ISSN 2150-5330 (online)

World Journal of *Gastrointestinal Pathophysiology*

World J Gastrointest Pathophysiol 2021 May 22; 12(3): 25-58

Published by Baishideng Publishing Group Inc

World Journal of Gastrointestinal Pathophysiology

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The primary aim of the *World Journal of Gastrointestinal Pathophysiology* (*WJGP*, *World J Gastrointest Pathophysiol*) is to provide scholars and readers from various fields of gastrointestinal pathophysiology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

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INDEXING/ABSTRACTING

The *WJGP* is now abstracted and indexed in Emerging Sources Citation Index (Web of Science), PubMed, PubMed Central, China National Knowledge Infrastructure (CNKI), and Superstar Journals Database.

RESPONSIBLE EDITORS FOR THIS ISSUE

NAME OF JOURNAL

World Journal of Gastrointestinal Pathophysiology

ISSN

ISSN 2150-5330 (online)

LAUNCH DATE

April 15, 2010

FREQUENCY

Bimonthly

EDITORS-IN-CHIEF

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https://www.wjgnet.com/2150-5330/editorialboard.htm

PUBLICATION DATE

May 22, 2021

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https://www.wjgnet.com/bpg/gerinfo/208

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https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS

https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION

https://www.f6publishing.com

World Journal of Gastrointestinal Pathophysiology

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World J Gastrointest Pathophysiol 2021 May 22; 12(3): 25-39

DOI: 10.4291/wjgp.v12.i3.25

ISSN 2150-5330 (online)

MINIREVIEWS

Neuroimmunomodulation by gut bacteria: Focus on inflammatory bowel diseases

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Author contributions: Aggarwal S conceptualized and wrote the paper; Ranjha R and Paul J performed the review and editing of the paper.

Conflict-of-interest statement: Authors declare no conflict of interests for this article.

Manuscript source: Invited manuscript

Specialty type: Gastroenterology and hepatology

Country/Territory of origin: India

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Abstract

Microbes colonize the gastrointestinal tract are considered as highest complex ecosystem because of having diverse bacterial species and 150 times more genes as compared to the human genome. Imbalance or dysbiosis in gut bacteria can cause dysregulation in gut homeostasis that subsequently activates the immune system, which leads to the development of inflammatory bowel disease (IBD). Neuromediators, including both neurotransmitters and neuropeptides, may contribute to the development of aberrant immune response. They are emerging as a regulator of inflammatory processes and play a key role in various auto-immune and inflammatory diseases. Neuromediators may influence immune cell's function *via* the receptors present on these cells. The cytokines secreted by the immune cells, in turn, regulate the neuronal functions by binding with their receptors present on sensory neurons. This bidirectional communication of the enteric nervous system and the enteric immune system is involved in regulating the magnitude of inflammatory pathways. Alterations in gut bacteria influence the level of neuromediators in the colon, which may affect the gastrointestinal inflammation in a disease condition. Changed neuromediators concentration *via* dysbiosis in gut microbiota is one of the novel approaches to understand the pathogenesis of IBD. In this article, we reviewed the existing knowledge on the role of neuromediators governing the pathogenesis of IBD, focusing on the reciprocal relationship among the gut microbiota, neuromediators, and host immunity. Understanding the neuromediators and host-microbiota interactions would give a better insight in to the disease pathophysiology and help in developing the new therapeutic approaches for the disease.

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: December 14, 2020
Peer-review started: December 14, 2020
First decision: February 14, 2021
Revised: March 1, 2021
Accepted: April 20, 2021
Article in press: April 20, 2021
Published online: May 22, 2021

P-Reviewer: Iizuka M
S-Editor: Fan JR
L-Editor: A
P-Editor: Yuan YY

Key Words: Inflammation; Gut microbiota; Inflammatory bowel disease; Enteric nervous system; Neuroimmunomodulation; Neuromediator

Core Tip: Dysbiosis in gut bacteria is a well-established factor, and the abnormality in the enteric nervous system is an emerging aspect that influences the gut inflammation. Both of them contribute to inflammatory bowel disease (IBD) pathogenesis by modulating the host immune response. Through this review, we linked the two pathological mechanisms and explained how neuroimmunomodulation by gut bacteria play a crucial role in IBD. We elaborated all the known neuromediators produced by gut bacteria and the role of each neuromediator as well as the respective gut bacteria in inflammatory signaling pathways especially in IBD.

Citation: Aggarwal S, Ranjha R, Paul J. Neuroimmunomodulation by gut bacteria: Focus on inflammatory bowel diseases. *World J Gastrointest Pathophysiol* 2021; 12(3): 25-39
URL: https://www.wjgnet.com/2150-5330/full/v12/i3/25.htm
DOI: https://dx.doi.org/10.4291/wjgp.v12.i3.25

INTRODUCTION

The gastrointestinal tract (GIT) is equipped with the most extensive immune system, and the largest network of neurons outside the central nervous system (CNS) called the enteric nervous system (ENS). Sometimes, ENS also referred to as "brain in gut" because it does not require any intermediate input from the brain for its functioning. The structure of ENS is organised into two Plexi, myenteric plexus and submucosal (Meissner's) plexus. Myenteric plexus is located between the longitudinal and circular muscle of muscularis propria and regulates the intestinal motility. Submucosal plexus is located in the submucosa of the intestine and regulates secretion, absorption, and blood flow[1]. Neurons of these Plexi releases various neurotransmitters that regulate the secretory and motor functions of GIT. During inflammatory bowel disease (IBD), there are morphological, histological, and immunohistochemical abnormalities in the ENS which causes neuronal hyperplasia, necrosis, ganglion, and axonal degeneration, alteration in synthesis and release of neurotransmitters. It leads to a defect in the secretory and motor functions of GIT[2].

The neurotransmitters and neuropeptides released from ENS can alter various immune cell functions. Immune cells residing in colon express various receptors for neurotransmitters, and once neurotransmitter binds to these receptors, there would be an initiation of signal transduction pathways of cytokine production[3]. These cytokines, in turn, bind to their specific receptors, expressed on sensory nerve fibers to trigger neuronal response, thus establishing a bidirectional communication. This bidirectional cross-talk between ENS and the enteric immune system is crucial to maintain visceral homeostasis. This cross-talk regulates the magnitude of inflammatory response *via* the production of cytokines, disruption of epithelial tight junctions, neutrophil recruitment, phagocytosis, modification in lymphocyte differentiation, and ultimately cell death ensues[4].

During the early postnatal life, ENS undergoes extensive development in parallel to the colonisation of gut microbiota and maturation of mucosal immune system in GIT. In germ-free mice, structural and functional abnormalities of the ENS have been observed, which suggests the role of gut microbiota in ENS development. Microbiota interacts with the nervous system through modulation of neurotransmitters production. Indeed, bacteria have been found to have the capability to produce a range of significant neurotransmitters in the gut. Therefore, gut microbiota fine-tunes the interaction between enteric nervous and immune system by altering the level of neuromediators (Figure 1).

A more thorough understanding of the interactions among neuromediators, inflammation, and neuromediators producing gut microbiota is required to ensure the effectiveness of neuromediators as a treatment option for IBD. Herein, we review the current knowledge of the role of neuromediators and bacteria that produce neurome-

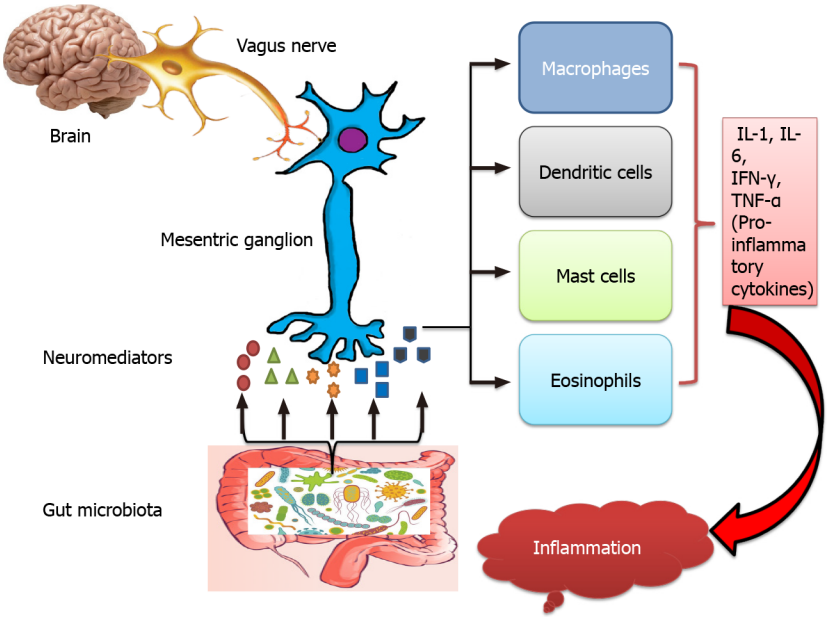

Figure 1 Modulation of cross-talk between the enteric nervous system and the enteric immune system *via* gut bacteria. Gut microbiota and vagus nerve stimulate mesenteric ganglion (enteric neuron) to produce neuromediators. Neuromediators act on various immune cells and influence their ability to release pro-inflammatory cytokines. During inflammatory bowel disease, dysbiosis in gut microbiota and abnormality in the enteric nervous system affect the level of neuromediators that results in overproduction of pro-inflammatory cytokines and promote inflammation. IL: Interleukin; TNF-α: Tumour necrosis factor-α; IFN-γ: Interferon-γ.

diators which might be a potential option in the treatment of IBD.

NEUROMEDIATORS AND IBD

A variety of neuropeptides and neurotransmitters are known to involve in the pathogenesis of IBD. Neuropeptides such as substance P (SP), neurotensin (NT), vasoactive intestinal peptide (VIP), neuropeptide Y (NPY), corticotrophin-releasing hormone (CRH), galanin (GAL) and calcitonin gene-related peptides (CGRP) and neurotransmitters like serotonin, nitric oxide (NO), acetylcholine, noradrenaline (NA) and γ-aminobutyric acid (GABA) regulates inflammatory processes by employing immunomodulatory pathways. Role of each of these neuromediators are briefly summarized in Table 1.

SP

SP is released from neurons and also from inflammatory cells like lymphocytes, macrophages, and dendritic cells. It acts by binding to the neurokinin-1 receptor (NK-1R). It plays a vital role in the amplification of inflammatory response by inducing the release of cytokines, reactive oxygen species, and stimulates leukocyte recruitment. Increased level of SP has been observed in the colon of IBD patients and, in the synovial fluid and serum of rheumatoid arthritis (RA) patients. Also, the enhanced expression of NK-1R was reported in the colon of IBD and synoviocytes of RA patients. SP has pro-inflammatory effects in epithelial and immune cells and contributes to many inflammatory diseases, including sarcoidosis, asthma, chronic bronchitis, RA, and IBD[5]. However, in murine models of colitis, SP plays a regulatory action[6]. In a recent study, SP was observed as an accelerator for healing the dextran sodium sulfate (DSS)-induced damaged intestine *via* inhibiting inflammatory responses through the modulation of cytokine expression[7].

NT

NT is a tridecapeptide, a pro-inflammatory neuropeptide widely distributed in the

Table 1 List of neuromediators and their role in gut inflammation

Neuromediator	Distribution	Binding receptor	Function
SP	Neurons and inflammatory cells like lymphocytes, macrophages, and dendritic cells	NK-1R	Exerts pro-inflammatory effects in epithelial and immune cells and contributes to inflammatory diseases. In murine model of colitis, it plays regulatory role
NT	Nervous system and intestine	NTR1	Recognized as an immunomodulator. By interacting with immune cells, it enhances the chemotaxis and induces the cytokine release to modulate the immune response. In IBD, it exerts its pro-inflammatory effects by promoting the expression of miR-210 in intestinal epithelial cells
NPY	Central and peripheral nervous system and immune cells	Out of five receptors of NPY, NPYY1 is known to play a crucial role in immunomodulation	Regulates various immune cell functions such as T helper cell differentiation, neutrophil chemotaxis, natural killer cell activity, and granulocyte oxidative burst and NO production. In the gut, NPY is known to exert pro-inflammatory effects
VIP	Neuronal and lymphoid cells	VIPR1 and VIPR2	Identified as an anti-inflammatory molecule. administration of VIP nanomedicine in the form of VIP-SSM are capable of alleviating the symptoms of DSS- induced mice model of colitis
GAL	Vasculature, immune cells and colonic epithelial cells	GAL (1-3) receptor	Exerts anti-inflammatory effects in TNBS induced colitis model by reducing the expression and activity of iNOS
CRH	Immune cells	CRH-R1 and CRH-R2	It acts as a pro-inflammatory peptide. The expression pattern of CRH 1 and CRH 2 varies in ulcerative colitis. Inhibition of CRH1 and overexpression of CRH2 may have the therapeutic potential in IBD
CGRP	Sensory nerves projecting to the lymphoid organs, airways, and pulmonary neuroendocrine cells	CGRP receptors	CGRP negatively regulates innate immune responses and thus has potential anti-inflammatory effects. Its expression reduced in the colon of an animal model of colitis
NA	Nerves innervating the peripheral lymphoid organs	Adrenergic α and β receptors	immunomodulatory effect of NA is administered *via* cAMP. Activation of NA receptors that stimulate cAMP resulting in a shift toward Th2 responses which are anti-inflammatory and neuroprotective whereas decreased cAMP stimulates Th1 responses resulting in cell destruction and inflammation
Acetylcholine	Central and peripheral nervous system, immune cells, keratinocytes, endothelial cells, urothelial cells of the urinary bladder, airways and epithelial cells of the placenta	Nicotinic and muscarinic receptors	Muscarinic receptors mediate pro-inflammatory responses and nicotinic receptors enhance anti-inflammatory responses. Treatment of UC *via* nicotine suggests the role of the cholinergic pathway in colonic inflammation
NO	Neuron synapses and immune cells	NO does not act *via* receptors. its specificity for target cell depends on its concentration, its activity and response, and territory of target cells	NO is oxidised to reactive nitrogen oxide species which mediate most of the immunological effects. It regulates the growth, functional activity, and death of immune cells. It acts as a biomarker for monitoring disease activity due to its increased serum concentration during the active phase of both UC and CD and reduced concentration during the inactive phase of the disease
Serotonin or 5-HT	Central nervous system and EC cells of GIT	5-HT receptor	It promotes activation of lymphocytes and secretion of pro-inflammatory cytokines. It activates the signalling molecules of the NF-kB pathway during gut inflammation
GABA	Nervous system and immune system	GABA- AR and GABA-BR	GABA has several effects on immune cells, including modulation of cytokine secretion, regulation of cell proliferation, and migration. Activation of GABA-A receptor aggravates DSS induced mice model of colitis

SP: Substance P; GABA: γ-aminobutyric acid; 5-HT: 5-hydroxytryptamine; EC: Enterochromaffin; NF-kB: Nuclear factor kB; NO: Nitric oxide; NA: Noradrenaline; CD: Crohn's disease; UC: Ulcerative colitis; cAMP: Cyclic adenosine monophosphate; CGRP: Calcitonin gene-related peptide; IBD: Inflammatory bowel disease; CRH: Corticotropin-releasing hormone; iNOS: Inducible nitric oxide synthase; GAL: Galanin; TNBS: 2,4,6-trinitrobenzenesulfonic acid; VIP: Vasoactive intestinal peptide; SSM: Sterically stabilised micelles; NPY: Neuropeptide Y; NK-1R: Neurokinin-1 receptor; NTR1: Neurotensi receptor 1; DSS: Dextran sodium sulfate.

nervous system and intestine. It binds to NT receptor 1 (NTR1) which is a high-affinity receptor and expressed in neurons, immune cells, colonic epithelial cells and colon cancer cell lines. It regulates various peripheral processes including gut motility, intestinal epithelial cell proliferation, secretion, and vascular smooth muscle activity, but recently it is recognized as an immunomodulator. NT interacts with leukocytes, dendritic cells and peritoneal mast cells, inducing the release of cytokines and enhancing chemotaxis in order to modulate the immune response. The elevated level

of NT and increased expression of NTR1 have been reported in the colonic mucosa of the experimental model of colitis and ulcerative colitis (UC) patients. NT is implicated in various acute and chronic inflammatory diseases, including lung and intestinal inflammation[8-11]. In IBD, NT exerts its pro-inflammatory effects by promoting the expression of miR-210 in intestinal epithelial cells[12].

NPY

NPY is a peptide of 36 amino acids and produced abundantly by the central and peripheral nervous system and also by immune cells. Neuronal functions of NPY include modulation of blood pressure, nociception, anxiety, and appetite. It also has diverse effects on innate and adaptive immunity, including immune cell migration, cytokine release from macrophages and T helper cells, and antibody production. Out of five receptors of NPY, NPYY1 is known to play a crucial role in immunomodulation. To modulate inflammation, NPY regulates various immune cell functions such as T helper cell differentiation, neutrophil chemotaxis, natural killer cell activity, and granulocyte oxidative burst and NO production. In the gut, NPY is known to exert pro-inflammatory effects. Several clinical studies reported the role of NPY in immune or inflammatory disorders such as arthritis, asthma, and IBD[4,13-15].

VIP

VIP is a 28 amino acid neuropeptide, produced by neuronal cells and lymphoid cells. It controls the homeostasis of the immune system by carrying out a wide range of immunological functions. Recently, it has been identified as an anti-inflammatory molecule. It is reported to inhibit pro-inflammatory cytokines and chemokines production from macrophages, dendritic cells, and microglial cells. Furthermore, VIP reduces the expression of costimulatory molecules on antigen-presenting cells, resulting in the promotion of Th2 type responses and reduction in Th1 type responses. VIP has been considered as a promising target for the treatment of autoimmune as well as acute and chronic inflammatory diseases such as multiple sclerosis, RA, Crohn's disease (CD), septic shock, or autoimmune diabetes[4,16-18]. Recombinant VIP analogue protects the intestinal mucosal barrier function effectively in rats. This analogue of VIP ameliorates 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colonic injury and inflammation through downregulating the expression of tumour necrosis factor-α and upregulating the interleukin (IL)-10 expression[15,19]. Though the administration of VIP shown anti-inflammatory effect but its therapeutic use is restricted due to its rapid degradation and continuous infusion. Recently, the administration of VIP nanomedicine in the form of sterically stabilized micelles has been observed to overcome the barriers and are capable of alleviating the symptoms of DSS-induced mice model of colitis[20].

GAL

GAL is a 30 amino acid long sensory neuropeptide known to attenuate neurogenic inflammation. Among the receptors (GAL1-3), GAL-3 is most abundantly expressed on the vasculature, and immune cells and GAL-1 is the only receptor expressed in colonic epithelial cells. Various studies indicate the role of GAL-3 in inflammatory disease conditions. GAL-1 has multiple recognition sites for nuclear factor kB (NF-kB), and its expression is increased in colonic tissues of IBD patients. NF-kB is a significant player in IBD; thus, specific antagonists of GAL-1 may be used in the treatment of IBD. Administration of GAL in the TNBS-induced colitis model exerts anti-inflammatory effects by reducing the expression and activity of inducible NO synthase (iNOS)[21]. GAL may act as an immunomodulatory peptide because of its ability to sensitize natural killer cells and polymorphonuclear neutrophils towards pro-inflammatory cytokines. In neutrophil-dominated autoimmune arthritis, activation of GAL-3 can be considered as a substantial anti-inflammatory pathway. In multiple sclerosis, GAL-2 agonist has been reported to be a promising therapeutic target[22-26].

CRH

CRH is 41 amino acid neuropeptide, produced by various immune cells to regulate immune/inflammatory responses. This locally produced CRH in the peripheral organs, also called peripheral CRH. Peripheral CRH is expressed in various inflamed sites where it acts as a pro-inflammatory peptide. It is also found in the testes, adrenal medulla, ovaries, GIT, cardiovascular system, spinal cord, pancreas, lung, endometrium, and placenta. It has also shown pro-inflammatory effects in the female reproductive system. CRH exerts its biological effects by CRH-Receptor R1 and CRH-R2. CRH and CRH-Rs are known to be expressed in several components of the immune system and regulates various inflammatory phenomena. Due to its pro-inflammatory properties, the antagonist of CRH has been proposed as a potential therapeutic target in the treatment of allergic conditions (asthma, eczema, urticaria) and also in the treatment of lower gastrointestinal inflammatory diseases (chronic inflammatory bowel syndromes, irritable bowel disease, and UC)[23,27]. The expression pattern of CRH-1 and CRH-2 is found to be altered in UC. Based on their differential expression, their therapeutic role is advocated in IBD. Inhibition of CRH-1 and overexpression of CRH-2 may have the therapeutic potential[28]. Activation of CRH-1 signaling upregulates the production of vascular endothelial growth factor-A *via* cyclic adenosine monophosphate (cAMP) response-element binding protein (CREB) transcriptional activity, which results in inflammatory angiogenesis in the gut. Therefore by targeting CREB inactivation, symptoms of colitis may be ameliorated[29]. CRH is also reported to enhance gut permeability by activating mast cells that worsen the IBD pathogenesis. Thus blocking CRH receptors with appropriate antagonists can inhibit mast cell activation and may be considered as a promising therapeutic target for chronic gastrointestinal inflammatory diseases, including IBD[30,31].

CGRP

CGRP is a 37 amino acid peptide that is expressed by sensory nerves projecting to the lymphoid organs, airways, and by pulmonary neuroendocrine cells. Peripheral CGRP is a vasodilator and responsible for acute neurogenic inflammation. It upregulates the expression of IL-10 and inhibits activation of NF-kB by acting on innate immune cells. It also inhibits the production of pro-inflammatory cytokines and presentation of antigens to T cells by directly acting on dendritic cells and macrophages. CGRP negatively regulates innate immune responses and thus has potential anti-inflammatory effects. Available pieces of evidence suggest CGRP contributes to limiting tissue damage in liver inflammation, joint inflammation, and also in chronic obstructive pulmonary disease. Decreased level of CGRP was observed in the colon of an animal model of colitis which suggests its role in intestinal inflammation[32-36].

NA

NA is a primary neurotransmitter of the sympathetic nervous system, released from nerves innervating the peripheral lymphoid organs. Some evidence suggests that the immunomodulatory effect of NA is administered *via* cAMP. NA influences immune response directly by alteration in expression of adrenergic β receptors on macrophages or indirectly by alteration in level of endogenous NA. Activation of α2 adrenoceptors located on sympathetic nerve terminals results in decreased extracellular NA concentration by a negative feedback effect. Activation of NA receptors that stimulate adenylate cyclase to produce cAMP resulting in a shift toward Th2 responses which are anti-inflammatory and neuroprotective whereas decreased cAMP stimulates Th1 responses resulting in cell destruction and inflammation[3,37]. The use of the α2-adrenoceptor antagonist might be a novel therapeutic approach for the management of colitis[38].

ACETYLCHOLINE

Previously it was thought that acetylcholine is synthesised by only neurons of the parasympathetic and sympathetic nervous system, but now it is established that

acetylcholine is also synthesized by immune cells, keratinocytes, endothelial cells, urothelial cells of the urinary bladder, airways and epithelial cells of the placenta. Acetylcholine released from these cells has been reported to modulate local inflammatory processes. Muscarinic and nicotinic are the two receptor subtypes of acetylcholine. T-cells express both subtypes and activation of each subtype exhibit differential effect. Muscarinic receptors mediate pro-inflammatory responses and nicotinic receptors enhance anti-inflammatory responses. Acetylcholine binds to α7 nicotinic receptors thus inhibits the release of pro-inflammatory cytokines from macrophages, and it is referred to as "cholinergic anti-inflammatory pathway". Acetylcholinesterase is an enzyme that catabolizes acetylcholine; thus, inhibitors of acetylcholinesterase may be considered for attenuating inflammation. In the murine model of sepsis, levels of pro-inflammatory cytokines can be brought down by injecting acetylcholinesterase inhibitors intraperitoneally. Reduced level of acetylcholine has been observed in multiple sclerosis, which is characterized by heightened inflammation. In mice, lacking the α7 subunit of the nicotinic acetylcholine receptor (α7nAChR-/-), the severity of colitis was found to be enhanced[39]. Treatment of UC *via* nicotine also suggests the role of the cholinergic pathway in colonic inflammation. Acetylcholine is well evident to play an essential role in acute or chronic inflammation or autoimmune diseases, including RA[40,41].

NO

NO is a major non-adrenergic non-cholinergic potent neurotransmitter at the neuron synapses. It is involved in the regulation of apoptosis. NO is a gaseous signaling molecule, synthesized by many cells that are involved in immunity and inflammation. However, low levels of NO gives an anti-inflammatory effect and maintain homeostasis but overproduction of NO induces inflammation and causes tissue destruction. The key enzyme involved in NO synthesis is iNOS-2. At high concentrations, NO is oxidized to reactive nitrogen oxide species which mediate most of the immunological effects. NO does not act *via* receptors, its specificity for target cell depends on its concentration, its activity and response, and territory of target cells. In the cardiovascular system, it induces vasodilation. It also regulates the growth, functional activity, and death of various cells including T lymphocytes, atrial premature complexes, neutrophils, mast cells, NK cells, and most importantly macrophages, which release NO in high concentration. Available information suggests that it contributes to the pathogenesis of inflammatory diseases of joint, gut and lungs[37,42,43]. NO may act as a biomarker for monitoring disease activity due to its increased serum concentration during the active phase of both UC and CD and reduced concentration during the inactive phase of the disease[44].

SEROTONIN OR 5-HYDROXYTRYPTAMINE

Five-hydroxytryptamine (5-HT) is a monoamine neurotransmitter and hormone which is traditionally recognized by its functions in the CNS where it is known to regulate sleep, appetite, mood, body temperature, metabolism, and sexuality. The majority of 5-HT is localized to the intestine and tryptophan hydroxylase (TPH1) enzyme catalysis the synthesis of serotonin in enterochromaffin (EC) cells of GIT. EC cells produce 5-HT more than all neuronal and other sources combined. 5-HT is reported to promote activation of lymphocytes and secretion of pro-inflammatory cytokines[45]. 5-HT is considered a potent immunomodulator and it can affect various immune cells including dendritic cells, macrophages, lymphocytes, enteric epithelial cells, and endothelial cells through 5-HT receptors and also *via* a process of serotonylation. During intestinal inflammation, 5-HT is known to mediate activation of signaling molecules of the NF-kB pathway[46]. Upregulated TPH1 and downregulated serotonin transporter (5-HT) expression leads to increased 5-HT availability resulting in enhanced 5-HT signalling, which is associated with inflammation in CD[47]. The role of 5-HT is not only limited to intestinal inflammation, but the alteration in its levels has also been observed in patients with RA and allergic airway inflammation[37,48].

GABA

GABA is an amino acid that is synthesised by decarboxylation of the glutamate with the help of enzyme glutamic acid decarboxylase. It is a classical neurotransmitter and best studied in CNS where it acts as an inhibitory neurotransmitter. Recently it has been found that the immune system is capable of synthesising GABA. GABA has several effects on immune cells, including modulation of cytokine secretion, regulation of cell proliferation, and migration. It can regulate immune responses in various autoimmune and inflammatory diseases such as multiple sclerosis, RA, psoriasis, and type 1 diabetes[49]. Reduced GABAergic signaling is reported to contribute in the pathogenesis of IBD[50]. However, a recent study demonstrated the aggravation of DSS-induced colitis through activation of GABA-A receptor[51].

NEUROMEDIATORS PRODUCING GUT MICROBIOTA AND IBD

Several commensal gut bacteria have emerged as the producers of a variety of neuromediators. These neuromediators are the result of the metabolism of indigestible fibres by gut bacteria. Many bacteria genera are recognised to produce different neuromediators. *Bacillus* family is reported to contribute to the synthesis of dopamine, various species of *Bacteroides*, *Parabacteroides*, *Lactobacillus* and *Bifidobacteria* are known to produce GABA. Similarly, serotonin is produced by *Enterococcus*, *Streptococcus*, and *Escherichia* families. Some species of *Lactobacilli* are involved in acetylcholine synthesis. Some species of *Bacillus* and *Escherichia* also produce noradrenaline[52].

Bacillus

Despite the low abundance of *Bacillus* species in the human gut, it has many beneficial effects, including probiotic features in GIT. Administration of *Bacillus subtilis* in DSS-induced mice model of colitis attenuated the gut inflammation and dysbiosis of gut microbiota[53]. It balances the pro and anti-inflammatory cytokines during disease conditions. It has also shown it's protective effects in IBD patients[54]. Bacillus is reported to produce bioactive metabolites, including neurotransmitters, that further affect the host inflammatory responses[55].

Bacteroidetes

Bacteroidetes is one of the most dominant genera of gut microbiota. It is comprised of *Bacteroides*, *Parabacteroides*, and *Alistipes*. In IBD patients, a low abundance of *Bacteroidetes* has been observed. *Bacteroidetes* confer protection against colitis by expressing polysaccharide A, which can induce the growth of regulatory T cell[56]. Various species of *Bacteroidetes* including *Bacteroides fragilis*, *Bacteroides vulgatus*, *Bacteroides ovatus*, *Bacteroides thetaiotamicron*, *Parabacteriodes*, *Alistipes indistinctus*, *Alistipes finegoldiiand*, *Alistipes putredinis* are evident to produce GABA[57,58]. Administration of these species in LPS induced intestinal epithelial cells and animal model of colitis ameliorated colonic inflammation[59,60]. Significant reduction in the severity of gut inflammation in DSS induced mice model of colitis have been observed after oral administration of *Parabacteroides distasonis*[61].

Bifidobacterium

Bifidobacteria is considered the early colonisers of human GIT. The beneficial effects of this genus are very well established[62]. It is widely used in the preparation of probiotics and reported to exert anti-inflammatory effects. Many species such as *Bifidobacterium dentium*, *Bifidobacterium breve*, *Bifidobacterium bifidum* are found to produce GABA[58]. These species, together with some other species like *Bifidobacterium longum*, *Bifidobacterium adolescentis* are known to confer beneficial effects to IBD patients by inhibiting the NF-kB activation, blocking pro-inflammatory cytokines expression and ultimately attenuating the inflammation[63,64].

Enterococcus

Enterococcus primarily resides in the small and large intestine of human GIT. The strains of *Enterococcus* represent approximately 1% of human faecal flora. *Enterococcus faecalis* and *Enterococcus faecium* are the two dominant species found in the human gut[65]. *Enterococcus* is comprised of both commensals as well as nosocomial pathogens. However, commensals have shown several beneficial effects including antimicrobial properties, by releasing bacteriocins and genetically they are very distinct from pathogenic but still, they are not considered safe due to its pathogenic strains. *Entero-*

coccus is found to be actively involved in the biosynthesis of serotonin[66]. Increased abundance of *Enterococcus faecalis* has been observed in IBD patients where it contributes toward pathogenesis[67]. In IL-10 knockout mice, *Enterococcus faecalis* can also induce IBD[68]. Daily administration of probiotic strain of *Enterococcus faecium* in combination with *Lactobacillus helveticus* 416 and *Bifidobacterium longum* ATCC 15707 is known to relieve the symptoms in DSS-induced colitis in rats[69].

Escherichia

Escherichia coli is the regular inhabitant of human GIT. It is the most diverse member of gut microbiota which can act like commensal, probiotic, and pathogenic as well. Increased abundance of *Escherichia* is evident in several mouse models of colitis[70]. A newly identified pro-inflammatory strain of *Escherichia coli* (*E. coli*), adherent-invasive *E. coli* is detected in UC, CD and colorectal cancer. It is highly prevalent and associated with CD pathogenesis as compared to UC[71,72]. *E. coli* Nissle 1917 (EcN) is reported to produce serotonin and also enhance its bioavailability by interacting with the host. Clinical trials demonstrated the beneficial role of EcN in maintaining the UC in remission phase[73-75]. Serotonin signalling was reported to be altered in IBD patients[76]. Some strains of *E. coli* are found to exacerbate the gut inflammation, which suggested the strain-specific effects of *E. coli*[77].

Lactobacillus

Despite having a low abundance, this genus is well known for its probiotic effects[74]. The population of *Lactobacillus* is either positively or negatively associated with many diseases, including IBD[78]. Significant reduction in the *Lactobacillus* population has been observed in UC patients, and there are reports suggested the improvement in clinical symptoms of UC patients after consuming food containing *Lactobacillus*. It showed a beneficial effect in intestinal inflammation by modulating Treg cells which maintain intestinal homeostasis by secreting anti-inflammatory cytokines[79]. Various species of *Lactobacillus* like *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus reuteri* and *Lactobacillus rhamnosus* are reported to produce GABA[57].

Additionally, acetylcholine is also produced by various strains of *Lactobacillus*, especially *Lactobacillus plantarum*[80]. In a recent study, the effect of dietary probiotics is investigated in IBD induced murine model where *Lactobacillus rhamnosus* is observed as a significant producer of IL-10 and interferon-γ[81]. Group of animal studies, human trials, and in vitro studies revealed that these species of Lactobacillus are involved in controlling inflammation either by inhibiting the NF-κB induced release of pro-inflammatory cytokines or by maintaining the intestinal barrier integrity[82-88].

Streptococcus

Streptococcus is a luminal microbial genus, dominant in the distal oesophagus, duodenum, and jejunum. The most common species are *Streptococcus salivarius*, *Streptococcus thermophilus*, and *Streptococcus parasanguinis*[89]. *Streptococcus* species, including *Streptococcus thermophilus* is reported to produce serotonin[90]. Increased abundance of streptococcus has been observed in IBD patients that indicated the involvement of this genus in the severity of IBD. *Streptococcus bovis* is found to be associated with colon cancer and IBD. Streptococcus is known to interact with immune cells and modulate the secretion of pro-inflammatory cytokines that could initiate the inflammatory response in different organs[91]. Recently, immunoglobulin enriched streptococcus is reported in IBD patients that implicate a prominent role of oropharyngeal bacteria in IBD pathogenesis by triggering host immune response[92].

SIGNIFICANCE OF NEUROIMMUNOMODULATION BY GUT BACTERIA

Gut bacteria have been known to be crucial for human health. It deliberate number of benefits to the host, including digestion of indigestible carbohydrates that leads to the production of short-chain fatty acids (SCFA) and prevent the colonisation of pathogenic bacteria by producing antimicrobial peptides. SCFAs are involved in various functions like protection from epithelial injury, synthesise vitamins (vitamin B12, vitamin K and folic acid) and essential amino acids, regulate fat metabolism, boost intestinal angiogenesis, cause intestinal motility and promote proper development of immune system[93-95]. Studies conducted in IBD patients and mice models have indicated the central role of gut bacteria in the gut inflammation[96]. The new research in the field opens up new avenues to understand the IBD pathogenesis. Through

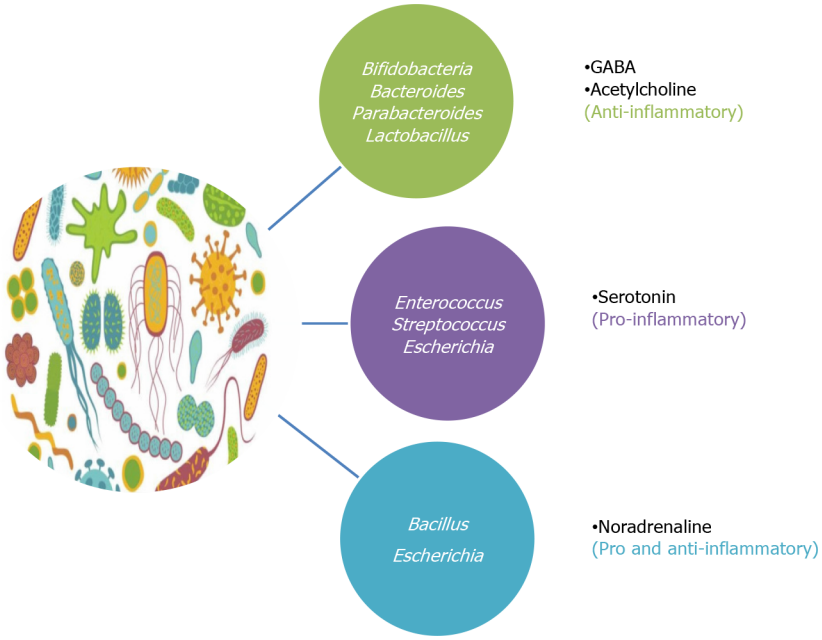

Figure 2 Inter-relation of diverse gut microbiota and their respective neuromediators with gut inflammation. Bacteria used as probiotics in inflammatory bowel disease (IBD) (green box) produces anti-inflammatory neuromediators (γ-aminobutyric acid, acetylcholine), bacteria having a detrimental role in IBD (purple box) releases pro-inflammatory neuromediator (serotonin) and bacteria having a debatable role in IBD (blue box) secrete neuromediator (noradrenaline) having both pro and anti-inflammatory properties. GABA: γ-aminobutyric acid.

numerous mechanisms, bacteria execute their part in disease pathogenesis. The revelation of secretion of neuromediators from gut microbes introduced a new area for research and a unique way of looking at the pathophysiology of IBD.

Neuromediators, apart from their classical neuronal functions, are currently being recognised as a pillar in maintaining the gut homeostasis. There are different sources of neuromediators in GIT, including enteric neurons, gut microbiota, immune cells and gut epithelial cells. Out of all the sources, microbial content is the only factor which can be extrinsically varied. Altering the neuromediators *via* gut bacteria can affect the gut physiology, signalling and immune cells secretions and function in GIT. The available literature on the signalling pathways of a variety of neuromediators and their respective gut bacteria in IBD indicated that the neuromediators released by bacteria being used as probiotic are having anti-inflammatory properties and bacteria which were reported to increase disease severity produce neuromediators with pro-inflammatory properties. For instance, GABA and acetylcholine are the anti-inflammatory neuromediators, produced by those bacteria which are very well established to attenuate gut inflammation in both DSS-induced mice model of colitis and IBD patients. Serotonin which is a pro-inflammatory neuromediator is produced by bacteria that are involved in the severity of IBD. Besides, noradrenaline, having both anti and pro-inflammatory properties, produced by two different types of bacteria, one having the beneficial role and other having the debatable role in IBD (Figure 2). This interrelation suggests that bacteria impart their effects in gut inflammation through releasing neuromediators as one of the mechanism.

CONCLUSION

Neuromediators are emerging as essential players in IBD pathogenesis. These are influenced by the complex interaction of gut microbiota, host immunity, and intestinal epithelium. During gut inflammation or IBD, dysbiosis in gut microbiota and alteration in neuromediators complicate the mechanism of gut homeostasis resulting in perturbed equilibrium (Figure 3). In-depth mechanism of neuroimmunomodulation due to gut bacteria needs to be explored more, to settle the gut homeostasis during disease. These neuromediators may prove to be a great tool to clinicians in treating inflammatory diseases. Through this review, we summarized various neuromediators produced by different gut microbiota and their significance as an immunomodulatory entity in the colon. Using gut bacteria that can produce neuromediators having anti-

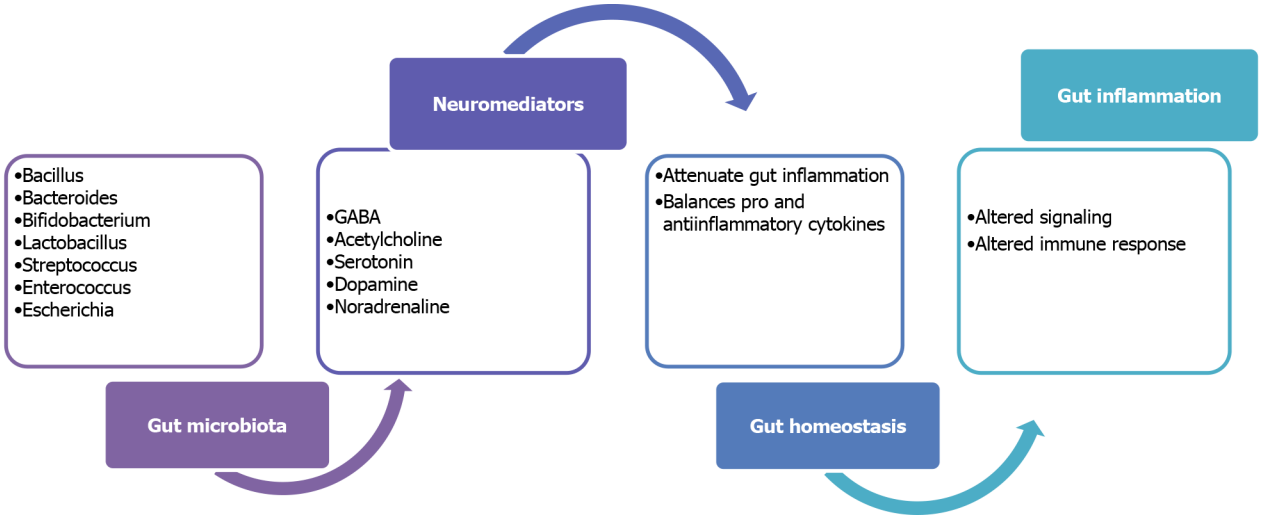

Figure 3 Role of neuromediators producing gut microbiota during gut inflammation. Gut microbiota produces various neuromediators that attenuate the gut inflammation by balancing the pro and anti-inflammatory cytokines to maintain gut homeostasis. During inflammation, dysbiosis in gut microbiota leads to alteration in respective neuromediators which may lead to altered the host immune response. GABA: γ-aminobutyric acid.

inflammatory properties for treating IBD patients may be a novel therapeutic approach and also the fertile area for future research.

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World Journal of Gastrointestinal Pathophysiology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Pathophysiol 2021 May 22; 12(3): 40-50

DOI: 10.4291/wjgp.v12.i3.40

ISSN 2150-5330 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

Platelet count as a screening tool for compensated cirrhosis in chronic viral hepatitis

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Author contributions: Surana P designed and performed the research and wrote the paper; Koh C designed the research and supervised the report; Takyar V performed the research; Hercun J performed the research and wrote the paper; Kleiner DE and Heller T provided clinical advice; all authors reviewed the manuscript for important intellectual content and approved the final version of the manuscript.

Institutional review board statement: This study was reviewed and approved by the National Institute of Diabetes and Digestive and Kidney Diseases Institutional Review Board.

Informed consent statement: Patients gave written informed consent to the study agreeing to the use of anonymous clinical data obtained under protocol 91-DK-0214.

Conflict-of-interest statement: We have no financial relationships related to this research to disclose.

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Abstract

BACKGROUND

Simple tools for clinicians to identify cirrhosis in patients with chronic viral hepatitis are medically necessary for treatment initiation, hepatocellular cancer screening and additional medical management.

AIM

To determine whether platelets or other laboratory markers can be used as a simple method to identify the development of cirrhosis.

METHODS

Clinical, biochemical and histologic laboratory data from treatment naive chronic viral hepatitis B (HBV), C (HCV), and D (HDV) patients at the NIH Clinical Center from 1985-2019 were collected and subjects were randomly divided into training and validation cohorts. Laboratory markers were tested for their ability to identify cirrhosis (Ishak ≥ 5) using receiver operating characteristic curves and an optimal cut-off was calculated within the training cohort. The final cut-off was tested within the validation cohort.

RESULTS

Overall, 1027 subjects (HCV = 701, HBV = 240 and HDV = 86), 66% male, with mean (standard deviation) age of 45 (11) years were evaluated. Within the training cohort (n = 715), platelets performed the best at identifying cirrhosis compared to other laboratory markers [Area Under the Receiver Operating Characteristics curve (AUROC) = 0.86 (0.82-0.90)] and sensitivity 77%, specificity 83%, positive predictive value 44%, and negative predictive value 95%. All other

Data sharing statement: No additional data are available.

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

Manuscript source: Invited manuscript

Specialty type: Gastroenterology and hepatology

Country/Territory of origin: United States

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C, C, C, C, C
Grade D (Fair): D, D
Grade E (Poor): E

Received: January 21, 2021
Peer-review started: January 21, 2021
First decision: February 9, 2021
Revised: February 25, 2021
Accepted: March 31, 2021
Article in press: March 31, 2021
Published online: May 22, 2021

P-Reviewer: Ferraioli G, Garbuzenko DV, Kreisel W, Liu N, Maslennikov R, Pluta M, Qi XS
S-Editor: Gao CC
L-Editor: A
P-Editor: Yuan YY

tested markers had AUROCs ≤ 0.77. The optimal platelet cut-off for detecting cirrhosis in the training cohort was 143 × 10^9/L and it performed equally well in the validation cohort (n = 312) [AUROC = 0.85 (0.76-0.94)].

CONCLUSION
The use of platelet counts should be considered to identify cirrhosis and ensure optimal care and management of patients with chronic viral hepatitis.

Key Words: Chronic hepatitis B; Chronic hepatitis C; Chronic hepatitis D; Platelets; Cirrhosis; Non-invasive assessment

Core Tip: Platelet count is a well-recognized surrogate marker for progression of liver disease, however a specific cut-off for cirrhosis has not been established. In this study, platelet counts can accurately stratify chronic viral hepatitis patients with cirrhosis; and a platelet count > 143 × 10^9/L appears to have the most clinical utility in ruling out cirrhosis across all chronic viral hepatitis. This widely available laboratory value may be useful in decision making for the management of patients with chronic viral hepatitis and represents a finding which may be of particular value in a primary care setting.

Citation: Surana P, Hercun J, Takyar V, Kleiner DE, Heller T, Koh C. Platelet count as a screening tool for compensated cirrhosis in chronic viral hepatitis. *World J Gastrointest Pathophysiol* 2021; 12(3): 40-50
URL: https://www.wjgnet.com/2150-5330/full/v12/i3/40.htm
DOI: https://dx.doi.org/10.4291/wjgp.v12.i3.40

INTRODUCTION

Globally, chronic hepatitis B, C, and D virus (HBV, HCV and HDV respectively) affect about 325 million people[1]. Progression of these viral infections is associated with serious complications including cirrhosis, hepatic decompensation, hepatocellular carcinoma, and death. With effective treatments for hepatitis B and C, the Centers for Disease Control and Prevention have advocated for widespread screening for viral hepatitis in adults[2,3]. There has also been a paradigm shift where primary care physicians are increasingly tasked with managing and treating these patients[4], and various programs have allowed for expanded care in areas with poor access to viral hepatitis care[5]. In addition, numerous efforts worldwide have aimed to increase the number of providers with the ability to manage chronic viral hepatitis, including the national viral hepatitis action plan 2017-2020 by the U.S. Department of Health and Human Services[6] and the Mukh-Mantri Punjab Hepatitis C Relief Fund program in India[7].

The decision of when and whom to treat in chronic viral hepatitis infections is often dependent upon the stage of liver disease[8,9]. Currently, liver biopsy is the gold standard for staging disease severity in patients with liver disease. However, liver biopsies are invasive, performed by a specialist and access may be limited in resource-poor regions. To date, no single routinely measured laboratory marker has been explored for the identification of cirrhosis. Although expert consensus suggests that thrombocytopenia, with a laboratory cutoff value of < 150 × 10^9/L, is a surrogate marker for cirrhosis, this has mostly been demonstrated in patients with chronic HCV[10,11]. More recently, platelet counts have been used in conjunction with other markers. Current hepatology guidelines state that clinically significant portal hypertension can be identified by "liver stiffness > 20-25 kPa, alone or combined with platelet count and spleen size"[12]. Unfortunately, ultrasound-based techniques [such as Vibration Controlled Transient Elastography (VCTE)] providing an assessment of liver stiffness and cirrhosis are not widely available in all regions and to all healthcare providers.

Common serum laboratory tests, including platelet counts, have been included in

non-invasive markers of liver fibrosis or cirrhosis and have demonstrated clinical utility in the management of hepatitis C[9,13]. However, these non-invasive markers have not been shown to be as useful in chronic HBV due to its complex natural history[14,15]. Nonetheless, these tools have provided a cost-effective method to identify disease progression in patients with chronic viral hepatitis. Unfortunately, these tests require an on-line calculator as well as interpretation of various cutoff values and although often used by hepatologists and gastroenterologists, they remain unknown to primary care providers. Additionally, while their use for diagnosis of advanced fibrosis is widespread, they are not as powerful in determining cirrhosis as ultrasound-based methods[16,17].

With the increasing role of primary care providers in the management of chronic viral hepatitis, the development of a widely available and versatile tool in identifying patients with cirrhosis is clinically necessary. In this group of patients, additional management and treatment considerations may be required, as well as a referral to a specialist. In this study, we explore whether platelets or other commonly measured laboratory markers, alone, can be used as a simple and effective way to characterize the progression of viral hepatitis and whether a threshold can be identified for the development of cirrhosis.

MATERIALS AND METHODS

Study population

This retrospective, cross-sectional study consisted of patients infected with HBV, HCV or HDV and who underwent liver biopsy at the National Institutes of Health Clinical Center between 1985 and June 2019. Chronic viral hepatitis infection was established if patients demonstrated viral positivity for at least six months and/or histology consistent with the respective chronic infection. Chronic hepatitis B infection was established with the presence of hepatitis B surface antigen (HBsAg) in serum and positive HBsAg or hepatitis B core antigen staining on histology. Chronic hepatitis D co-infection was established with the presence of anti-HDV antibodies and HDV RNA in serum or positive hepatitis D antigen staining on histology in patients with chronic HBV. In patients who underwent biopsy after 1991, chronic hepatitis C was established using the presence of HCV RNA in serum for six months. In those who underwent biopsy prior to 1991, patients with presence of clinical and histologic features of non-A non-B hepatitis were later confirmed to have HCV infection by testing for HCV RNA using stored serum.

Patients with concomitant chronic non-viral liver diseases, multiple viral hepatitis (besides HBV/HDV co-infection), or HIV co-infection were excluded. In addition, patients were judged to be in adequate overall health to undergo liver biopsy and had no severe systemic diseases. All patients were enrolled in clinical research protocols approved by the National Institute of Diabetes and Digestive and Kidney Diseases Institutional Review Board and gave written, informed consent for participation. Pre-treatment liver biopsies were reviewed, and concurrent laboratory values were also collected using the NIH Biomedical Translational Research Information System. Laboratory results within two months prior to the liver biopsy and initiation of any treatment were utilized for analysis.

Liver histopathology

All liver biopsies were scored and analyzed by a single hepatopathologist (DEK). Ishak fibrosis scores were used to score hepatic fibrosis, ranging from 0 (no fibrosis) to 6 (cirrhosis)[18]. Cirrhosis was defined as a score ≥ 5. Inflammation was scored using the modified histologic activity index (HAI), ranging from 0-18[19]. The total HAI score comprised of the summation of periportal inflammation, lobular inflammation, and portal inflammation.

Statistical methods

Training and validation cohorts: The entire cohort was randomly divided into training and validation cohorts using simple random sampling and a sample rate of 0.3. Selection was stratified by gender and virus type. Univariate comparisons of the two cohorts were conducted using student *t*-tests and chi-square tests where appropriate. Based on this analysis the training and validation cohorts were similar.

Biomarker selection: The training cohort was used to single out the best performing biomarker to identify cirrhosis status. Spearman's correlations were calculated in the

training cohort to determine the association between fibrosis and selected laboratory markers. Of the significantly correlated laboratory parameters, those with an absolute value of Spearman's R greater than 0.3 (moderate correlation) were selected for further analysis within the training cohort[20]. Logistic regression was used to create receiver operating curves and calculate the area under the curve (AUROC) of each selected laboratory parameter within the training cohort. Laboratory markers were log transformed to assure normality of the data. Sensitivity, specificity, positive predictive value, and negative predictive value were also used to measure performance. Delong Test was used to compare ROC curves for different laboratory parameters within the same sample group. Youden's index, as well as sensitivity, specificity, positive predictive value, and negative predictive value were all used to determine the optimal platelet cut-off point to predict cirrhosis. Once this analysis was completed in the training cohort, the most significant factor in the training cohort was tested in the validation cohort and by virus within the validation cohort through AUROC values, sensitivity, specificity, positive predictive value, and negative predictive value. Fibrosis-4 index (Fib-4) and AST (aspartate aminotransferase) to Platelet Ratio Index (APRI) were calculated using the established formulas[9,13]. All analysis was conducted using SAS 9.4 (Cary, NC, United States).

RESULTS

Study demographics

A total of 1027 untreated subjects with viral hepatitis were evaluated (HCV = 701, HBV = 240, HDV = 86). The mean age of the cohort was 45 years (SD: 11) and 66% of subjects were male. Baseline demographics for the training and validations cohort are displayed in Table 1. In the training cohort, the mean Ishak fibrosis score was 2.4 (SD: 1.8) and 15% of patients were cirrhotic.

Mean platelet count in the training cohort was 187 × 10^9/L (SD: 64). Mean alanine aminotransferase (ALT) and AST values were elevated within the training cohort [104 IU/mL (SD: 88); 70 IU/mL (SD: 55) respectively]. Mean albumin, prothrombin time, total bilirubin, and alkaline phosphatase values were within normal limits.

Using a single laboratory marker to identify cirrhosis

Laboratory markers commonly used to characterize liver disease were tested for their ability to identify cirrhosis within the training cohort (Table 2). These markers included transaminases, platelet count, total bilirubin, prothrombin time, albumin, and alkaline phosphatase. On Spearman's correlation of the training cohort, all tested laboratory markers appeared to be significantly correlated with Ishak fibrosis stage; however, only platelets, ALT, AST, alkaline phosphatase, and prothrombin time had Spearman correlations > 0.3 (Table 2).

Out of all of these laboratory markers, platelets performed the best at identifying cirrhosis compared to other laboratory markers (AUROC = 0.86, 95%CI 0.82-0.90), with all other markers with AUROCs ≤ 0.77 (Table 3). Prothrombin time had the next highest AUROC in the entire cohort (0.76, 95%CI 0.71-0.82). When comparing the ROC curves by the Delong test, platelets performed significantly better than all other tested laboratory markers in the training cohort (P < 0.002). Platelet counts compared favorably to both APRI [AUROC 0.84 (95%CI 0.80-0.88)] and Fib-4 [AUROC 0.88 (95%CI 0.85-0.91)].

Calculating a platelet cut-off for cirrhosis

The optimized platelet cut-off for detecting cirrhosis in the training cohort was 143 × 10^9/L (sensitivity: 77%, specificity: 83%, positive predictive value: 44%, negative predictive value: 95%). Figure 1 shows an overall decrease in the distribution of platelet count by Ishak fibrosis in the training and validation cohorts. Additionally, the demarcated, calculated platelet cut-off of 143 × 10^9/L appears to separate a majority of subjects with Ishak fibrosis ≥ 5 (Figure 1).

Platelet performance in validation cohort

The cutoff calculated in the training cohort was applied to the entire validation cohort and was also evaluated for each viral hepatitis. The performance of platelets to identify cirrhosis is demonstrated in Figure 2; platelets performed adequately in each virus (AUROC ≥ 0.81) and performed best in the HDV/HBV co-infection subset of the validation cohort (AUROC = 0.87). In the entire validation cohort, platelets performed

Table 1 Baseline demographics

	Training (*n* = 715)	Validation (*n* = 312)	*P* value
Age (yr)	45.6 (10.7)	44.5 (11.1)	0.1
Male/female (%)	66/34	66/34	1.0
Platelets ($\times 10^9$/L)	186.7 (64.4)	190.6 (68.2)	0.4
Alanine aminotransferase (IU/L)	103.8 (88.1)	105.1 (89.1)	0.8
Aspartate aminotransferase (IU/L)	69.9 (55.0)	68.0 (53.3)	0.6
Albumin (g/dL)	3.9 (0.46)	3.9 (0.39)	0.2
Alkaline phosphatase (IU/L)	82.3 (39.2)	79.0 (29.2)	0.1
Prothrombin time (s)	13.0 (1.3)	12.9 (1.1)	0.3
Total bilirubin (mg/dL)	0.81 (0.48)	0.77 (0.45)	0.2
Ishak fibrosis	2.4 (1.8)	2.3 (1.7)	0.3
HAI inflammation	8.0 (3.0)	7.9 (3.1)	0.5
HBV/HCV/HDV (%)	23/68/8	23/68/9	1.0

Values presented as mean (SD) unless otherwise noted.

Table 2 Spearman correlations between Ishak fibrosis and liver tests within training cohort

	R	*P* value
Platelets	-0.49	< 0.0001
AST	0.51	< 0.0001
ALT	0.37	< 0.0001
Alkaline phosphatase	0.35	< 0.0001
Prothrombin time	0.33	< 0.0001
Albumin	-0.30	< 0.0001
Total bilirubin	0.18	< 0.0001

Table 2 shows the calculated Spearman *R* and *P* value for the correlations between Ishak fibrosis and the indicated laboratory value. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

Table 3 Area under the curve using selected liver tests within the training cohort

Platelets	ALT	AST	Alkaline phosphatase	Prothrombin time
0.86 (0.82, 0.90)	0.65 (0.59, 0.71)	0.76 (0.71, 0.81)	0.76 (0.71, 0.81)	0.77 (0.71, 0.82)

Values presented as Area Under the Receiver Operating Characteristics curve (AUROC) (95% Wald confidence interval). Table 3 displays the calculated AUROC and 95% Wald confidence interval for each selected laboratory marker in identifying cirrhosis (Ishak ≥ 5) in the training cohort and the entire cohort. Overall, when compared by Delong test, platelets have a significantly greater AUROC value than each of the other laboratory values ($P > 0.002$). ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

with an AUROC of 0.85 (95%CI 0.76-0.94) and performed as well as APRI [AUROC 0.82 (95%CI 0.74-0.90)] and Fib-4 [AUROC 0.86 (95%CI 0.80-0.93)]. In general, the optimal platelet cut-off had a higher negative predictive value than positive predictive values (Table 4).

For simplicity, it may be suggested that a platelet cut-off of 143 × 10^9/L be rounded to 140 × 10^9/L instead. The sensitivity, specificity, positive predicative value, and negative predictive values were not greatly altered in the validation cohort (73%, 86%, 48%, 95% respectively) (Table 5).

Table 4 Performance of optimal platelet cut-offs in validation cohort

	Platelet cut-off (× 10^9/L)	AUROC	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Entire validation cohort	143	0.85 (0.76-0.93)	79	84	33	98
HBV	143	0.81 (0.53-1.00)	83	82	29	98
HCV	143	0.83 (0.72-0.94)	75	86	31	98
HDV	143	0.87 (0.74-1.00)	100	60	47	100

Table 4 displays the calculated cut-offs and sensitivity, specificity, positive predictive values, and negative predictive values for each the calculated optimal cut-off within the validation cohort. AUROC: Area Under the Receiver Operating Characteristics curve. HBV: Hepatitis B virus; HCV: Hepatitis C virus; HDV: Hepatitis D virus.

Table 5 Performance of platelet cut-offs in training cohort

Platelet counts (× 10^9/L)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
130	67	91	57	94
140	73	86	48	95
143	74	83	44	94
150	78	78	38	95

Table 5 displays the calculated sensitivity, specificity, positive predictive values, and negative predictive values for four cut-off platelet counts within the training cohort.

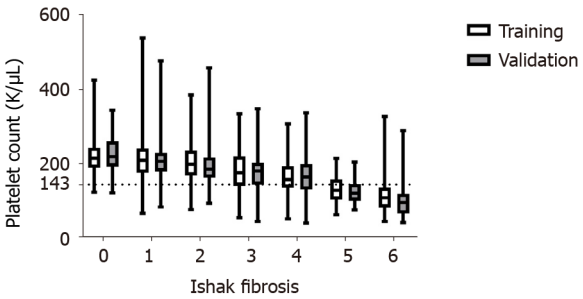

Figure 1 Platelet count distribution by Ishak fibrosis. This figure displays the distribution of platelets in the training and validation cohorts by Ishak fibrosis. The dotted line indicates the calculated optimal platelet cut-off (143 × 10^9/L).

DISCUSSION

In the largest reported cross-sectional retrospective study of patients with chronic viral hepatitis evaluating routinely measured laboratory tests, platelet counts were identified as a surrogate marker for the development of cirrhosis. In comparison to other commonly performed clinical tests in a primary care setting, platelet counts performed the best and had the highest AUROC in identifying patients with cirrhosis. An optimized platelet cut-off value of 143 × 10^9/L across all chronic viral hepatitis infections suggesting cirrhosis was validated. A rounded platelet count of 140 × 10^9/L appears to show similar performance in identifying cirrhosis as well. Given that primary care providers are uniquely positioned in managing patients with chronic viral hepatitis, these results offer a simple and effective method to determine severity of liver disease in a primary care setting without additional testing. The ability to rule out cirrhosis through a simple surrogate marker may provide a simplified approach to connecting patients to treatment and optimal medical management.

Thrombocytopenia is often recognized as a complication of liver disease and has been used as a surrogate marker for varices, portal hypertension, and increased risk of hepatocellular carcinoma; typical complications of cirrhosis[21-23]. Mechanistically,

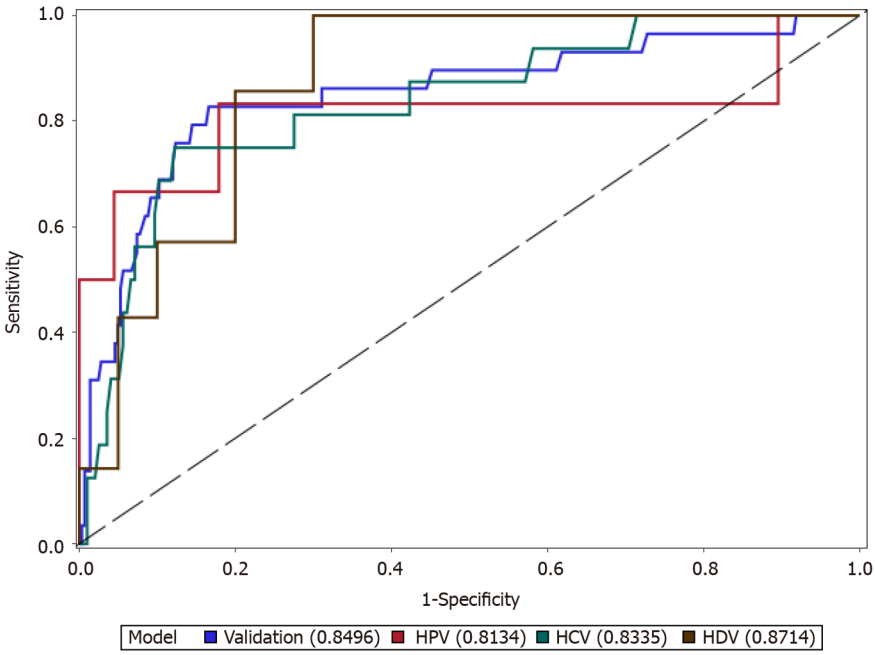

Figure 2 Receiver operating characteristic curves for platelet performance. Receiver operating characteristic curves testing the performance of platelets in identifying cirrhosis in chronic viral hepatitis patients. Area Under the Receiver Operating Characteristics curves (AUROC) were calculated for the entire validation cohort and by virus subgroups within the validation cohort. AUROC values are displayed in the figure key. HBV: Hepatitis B virus; HCV: Hepatitis C virus; HDV: Hepatitis D virus.

there are several possible explanations for the thrombocytopenia in chronic liver disease; such as, splenic sequestration of platelets, decreased platelet production, and decreased thrombopoietin activity[22,24]. Historically, only thrombocytopenia below 50 × 10^9/L has demonstrated clinical relevance[25]. Recently, various scores incorporating platelet counts have been proposed as a surrogate screening tool for complications of portal hypertension, most notably high-risk varices, including the Baveno VI criteria, the expanded Baveno VI criteria, and the albumin, bilirubin and platelet criteria (ABP criteria)[21,26,27]. In these scores, the suggested platelet count cut-offs range from 110 × 10^9/L to 150 × 10^9/L. Nonetheless, these models are restricted to patients with an established diagnosis of cirrhosis.

Additionally, platelets have been incorporated into non-invasive biomarkers of fibrosis such as Fib-4 and APRI, formulas typically utilized by sub-specia-lists[9,13]. Non-invasive biomarkers have been gaining interest as a useful tool in risk stratification in liver disease. However, transaminases, including AST are required for their calculation. This represents a significant drawback in the primary care setting due to increased evidence in certain regions of the world advocating for limiting hepatic screening panels to ALT and alkaline phosphatase[14,28]. Likewise, the cost-effectiveness of this strategy has also been described[29]. Over time, this approach has become an integral part of guidelines, including from the British Society of Gastroenterology[30]. Additionally, these indexes do not perform as well as patented biomarkers (FibroTest, FibroSure, Enhanced Liver Fibrosis) which are not widely available and are costly[31,32]. Therefore, in this context the use of a simple tool, such as platelet counts alone, can be a valuable tool for following patients with viral hepatitis prior to developing cirrhosis. In our cohort, platelet counts alone performed similarly to calculated non-invasive markers. This study demonstrates that thrombocytopenia below 143 × 10^9/L on its own is of clinical importance in viral hepatitis and is a useful single laboratory test to rule out cirrhosis.

According to the World Health Organization, health equity has still not been achieved by countries of all socioeconomic levels. In order to breach this gap in care, an increasing number of primary care physicians are being trained to care for patients with chronic liver disease through programs and resources such as Project ECHO, HepCCaTT (offering care for HCV), and the HBV Primary Care Workgroup[5,33-35] (all in the United States) or the Mukh-Mantri Punjab Hepatitis C Relief Fund in India[7]. However, chronic liver disease is just one of many chronic illnesses that primary care physicians are called upon to manage in these settings. The utility of other non-invasive markers may be limited in resource poor-settings. Both Fib-4 and APRI require multiple laboratory marker measurements, calculations, and knowledge

of validated cut-offs for correct interpretation[9,13,32]. VCTE, while simple and useful technology, is expensive and may not be available at all centers of care. In addition, complex algorithms including a sequential use of non-invasive markers to improve their accuracy have also been suggested[36,37]. These non-invasive markers are useful in specialist care settings, but might not be optimal in resource limited settings where primary-care physicians are the main point of care.

While platelet count has been proven to be an important indicator of liver disease progression, it is important to note that the platelet counts represented in this retrospective single center study's cohort may differ from those seen in a typical primary care setting. Given the specialized setting of the National Institutes of Health, this population may have a higher prevalence of cirrhosis than the typical primary care setting, and this may enhance the performance of platelet count as a marker of cirrhosis within this study. This study proposes the use of a single, commonly measured laboratory marker to monitor the progression of chronic viral hepatitis and identifies a clinically relevant cut-off for clinical decision making and to rule-out cirrhosis. Further studies would provide more information about the clinical outcomes of these patients, on what the degree of thrombocytopenia may imply for these patients and how platelet counts should be included in non-invasive monitoring algorithms. The strength of this study lies in the large cohort of chronically infected patients with histology and three etiologies of viral hepatitis with the inclusion of patients with chronic delta hepatitis.

CONCLUSION

While platelet count has been established as a surrogate marker for disease progression, a specific cut-off for cirrhosis has not been established. Platelet counts can accurately stratify chronic viral hepatitis patients with cirrhosis, a finding which may be of particular value in a primary care setting. As a potential non-invasive biomarker, a platelet count > 143 × 10^9/L or the rounded value 140 × 10^9/L appear to have the most clinical utility in ruling out cirrhosis across all chronic viral hepatitis. This routine and widely available laboratory value may be useful in the identification of patients with cirrhosis from chronic viral hepatitis which has downstream consequences related to their treatment and management and should be further explored for these purposes.

ARTICLE HIGHLIGHTS

Research background

The diagnosis of cirrhosis in patients with chronic viral hepatitis has both treatment and management implications. Identifying these patients is crucial in order to ensure proper care, prevent complications of cirrhosis and for judicious allocation of resources.

Research motivation

With an increasing reliance on primary care in management of chronic viral hepatitis, reliable simple non-invasive assessments of cirrhosis are needed in order to identify cirrhosis and to determine requirement of referral to specialized care.

Research objectives

To evaluate the performance of single laboratory markers, with an emphasis on platelet counts, to identify development of cirrhosis in patients with chronic hepatitis B virus, hepatitis C virus, and hepatitis D virus infection.

Research methods

Retrospective study comparing the accuracy of single laboratory markers in determining cirrhosis (defined as Ishak fibrosis score ≥ 5). Area Under the Receiver Operating Characteristics curve (AUROC), sensitivity, specificity, positive predictive value and negative predictive value were measured first in a training cohort and then in a validation cohort.

Research results

In a cohort of 1027 subjects, compared to other single laboratory markers, platelet counts performed the best at identifying cirrhosis [AUROC 0.86 (0.82-0.90)] and sensitivity 77%, specificity 83%, positive predictive value 44%, and negative predictive value 95%. The optimal cut-off point was 143 × 10^9/L. This performed equally well in a validation cohort.

Research conclusions

Platelet counts are the most reliable single serological marker in ruling out cirrhosis in patients with chronic viral hepatitis. Thrombocytopenia can potentially be used in the primary care setting for management of patients with viral hepatitis.

Research perspectives

Future research directions include validation of this cut-off value of platelet counts in other cohorts of patients with liver disease and evaluation of longitudinal trends of thrombocytopenia.

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World Journal of Gastrointestinal Pathophysiology

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World J Gastrointest Pathophysiol 2021 May 22; 12(3): 51-58

DOI: 10.4291/wjgp.v12.i3.51

ISSN 2150-5330 (online)

ORIGINAL ARTICLE

Prospective Study

Impact of cytomegalovirus reactivation just before liver transplantation: A prospective cohort study

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Author contributions: Stadnik CMB performed the research, collected and analyzed the data, and wrote the paper; Pasqualotto AC wrote the paper and reviewed of manuscript; Caurio CFB collected data and performed laboratory analyses; Rodrigues-Filho EM, Cantisani GP and Zanotelli ML designed the study and reviewed the manuscript; all authors have read and approved the final manuscript.

Supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)–Brazil, No. 479880/2012-3.

Institutional review board statement: This study was approved by the Research Ethics Committee at Santa Casa de Misericordia of Porto Alegre (294/2010).

Conflict-of-interest statement: The

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Abstract

BACKGROUND

Cytomegalovirus (CMV) is the most common viral pathogen after liver transplantation (LT). Although reactivation of CMV infection is generally described in the context of immunosuppression, it has also been described in critically ill immunocompetent patients including cirrhotic patients.

AIM

To determine the incidence of reactivated CMV prior to LT.

METHODS

This was a prospective cohort study evaluating adult patients who underwent LT between 2014 and 2016. A plasma sample was obtained from all patients for CMV quantitative real-time PCR testing right before transplantation. Patients were followed for at least 1 year to assess the following outcomes: Incidence of CMV infection, organ rejection and overall mortality.

authors declare no conflict of interest.

Data sharing statement: No additional data are available.

Manuscript source: Unsolicited manuscript

Specialty type: Transplantation

Country/Territory of origin: Brazil

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

Received: September 23, 2020
Peer-review started: September 23, 2020
First decision: November 16, 2020
Revised: November 30, 2020
Accepted: February 25, 2021
Article in press: February 25, 2021
Published online: May 22, 2021

P-Reviewer: Akbulut S, Chiu KW
S-Editor: Fan JR
L-Editor: Webster JR
P-Editor: Yuan YY

RESULTS

A total of 72 patients were enrolled. Four patients died before transplantation, thus 68 patients were followed up for a median of 44 mo (20-50 mo). In 23/72 patients (31.9%) CMV was reactivated before transplantation. Post-transplantation, 16/68 (23.5%) patients had CMV infection and that was significantly associated with the recipient being CMV negative and a CMV-positive donor. Pre-transplant CMV reactivation was not associated with overall mortality (log rank: 0.9).

CONCLUSION

This study shows that CMV infection is common in patients with chronic liver disease just before LT, but the clinical impact of this infection seems to be negligible.

Key Words: Liver transplantation; Cytomegalovirus infection; Quantitative real-time PCR; Risk factors; Liver cirrhosis; Molecular biology

Core Tip: Cytomegalovirus (CMV) commonly reactivates before liver transplantation in patients with chronic liver conditions. This prospective cohort study demonstrates for the first time that although frequent, CMV reactivation has limited clinical impact when occurring just before liver transplantation.

Citation: Stadnik CMB, Caurio CFB, Rodrigues-Filho EM, Nedel WL, Cantisani GP, Zanotelli ML, Pasqualotto AC. Impact of cytomegalovirus reactivation just before liver transplantation: A prospective cohort study. *World J Gastrointest Pathophysiol* 2021; 12(3): 51-58
URL: https://www.wjgnet.com/2150-5330/full/v12/i3/51.htm
DOI: https://dx.doi.org/10.4291/wjgp.v12.i3.51

INTRODUCTION

Cytomegalovirus (CMV) is the most common viral pathogen after liver transplantation (LT). Most infections occur between the 3rd and the 12th postoperative week, reaching the highest incidence around the 5th post-transplant week. The overall incidence of CMV infection is between 50%-60% in liver transplant recipients, with 20%-30% of patients demonstrating symptomatic infection[1]. The incidence of post-transplant CMV infection depends mainly on the recipient and donor serological profile. Accordingly, it is more frequent in the context of positive immunoglobulin G (IgG) CMV serology in donors, and negative recipients (*i.e.*, D+/R- status), with more than half of these patients developing visceral disease, in the absence of antiviral prophylaxis[2]. The lowest-risk groups include positive serology for both donors and recipients (D+/R+ status) and a negative status for both donors and recipients (D-/R-). The incidence of CMV infection in such low-risk groups ranges between 5%-40%[3]. Intense immunosuppression and fulminant hepatitis transplantation are also important risk factors for infection.

Although reactivation of CMV infection is mostly described in the context of overt immunosuppression, reactivation may also occur in critically ill immunocompetent patients[4-7] associated with increased mortality[8,9]. A subgroup of particular interest is patients with chronic liver diseases[10,11]. Whether CMV reactivation in these individuals that are listed for LT has any impact on post-transplant outcomes has not been determined[12]. Therefore, here we investigate the frequency and impact of CMV reactivation in patients with chronic liver disease on the waiting list for LT. In particular, we were interested to study the impact of plasma circulating CMV DNA in terms of organ rejection, reactivation of CMV post-transplantation and overall mortality.

MATERIALS AND METHODS

This was a prospective cohort study that evaluated adult (≥ 18 years of age) patients with chronic liver disease listed to undergo LT at Santa Casa de Misericórdia de Porto Alegre. Santa Casa is a referral hospital for organ transplantation in Latin America, and performs approximately 60 liver transplant procedures every year. Patients were non-consecutively enrolled between the years 2014 and 2016.

Clinical and demographic data obtained in this study included age, gender, presence of comorbidities, Model for End-Stage Liver Disease (MELD) score, donor and recipient IgG serostatus for CMV infection, presence of hepatitis B virus (HBV) infection, hepatitis C virus (HCV) infection, renal insufficiency, hepatocarcinoma, fulminant hepatitis and re-transplantation.

Patients were followed for a minimum of 1 year after LT. During this period, all episodes of CMV reactivation [detected by either quantitative real-time PCR (qRT-PCR) and/or pp65 antigenemia] were documented, as well as events of CMV disease, organ rejection and overall mortality. Screening for CMV reactivation was performed monthly for the first three months after transplantation or whenever the patient presented with clinical symptoms such as fever, fatigue, organ rejection or in the case of diagnostic uncertainty (according to the institutional protocol of low resource countries). Antiviral prophylaxis was not used, instead preemptive treatment against CMV was applied to all patients, including sero-discordant patients.

Molecular tests

At the time the enrolled participants were called in for LT, 4 mL of plasma was collected in an ethylene diamine tetraacetic acid tube centrifuged at 1300 g for 15 min and frozen at -80°C until nucleic acid extraction for analysis of CMV qRT-PCR.

DNA was extracted using the Qiagen DNA Mini Kit (Qiagen Inc., Valencia, United States) following the manufacturer's instructions. qRT-PCR reactions were performed using an in-house assay calibrated with the 1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques NIBSC code: 09/162 that targets the genes UL 34 and UL 80.5. Primers and probes used in this study were described by Ho and Barry and the sequences are shown in the supplementary material with some modifications in the probe design[13]. The reagents and concentration of the qRT-PCR reaction are shown in the supplementary material. Amplification was performed in an 7500 real-time PCR system (Thermo Scientific, United States), the thermocycling conditions for the qRT-PCR reaction were: 1 cycle of 2 min at 50°C; 2 min at 95°C; followed by 40 cycles of 15 s at 95°C, and 1 min at 60°C. The results are reported in International Units (IU/mL) according to CMV World Health Organization standards[14]. The limit of detection and quantification of the test was 60.26 IU/mL, and the results were considered positive only above this cut-off value.

Statistical analysis

Statistical calculations were performed using SPSS 20.0 software. The Chi-square test or Fisher's exact test compared categorical variables, as appropriate. For continuous variables, we used the Student *t*-test or Mann-Whitney test, as appropriate. Multivariate analysis with a logistic regression model was used to estimate the probability of an association between active CMV infection immediately before the procedure and post-transplant reactivation. All variables demonstrating $P < 0.20$ at univariate analysis were considered for multivariate analysis, in addition to the variables of known biological significance. Kaplan Meier and Cox regression tests were used to evaluate predictors of mortality. For all statistical tests used, a value of $P < 0.05$ was considered statistically significant.

Sample size calculation

Considering the primary endpoint of the study and based on studies showing that approximately 50% of cirrhotic patients have detectable plasma CMV DNA[15], 64 patients would need to be studied, considering an alpha error of 0.05 and 80% of power. Thus, respecting a confidence interval of 95%, and to account for possible losses (10%), we estimated to include 70 patients.

Ethical aspects

This study was approved by the Research Ethics Committee at Santa Casa de Misericordia of Porto Alegre, No. 294/2010. All patients signed an informed consent form and agreed to participate in the study.

RESULTS

A total of 72 patients were enrolled in the study. Four patients died before transplantation; thus, 68 patients were followed up for a median of 44 mo (25%-75% percentile: 20-50 mo). Clinical and demographic characteristics of the patients are shown in Table 1. The majority of patients were female (70.8%) had active chronic hepatitis C infection (63.9%) and hepatocellular carcinoma (58.3%). Only 5 patients (6.9%) were CMV sero-discordant (D+/R-).

CMV reactivation was demonstrated in 31.9% (23/72) of patients before transplantation. Median plasma CMV DNA concentration in these patients was 1.212 IU/mL (25%-75% percentile: 560-4.197 IU/mL). In addition, two IgG negative patients had CMV reactivation but none received treatment at that time (7.486 and 7.917 UI/mL). Following LT, CMV infection occurred in 16/67 patients (23.8%) including two patients with IgG negative/PCR positive. At univariate analysis, the only statistically significant factor associated with post-transplant CMV infection was a CMV negative recipient with a positive CMV donor (Table 2). Multivariate analysis confirmed this as the only statistically significant factor for the prediction of post-transplant CMV infection [Odds ratio (OR): 11.5; 95% confidence interval (CI): 1.1-120; $P = 0.04$].

The crude mortality rate was 20/68 (29.4%), median 7.7 mo (perc 25-75: 1-12), and 7/22 (31.8%) in patients with pre-transplant CMV reactivation ($P = 0.763$). In Kaplan-Meier analyses, pre-transplant CMV reactivation had no impact on mortality following LT (log rank: 0.92) (Figure 1). Cox regression analysis also identified no statistically significant factor for mortality in this cohort.

DISCUSSION

This is the first study to document the frequency of CMV infection just before LT in patients with chronic liver disease, using a very sensitive diagnostic tool (qPCR). We observed a high frequency of CMV infection in these patients (31.9%), even though it had no impact on clinically significant variables in the post-transplant period, including CMV infection/disease, organ rejection and mortality. CMV viremic patients usually had a low CMV viral load (median: 1212 IU/mL).

Our results were probably influenced by the profile of patients being transplanted in our institution, which follows the modified Milan criteria[16], together with the proportion of patients with hepatocellular carcinoma (58.3%), as these patients usually have better performance with a lower MELD, which could induce a lower CMV reactivation rate. Nevertheless, in a similar study, a pre-LT reactivation incidence of 0.7% was found, much lower than that in our study[12]. Our findings were similar to the incidence of reactivation in intensive care patients (31%; 95%CI: 24%-39%) as shown in a recent meta-analysis[9].

When comparing with the findings in the literature, Lapiński *et al*[17] evaluated 123 patients with chronic HCV hepatitis for the presence of CMV infection, also determined by qPCR. CMV DNAemia, predominantly at low levels, was detected in 18 (14.6%) patients. Similar to our study, there was no correlation with HCV viral load, and detection of CMV DNA did not result in clinical and laboratory changes[17]. Bayram *et al*[15] quantitatively evaluated the presence of CMV infection in liver biopsy samples from 44 patients with chronic HBV and 25 patients with chronic HCV infection. CMV infection was demonstrated by qPCR in 52.3% of patients with HBV and in 36% of patients with HCV. Histological activity scores (necroinflammation and fibrosis) were worse in patients who were infected with CMV[15].

We observed that CMV was reactivated in 23% of patients in the post-transplant period, which is comparable to other studies[1-3] as most of them were low or moderate risk for infection (CMV receptor positive in 93%). Moreover, we did not find any association between reactivation before transplantation and reactivation after transplantation in both univariate and multivariate analyses. According to the literature, only a high risk for CMV infection (D+/R-) was statistically associated with CMV reactivation following LT (OR:11.5, 95%CI: 1.1-120, $P = 0.04$). We also did not identify pre-transplant CMV reactivation as a risk factor for organ rejection or overall mortality when both 30 d and 1-year mortality were considered.

This investigation has several limitations, including being a single-center study. In addition, patient selection occurred by convenience (sampling was not consecutive), which may have added some selection bias. Given that the reactivation rate was lower than initially expected (32% *vs* 50%), despite the sample calculation, we had small

Table 1 Patient characteristics and frequency of cytomegalovirus reactivation before liver transplantation

	Total (%)	Reactivation (%)	RR (95%CI)	*P* value
Number of patients (%)	72 (100)	23 (32)		
Gender (male)	21 (29.2)	7 (33.3)	1.09 (0.37-3.23)	0.871
Mean age, years (SD)	56.3 (9.6)	57.3 (9.2)	NA	0.900
MELD, median (IqR)	12 (14)	12 (12)	NA	0.712
Lymphocyte count, median (IqR)	929 (808)	929 (770)	NA	0.471
CMV receptor IgG-negative	5 (8.7)	2 (40)	0.68 (0.11-4.40)	0.652
HCV	46 (63.9)	15 (32.6)	1.09 (0.39-3.1)	0.872
HBV	5 (6.9)	1 (20)	0.51 (0.05-4.9)	1.000
Hepatocarcinoma	42 (58.3)	14 (33.3)	1.17 (0.42-3.2)	0.765
Fulminant hepatitis	2 (3)	0	NA	NA
Diabetes mellitus	24 (33.3)	8 (33.3)	1.1 (0.38-3.13)	0.858
Renal failure	8 (11.1)	5 (62.5)	4.26 (0.92-19.7)	0.100
Re-transplant	2 (3)	0	NA	NA

CI: Confidence interval; IqR: Interquartile range; MELD: Model for end-stage liver disease; NA: Not applicable; RR: Relative risk; SD: Standard deviation; CMV: Cytomegalovirus; HCV: Hepatitis C virus; HBV: Hepatitis B virus; IgG: Immunoglobulin G.

Table 2 Predictors of cytomegalovirus infection after liver transplantation

	CMV (%)	No CMV (%)	RR (95%CI)	*P* value
Number of patients (%)	16/68 (23.5)	52/68 (76.5%)		
CMV reactivation before transplantation	7/16 (43.8)	15/52 (28.8)	1.91 (0.6-6.1)	0.265
Quantitative PCR pre-transplant (IU/mL), mean (SD)	2862 (5696)	868 (2756)	NA	0.154
Gender (male)	4/16 (25)	16/52 (30.8)	0.75 (0.2-2.7)	0.762
Mean age, years (SD)	55 (10.3)	57.3 (8)	NA	0.373
MELD score, median (IqR)	11 (4)	12 (11)	NA	0.254
Lymphocyte count, median (IqR)	1101 (1109)	918 (754)	NA	0.580
Organ rejection	3/16 (18.7)	8/52 (15.3)	1.27 (0.3-5.5)	0.716
CMV-negative receptor	3/16 (18.7)	1/52 (1.9)	11.7 (1.1-122.6)	0.038
Hepatitis C infection	9/16 (56.2)	34/52 (65.4)	0.7 (0.2-2.1)	0.508
Hepatitis B infection	1/16 (6.2)	4/52 (7.7)	0.8 (0.1-7.1)	0.100
Hepatocarcinoma	9/16 (56.2)	30/52 (57.7)	0.9 (0.3-2.9)	0.919
Fulminant hepatitis	0	1/52 (1.9)	NA	NA
Diabetes mellitus	6/16 (37.5)	16/52 (30.8)	1.3 (0.4-4.3)	0.615
Renal failure	2/7 (12.5)	5/52 (9.6)	1.3 (0.2-7.7)	0.664
Re-transplantation	1/16 (6.2)	1/52 (1.9)	3.4 (0.2-57.7)	0.418

CI: Confidence interval; IqR: Interquartile range; IU: International units; MELD: Model for end-stage liver disease; NA: Not applicable; RR: Relative risk; SD: Standard deviation; CMV: Cytomegalovirus.

numbers of some of the events, which may have mainly affected the multivariate analysis.

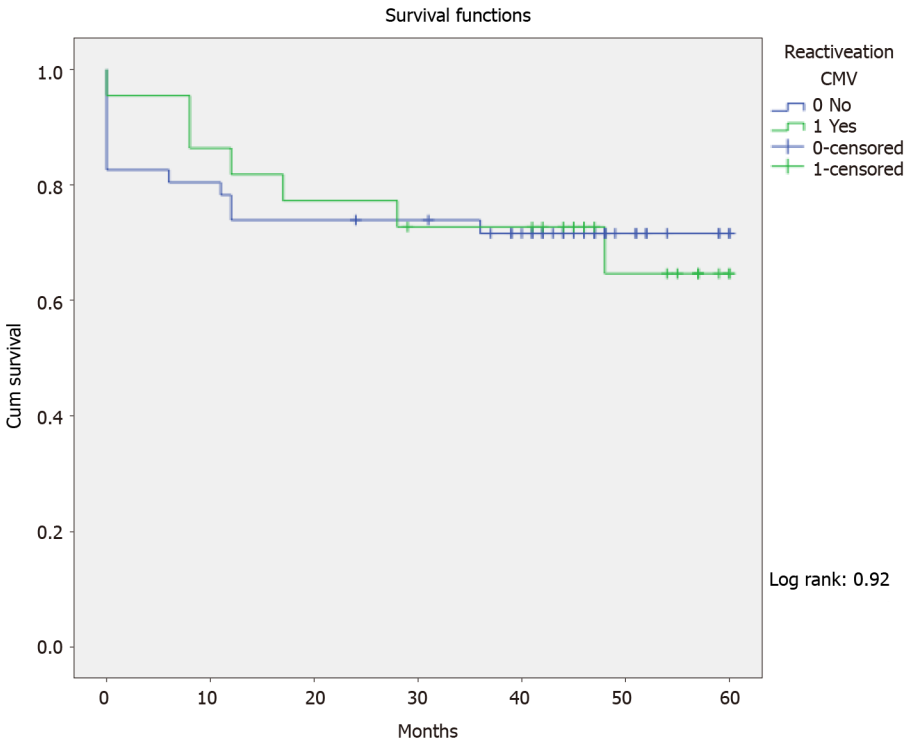

Figure 1 In Kaplan-Meier analyses pre-transplant cytomegalovirus reactivation had no impact on mortality following liver transplantation (log rank: 0.92). CMV: Cytomegalovirus.

CONCLUSION

The findings of this study suggest that pre-transplant CMV reactivation has no influence on LT results, and has no impact on post-transplant CMV reactivation or overall mortality. Based on this study, screening for CMV DNAemia before LT does not seem justified. A larger sample size, better quality and multicenter studies are required to fully elucidate this issue.

ARTICLE HIGHLIGHTS

Research background

The overall incidence of cytomegalovirus (CMV) infection is between 50%-60% in liver transplant recipients, with 20%-30% of patients demonstrating a symptomatic infection[1]. The incidence of post-transplant CMV infection depends mainly on the recipient and donor serological profile. The lowest-risk groups include positive serology for both donors and recipients (D+/R+ status) and a negative status for both donors and recipients (D-/R-). Although reactivation of CMV infection is mostly described in the context of overt immunosuppression, reactivation may also occur in critically ill immunocompetent patients[4-7] associated with increased mortality[8,9].

Research motivation

A subgroup of particular interest is patients with chronic liver diseases[10,11]. Whether CMV reactivation in these individuals that are listed for liver transplantation has any impact on post-transplant outcomes has not been determined[12].

Research objectives

To determine the incidence of reactivated CMV prior to liver transplantation.

Research methods

This was a prospective cohort study that evaluated adult (≥ 18 years of age) patients with chronic liver disease listed to undergo liver transplantation at a referral hospital for organ transplantation in Latin America. Patients were followed for a minimum of 1 year after liver transplantation. During this period, all episodes of CMV reactivation

[detected by either quantitative real-time PCR (qRT-PCR) and/or pp65 antigenemia] were documented, as well as events of CMV disease, organ rejection and overall mortality. Screening for CMV reactivation was performed monthly for the first three months after transplantation or whenever the patient presented with clinical symptoms. At the time the enrolled participants were called in for liver transplantation, plasma was collected for analysis of CMV qRT-PCR.

Research results

A total of 72 patients were enrolled in the study. Four patients died before transplantation, thus 68 patients were followed up for a median of 44 mo (25%-75% percentile: 20-50 mo). CMV reactivation was demonstrated in 31.9% (23/72) of patients before transplantation. Median plasma CMV DNA concentration in these patients was 1.212 IU/mL (25%-75% percentile: 560-4.197 IU/mL). Following liver transplantation, CMV infection occurred in 16/67 patients (23.8%).

The crude mortality rate was 20/68 (29.4%), median 7.7 mo (perc 25-75: 1-12), and 7/22 (31.8%) in patients with pre-transplant CMV reactivation (P = 0.763). In Kaplan-Meier analyses, pre-transplant CMV reactivation had no impact on mortality following liver transplantation (log rank: 0.92) (Figure 1). Cox regression analysis also identified no statistically significant factor for mortality in this cohort.

Research conclusions

The findings of this study suggest that pre-transplant CMV reactivation has no influence on liver transplantation results, and has no impact on post-transplant CMV reactivation or overall mortality.

Research perspectives

Based on this study, screening for CMV DNAemia before liver transplantation does not seem justified. A larger sample size, better quality and multicenter studies are required to fully elucidate this issue.

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