* 0.59%) probably because of higher levels of ischemia reperfusion injury in the former group. Other large solid organs, such as livers may have more cell turnover and larger proportions of dd-cfDNA released in the recipient. Beck *et al*[10] found dd-cfDNA fractions of 90% immediately after transplant with steady state levels below 15% by day 10.

MEASURING DD-CFDNA

The technology of measuring dd-cfDNA initially had some limitations as the assays required prior recipient and donor genotyping and were time consuming and expensive[8,9]. Newer technologies that have been validated as clinical-grade assays measure dd-cfDNA in transplant recipients by polymerase chain reaction (PCR) such as real-time quantitative PCR, droplet digital PCR, or next generation sequencing (NGS) as described by Grskovic *et al*[19]. Droplet digital PCR and NGS have been clinically investigated and validated over a wide range for detecting rejection in transplant recipients[31,32]. The basic principle of measuring dd-cfDNA is by measuring single nucleotide polymorphisms (SNPs) that are homozygous in the recipient and differ from those of the donor. That can be accomplished in the absence of donor genotyping[33]. There are no standardized assays to be used for transplantation, in terms of the number of SNPs. The commercially available assays using NGS technology so far are Allosure, (CareDx, Brisbane, CA, United States), which targets 266 SNPs[34]; Prospera, (Natera Inc, San Carlos, CA, United States), which targets 13392 SNPs[35], Viracor Transplant Rejection Allograft Check (TRAC) combined with TruGraf, and (Eurofins Viracor, (Lee's Summit, MO, United States), which targets 70000 SNPs[36]. There is one study that compared the results of two commonly available commercial assays in United States; Allosure and Natera, involving 76 kidney transplant recipients. It found no significant differences in the test results for predicting rejection or other test characteristics, but found some differences in the test result turnaround time[36,37]. The recipient genotype is determined at each SNP and the relative fraction of dd-cfDNA is computed using custom bioinformatics tools. The performance of the assay was validated in 1117 samples from related and unrelated transplant recipients with reliability and precision. The turnaround time of the test was 3 d, which was considered as a practical time frame for transplant recipients.

REPORTING DD-CFDNA AS A FRACTION *VS* ABSOLUTE VALUE

In clinical application, the dd-cfDNA value is expressed as a fraction of background circulating cell-free DNA fragments. This assumes that the recipient's DNA fragments remain constant. However the host's cell-free DNA fragment levels can vary in different scenarios such as exercise, inflammatory state, and body size[22,38,39]. In a recent report involving 121 stable kidney transplant recipients, there was a significant negative correlation of the average baseline dd-cfDNA fractions between 4-12 wk post-transplantation and increasing recipient BMI[22]. That indicates that dd-cfDNA fractions are influenced by recipient body size.

Previous studies have compared absolute dd-cfDNA values to fractional values[40-42]. The analysis by Whitlam *et al*[40] included 61 samples and reported similar areas under the curve (AUC) for diagnosing ABMR, with an absolute dd-cfDNA value of 0.91 [95% confidence interval (CI): (0.82-0.98)] and a dd-cfDNA fraction of 0.89 (95%CI: 0.79-0.98). Neither measure was very useful in diagnosing 1A and borderline TCMR rejection. In a prospective observational study, Oellerich *et al*[42] compared dd-cfDNA quantification of copies/mL plasma to dd-cfDNA fraction at prespecified visits in 189 patients over 1 yr post kidney transplant. Median dd-cfDNA (copies/mL) was 3.3-fold and the median dd-cfDNA fraction was 2.0 fold higher in patients with biopsy-proven rejection (n = 15 with 22 samples) compared with the median in stable patients (n = 83 with 408 samples). Measuring dd-cfDNA (copies/mL) showed superior performance (P = 0.02) with an AUC of 0.83 compared with the dd-cfDNA fraction, which had an AUC of 0.73. A subset analysis found a significant inverse correlation between tacrolimus levels and dd-cfDNA (copies/mL), implying that dd-cfDNA may be useful

in evaluating adequacy of immunosuppression. A subsequent study from the same group evaluated the longitudinal time-dependent changes in total cfDNA (copies/mL), dd-cfDNA (copies/mL) and dd-cfDNA fraction in 303 clinically stable kidney transplant recipients 12-60 mo post-transplantation[41]. Total cfDNA showed a significant decline over time, resulting in increasing dd-cfDNA fractions, with doubling of the 85th percentile value by 5 yr. In contrast, dd-cfDNA (copies/mL) values remained stable during the same period. The authors concluded that measurement of absolute dd-cfDNA concentrations minimize false positive results compared with dd-cfDNA fractions and were hence superior for long-term allograft monitoring. Further large scale studies are still needed to define the ideal method of dd-cfDNA monitoring.

DD-CFDNA IN DIAGNOSING AR IN KIDNEY TRANSPLANTATION

The Diagnosing AR in Kidney Transplant Recipients (DART) study by Bloom *et al*[33] focused more on dd-cfDNA (Allosure, CareDx, Brisbane, CA) as a novel biomarker in discriminating subclinical rejection from no rejection at an early stage, which could allow early intervention and hopefully better outcomes. It was a prospective multicenter study of renal allograft recipients (n = 102) that used targeted amplification of dd-cfDNA by sequencing of SNPs to quantify donor and recipient DNA contributions in the plasma without the need of donor genotyping. A dd-cfDNA level of < 1% had an AUC of 0.87 (95%CI: 0.75-0.97) for discriminating ABMR from no rejection. The positive predictive value (PPV) and negative predictive value (NPV) with a cutoff of < 1% were 44% and 96% respectively, which was quite significant, suggesting a dd-cfDNA value of > 1% may indicate active rejection (TCMR type ≥ 1 b or ABMR) where the sensitivity and specificity were 59% and 85% respectively. The hope is that this noninvasive biomarker could replace the need of surveillance biopsies done at some centers to monitor for rejection. A limitation of the study was that the test failed to pick up borderline TCMR type Ia rejection. Measurement of dd-cfDNA as a steady state fraction of recipient cfDNA in kidney transplants was first described by Bromberg *et al*[32], using the Allosure test. The study established that in steady state, a dd-cfDNA fraction above 1.2% could be abnormal and potentially predict AR. The results of the Prospera test were reported by Sigdel *et al*[35] in a single center retrospective study from a curated biobank. Along the same lines, a study by Jordan *et al*[34] combining the use of elevated DSA with dd-cfDNA > 1% increased the pro-bability of diagnosis of ABMR. That study involved 87 kidney transplant recipients, 16 had ABMR, and the PPV of a 1% threshold level of dd-cfDNA to detect active ABMR in DSA positive patients was 81%, whereas the NPV was 83%. The PPV for DSA positivity alone was 48%.

Based on pivotal validation studies, dd-cfDNA became Medicare reimbursable in October 2017 for noninvasive monitoring of rejection in transplant recipients. A subsequent external validation study by Huang *et al*[43] in 63 kidney transplant recipients with suspicion of rejection, revealed that the dd-cfDNA test did not discriminate TCMR from no rejection. The AUC for TCMR was 0.42 (CI: 0.17-0.66), although performance for diagnosing ABMR was much better, with an AUC of 0.82 (CI: 0.71-0.93). To better understand the long-term outcomes based on dd-cfDNA, a large prospective multicenter observational cohort study, the Kidney Allograft Outcomes AlloSure Registry (KOAR, ClinicalTrials.gov Identifier NCT03326076) is underway and plans to enroll 4000 kidney transplant recipients. KOAR is sponsored by CareDx, and will complete enrollment in December 2021. The ProActive study utilizing the Prosepra test and sponsored by Natera, Inc. (NCT04091984) is also underway and is targeting to enroll 3000 kidney transplant recipients prospectively from the time of transplant surgery. It will assess changes in the utilization of allograft biopsy and clinical outcomes based on physician-directed use of the Prospera test to rule in and rule out active rejection. The planned follow up for the study is 3 yr for most patients and 5 yr for a subset of patients at high immunologic risk.

The utility of dd-cfDNA in first time single kidney transplant recipients (SKTR) was clearly shown in the above mentioned studies, but the validity of the test in repeat kidney transplant recipients (RKTR) was unclear until Mehta *et al*[38] reported a median dd-cfDNA of RKTR (n = 12) in the surveillance group that was higher than in the SKTR group (0.29% *vs* 0.19%, P < 0.001). However, both were significantly lower than the established 1% dd-cfDNA rejection threshold[44]. Another study by Sureshkumar *et al*[45], showed that there were no significant differences in dd-cfDNA values for either deceased *vs* living donor (0.39% ± 0.42% *vs* 0.37% ± 0.20%, P = 0.35) or

repeat *vs* first time (0.34% ± 0.07% *vs* 0.39% ± 0.43%, P = 0.36) kidney transplant recipients. One possible reason for the latter observation could be that the limited number of viable cells in a failed allograft is insufficient to generate enough cell-free DNA fragments.

Using a slightly different platform from Natera to detect dd-cfDNA, Sigdel *et al*[35] have shown promising results. They measured plasma dd-cfDNA with a single SNP-based cell-free assay targeting 13392 SNPs using a massively multiplexed PCR method to detect allograft injury or rejection without knowing the donor genotype. Altuğ *et al* [31] further validated the performance of this method to detect the dd-cfDNA fraction with improved precision over other currently available tests, regardless of donor-recipient relationships. A major limitation was that the study was a retrospective analysis of archived samples from a single center comparing outcomes of patients who underwent for-cause biopsies, with an increased risk of rejection. The superiority in the technique of measuring dd-cfDNA and methodology of those studies was questioned in an editorial by Grskovic *et al*[46]. More studies are needed to prove the superiority of this technique over the other available techniques used to measure dd-cfDNA.

There have been multiple recent meta-analyses compiling the data from studies of the potential of dd-cfDNA as a biomarker to distinguish between different types of allograft rejection in kidney transplant recipients. A meta-analysis by Wijtvliet *et al*[47] included seven studies and one by Xiao *et al*[48] included nine studies. Both revealed significantly higher levels of dd-cfDNA in patients with ABMR compared with those with no rejection. The diagnostic accuracy was less for early TCMR, particularly Banff 1A and borderline. The meta-analysis by Xiao *et al*[48] revealed that the incidence of ABMR was 12%-37% in patients with elevated dd-cfDNA, with a pretest probability of 25%, positive likelihood of 58%, and negative likelihood of 6%, suggesting it may be a good test to rule out rejection. The presence of DSA can enhance the ability of dd-cfDNA to diagnose ABMR[34]. Zhang *et al*[49] showed that patients with positive DSA but without ABMR on biopsy had a higher baseline dd-cfDNA value compared with transplant recipients with neither DSA nor ABMR. The study suggests that the dd-cfDNA level may help in differentiating possibly "benign" DSA from the more damaging DSA that can cause ABMR. The majority of stable kidney transplant recipients have a median dd-cfDNA value of 0.21% with an NPV of 95%; suggesting that dd-cfDNA could be a reasonably accurate marker to rule out active rejection [33]A recent meta-analysis reported similar results[48].

DD-CFDNA IN SUBCLINICAL REJECTION

Subclinical rejections are usually diagnosed in protocol biopsies, and there has been some data to suggest that subclinical rejections portend worse long-term graft outcomes; yet there is no data to suggest that treatment of this improves outcomes [50]. A study by Gielis *et al*[51] using dd-cfDNA measured by NGS in 43 patients who had 107 protocol biopsy specimens did not differentiate subclinical rejection from pyelonephritis or acute tubular injury. Bloom *et al*[33] reported that in the DART study, dd-cfDNA did not predict early TCMR, the majority of which were subclinical rejections. Even though the efficacy of diagnosing subclinical rejection is low, use of dd-cfDNA in combination with other markers of graft dysfunction such a DSA, chemokines, gene transcripts, and other novel biomarkers, might be able to predict rejection in immunologically high risk recipients[50]. In a recently published multicenter study involving 79 patients with steroid-treated borderline/1A TCMR, those with dd-cfDNA ≥ 0.5% had a steeper decline in glomerular filtration rate (median 8.5% *vs* 0%), more frequent development of DSA (40.5% *vs* 2.7%) and recurrent rejection rates (21.4% *vs* 0%) at 3-6 mo post-initial diagnosis than patients with a value < 0.5%[52].

DD-CFDNA FOR SURVEILLANCE AND MONITORING

The ideal frequency of monitoring dd-cfDNA has not been established, but studies have shown that, depending on the type of donor organ (*i.e.* living or deceased with or without DGF), the dd-cfDNA value nadirs at 2 wk post transplant, from the ischemia reperfusion injury. Hence, the monitoring should begin at 2 wk post transplant[30]. Some studies, like as the DART study[33], measured dd-cfDNA monthly for 3 mo and quarterly thereafter for a year, which might be a good frequency to follow. Various

other studies to look at the outcomes of using this biomarker as a tool for surveillance to monitor rejection in all transplant recipients or a subset of those with high immunological risk are ongoing and are described in Table 2. Interestingly, dd-cfDNA was elevated in pathologies other than rejection, such as BK nephritis[53] and infection[54].

LIMITATIONS OF DD-CFDNA AS A BIOMARKER

The use of the dd-cfDNA assay has limitations that need to be kept in mind. The test may give inaccurate results if performed within first 2 wk of transplant, in pregnant women, within 24 h of kidney biopsy, in patients who received whole blood or WBC components within a month of testing, in those with history of allogenic bone marrow transplantation, kidney transplant from monozygotic twin and in multiorgan transplant recipients. In dual organ transplants from a single donor, a cutoff value above which one could anticipate an increased risk of rejection has not been defined, and an increased value will not distinguish which organ is experiencing the injury. A positive result in single organ recipients does increase the risk of rejection, but cannot distinguish the grade and type of rejection. Confirmatory diagnosis of type and intensity of rejection is still based on biopsy findings. Occasionally increased levels of dd-cfDNA were be seen in BK nephritis or other causes of allograft injury other than rejection.

FUTURE DIRECTIONS

The availability of dd-cfDNA for clinical use in recent years is a step in the right direction toward noninvasive monitoring of allograft health, especially following kidney transplantation. A number of recent publications have described the utility of dd-cfDNA in kidney transplant recipients. In general, studies have found that dd-cfDNA was more useful in diagnosing ABMR, with less clear impact toward diagnosing milder forms of TCMR. One possible reason for the early rise in dd-cfDNA levels in ABMR is the associated microvascular injury in the allograft, with earlier release of cell-free DNA fragments into the circulation. Emerging reports suggest that dd-cfDNA is predictive of short-term adverse graft outcomes in TCMR1A at a lower threshold dd-cfDNA level. Despite being clinically available as an attractive option for noninvasive allograft evaluation, there are still many unanswered questions on the optimal utilization of these biomarkers. More large studies and experience are needed. Some of these questions are: (1) Should we use absolute dd-cfDNA levels or dd-cfDNA fractions? (2) What is the role of surveillance using dd-cfDNA in stable kidney transplant recipients, and would there be a favorable impact on long-term transplant outcomes? And (3) Is it cost effective to perform serial dd-cfDNA measurements? Puttarajappa *et al*[55] used a Markov model to perform an economic analysis comparing noninvasive biomarker monitoring to protocol biopsy during the first 12 mo following kidney transplantation. Assuming an incidence of 12% subclinical TCMR and 3% subclinical ABMR, protocol biopsy yielded more quality-adjusted life years at a lower cost compared with biomarkers. Hopefully many of these questions will be answered once the results of large database studies such as KOAR and ProActive become available.

CONCLUSION

Noninvasive monitoring of early diagnosis of kidney allograft injury is a need of the hour. Among the various biomarkers that have been studied, dd-cfDNA captured the most attention and data is emerging. The available literature finds dd-cfDNA to be valuable for the early diagnosis of ABMR, but its role in milder forms of TCMR is less clear. Similarly, the favorable impact of dd-cfDNA in allograft surveillance on long-term outcomes is also not clear. Results from ongoing large outcome studies could shed further light onto this.

Table 2 Trials of donor-derived cell-free DNA in kidney transplantation

NCT02424227	Noninvasive blood test to diagnose acute rejection after kidney transplantation (DART)	Completed
NCT03765203	Utility of a novel dd-cfDNA test to detect injury in renal posttransplant patients (QIDNEY)	Completed
NCT03326076	Evaluation of patient outcomes from the kidney allograft outcomes allosure registry (KOAR)	Recruiting
NCT04091984	The Prospera kidney transplant active rejection assessment registry (ProActive)	Recruiting
NCT04057742	Allosure for the monitoring of antibody-mediated processes after kidney transplantation (All-MAP)	Recruiting
NCT03759535	Study in detection cfDNA for the early stage diagnosis of acute rejection post-renal transplantation	Not yet recruiting
NCT03984747	Study for the prediction of active rejection in organs using donor-derived cell-free DNA detection (SPARO)	Recruiting
NCT04130685	Donor-derived cell-free DNA for surveillance in simultaneous pancreas and kidney transplant recipients	Recruiting
NCT04166149	Eliminating the need for pancreas biopsy using peripheral blood cell-free DNA (PancDX)	Recruiting
NCT03859388	Longitudinal changes in donor-derived cell-free DNA with tocilizumab treatment for chronic antibody-mediated rejection	Enrolling
NCT04225988	Comparison of tacrolimus extended-release (envarsus xr) to tacrolimus immediate-release in HLA sensitized kidney transplant recipients	Recruiting
NCT04177095	Immune monitoring to facilitate belatacept monotherapy	Recruiting
NCT04239703	Intercomex donor-derived cell-free DNA study	Recruiting

dd-cfDNA: Donor-derived cell-free DNA; HLA: Human leukocyte antigen.

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MINIREVIEWS

Potential role of intermittent fasting on decreasing cardiovascular disease in human immunodeficiency virus patients receiving antiretroviral therapy

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Abstract

Cardiovascular disease (CVD) has become one of the commonest causes of comorbidity and mortality among People living with human immunodeficiency virus (HIV) (PLWH) on antiretroviral therapy (ART). Nearly 50% of PLWH are likely to

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have an increased risk of developing CVD, including coronary heart disease, cerebrovascular disease, peripheral artery disease and aortic atherosclerosis. Aside from the common risk factors, HIV infection itself and side effects of antiretroviral therapy contribute to the pathophysiology of this entity. Potential non-pharmacological therapies are currently being tested worldwide for this purpose, including eating patterns such as Intermittent fasting (IF). IF is a widespread practice gaining high level of interest in the scientific community due to its potential benefits such as improvement in serum lipids and lipoproteins, blood pressure (BP), platelet-derived growth factor AB, systemic inflammation, and carotid artery intima-media thickness among others cardiovascular benefits. This review will focus on exploring the potential role of intermittent fasting as a non-pharmacological and cost-effective strategy in decreasing the burden of cardiovascular diseases among HIV patients on ART due to its intrinsic properties improving the main cardiovascular risk factors and modulating inflammatory pathways related to endothelial dysfunction, lipid peroxidation and aging. Intermittent fasting regimens need to be tested in clinical trials as an important, cost-effective, and revolutionary coadjutant of ART in the fight against the increased prevalence of cardiovascular disease in PLWH.

Key Words: Human immunodeficiency virus; Intermittent fasting; Antiretroviral therapy; Metabolism; Cardiovascular disease; Mortality and morbidity

Core Tip: Intermittent Fasting of 14-18 h/d (Time Restrictive Feeding) or 2 d fast/5 d fed (Alternate d Fasting) is a widespread practice that has aroused great interest in the scientific community. Many reviews have postulated the potential benefits of intermittent fasting in different diseases. It has been shown to improve weight loss, cardiovascular effects, and glucose metabolism. It consists of periods of strict caloric restriction alternating with variable feeding schedules. Hence, we aimed to present the first literature review regarding the role of intermittent fasting as a potential nonpharmacological and cost-effective strategy in decreasing the burden of cardiovascular disease among human immunodeficiency virus patients on antiretroviral therapy.

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INTRODUCTION

The 2019 Heart Disease and Stroke Statistics update of the American Heart Association (AHA) reported that 48 percent of persons ≥ 20 years of age in the United States have some form of Cardiovascular Disease (CVD)[1]. In USA, roughly 16.3 million of people have Coronary Heart Disease (CHD)[2], secondly with approximately 7 million of Americans had at least one episode of stroke. Moreover, almost 82.6 million US citizens present at least one or more forms of CVD[2], which encompasses four major areas: CHD, cerebrovascular disease, peripheral artery disease and aortic atherosclerosis as well as thoracic or abdominal aortic aneurysm[1].

Current data suggest that every 36 s Americans die from CVD, accounting for 1 in 4 deaths in the country[3]. Furthermore, this illness it is characterized as a chronic low grade inflammatory condition that has atherosclerosis as its most common pathological substrate. In People living with HIV (PLWH), CVD risk has been shown to be 50% higher than in uninfected individuals[4]. Aside from the well-known risk factors for CVD such as smoking, changes in lipid profile and insulin resistance; HIV infection itself and some side effects of antiretroviral therapy (ART), especially protease

inhibitors, are further contributing factors among this population[5-7]. In that sense, Cardiovascular disease (CVD) has become one of the commonest causes of death in the PLWH under treatment with virological and immunological control[8].

Intermittent fasting (IF), consisting of periods of strict calorie restriction (CR) alternating with variable feeding schedules, is a widespread practice gaining high level of interest in the scientific community and the media followed by millions of people around the globe[9,10]. Different regimens of intermittent fasting have been reported in the literature with two of them being the most notorious: *Time Restrictive Feeding (TRF)*, where the fasting period is about 14-20 h/d, and *Alternate d Fasting*, traditionally 2 d fast/5 d fed[9,11,12]. It is important to remark that intermittent fasting does not necessarily involve limiting the total number of daily calories as in a typical caloric restriction regimen; therefore, it may be implemented in pathologies that do not require a reduction in the number of calories ingested[12]. Multiple potential benefits of IF have been described such as improvement in glucose metabolism and insulin sensitivity, weight loss, delayed aging, systemic inflammation, beneficial neurocognitive effects and cardiovascular benefits[12,13]. Additional metabolic benefits are still being investigated with promising paths for future research[12].

To the best of our knowledge, there is a large literature on the benefits of IF in cardiovascular disease, but none on the case of PLWH. Therefore, we aimed to explore the potential role of intermittent fasting as a non-pharmacological and cost-effective strategy in decreasing the burden of cardiovascular diseases among HIV patients on ART due to its intrinsic properties improving the main CVD risk factors and modulating the systemic inflammatory state.

EPIDEMIOLOGY

People living with HIV are almost 38 million distributed throughout all the continents [14]. PLWH on ART are disproportionately affected by an increase in the incidence of CVD compared with age-matched HIV-negative controls[4]. To date, it is known that people living with HIV present more than twice increased risk of cardiovascular disease in general[4,14]. For instance, from 1999 to 2013 the rate of deaths in the US caused by CVD in PLWH increased from 2% to almost 5%[15]. Furthermore, CVD is one of the main non-AIDS- related complications, since between 9% and 20% of PLWH in developed countries are at moderate to high risk of suffering a myocardial infarction (MI)[16].

Lately, there has been an increase prevalence of smoking in the HIV population which could be explained by a variety of factors including anxiety and other mental illnesses, alcohol and illicit drug use, sociodemographic stressors due to social discrimination, increased risk-taking behaviors and impulsiveness, or false perception of smoking risks[17,18]. It was seen in a Danish study that HIV smokers had a higher relative risk of suffering a Myocardial infarction (MI) compared to negative controls [19]. Furthermore, some of the ART regimens that include protease inhibitors (PIs) can also contribute to the increase in the incidence of CVD[7]. On a different note, the fact that the Framingham Score underestimates the MI risk in PLWH, which was clearly observed in a cohort study, complicates even more the early detection and treatment [20]. The intensity of CVD in HIV patients (measured objectively as Intimal Media Thickness = IMT) may also be directly related to the HIV duration, meaning that the arterial damage is most likely accumulative over the years[21]. The accelerated atherosclerosis formation is thought to be independent of viral replication (at least in plasma) and multifactorial[22-26] being the microbial translocation at the level of the Gut-mucosa one of the main culprits and generators of chronic inflammation[21,27-30].

PATHOPHYSIOLOGY

The increase in the CVD risk on PLWH can be explained due to the significant increase of systemic inflammation and immune-activation compared to HIV uninfected controls even in the presence of effective ART (Figure 1). Other identified contributing factors are increased clotting, altered lipid metabolism, macrophage/T-cell infiltration of arteries, residual viral replication, direct toxicity of ART, and immune-senescence [29,31]. Early immune senescence may contribute directly to accelerated CVD since senescent cells promote the secretion of pro-inflammatory cytokines (termed "senescent-associated secretory phenotype or SASP")[32]. In that sense, it was found that elimination of senescent cells from prematurely aged mice prevented aging of

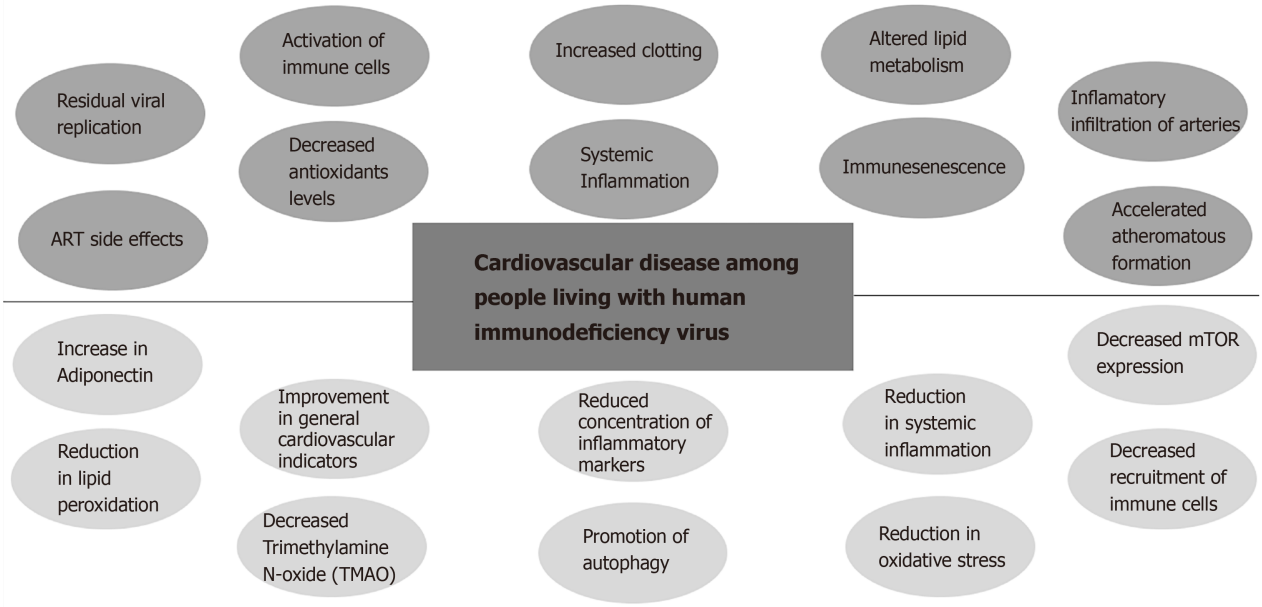

Figure 1 Summary of the interplay of the human immunodeficiency virus- antiretroviral therapy related contributing factors to cardiovascular disease and intermittent fasting potential benefits among People living with human immunodeficiency virus.

some organs[32]. Also, HIV is associated with decreased levels of antioxidants such as ascorbic acid, tocopherols, selenium, superoxide dismutase, and glutathione[33,34] along with an increase in the levels of hydroperoxides and malondialdehyde[35]. In addition, peroxides and aldehydes are not only passive markers of oxidative stress, but also really toxic compounds for cells being lipid peroxidation and LDL oxidation involved in the pathophysiology of CVD[36]. Endothelial dysfunction is associated with many of the traditional risk factors for atherosclerosis described above. The endothelial dysfunction is induced by oxidized low density lipoprotein (LDL) and should be considered as a common final pathway of multiple vascular insults[37]. On the other hand, metabolic side effects of ART are continuously being updated. Besides the well described metabolic side effects of some Protease inhibitors, new concerns regarding weight gain and subsequent metabolic disturbances are raising with the use of first line drugs such as Tenofovir Alafenamide (TAF) and Integrase strand transfer inhibitors (Raltegravir, Elvitegravir (EVG), Dolutegravir (DTG), and Bictegravir (BIC) [38]. The combination of the later generation ISTIs (Dolutegravir and Bictegravir) along with TAF presents the highest risk[38] (Figure 1).

The genesis of inflammation and immune-activation in PLWH most likely starts at the Gut-mucosal level early after the infection. It has been extensively studied that simian immunodeficiency virus SIV (in non-natural hosts) and HIV infection lead to breaches in the tight junctions between epithelial cells in the gut mucosa that allow microbial products, and chemokines to cross over[36,39-41]. These abnormalities are not only anatomical but functional as well. It is well known that bacterial products from the "gut-microbiome" like lipopolysaccharides (LPS) can stimulate the innate immune system through the pattern recognition receptors such as toll-like receptors (TLRs) mainly TLR-4 generating a local and systemic proinflammatory state[36,39]. Actually, it has been shown that an increase in the sCD14 (a soluble marker of monocyte activation after binding to LPS) predicts early mortality in HIV patients[42]. This finding is the first link between microbial translocation and mortality on HIV individuals particularly related to CVD.

The increased systemic inflammation and immune-activation in PLWH can be objectively measured through a specific cytokine profile. In HIV patients on effective ART with excellent immunological response (CD4 cell count > 500), fibrinogen and C-reactive protein (CRP) still remain strong and independent predictors of mortality[43]. In addition, interleukin 6 (IL-6), CRP, tumor necrosis factor (TNF), interferon gamma (IFN-gamma) and D-dimer all remain elevated even after effective ART[44]. It was shown that elevated CRP and HIV are independently associated with increased myocardial infarction (MI) risk, and that patients with HIV with increased CRP have a markedly increased relative risk of MI. Similarly, IL-6 and D-dimer were strongly related to all-cause mortality in this population[45]. Also, chemokines like interleukin 8 (IL-8), Regulated upon Activation Normal T Cell Expressed and Presumably

Secreted (RANTES), C-C motif ligand 2 (CCL2), and interferon gamma- induced protein 10 (IP10) remain elevated in PLWH[46], which is evidence of active recruitment of immune cells to the plaque. The above points toward a well-defined mechanism of accelerated atheromatous formation in PLWH related to systemic inflammation and local recruitment of inflammatory cells to the atheromatous plaque, a process that starts off at the level of HIV-associated gut mucosal dysfunction.

INTERMITTENT FASTING AND PREVENTION OF CARDIOVASCULAR DISEASE IN HUMAN STUDIES: TRANSLATION TO PLWH

Multiple strategies directed to decrease inflammation and immune-activation in PLWH on effective ART have shown partial and non-definitive results. In an attempt to look for nutritional and non-pharmacological approaches to face this problem, IF looks extremely attractive. IF has shown to decrease the CVD risk either directly (through improvement on the main CV risk factors) or indirectly (decreasing inflammation, immune-activation, immune cells migration, Trimethylamine N-oxide (TMAO) formation, and local oxidative stress)[12,47,48] (Figure 1). Multiple animal studies of IF have consistently proven to increase lifespan, decrease inflammation, treat diabetes and other metabolic diseases, improve cardiovascular health, and promote innumerable neurocognitive benefits (including neuro-protection against stroke) which has been described in detail in previous reviews by Mattson, M. and Longo[47,48]. Even though there is less robust evidence in human studies, multiple recent clinical trials have proven that IF decreases the overall CV risk through the improvement of each of its main modifiable risk factors. There is some discussion as to whether the decrease in the CVD risk with IF is due to its intrinsic characteristics or due to the weight loss secondary benefits. Of note, a very recent clinical trial showed the health benefits regardless of the daily calorie intake in a group of patients with metabolic syndrome[49]. As explained before, the health benefits are beyond weight loss since IF not necessarily implies a decrease in the daily caloric intake.

Direct Mechanism: improving modifiable traditional CVD risk factors

A recent study showed that a scheduled calorie restriction and IF (24 mo) in healthy, non-obese individuals was proven to be beneficial in improving risk factors for cardiovascular and metabolic disease such as visceral adipose tissue mass, ectopic lipid accumulation, blood pressure, and lipid profile, but improvements in insulin sensitivity were only transient[50]. Individuals that had been in a prolonged calorie restriction (CR) program had better outcomes in terms of serum lipids and lipoproteins, fasting plasma glucose and insulin, blood pressure (BP), high-sensitivity C-reactive protein (CRP), platelet-derived growth factor AB (PDGF-AB), body composition, and carotid artery intima-media thickness (IMT). Importantly, patients that were in the CR group had 40% less IMT, which is an important surrogate for coronary artery disease[51] (Figure 2). A very recent comprehensive review by Mattson M *et al*[52] showed that IF improves multiple indicators of cardiovascular health including blood pressure, resting heart rate, LDL and HDL levels, cholesterol, triglycerides, glucose and insulin resistance. The same review encouraged practitioners to start applying this strategy to patient care always under close professional supervision and progressively over weeks or months. Another recent study (single-arm, paired-sample trial) showed that 19 participants with metabolic syndrome who were exposed to a TRF (Time Restricted Feeding) protocol on which they ate for only 10 h, showed significant improvements in health indicators including: weight loss; reduced waist circumference, percent body fat, and visceral fat; reduced blood pressure, atherogenic lipids, and glycated hemoglobin[49]. Since PLWH are disproportionately affected by the traditional reversible CV risk factors IF could provide a significant improvement of health indicators, improvement in quality of life, and a marked reduction in the risk of CVD (Figure 2).

Indirect mechanisms

Indirectly, IF can decrease the CVD risk in PLWH through the decrease in systemic inflammation, reduction of lipid peroxidation, decrease in Trimethylamine N-oxide (TMAO), promotion of autophagy of cellular debris, and decrease in oxidative stress which in turn, shall decrease the accelerated atheroma plaque formation (Figure 3). It is important to clarify that even though IF showed much of its anti-inflammatory properties in animal studies, HIV patients present inflammatory levels way above the

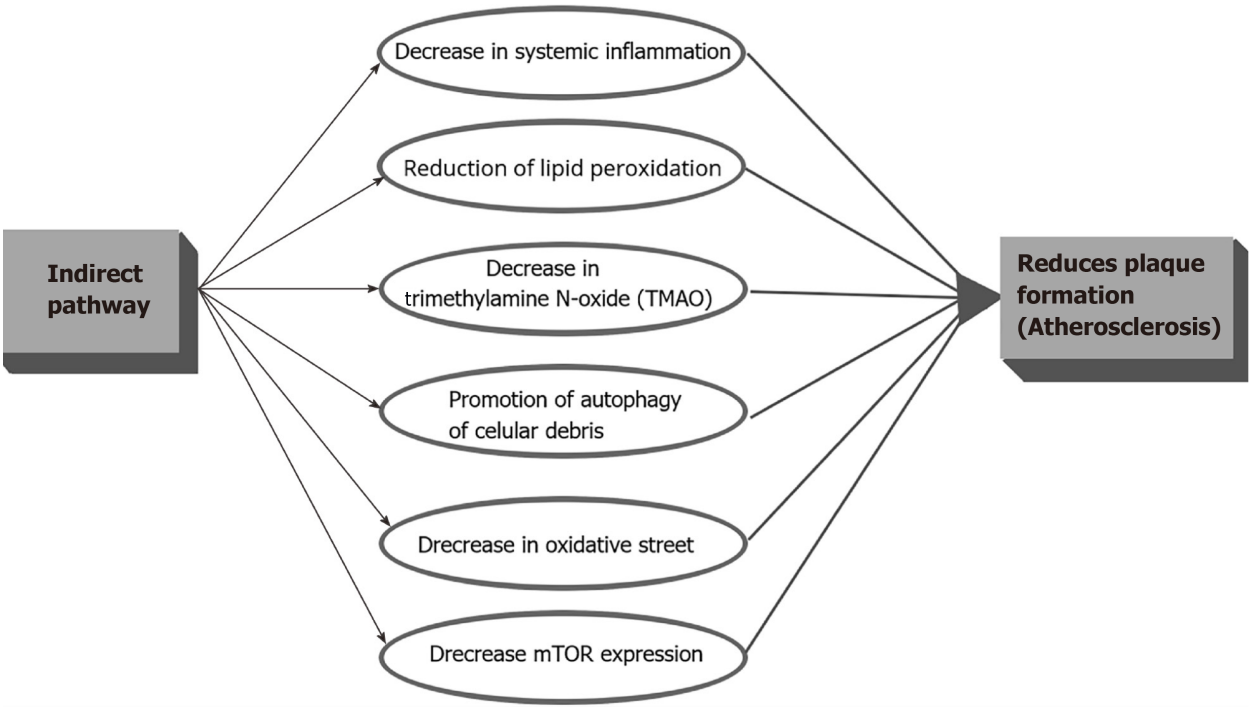

Figure 2 Summary of the potential benefits of the direct intermittent fasting pathway among People living with human immunodeficiency virus.

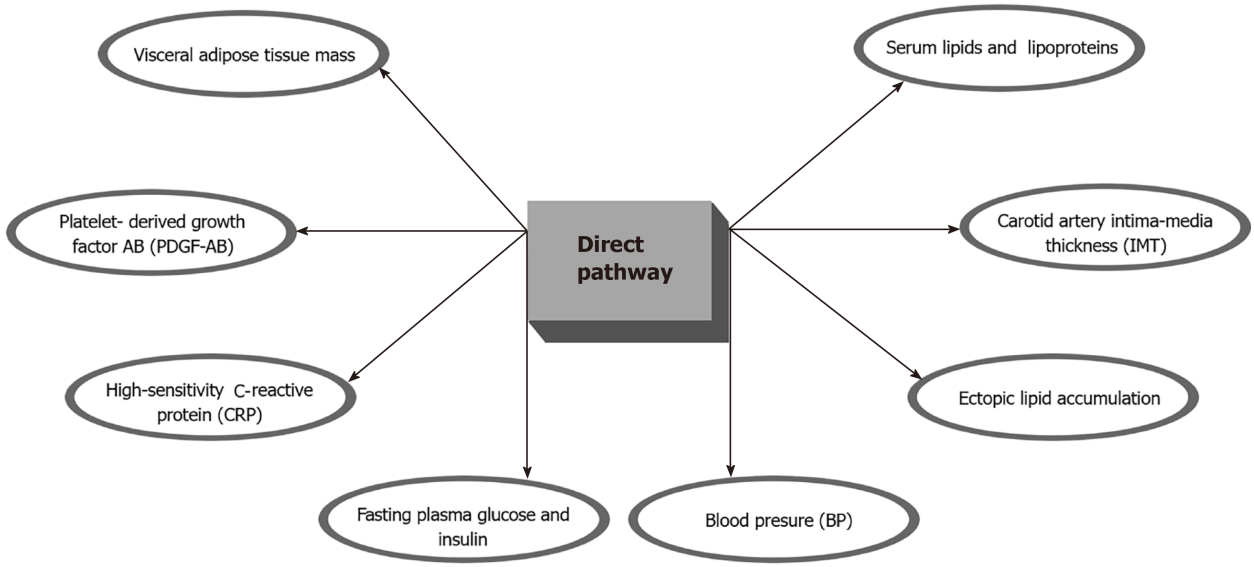

Figure 3 Summary of the potential benefits of the indirect intermittent fasting pathway among People living with human immunodeficiency virus.

mean levels compared with HIV negative controls which means that any change may correlate with a significant decrease in the CVD risk and clinical events. Trimethylamine N-oxide is an amine oxide produced in humans by intestinal microbiota from excess trimethylamine (TMA), and intermediate of choline metabolism. It has been linked to increase inflammation in adipose tissue and accelerate atherosclerosis [53]. A mean level of 14.3 ng of TMAO during fasting versus a baseline mean of 27.1 ng in control subjects (P = 0.019) was found in an IF study in humans[54], which means than IF can have implications on decreasing inflammation in the atheromatous plaque not only by decreasing the recruitment of activated monocytes but by decreasing the TMAO levels.

This ancient mechanism was probably not only created to use alternative sources of energy when food is lacking but also to clear cells from toxic molecules, reactive oxygen species (ROS), deoxyribonucleic acid (DNA) damage, and cellular debris probably through autophagy. As we explained above, oxidative stress and decreased antioxidants with lipid peroxidation is important for the plaque formation (Figure 3). The anti-atheroma formation mechanisms of IF may be mediated through: Possible endothelial improved cellular stress adaptation to ischemia and inflammation (mainly against ROS generation), decreased DNA damage, decreased inflammation, decrease recruitment of immune cells, decrease mammalian target of rapamycin (mTOR) expression[47], and promoting autophagy. In rats exposed to IF in stroke experimental models (which causes brain inflammation), decreases of Interleukin 1 beta (IL1-b), TNF-alpha, IL-6, and suppression of the "inflammasome" was observed[55]. IF also resulted in reduced levels of messenger ribonucleic acid (mRNAs) encoding the LPS receptor TLR4 and inducible nitric oxide synthase (iNOS) in the hippocampus of rats exposed to systemic LPS. Moreover, in another study IF prevented the LPS-induced elevation of IL-1α, IL-1b, IFN-γ, RANTES, TNF-α and IL-6[56]. Those two studies could have implications to decrease the LPS-driven activation of TLRs in innate immune cells, and, hence, gut inflammation in PLWH. The decrease in the gut inflammation shall decrease monocyte activation, migration, and generation of CD14's, which is directly implicated in the accelerated atheromatous plaque formation (Figure 3). IF could interrupt the "Gut-Heart axis" and significantly decrease the endothelial dysfunction. Following the same line of thoughts, IF may also inhibit the development of the atheroma plaque in HIV patients by reducing the local concentration of inflammatory markers, such as IL-6, homocysteine, and CRP, and, at the same time, decreasing the migration of immune cells to the subendothelial area through the increase of adiponectin[57]. Recently was shown that isocaloric TRF (Time Restricted Feeding) during 8 wk in males, reduced many markers of inflammation such as TNF alpha, IL-6, and IL-1b, and, increased adiponectin (an anti-inflammatory cytokine) [58]. Considering that this was a study in healthy human subjects and due to the fact that the HIV patients on ART have much higher levels of inflammation, the decrease in the CVD risk could be clinically significant. There are no theoretical biological barriers for which the above physiologic events would not happen in PLWH exposed to IF.

To understand the pathophysiology of chronic inflammation some big players need to be explained more in detail. The NLRP3 inflammasome is a multiprotein platform which is activated by infection (including HIV) or some sort of cellular stress (including ischemia). Its activation leads to caspase-1-dependent secretion of proinflammatory cytokines like interleukin-1β (IL-1β) and IL-18, and leads to an inflammatory form of cell death termed as "Pyroptosis"[59]. The inflammasome activation as a generator of inflammation will contribute to the increased CVD risk. The inflammasome can be activated directly by HIV through TLR8 activation after contact with viral RNA[46] but also by other TLRs-mediated pathways (like TLR4 with LPS in the gut mucosa as explained above). It was proved that the ketone bodies β-hydroxybutyrate (BHB) and acetoacetate, both elevated during starvation, inhibits the NLRP3 inflammasome. BHB and acetoacetate were shown to reduce the NLRP3 inflammasome-mediated interleukin (IL)-1β and IL-18 production in human monocytes[60] which will be extremely important for latently-HIV-infected monocytes to prevent activation and further recruitment with migration to the atheroma plaque. In another experimental model in rats with an experimentally induced stroke (which causes local inflammation), IF could attenuate the inflammatory response and tissue damage by suppressing NLRP1 and NLRP3 inflammasome activity[61]. A stressed Endoplasmic Reticulum (ER) is known to generate ROS which, in turn, activates the NLRP3 inflammasome and secretion of IL-1b. A recent study in rats also showed a potential therapeutic role of β-hydroxybutyrate in suppressing the ER (stressed)-induced inflammasome activation[62]. It was revealing the study that showed that patients with Rheumatoid Arthritis (RA) had significant clinical improvement (pain and inflammation) after a period of fasting if a vegetarian diet was followed thereafter [63]. Another study in overweight asthmatic female patients exposed to IF showed a significant decrease in the levels of TNF-alpha and markers of oxidative stress (8-isoprostane, nitrotyrosine, protein carbonyls, and 4-hydroxynonenal adducts) with improved clinical response. It showed that prolonged fasting blunted the NLRP3 inflammasome and T Helper 2 (Th2) cell activation in steroid-naive asthmatics as well as diminished the airway epithelial cell cytokine production[64]. These two studies highlight the possibility of using the "survival-mode" of IF to fight chronic inflammatory conditions, which, in turn, promote accelerated aging and CVD. In fact, HIV is a perfect example of a chronic inflammatory disease. We think that in all these

conditions (RA, Asthma, and HIV) the baseline level of inflammation is so high that any change will have significant clinical implications. There is no reason to think that the decreased levels of inflammation seen in these two studies will not be translated to PLWH, and, actually, it may be exacerbated. The decrease in the migration of inflammatory cells to the atheromatous plaque during IF is due to the decrease in the expression of the vascular cell adhesion molecule 1 (VCAM-1), endothelial-leukocyte adhesion molecule 1 (ELAM-1), and intercellular adhesion molecule 1 (ICAM-1) on vascular endothelial cells - all molecules highly implicated in the pathogenesis of atherosclerosis-[57]. Migration and trafficking of activated immune cells are highly involved in the pathogenesis of CVD in PLWH (Figure 4). Interestingly, Proteobacteria was identified as one of the main producers of TMAO which is increased in the dysbiosis caused by HIV[53]. IF may in fact cause a reversal of the HIV- associated - dysbiosis with decrease in the Proteobacterias (mainly inflammatory and Pro-glycolytic) with possible switch to a healthier microbiome (with less production of TMAO) like Lactobacillus and Firmicutes (Figures 3 and 4).

OTHER DIETARY REGIMENS AND HIV

Different dietary regimens have been evaluated with mixed results in PLWH on ART. A recent systematic review explored the potential benefits of micronutrients including but not limited to Vitamin A, D, Zinc, and Selenium[65]. The administration consisted in either each macronutrient or in combination. However, after a period of follow up to 6-18 mo, the study revealed minimal or no relevant benefits[65]. Another study compiled the interventions of some diets such as low-fat diet, hypocaloric diet, omega-3 fatty acids, carnitine, micronutrient supplements, formula, amino acids, uridine, among others, on HIV-infected patients receiving combination antiretroviral therapies. Where oral nutrition support (protein and energy intake) has been demonstrated to promote weight gain and fat mass overall[66]. Besides this, formula supplementation has not demonstrated further benefits. Whereas amino acids in combination showed to increase lean body mass in HIV-infected patients undergoing weight loss. The use of a low-fat diet was suggested to be implemented carefully and tailored accordingly in order to avoid a severe reduction in body mass[66,67]. Despite the paucity of controlled randomized trials with larger sample sizes, above results in small but significant findings. Further larger randomized blinded clinical trials are needed to ensure confirmatory results.

When it comes to assessing diet adherence among PLWH, it was previously seen in a study that overweighted HIV positive individuals tend to have a higher adherence to Mediterranean diet compared to the rest of the group[68]. It is hypothesized that due to the moderate risk of CVD and a diagnosis of metabolic syndrome, there is an increased awareness towards a healthier food pattern to avoid further complications [68]. In that sense, when introducing IF to PLWH we believe that adherence will not be a real problem indeed and PLWH with higher risk factors would be more prone to adhere to the new dietary regimen. Nevertheless, nutritional education strategies should be implemented early and routinely to optimize adherence among patients.

FUTURE TRIALS IN PLWH AND POTENTIAL CLINICAL IMPLICATIONS

To the best of our knowledge this is the first review addressing the possibility of applying IF in PLWH on effective ART. Due to the evidence presented above and due to the fact that PLWH are aging with increased prevalence of CVD, IF strategies need to be tested in clinical trials through proof-of-concept studies or large prospective randomized clinical trials. There are no obvious absolute contraindications that we can think of besides the obvious harm associated with extreme weight loss in patients with AIDS and wasting syndrome being off ART. Inclusion and exclusion criteria will need to be carefully defined in prospective clinical trials in order to be safe.

IF studies did not include pregnant women and were not tested in the extremes of age (pediatrics or frail elderly subjects) in which case its use is discouraged and the possible consequences are unknown. One caveat is that some HIV medications needs to be ingested with food and not on an empty stomach, but given the posology of current antiretrovirals (1 or two pills a d usually once daily in naïve patients) the recommendation would be to take the medication when the patient ingest the first meal of the d (when the patient "*breaks the fast*"). In the case of more complex regimens in PLWH with multidrug resistance and twice daily regimens personal accommod-

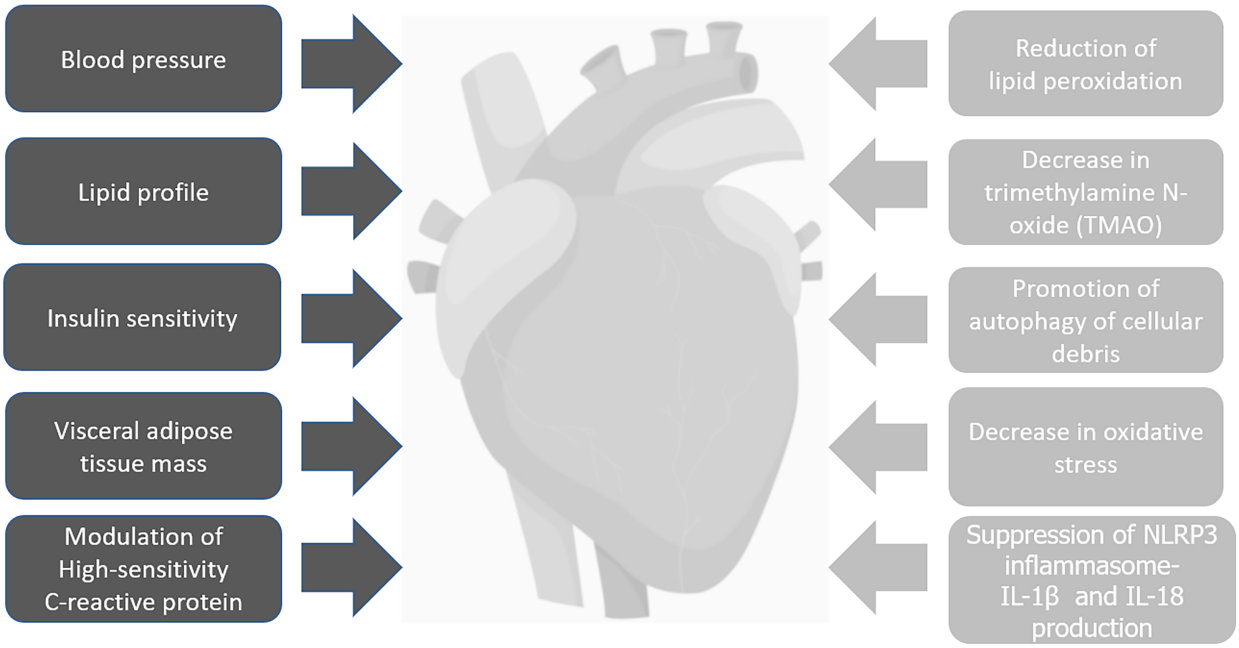

Figure 4 Summary of the direct (black) and indirect (gray) mechanisms of intermittent fasting in cardiovascular disease in People living with human immunodeficiency virus.

ations will need to be taken into account. Monthly injections of Cabotegravir and Rilpivirine were recently approved on which case IF protocols will be easier. However, first line initial regimens for most people with HIV -which generally consists of the combination of two nucleoside reverse transcriptase inhibitors (NRTIs) with an integrase strand transfer inhibitor (INSTI)-, suggest to use the combination of Bictegravir, Tenofovir alafenamide and Emtricitabine (BIC/TAF/FTC)[69]. Current indications from the Food and Drug Administration (FDA) suggest taking the drugs with or without food[70-72]. Rilpivirine (oral formulation only) regimens, in the contrary, will require a high caloric meal to increase its absorption when the feeding window starts.

Ruling out any impediment with the practice of IF within these patients. Of note, this strategy that we propose will need to be applied only to PLWH on stable ART with immunological and virological response (< 20 copies in two different occasions at least 6 mo apart in a stable regimen with good CD4 response which is not well defined but definitely more than 200 cells or more than 14%), without active opportunistic infections, active malignancy, malnourishment, or any other chronic debilitating disease. The inclusion and exclusion criteria will need to be clarified in detail by future investigations since this is a new concept so far unexplored. For sure, pregnancy and extremes of age with frailty and weight loss will be excluded during the initial trials.

CONCLUSION

The burden of Cardiovascular Diseases among HIV patients on ART is continuously growing. Intermittent fasting, through direct and indirect mechanisms, could play a role in the management and prevention of CVD among PLWH on ART. If these concepts are proven to be true in future clinical trials IF could be considered as an extremely important, cost-effective and revolutionary coadjutant of ART in the fight against the increased prevalence of CVD in PLWH which could, in turn, improve survival, decrease CV clinical events, and improve quality of life. Therefore, we recommend further longitudinal and experimental studies to ensure the safety, efficacy and effectiveness of IF on CVD among PLWH.

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MINIREVIEWS

Differential diagnosis and management of immune checkpoint inhibitor-induced colitis: A comprehensive review

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Abstract

Immune checkpoint inhibitors (ICIs) are a new class of cancer pharmacotherapy consisting of antibodies that block inhibitory immune regulators such as cytotoxic T lymphocyte antigen 4, programmed cell death 1 and programmed death-ligand 1. Checkpoint blockade by ICIs reactivates a tumor-specific T cell response. Immune-related adverse events can occur in various organs including skin, liver, and gastrointestinal tract. Mild to severe colitis is the most common side effect with some experiencing rapid progression to more serious complications including bowel perforation and even death. Prompt diagnosis and management of ICI-induced colitis is crucial for optimal outcome. Unfortunately, its clinical, endoscopic and histopathologic presentations are non-specific and overlap with those of colitis caused by other etiologies, such as infection, medication, graft-*versus*-host disease and inflammatory bowel disease. Thus, a definitive diagnosis can only be rendered after these other possible etiologies are excluded. Sometimes an extensive clinical, laboratory and radiologic workup is required, making it challenging to arrive at a prompt diagnosis. Most patients experience full resolution of symptoms with corticosteroids and/or infliximab. For ICI-induced colitis that is treatment-refractory, small scale studies offer alternative strategies, such as vedolizumab and fecal microbiota transplantation. In this review, we focus on the clinical features, differential diagnosis, and management of ICI-induced colitis with special attention to emerging treatment options for treatment-refractory ICI-induced colitis.

Key Words: Immune checkpoint inhibitor; Immune checkpoint inhibitor-induced colitis; Infliximab; Vedolizumab; Graft-*versus*-host disease; Inflammatory bowel disease

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Core Tip: Colitis is the most common adverse effect associated with immune checkpoint inhibitor (ICI) therapy. Its clinical, endoscopic and histopathologic presentations overlap with those of colitis caused by other etiologies, including infection, other medications and graft-*versus*-host disease. Patients often present with diarrhea, abdominal pain and variable endoscopic findings ranging from normal or mild inflammation to ulcerations. Microscopically, acute colitis pattern of injury is the most common finding. ICI-induced colitis is a diagnosis of exclusion. Its current first-line treatment is corticosteroids, followed by infliximab for steroid-refractory colitis. Vedolizumab and fecal microbiota transplantation are promising options for treatment-refractory ICI-induced colitis.

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INTRODUCTION

Our immune system can recognize some cancers with a high frequency of mutations as foreign and stimulate a tumor-specific immune response. Immune checkpoint inhibitors (ICIs) are antibodies that block inhibitory immune regulators such as cytotoxic T lymphocyte antigen 4 (CTLA-4), programmed cell death 1 (PD-1) and programmed death-ligand 1 (PD-L1). Checkpoint blockade by ICI enhance the body's defense against cancer[1]. Since 2011, the Food and Drug Administration has approved several ICIs including CTLA-4 inhibitor (ipilimumab), PD-L1 inhibitors (atezolizumab, avelumab and durvalumab), and PD-1 inhibitors (nivolumab and pembrolizumab). These ICIs demonstrated tremendous efficacy for a broad range of cancers, including advanced-stage melanoma[2], renal cell carcinoma[3], and non-small-cell lung cancer [4].

Compared to chemotherapy, immune-related adverse events involving skin, liver and gastrointestinal (GI) tract are more common with ICIs[5]. Diarrhea/colitis represent the most common adverse effect[5,6] and is the most common reason for discontinuation of ICI therapy[2,7]. Its clinical presentations range from mild to severe colitis with some patients experiencing rapid progression to serious complications including bowel perforation and even death. The Common Terminology Criteria for Adverse Events version 5 is commonly used to access and grade disease severity. It is graded from 1 to 5 with grade 1 representing mild symptoms and grade 5 patient's death related to ICI-induced colitis[8]. Prompt diagnosis and management of ICI-induced colitis is crucial for optimal outcome[9-11]. Unfortunately, its clinical, endoscopic and histopathologic presentations are non-specific and overlap with those of colitis caused by other etiologies, such as infection, other medications and graft-*versus*-host disease (GvHD). Thus, it may be challenging to make a timely diagnosis of ICI-induced colitis. For these reasons we believe it is important to raise awareness for this newer entity. In this review, we focus on the clinical features, differential diagnosis and management of ICI-induced colitis. Especially, we provide detailed histological differential diagnoses and supply ample microscopic images.

CLINICAL FEATURES

Incidence

The frequency of colitis complicating ICI therapy is variable depending on the ICI regimen and individual patient characteristics[5]. The highest incidence has been reported in patients treated with anti-CTLA-4 antibodies, ranging from 3.4%-15.5% for all grade colitis and 2.3%-8.3% for grade 3-4 colitis, followed by combination of anti-CTLA-4 and PD-1 with an incidence rate of 0.7%-12.8% for all grade colitis and 0.5%-8.3% for grade 3-4 colitis. The lowest incidence was in patients treated with anti-PD-1/L1 checkpoint inhibitors, ranging from 0.7%-2.6% for all grade colitis and 0.3%-1.0%

for grade 3-4 colitis[12]. Patients with melanoma receiving anti-PD-1 agents seem to have a higher risk of developing ICI-induced colitis than those who receive anti PD-1 agents for non-small cell carcinoma [odds ratio (OR): 4.2; 95% confidence interval (CI): 1.3-14.0][7]. Although the mechanism is unclear, stage IV malignancies were associated with a lower incidence of diarrhea and colitis when compared to patients with stage III malignancies (35.3% *vs* 72.0%; $P = 0.001$)[13]. Caucasian patients have high risk of developing diarrhea/colitis (OR: 5.76; 95%CI: 2.03-16.36), while patients' age and sex have no association with the incidence of diarrhea and colitis[13].

Interval from drug infusion to colitis

The median interval to onset of diarrhea is approximately 4-8 wk[5] after the first infusion. However, the range is broad with some patients experiencing symptoms as early as 1 wk after the exposure. Some patients developed symptoms months or even two years after discontinuation of the therapy. In these rare cases, the underlying mechanism was unclear[14,15].

Clinical presentations

Clinical presentations are usually non-specific and include diarrhea (92%), abdominal pain (82%), hematochezia (64%), fever (46%) and vomiting[16]. Disease severity is variable and can range from mild diarrhea to life-threatening colitis. In a meta-analysis, the overall mortality rate associated with ICI-induced colitis was 5% (225/3905). In this study, the correlation between the mortality and the grades of colitis was not analyzed. Sixty percent (135/225) of the fatality was from CTLA-4 inhibitor, 25.8% (58/225) from anti PD-1 or PD-L1 and 14.2% (32/225) from combined therapy[17]. Toxicities leading to fatal outcomes tend to occur early in the disease course and evolve rapidly, especially in patients receiving combination of agents. The median time to the onset of a fatal event is -14.5 d for ICI combination therapy, *vs* 40 d for ICI monotherapy ($P < 0.001$)[17]. ICI-induced colitis should be considered in the differential diagnosis in any patient treated with ICIs who presents with abdominal pain and or diarrhea.

ENDOSCOPIC FEATURES

Endoscopic presentation of ICI-related colonic inflammation varies from normal appearance, to edema, erythema, inflammatory exudate, erosions, aphthae, and ulcerations[18,19]. In the study published by Wang *et al*[18], ulceration was found in 40% (21/53), non-ulcerative inflammation in 42% (22/53) and no gross inflammation in 19% (10/53) of patients. Left-sided colitis was seen in 42% (18/43), left and right-sided colitis in 40% (17/43), ileocolonic disease in 14% (6/43) and 2% (1/43) had inflammation confined to the ileum. The distribution of the inflammation was diffuse (22/43, 51%), patchy (18/43, 42%) and less commonly, segmental (3/43, 7%)[18].

PATHOLOGICAL FINDINGS

The morphological features of ICI-induced colitis are similar between patients receiving anti-CTLA-4, anti-PD-1 and anti-PD-L1 regimens. The spectrum of abnormalities ranges from minimal to severely active colitis. The histologic features may resemble those of infectious colitis characterized by increased inflammatory infiltrate in the lamina propria with lymphocytes, plasma cells, neutrophils, and intraepithelial neutrophils (Figures 1A and B)[18,19]. Cryptitis and crypt abscess are commonly seen (Figures 1C and D)[18,19]. Mild to prominent intraepithelial lymphocytosis (17%) and apoptotic cells (42%) may be evident[20]. Crypt architecture irregularities (36%) such as shortening of the crypts, loss of crypts, or slight irregularities in diameter and shape of the crypts (Figure 1C) may also be seen[19]. In addition, the presence of crypt irregularities might lead to a misdiagnosis of inflammatory bowel disease (IBD). Patterns of microscopic colitis (*e.g.*, lymphocytic colitis and collagenous colitis) have been reported in about 10% of the cases[21,22].

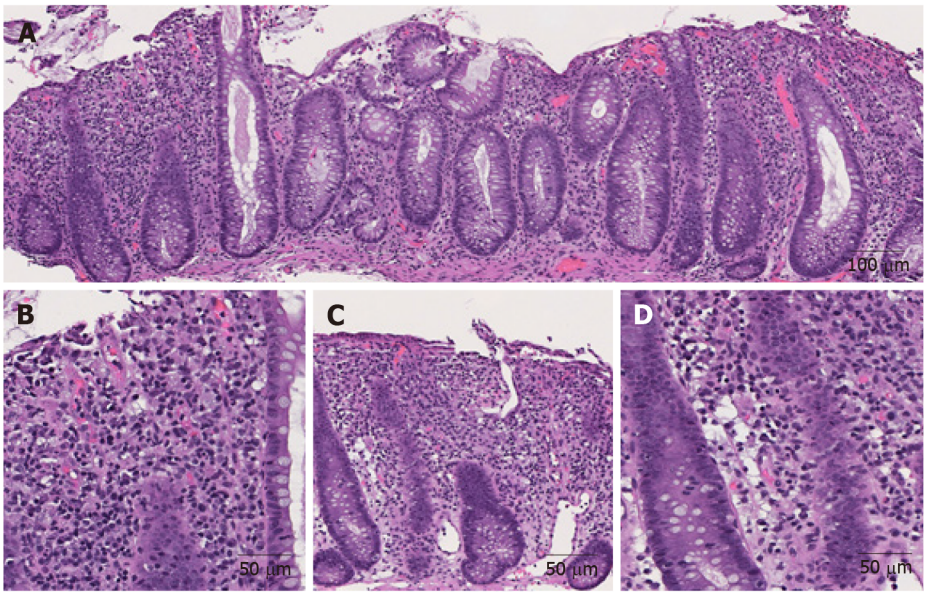

Figure 1 Representative images of immune checkpoint inhibitor-induced colitis in a patient with metastatic melanoma treated with nivolumab and ipilimumab for 2 mo (Hematoxylin and eosin). A: Active colitis characterized by mixed inflammatory cell infiltrates in the lamina propria and surface erosion (100 ×); B: High magnification of A. Note the neutrophils, lymphocytes, and plasma cells in the lamina propria (200 ×); C: Active colitis with mild crypt architectural irregularity (100 ×); D: Active colitis with neutrophilic cryptitis (200 ×).

PATHOPHYSIOLOGY

It is hypothesized that autoimmune related events are responsible for ICI-induced colitis. For example, the blockage of ICI leads to the release of T cells that had been previously suppressed. Likewise, immunosuppression therapy is usually effective for ICI-induced colitis[8,23,24]. In a study using endoscopic colon biopsies of ICI-induced colitis, marked activation and proliferation of cytotoxic effector CD8+ T cells was observed in the colonic tissue. T cell receptor sequence analysis showed that a substantial subset of these colitis-associated CD8+ T cells had originated from tissue-resident CD8+ T-cell populations. The authors speculated that following the activation of CD8+ T cells in the tissue, additional CD8+ and CD4+ T cells are recruited from the blood, leading to clinical progression of the colitis[25].

There is increasing evidence that gut microbiota plays an important role in the pathogenesis of ICI-induced colitis. For example, Chaput *et al*[26] reported that *Bacteroidetes* transplantation is associated with worse cancer outcome but lower incidence of ICI-induced colitis, whereas *Faecalibacterium*, in particular *F. prausnitzii* L2-6, butyrate-producing bacterium L2-21 and *G. formicilis* ATCC 27749, are associated with the development of ICI-induced colitis but favorable oncologic outcome. Currently the underlying mechanisms remain unclear. However, an animal study demonstrated that intestinal microbiome could mediate immune-related inflammation through host immune system[27].

DIFFERENTIAL DIAGNOSIS

A presumptive diagnosis of ICI-induced colitis can be considered in patients who develop diarrhea and abdominal pain while taking ICIs and have supportive endoscopic and/or histologic findings on GI biopsies. However, these clinical, endoscopic and histologic findings are non-specific; they can be seen in colitis caused by other etiologies. The diagnosis of ICI-induced colitis is one of exclusion and requires exclusion of other competing etiologies.

Infectious colitis

Colonic infection by bacteria, viruses, or parasites accounts for the majority of cases of patients presenting with acute diarrhea, fever, tenesmus and abdominal pain[24]. Common clinical presentation of infectious colitis is indistinguishable from that of ICI-induced colitis. Given that ICI-induced colitis patients are at increased risk for infectious colitis and ICI-induced colitis requires immunosuppressive therapy[8,23,

24], microbiological studies and/or stool culture should be performed first to exclude the common infectious etiologies. The most common food borne pathogens in United States include *Campylobacter*, *Salmonella*, *Escherichia coli* O157.H7 and *Norwalk virus*. Common non-foodborne agents include *Shigella*, *Yersinia, Coxsackie virus, rotavirus, enterovirus,* and *adenovirus*[28].

For infectious colitis, endoscopic findings are usually non-specific, and show edema, erythema, erosion and ulceration. Microscopically, inflammatory infiltration of the lamina propria and neutrophil-mediated cryptitis and/or crypt abscess are often evident[30]. Unfortunately, endoscopic and microscopic findings are usually non-specific for distinguishing ICI-induced colitis from different infectious etiologies[28]. However, some specific histologic patterns may be helpful in identifying the infectious etiologies.

Cytomegalovirus: Cytomegalovirus (CMV) is an important opportunistic infectious agent in frankly immunosuppressed patients, immunocompetent patients undergoing chemotherapy, and the elderly[29]. CMV-associated colitis has been reported in patients with corticosteroid- refractory ICI-induced colitis[30-32]. It causes an active colitis injury pattern (Figure 2A). The diagnosis is made by identifying the typical large cells with basophilic cytoplasm and pathognomonic large, oval, eosinophilic intranuclear inclusions (owl-eye inclusions), usually seen at the base of the ulcer (Figure 2B). However, the sensitivity of detecting viral inclusions on histologic examination is low[33]. Immunohistochemical staining can be very helpful when the inclusions are poorly formed, rare, or obscured by inflammation[34].

***Clostridium difficile*:** *Clostridium difficile* (*C. difficile*) is the most common cause of hospital-acquired infectious diarrhea and is strongly associated with the use of clindamycin, fluoroquinolones, cephalosporins, monobactams, and carbapenems[35]. The clinical symptoms associated with *C. difficile* infection range from mild, self-limiting diarrhea to fulminant colitis and toxic megacolon, leading to bowel perforation, sepsis and/or multisystem organ failure[36]. *C. difficile* colitis causes pseudo-membranous colitis. Endoscopically, pseudomembranous colitis is characterized by elevated, discontinuous, yellow-white nodules or plaques. Microscopically, these nodules or plaques consist of mushroom-like laminated lesions on the surface of mucosal glands, composed of fibrin-rich exudates and mucus with embedded neutrophils and necrotic epithelial cells (Figure 2C)[37]. Pseudomembranous colitis can also be seen in other infections such as with *E. coli* O157, *Shigella*, and other Shiga toxin-producing organisms as well as acute ischemia, acute radiation injury, and in association with drugs, such as albendazole[38-40]. Superimposed[41] and concurrent [42] *C. difficile* infections have been documented in patients with ICI-induced colitis. Laboratory testing for either free toxins or toxigenic *C. difficile* in stool is required for confirmation of *C. difficile* colitis[43].

***Yersinia*:** *Yersinia* enterocolitis is caused by *Y. enterocolitica* or *Y. pseudotuberculosis*, which are gram-negative coccobacilli. It is transmitted mostly by contaminated food and water[37]. *Yersinia* is an entero-invasive organism that primarily involves Peyer's patches and the surrounding mucosa, forming aphthous and linear ulcers often mimicking Crohn's disease (CD)[37]. Microscopically, *Yersinia* infection is characterized by epithelioid granulomas with associated prominent lymphoid tissue and mucosal ulceration[44]. Microbiologic cultures or molecular testing may be required to confirm the diagnosis.

Other medication-mediated colitis

A broad spectrum of drugs can cause GI toxicity. Symptoms are non-specific and include bloating, abdominal pain, cramping, diarrhea, weight loss, mucosal bleeding or anemia[45]. Due to the clinical and histological similarity with ICI-induced colitis, it should be considered in the differential diagnosis for ICI-induced colitis. Drugs that may lead to clinically significant colitis are listed herein:

Chemotherapy: Chemotherapy-induced GI mucosal injury oftentimes manifests as diarrhea, odynophagia, nausea, emesis, anorexia, malabsorption, abdominal pain and cramping. Common endoscopic findings include mucosal erythema, erosions and ulcers. Microscopically, the crypts are attenuated and or dilated with minimal inflammation and the epithelium may undergo apoptosis and show some degree of atypia, such as hyperchromatic nuclei[46]. This finding is not specific and can be seen in other disorders, such as ischemic enterocolitis and GvHD. Some chemotherapeutic agents produce characteristic mucosal alterations. For example, taxanes prevent depolymerization[47], resulting in ring mitotic figures in the proliferative compartment of the

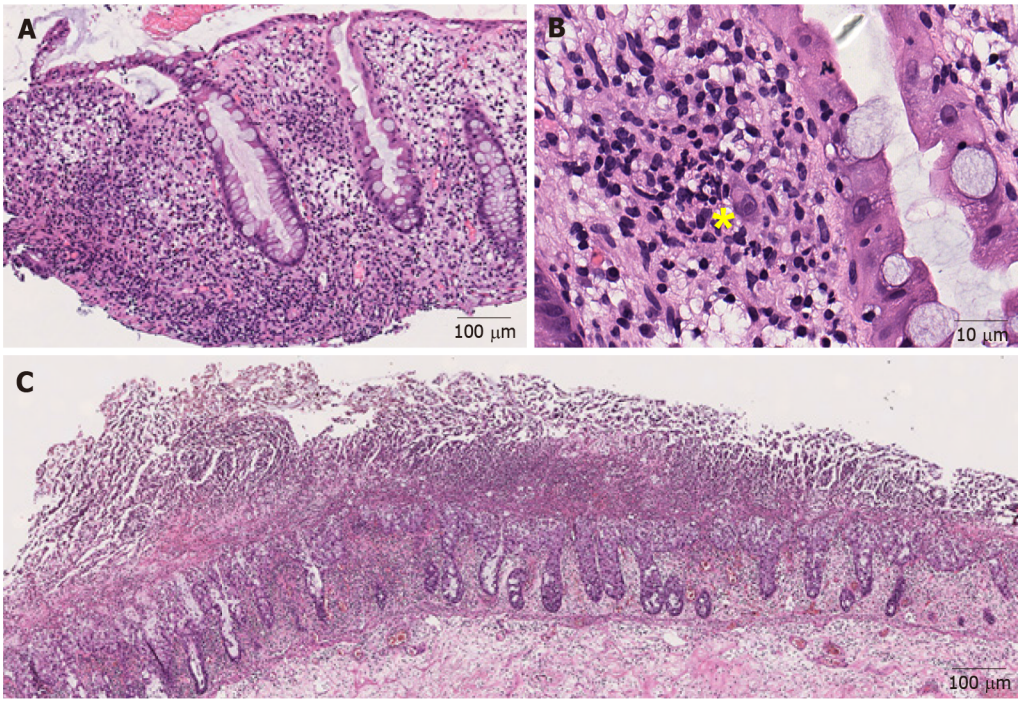

Figure 2 Representative images of infectious colitis (Hematoxylin and eosin). A: Low magnification view of cytomegaloviral (CMV) colitis. Notice the lymphocytes and neutrophils in the lamina propria (100 ×); B: Note an owl-eye inclusion characterized by enlarged nucleus with oval, eosinophilic intranuclear inclusion surrounded by clear halo, consistent with CMV inclusion (400 ×); C: *Clostridium difficile* colitis. Pseudomembranes composed of fibrin, neutrophils and necrotic epithelial cells are on the surface of the mucosal glands (40 ×). Yellow sign notes a viral inclusiona.

mucosa throughout the GI tract (Figure 3A). Some patients are treated with traditional chemotherapy prior to ICIs, or receive ICIs combined with chemotherapy[48,49]. In these patients, chemotherapy-induced GI mucosal injury should be considered, though admittingly it may be very difficult to distinguish that with ICI-induced colitis. Medication history and clinical correlation are necessary to sort out the specific cause of colitis on an individual basis.

Nonsteroidal antiinflammatory drugs: Nonsteroidal antiinflammatory drugs (NSAIDs)-induced pathology can be seen throughout the GI tract. However, the only pathognomonic NSAIDs-associated lesion is diaphragm. Diaphragm is formed when the lumen of the small bowel is divided into short compartments by circular membranes of mucosa and sub-mucosa protruding into and obstructing the lumen (Figure 3B)[45]. Reactive gastropathy is highly suggestive of their usage but is not specific[45] and is seen in other conditions such as bile reflux[50]. Several forms of colitis associated with NSAIDs have been documented. The most common microscopic injury is epithelial erosion with mixed infiltration of lymphocytes and neutrophils[51, 52]. NSAIDs-induced colitis may resemble lymphocytic colitis[51] and collagenous colitis[53], although the lymphocytosis and collagen deposition are usually patchier and less pronounced in NSAIDs-induced colitis.

All the histological presentations associated with NSAIDs use overlap with those of ICI-induced colitis. Given the widespread use of NSAIDs, NSAIDs-induced colitis should be always considered in the differential diagnosis for ICI-induced colitis. ICI induced adverse effect often involves multiple organs simultaneously[5], thus the history of NSAIDs use and the absence of other organ involvement would favor NSAIDs-induced colitis.

Mycophenolate mofetil: Mycophenolic acid is an immunosuppressive medication that is frequently used in solid organ transplant patients. One of the two forms, mycophenolate mofetil is well known to cause significant GI mucosal toxicity[54]. Nausea, vomiting, abdominal pain and watery diarrhea are frequent symptoms[54]. Endoscopic findings range from normal (47%), erythema (33%) to erosions/ulcers (19%)[55]. Histological findings include acute colitis pattern of injury with neutrophilic cryptitis or crypt abscesses (Figures 3C and D) (50%), crypt architecture distortion with lymphoplasmacyte-predominant lamina propria inflammation (36%), the presence of enterocyte apoptosis (Figure 3D) without lamina propria inflammation (8.3%), and

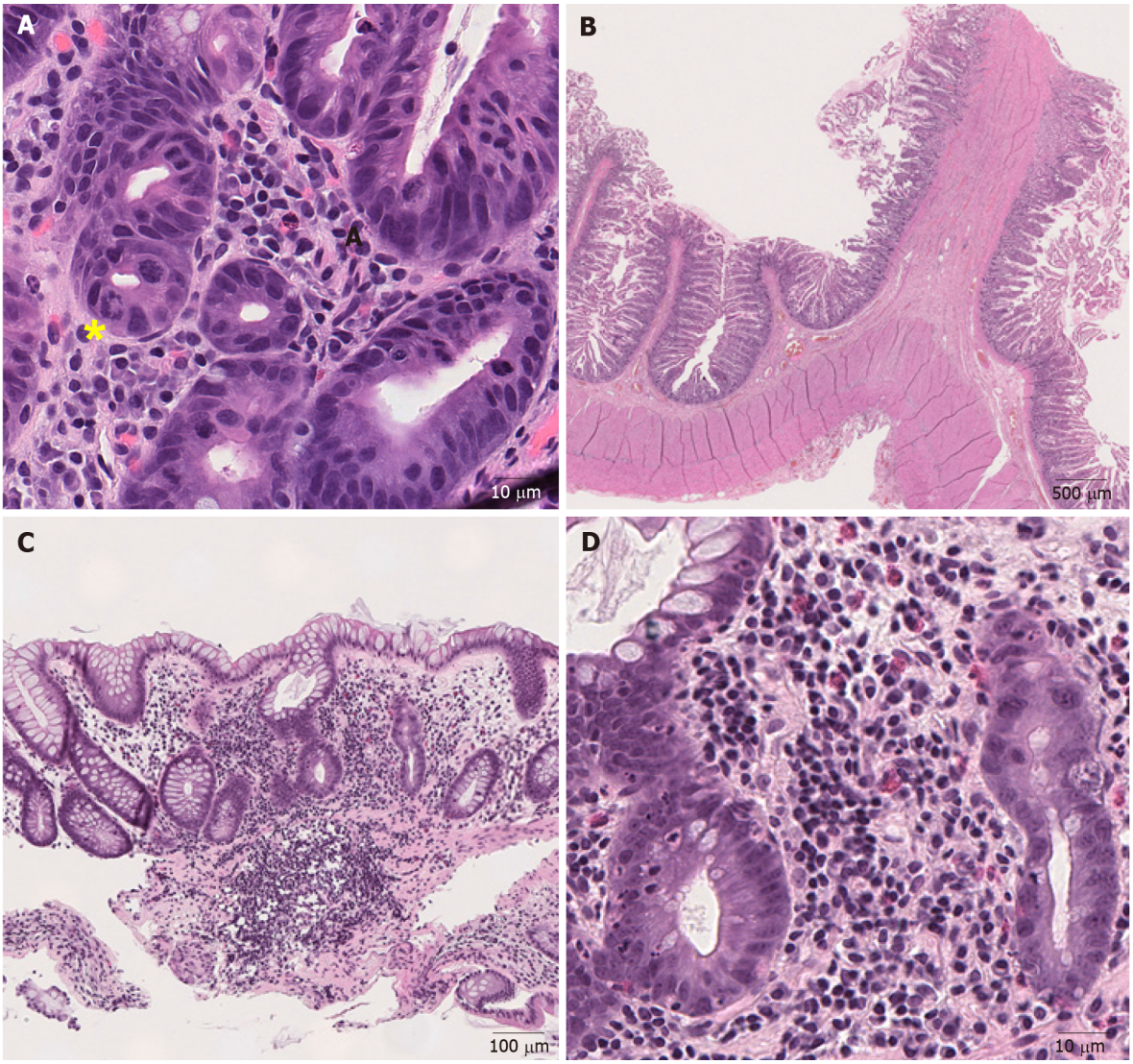

Figure 3 Representative images of medication-induced colitis (Hematoxylin and eosin). A: Ring mitosis caused by docetaxel in duodenum (400 ×); B: Small bowel diaphragm. Fibrotic submucosa protrudes into the intestinal lumen and forms a diaphragm (8 ×); C: Low magnification view of mycophenolate mofetil-induced colitis. (100 ×); D: Higher magnification view of mycophenolate mofetil-induced colitis. There is an inflammatory cell infiltrate consisting of lymphocytes, plasma cells and eosinophils in the lamina propria with neutrophilic cryptitis (400 ×). Yellow sign notes a ring mitosis.

mucin-depleted crypts with no or minimal lamina propria inflammation and crypt dropout (5.6%)[55]. All these features overlap with those of ICI-induced colitis. History of drug use is required to distinguish these two.

Many other drugs can damage the GI tract. FDA Adverse Event Reporting System Public Dashboard is a great platform to report and search for adverse events related to specific drugs and therapeutic biologic products (https://www.fda.gov/drugs/questions-and-answers-fdas-adverse-event-reporting-system-faers/fda-adverse-event-reporting-system-faers-public-dashboard). Awareness of adverse effects related to drugs, careful review of the medication history and clinical correlation are essential to recognize and distinguish drug-induced GI injury from other causes.

IBD

IBD is a form of chronic immunologically mediated intestinal disorders, consisting of CD and ulcerative colitis (UC)[56]. UC involves the rectum and continuously extends to involve proximal colonic mucosa. CD is distinguished from UC by skipped transmural inflammation of any part of the GI tract. Fissures/fistula, noncaseating sarcoidal granulomas, and transmural involvement are more characteristic of CD[37, 57]. Some ICI-induced colitis resemble IBD with its patchy or segmental distribution [5], left colon involvement[5], and crypt architecture irregularities (Figures 4A and B) [19]. Usually, IBD has an insidious onset in contrast to ICI-induced colitis which often

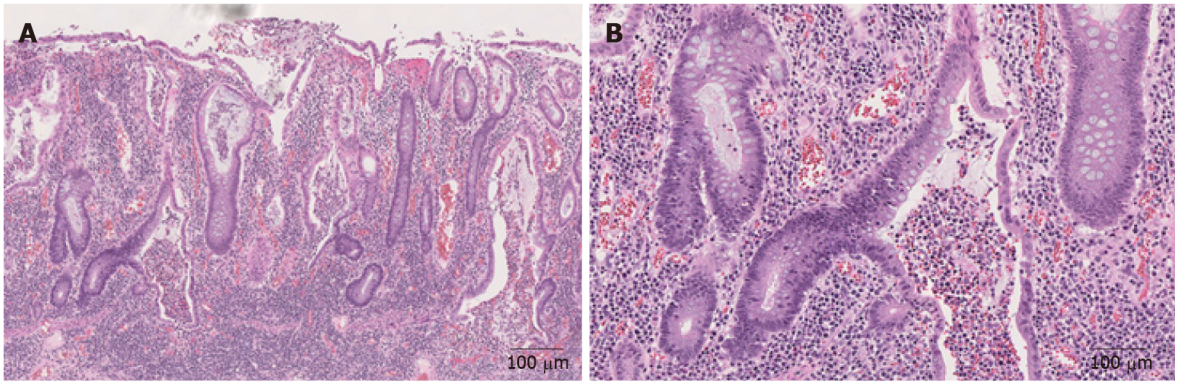

Figure 4 Representative images of inflammatory bowel disease (Hematoxylin and eosin). A: Mucosal inflammation with marked crypt distortion and neutrophilic cryptitis and abscesses in ulcerative colitis (40 ×); B: Higher magnification view shows crypt abscess (100 ×).

has a quick onset of symptoms after initiation of therapy[5]. Other features may aid in differentiating these two entities. Multiple organ involvement would favor ICI-induced colitis[5], fissures/fistula, noncaseating sarcoidal granulomas, and transmural involvement would favor CD and basal lymphoplasmacytosis would favor both UC and CD[37]. It may be challenging to distinguish superimposed ICI-induced colitis from IBD flare up only, as both show active colitis[18,37,55]. In this setting, the presence of crypt apoptosis would favor ICI-induced colitis as apoptosis is unusual in IBD[20].

GvHD

GvHD refers to a phenomenon wherein the donor's immune cells recognize the recipient's cells as foreign and attack and damage them. It is a common and serious complication of allogeneic hematopoietic cell transplantation, occurring in 30% to 70% of patients[58]. GI tract is the second most common site affected by GvHD following skin. Although esophageal web, stricture or stenosis in the upper to mid third of the esophagus is sufficient to establish the diagnosis of chronic GvHD, neither clinical nor endoscopic presentations of colonic involvement is specific for GvHD[59]. In GvHD, crypt injury, loss and ulcer may be found in severe disease (Figure 5A). Apoptotic bodies are commonly found at the deeper portion of the crypts in small and large intestinal mucosa (Figure 5B)[59] closely resembling ICI-induced colitis, although lamina propria inflammation is usually sparse in GvHD (Figure 5A). Both ICI-induced colitis and GvHD involve variable organs other than GI tract, such as skin and liver[5, 59], which make it even more challenging to distinguish them. Clinical history and medication history are necessary to sort out the specific cause of colitis on an individual basis.

TREATMENT

Comprehensive diagnostic protocol and management guidelines/recommendations regarding ICI-induced colitis were recently published[8,23,24]. Management varies according to the grade of colitis. In patients with only mild diarrhea (CTCAE grade 1), ICI therapy should be continued with close monitoring for dehydration. Once colitis reaches grade 2 or 3, ICI therapy should be suspended, but may restart anti PD-1 or PD-L1 agents if the patients recover to grade 1 or less following treatment. All ICI treatment should be permanently discontinued for patients with grade 4 colitis[24]. Although there is no definitive evidence to support their use, current guidelines universally recommend corticosteroids as initial management for ICI-induced colitis that is grade 2 or of higher grade[8,23,24]. Immunosuppressant maintenance therapy (< 10 mg prednisone equivalent dose) may be offered for initial treatment for grade 2 colitis if the infectious work-up in stool is negative. If diarrhea persists, 1 mg/kg/d prednisone or equivalent should be administered. Patients with grade 3 colitis generally start with high-dose corticosteroids (prednisone 1 to 2 mg/kg/d or methylprednisolone 1 to 2 mg/kg/d). If symptoms persist ≥ 3 to 5 d or recur after improvement, IV corticosteroid or stronger immunosuppressive agents are recommended, such as tumor necrosis factor-α blocker infliximab. Grade 4 colitis patients should start with IV corticosteroid and start infliximab 5 to 10 mg/kg if the symptoms are

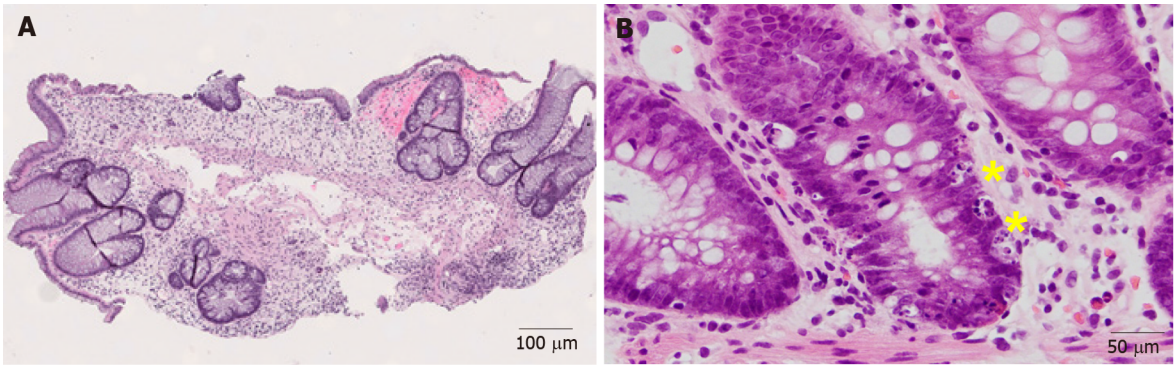

Figure 5 Representative images of graft-*versus*-host disease (Hematoxylin and eosin). A: Colonic graft-*versus*-host disease characterized by marked crypt architectural distortion and paucity of lamina propria inflammation (40 ×); B: On higher magnification, enterocyte apoptosis (yellow sign) are readily identified (200 ×).

refractory to corticosteroid within 2 to 3 d[8,24]. Mucosal ulceration or extensive colitis is an indication for early escalation to infliximab[8].

Approximately two-thirds of patients respond to initial management with corticosteroids and do not require any further treatment[24,60,61]. Steroid tapers are typically performed over 4-6 wk, depending on the severity of the initial inflammation and the rapidity of the initial response[24,60,61]. A small fraction of patients fails to respond to corticosteroids as well as infliximab. In these patients, confirmation of ongoing inflammation and exclusion of opportunistic infections is essential. Based on patients' risk factors, investigations should consider repeat stool cultures, *C. difficile* and CMV testing, and ova and parasite testing[23,32].

ALTERNATIVE STRATEGIES FOR TREATMENT-REFRACTORY ICI-INDUCED COLITIS

Currently there are case reports and small series reporting the use of alternative strategies for treatment-refractory ICI-induced colitis, including vedolizumab, fecal microbiota transplantation (FMT), and extracorporeal photopheresis (ECP). These will be briefly explored below.

Vedolizumab

Vedolizumab is a humanized monoclonal antibody that specifically recognizes the α4β7 heterodimer and modulates inflammation in the GI tract without inducing systemic immunosuppression[62]. Vedolizumab may benefit ICI-induced colitis patients who are refractory to infliximab and/or those with contraindication for its use. In a retrospective study of 28 patients with ICI-induced colitis who were refractory to corticosteroids, 32% of them did not respond to infliximab. Vedolizumab was administered using the same protocol for IBD. After 15 mo of follow up, 86% of the patients achieved and sustained clinical remission. Endoscopic remission was achieved in 54% (7/13) of the patients and 29% (5/17) achieved histologic remission [63]. Another small series also showed favorable outcome of vedolizumab use. Six out of seven steroid-dependent and/or partially refractory ICI-induced colitis patients experienced steroid-free remission of enterocolitis without related side effects[64]. It seems that a larger prospective study to evaluate the efficacy of vedolizumab is warranted.

FMT

FMT is the transfer of stool from a healthy donor into the colon of a patient whose disease is a result of an altered microbiome. The goal of FMT is to restore the normal microbiota. The most effective and well-studied indication for FMT is recurrent *C. difficile* infection[65]. Given the potential association between ICI-induced colitis and altered gut microbiota[26,27], FMT could be an effective treatment for treatment-refractory ICI-induced colitis. Wang *et al*[66] reported the first case series wherein ICI-induced colitis was successfully treated with FMT. Both patients achieved complete resolution of clinical symptoms and eventually returned to normal, daily solid bowel movements. Endoscopic evaluation demonstrated reduced inflammation and

resolution of ulcerations. Additional clinical trials are needed to validate the utility of this approach.

ECP

ECP is an immunomodulatory therapy wherein white blood cells are isolated and are exposed to 8-methoxypsoralenand and ultraviolet A-irradiation *ex vivo* before being re-infused to the patient[67]. ECP has been used for the treatment of chronic GvHD and in clinical trial for acute GvHD[67]. Apostolova *et al*[68] reported a patient with ICI-induced colitis who had a complete response following ECP. A 29-year-old man developed symptoms of dermatitis, thyroiditis, hepatitis, and colitis after two doses of ipilimumab and nivolumab combination therapy. The dermatitis, thyroiditis, and hepatitis resolved after the discontinuation of ICI and the initiation of glucocorticoid treatment. However, the colitis did not show durable response with glucocorticoids for a total of 23 wk, infliximab (two single doses during a 4-wk period), and cyclosporine (during a 14-wk period). During the next 8 mo, he underwent two cycles of ECP on two consecutive days every 2 to 4 wk, which resulted in a complete resolution of his colitis. Immunosuppression was tapered without a rebound of the colitis symptoms [68]. This preliminary result is promising and warrants further studies.

CONCLUSION

Colitis constitutes the most common adverse effect of ICI therapy. Its clinical, endoscopic and histopathologic manifestations are not specific and resemble those of infectious colitis, other medication-mediated colitis, GvHD, and IBD. ICI-induced colitis can rapidly progress to cause ulceration, perforation and even death when there is a delay in diagnosis and appropriate treatment. The diagnosis of ICI-induced colitis is one of exclusion and requires exclusion of all other competing etiologies, including infectious colitis, medication-mediated colitis, GvHD and IBD.

Currently high dose corticosteroids are used as initial management followed by infliximab for steroid-refractory colitis. When patients do not respond to corticosteroids or infliximab, concurrent infectious colitis such as *C. difficile* and CMV colitis should be considered and excluded. Vedolizumab and FMT are promising treatment options for treatment-refractory ICI-induced colitis.

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