GUT MICROBIOTA DYSBIOSIS, IMMUNOLOGICAL RESPONSE AND THE PERFORMANCE OF NON-INVASIVE MODELS FOR ASSESSING LIVER FIBROSIS IN PATIENTS WITH CHRONIC HEPATITIS B VIRUS

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ABSTRACT

Gut microbiota dysbiosis and lipopolysaccharide-mediated immune response has been linked with pathogenesis of complications and liver injuries in subjects with chronic hepatitis B virus infection. The levels of C-reactive protein (CRP), lipopolysaccharides (LPS), serum protein, platelet count (PLT), aspartate amino transferase (AST), and the performance of non-invasive models globulin to-platelet index (GPI), C-reactive protein to-albumin ratio (CAR), and aspartate to-platelet ratio index (APRI) in assessing fibrosis in patients with chronic hepatitis B virus infection (CHBV) were assessed in this case-control study. The study enrolled 60 subjects with CHBV and 40 healthy controls. Platelet count was determined by a 5 parts Sysmex XS-1000 haematology automated analyzer, lipopolysaccharide was determined by sandwich-ELISA method, CRP was determined by latex Reagent agglutination method, AST, ALB, TP, were determined by commercial colorimetric methods, C-reactive protein-albumin ratio (CAR), globulin-platelet index (GPI), aspartate-platelet ratio index (APRI), were computed. Data analysis was performed using analysis of variance, Pearson's and DeLong's test to compare the area under the receiver operating characteristic curve (AUROC) for the noninvasive markers, at α=0.05. Subjects with liver fibrosis (LF) had significantly higher LPS, CRP, AST, GLO, CAR, GPI and APRI and lower PLT, ALB, when compared with CHBV and control subjects. Log10 CAR correlated positively with Log10 GPI r= 0.464, P=0.000) respectively, CRP correlated positively with LPS and negatively with PLT (r=0.626, P=0.000 and r= -0.393, P=0.002) respectively, in the test subjects. The area under the curve for GPI, CAR and APRI were 0.923, 0.940, and 1.000 respectively. This study has shown that dysbiosis of the gut microbiota and LPS-mediated immune activation may underlie the pathogenesis of liver damage in subjects with chronic hepatitis B virus. The GPI, CAR and APRI models are good test instruments in predicting significant fibrosis and their use may represent simple and low-cost options in assessing liver injury in patients where FibroScan, transient elastography or liver biopsy is not accessible.

KEYWORDS: chronic hepatitis B virus, gut microbiota dysbiosis, immune response, noninvasive models,

INTRODUCTION

Hepatitis B virus (HBV) a non-cytopathic virus but chronic infection induces immune-mediated inflammation that is responsible for liver damage.

The gut microbiota is essential in preserving overall health and immune function. Nonetheless, dysbiosis, an imbalance in microbial composition, can deeply affect numerous aspects of human health, including a predisposition to development of complications in

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chronic hepatitis B virus infection. Chronic hepatitis B virus infection is associated with activated immune responses and dysbiosis of gut microbiota, leading to elevated circulating levels of C-reactive protein and lipopolysaccharide (Han et al., 2021, Li et al., 2022, Shen et al., 2023, Maciel-Fiuza et al., 2023). Prompt diagnosis and treatment of chronic hepatitis B (CHB) disease are significant for the avoidance of complications such as liver fibrosis, cirrhosis and hepatocellular cancer (Kaya et al., 2023). The host immune response is not only essential to control the spread of virus infection, but it is also responsible for the inflammatory events causing liver pathologies (Walter et al., 2022, Lopez-Scarim et al., 2023, Lu et al., 2023, Nevola et al., 2023). C-reactive protein-to-albumin ratio (CAR), aspartate-to-platelet ratio index (APRI) and Globulin-to-platelet ratio index (GPI) are noninvasive models derived from simple routine laboratory tests used in the evaluation of liver fibrosis in patients with chronic hepatitis B virus infection with reference to liver biopsy: the gold standard for detecting and grading liver fibrosis (Huang et al., 2017). The outcome of chronic HBV infection is defined by virus–host interactions.

During chronic HBV infection, host immune response acts as a double-edged sword; it provides defense against infection by destroying the virus infected cells, whereas inducing hepatic inflammation that worsen liver injury (Khanam et al., 2021). The C-reactive protein-to-albumin ratio (CAR) is a novel inflammation index that has recently been used as a marker for poor prognosis or mortality in various patient groups (Atas et al., 2023, Shi et al., 2023). The CAR was observed to be significantly higher in the non-surviving patients compared to the surviving patients (Wang et al., 2019, Zavalaga-Zegarra et al., 2022, Katkat et al., 2022, Jang et al., 2022). Moreover, CAR was observed to correlate positively with the model for end-stage liver disease (MELD) score and Child-Pugh score (Oikonomou et al., 2020, Wang et al., 2019). In multivariate analysis, the CAR and the MELD score were independent prognostic factors for hepatitis B related decompensated cirrhosis (HBV-DeCi) patients (Wang et al., 2019).

The bi-directional interaction between the liver and the gut (gut-liver axis) is yet to be fully understood, a growing consensus suggest that the human health closely relates to the composition of the gut microbiota (Ding et al., 2020, Vajro et al., 2013, Hou et al., 2022). The microbial ecosystem functions in symbiotic relationship with the host, bringing about homeostasis and regulation of immune function (Rastogi et al., 2022). But, microbiota dysbiosis can lead to abnormal regulation of bodily functions and diseases including hepatitis B virus, respiratory diseases, cancers (Wang et al., 2022, Neag et al., 2021). Supported by animal and human studies, the gut microbiota alters as the HBV-related liver fibrosis is initiated and progresses, typified by the decrease in the ratio between "good" and "potentially

pathogenic" microbes (Song et al., 2023, Lu et al., 2023). Improvements in primary disease via antiviral treatment have been reported to be accompanied with an attendant amendment of the gut microbiota dysfunction (Yang et al., 2021). Thus, the recovery of gut microbiota can promote the regression of liver fibrosis.

Lipopolysaccharides (LPSs) are bacterial surface glycolipids, derived from Gram-negative bacteria cell wall as a result of changes in the composition of the gut microbiota. The LPS itself is not intrinsically harmful, but, is known to determine both acute and chronic inflammatory reactions. It acts by inducing myeloid and/or non-myeloid cells to produce a number of proinflammatory cytokines, including interleukins, tumor necrosis factor and cyclooxygenase-2 (Chaiwut & Kasinrerk, 2022). Thus, LPS is a potent activator of the inflammatory responses. Dysbiosis of the intestinal microbiota can be exploited by viral hepatitis as an escape mechanism of the immune system (Fakharian et al., 2023, Li et al., 2022). It has been suggested that cirrhosis could impact the dysbiosis process, leading to a worsening of the patient's clinical condition (Nevola, et al., 2023). When impairment of intestinal barriers and disturbances of the gut microbiota occur, gut-derived microbial antigen translocation may lead to invasion of the liver. The association between gut microbiota alterations and chronic liver diseases (CLDs) has received great attention (Spanu et al., 2022). The LPS interacts with lipopolysaccharide binding protein (LBP) to form LPS-LBP complex which binds to cluster of differentiation 14 (CD14). However, CD14 cannot mediate LPS signaling through the cell membrane, because it does not have a transmembrane domain. Thus, it is believed that CD14 functions as the co-receptor for toll-like receptor 4 (TLR4). The gut microbiota controls the development of the immune system by setting systemic threshold for immune activation (Kartjito et al., 2023, Lozenov et al., 2023, Mazziotta et al., 2023). Lipopolysaccharides have been shown to be able to elicit both systemic proinflammatory and immunomodulatory responses (Xi et al., 2023, Thapa et al., 2023). This occurrence is particularly exciting considering that the immune system is charged with the task to distinguish the beneficial microbes from the pathogens, even if the commensal bacteria have molecular patterns resembling those of the pathogenic counterparts (Mazziotta, et al., 2023). Therefore, the importance of the chemical structure of these macromolecules in fine tuning this delicate equilibrium is beyond question (Fux, et al., 2023). Gut helps in the digestion of food by absorbing energy and nutrients. The portal system carries intestinal microbial products such as microbe-associated molecular patterns and endotoxins to the liver, facilitating the generation of a proinflammatory state.

Chronic hepatitis B virus infection is associated with activated immune responses and dysbiosis of gut microbiota. A non-invasive biomarker that accurately defines liver disease severity, relating the state of inflammation to the severity of liver damage is yet to be fully appreciated. This study determined the levels of CRP, LPS, serum protein, platelet count (PLT), aspartate amino transferase (AST), and the performance of non-invasive models GPI, CAR, APRI in assessing fibrosis in patients with chronic hepatitis B virus infection.

MATERIALS AND METHODS

Study design: This case-control study was conducted in Calabar Metropolis to determine the association between serum levels of of CRP, LPS, serum protein, platelet count (PLT), aspartate amino transferase (AST), and the performance of non-invasive models GPI, CAR, APRI in assessing fibrosis in patients with CHBV and hepatitis B related liver fibrosis, attending Clinics in Internal Medicine Department, University of Calabar Teaching Hospital (UCTH), Calabar, Nigeria. Metavir system was used to determine the level of fibrosis. The test subjects were further categorized based on the Metavir score into those with significant liver fibrosis of stages F2 (n=7), F3 (n=8) and those without significant fibrosis of stages F0 (n=25), F1 (n=20), F4=0.

Study setting/subject selection: The study population consists of patients with hepatitis B virus infection and associated complications in Calabar Metropolis. The study population comprised of 100 subjects (60 test and 40 control), aged 20-50 years. All the participants were screened for HIV and HCV infections. **Inclusion criteria**: Patients without a history of substance abuse, smoking, alcohol consumption and any known chronic organ or systemic illness or medication were recruited into the study. **Exclusion criteria:** Individuals with a history of smoking, diabetes or any known chronic organ or systemic illness, alcohol consumption and substance abuse or medication were excluded from the study.

Data collection: Socio-demographic data, (education, age, marital status, work), family and medical history of past illness, current medication use, social lifestyle (smoking habit, alcohol use, drug addiction, and substance abuse) were obtained from each participant using a structured interviewer questionnaire.

Sample size: Sample size was determined according to the method of Sullivan (2016) (39**)**, using the formula $\frac{(Z_\alpha+Z_\beta)^2 \cdot \bar{p}(1-\bar{p})}{(p0 - p1)2}$. The power of 0.84 was calculated at beta error of 80%, and attrition ratio of 10% added, giving a total of 60 test participants (60 test and 40 controls).

Sample collection
A standard venepuncture method was used to obtain 7 mL of blood from all the participants by a trained phlebotomist. Three milliliters of blood was

dispensed into K2 EDTA samples bottle for platelet estimation and 4 mls into plain bottles, allowed to clot for 1 hour and then centrifuged at 3 000 rpm for 5 mins at 25°C. The sera were separated immediately into aliquots using sterile Pasteur pipettes and stored at −20 °C until analysis.

Laboratory Methods
Estimation of platelet count
Platelet count was determined by a 5 parts Sysmex XS-1000 haematology automated analyzer. The sysmex-XS-1000 can analyse and output the results for 32 parameters of blood samples. It utilizes technology of fluorescence flow cytometry to quantitate the standard five part differentials, immature granulocytes (metamyelocytes, myelocytes and promyelocytes), nucleated red blood cells (NRBC), reticulocyte count, immature reticulocyte fraction and "optical" fluorescent platelet count. The combination of side scatter (inner complexity of the cell), forward scatter (volume) and fluorescence intensity of nucleated cells gives a concise and precise image of each cell detected in the peripheral blood. A well-defined physical description of the different leucocyte populations (clusters) is obtained. Abnormal and immature cells, with their larger nuclear volume show much higher fluorescence intensity than normal cells, and are easily distinguishable in the different scattergrams.

Estimation of serum lipopolysaccharide
Serum lipopolysaccharide was determined by sandwich-ELISA method using a kit obtained from Sunlong Biotech CO.,LTD., Zhejiang, Hangzhou, Yuhang, China.

Estimation of C-Reactive protein
The CRP was determined by latex Reagent agglutination method using a latex kit obtained from Atlas Medical, Cambridge, CB4 4WX, UK.
The CRP reagent kit is based on an immunological reaction between CRP antisera bound to biologically inert latex particles and CRP in the test specimen. (Pepys, 1981)

Estimation of serum aspartate aminotransferase (AST)
Serum AST was determined by commercial colorimetric kit produce by Randox Laboratories Ltd, United Kingdom according to the method of Reitman and Frankel 1957 (Reitman & Frankel, 1957)

Estimation of albumin
Albumin was determined by the Bromocresol green (BCG) colorimetric method using a reagent kit obtained from Atlas Medical, Cambridge, CB4 4WX, UK. (Doumas et al., 1971)

Estimation of total protein
Total protein was determined by the Biuret method using a reagent kit obtained from Anamol Lab. PVT. LTD, India. (Tietz, 1995)

Computation of C-reactive protein to albumin ratio
The CRP/Alb ratio was calculated by dividing the CRP level by the Albumin level

Computation of globulin

Globulin was computed using the formula: Globulin = Total protein – albumin

Computation of Globulin to platelet ratio GP index

GPI =   globulin x 100
        Platelet count (109/l)

Computation of aspartate to platelet ratio index (APRI)

Aspartate to platelet ratio index APRI was computed using the formula

APRI score = (AST/upper limit of the normal AST range) x 100
                         Platelet Count.

Calculation of the predictive values of the CAR and GPI

Positive predictive value (PPV) =   TP   x 100
                               (TP+FP)

Negative predictive value   (NPV)   =   TN   x 100
                                  (TN+FN)

The overall efficiency of a test   TP+TN x 100
                                   (TP+FP+TN+FN)

Statistical analyses

Data generated were presented as mean ± standard deviation. Data were analyzed using the Statistical Package for Social Sciences (SPSS version 23.0, IBM, USA). Analysis of variance (ANOVA) was used to test the variations within and among group means and Fisher's least significant difference (LSD) post-hoc analysis was used for the comparison of multiple group means. Pearson's correlation was used to determine the associations between variables. DeLong's test was done to compare the area under the receiver operating characteristic curve (AUROC) for the non-invasive markers using histology results as reference. The data was subjected to test of normality using the Shapiro-Wilk Test and Box and whisker plots, data found not normally distributed were Log transformed. The confidence interval was set to 95%. The significance level of the tests was set at α=0.05.

RESULTS

Among the subjects (60) with chronic hepatitis B virus, 35% (n=21) were females while 66% were males (n=39). Metavir score was used to determine the level of fibrosis. Fifteen (n=15) 25 % of the patients with chronic hepatitis B virus infection had significant fibrosis of stage F2 (n=7) and F3 (n=8) on biopsy. Subjects with chronic hepatitis B virus infection without fibrosis = 45, those with F0 = 25, those with F1=20 and F4=0.

Comparison of age, LPS, CRP, TP, ALB, GLO, PLT, AST, CAR, GPI and APRI in subjects with chronic hepatitis B (CBHV), Liver fibrosis (LF) and controls

The Comparison of age, LPS, CRP, TP, ALB, GLO, PLT, AST, CAR, GPI and APRI in subjects with CBHV, LF and controls is depicted in **table 1**. Subjects with LF had significantly higher LPS, CRP, AST, GLO, CAR, GPI and APRI and lower PLT, ALB when compared with CHBV and control subjects (P<0.05).

Association between serum LPS, CRP, PLT, CAR, GPI and APRI in subjects with CBHV, LF and controls.

A correlation plot of Log10 CAR against Log10 GPI in the test subjects is represented in **figure 1**. Log10 CAR correlated positively and significantly with Log10 GPI (r= 0.464, p=0.000) in the test subjects. A correlation plot of CRP against LPS levels in test subjects is represented in **figure 2**. The CRP correlated positively and significantly with LPS (r=0.626, p=0.000) in the test subjects. A correlation plot of CRP against PLT levels in the test subjects is depicted in **figure 3**. The CRP correlated negatively and significantly with PLT (r= -0.393, p=0.002) in the test subjects.

The performance of GPI, APRI and CAR in assessing fibrosis in subjects with CHBV by De Long test (area under the receiver operating characteristic curve AUROC).

The area under the curve (AUC) receiver operator characteristic curve of GPI, APRI and CAR in subjects with chronic hepatitis B without fibrosis and Liver fibrosis is represented in figure 4. GPI (AUC=0.923, SE=0.028, p=0.000, CI=0.868-0.978), CAR (AUC=0.940, SE=0.024, p=0.000, CI= 0.892-0.988), APRI (AUC=1.000, SE=0.000, p=0.000, CI=1.00-1.00). Using a potential cutoff value of 1.66, the sensitivity and specificity of GPI were 93.3 % and 24.7 % respectively. The positive and negative predictive values and overall efficiency of GPI were PPV=79 %, NPV=91.8 % and overall efficiency = 84 % respectively. Taking a potential cutoff value of 0.81, the sensitivity and specificity of CAR were 86.7 % and 20.0 % respectively. The positive and negative predictive values and overall efficiency (OE) of CAR were PPV=81 %, NPV=86 % and OE=83 % respectively. Using a potential cutoff value of 0.50, the sensitivity and specificity of APRI score were 100 % and 1.2 % respectively. The positive and negative predictive values and overall efficiency of APRI were PPV=99.8 %, NPV=100 % and overall efficiency (OE) of APRI= 99 % respectively

Table 1: The Comparison of age, LPS, CRP, TP, ALB, GLO, PLT, AST, CAR, GPI and APRI in subjects with CBHV, LF and controls is depicted in table

Parameters	CHBV without fibrosis (n=45)	Liver Fibrosis (n=15)	Controls (n=40)	Cal. F	P-Value
Age (years)	40.4±9.5 7	41.33±7.65	39.70±7.53	1.321	0.130
LPS (3.6 -180) ng/ml	222.38±15.97	301.64±17.76a	16.56±3.90	3861.66	<0.0001
CRP(0.02-1.35)mg/dL	1.72±0.41	2.62±0.48b	1.15±0.41	69.909	<0.0001
TP (6.2-8.5) g/dL	5.61±0.77	5.61±0.78	6.0±0.97	2.086	0.130
ALB (3.5-5.0) g/dL	2.46±0.55	2.21±0.57c	4.73±0.80	144.388	<0.0001
GLO (2.0-3.5) g/dL	3.21±0.77	3.33±0.77d	1.25±0.76	88.203	<0.0001
PLT (100-300) 109/l	183.82±39.84	122.53±13.93	254.0±43.96e	71.424	<0.0001
AST (5.0 – 12.0) IU/L	20.71±4.05	47.80±13.05f	9.0±4.74	200.33	<0.0001
GPI	1.84±0.63	2.75±0.52	0.5±0.29g	132.865	<0.0001
CAR	0.75±0.34	1.24±0.37h	0.25±0.10	79.077	<0.0001
APRI	0.29±0.08	0.94±0.21i	0.19±0.05	387.478	<0.0001

 Key: results expressed as mean±SD. a, b, e, f, g, h & i = significant difference between patients with CHBV without fibrosis, liver Fibrosis and controls, c & d = significant difference between patients with CHBV without fibrosis and liver fibrosis & between liver fibrosis and controls, CHBV=chronic hepatitis B virus infection without fibrosis, LF = patients with liver fibrosis, AST, aspartate amino transferase, LPS=lipopolysaccharide, CRP= C-Reactive protein, PLT= platelets count, ALB= albumin, GPI = globulin to platelets ratio index, CAR= C-Reactive protein to albumin ratio, APRI= aspartate to platelet ratio index.
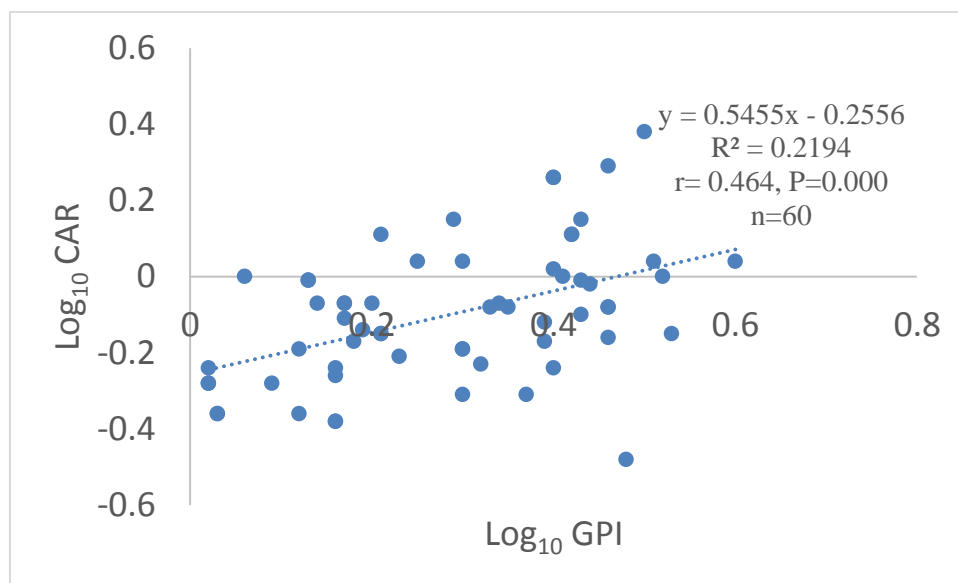

The plot shows:
$$y = 0.5455x - 0.2556$$
$$R^2 = 0.2194$$
$$r = 0.464, P=0.000$$
$$n=60$$

Figure 1, A correlation plot of Log10 CAR against Log10 GPI in test subjects.

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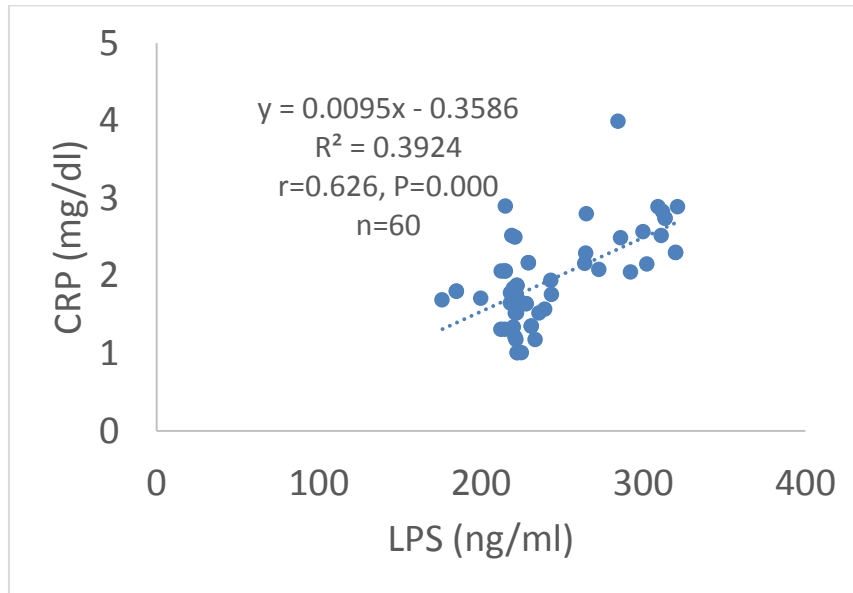
Figure 2, A correlation plot of CRP against LPS levels in test subjects

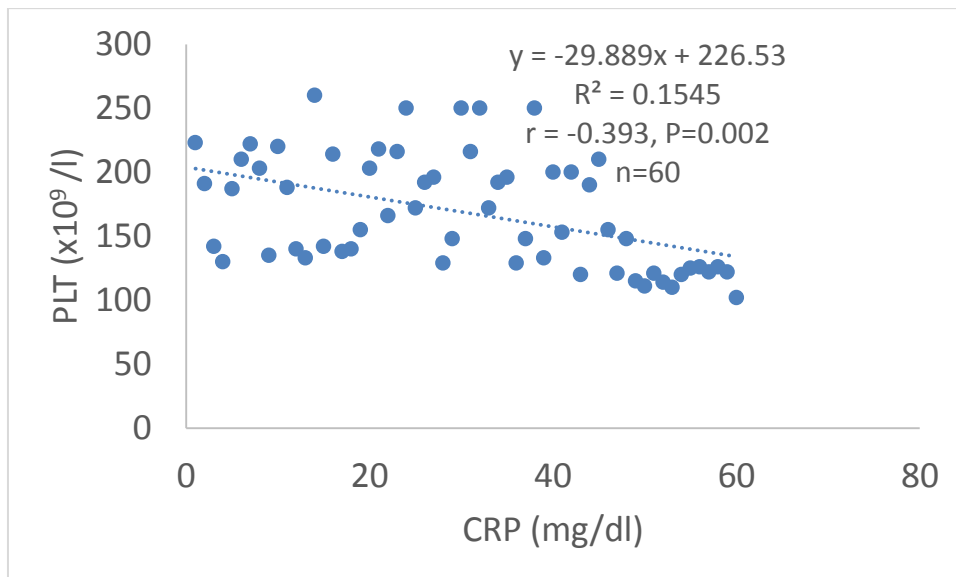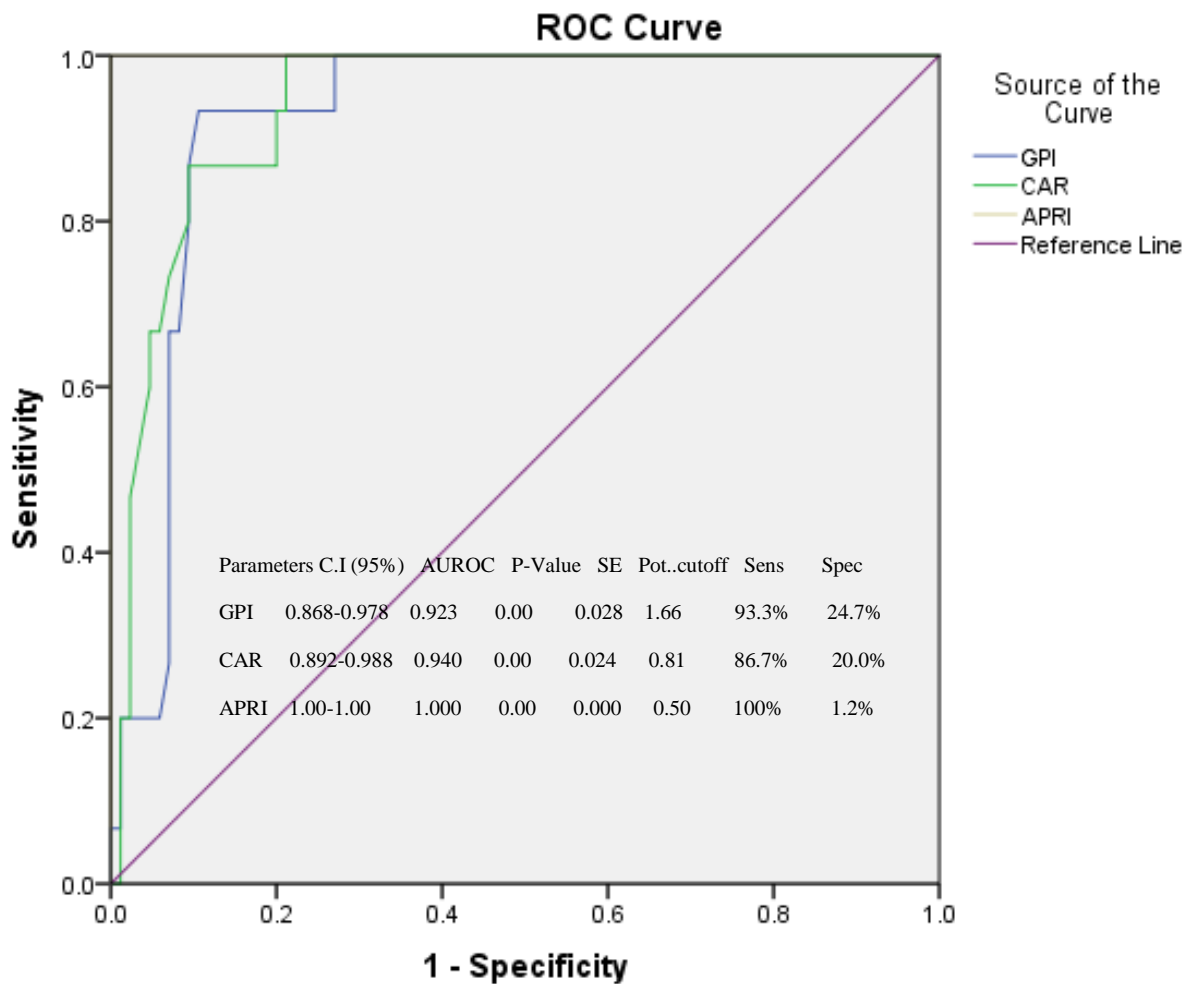**Figure 3, A correlation plot of CRP against PLT levels in the test subjects**

ROC Curve

Parameters	C.I (95%)	AUROC	P-Value	SE	Pot..cutoff	Sens	Spec
GPI	0.868-0.978	0.923	0.00	0.028	1.66	93.3%	24.7%
CAR	0.892-0.988	0.940	0.00	0.024	0.81	86.7%	20.0%
APRI	1.00-1.00	1.000	0.00	0.000	0.50	100%	1.2%

Diagonal segments are produced by ties.

SE = Standard error, Pot.cutoff = Potential cutoff, Sens= sensitivity, Spec = specificity

Figure 4, Receiver operator curve of GPI, CAR and APRI in subjects with CHBV and LF.

DISCUSSION

Dysbiosis of the gut microbiota repertoire and consequent release of lipopolysaccharides into the circulation helps in the activation of the innate immune reaction, responsible for secondary liver injury and pathogenesis of the disease in chronic hepatitis B virus infection.

In this study, LPS levels of subjects with LF were significantly higher than those of CHBV and control. The higher levels of LPS in subjects with LF compared to CHBV and control counterparts may be attributed to changes in the gut microbial bionetwork, which aids the progression of the disease through innate immune activation. This finding is similar to that of a previous study, that demonstrated a loss of diversity and increase in potential pathobionts (any potentially pathological organism originating from within the gut which, under normal circumstances, lives as a non-harming symbiont), well before cirrhosis in stool microbiome of subjects with viral hepatitis (hepatitis B and hepatitis C)(Zeng et al.,

2020). Also, a study had reported gut microbiota dysbiosis to be associated with a negative regulation of liver dysfunction and T cell immune response (Yan et al., 2023). Similarly, it has been observed that the gut microbiota alters as the HBV-related liver fibrosis is initiated and progresses, characterized by the decrease in the ratio between "good" and "potentially pathogenic" microbes, and that when the primary disease is contained by means of antiviral treatment, the gut microbiota dysfunction tends to improved (Li et al., 2022). This suggests that the recovery of a normal gut microbiota community can promote the regression of liver fibrosis. More so, a study observed that dysbiosis of gut microbiota in chronic hepatitis B infection affects disease pathogenesis, and LPS helps in the activation of innate immune response by recognizing TLRs, especially TLR2 and 4 (Zhou et al., 2022). Lipopolysaccharide mediated immune activation may underlie the pathogenesis of fibrosis in CHBV. Several studies have reported the occurrence of a compositional shift in microbiome

with enriched LPS-producing genera in HBV infection (Shen et al., 2023, Yang et al., 2023, Liu & Yang, 2023). The shift in conformation of the gut microbial ecology and the release of LPS may be associated with immune activation linked with liver injury in CHBV. It has been demonstrated that the active receptor for LPS is CD14/TLR4/MD2 receptor complex, which on induction secretes many pro-inflammatory cytokines including tumor necrosis factor-α, IL-1, IL-6, and chemokines through the NF-κB signaling to cause liver injury (Ciesielska et al., 2021). Furthermore, LPS/LBP/CD14 complex interacts with TLR4. The LPS responses mediated through TLR4 require the presence of Myeloid differentiation protein 2 (MD2), a cell surface protein. All these components form the complex of LPS/LBP/CD14/MD2/TLR4. Upon the interaction of LPS with TLR4 and associated receptor components, TLR4 exploits the combination of (Mal/TIRAP) the adaptor protein, Toll-interleukin-1 Receptor domain-containing adaptor protein (TIRAP), also known as MAL (MYD88 adapter-like), essential for MYD88-dependent signalling downstream of TLR4 and MyD88 at the plasma membrane to induce inflammatory gene expression (called MyD88-dependent pathway) (Rajpoot et al., 2021). The LPS induces the up-regulation of cluster of differentiation 14 protein (CD14) in the intestinal tissues via the TLR4 pathway, which decreases the relative epithelial resistance and increases its permeability. In the intestinal tract, LPS down-regulates the expression of various tight junction proteins (ZO-1 and closed protein) by increasing the permeability of the intestinal mucosa and enters the blood flow through the portal venous system. Thus, LPS plays an important role in conducting liver damage through the LPS/LBP/CD14/MD2/TLR4/Mal/TIRAP signaling pathway (Ciesielska et al., 2021).

The higher levels of CRP in subjects with LF compared to the CHBV and control may be due to virus induced hepatic inflammation and increased hepatocyte production of CRP essential for phagocytosis and viral clearance. This finding is similar to previous studies, which observed that CRP was significantly higher in nonsurviving HBV infected patients than in surviving patients, and that CRP was able to predict early mortality in HBV patients with decompensated cirrhosis (State, 2021, Zhu et al., 2017). A study also reported a significantly higher CRP levels in patients with HBV compared to the controls which correlated with the ability of the virus to cause liver damage (Bayram et al., 2021). Another study demonstrated that hepatitis B Virus up-regulates the expression of C-reactive protein both in vivo and in vitro (Hao et al., 2017). C-reactive proteins is an acute phase reactant protein synthesized by hepatocytes in response to inflammatory reactions as a result of the innate immune response of the host that facilitates both apoptosis and phagocytosis. Furthermore, CRP can

also activate the classical complement pathway through binding to C1q protein leading to opsonization of infected hepatocytes.

The significantly lower levels of platelet count in patients with liver fibrosis and chronic hepatitis B virus infection when compared to CHBV and controls may imply progressive liver damage caused by the virus with impaired liver ability to synthesize proteins including thrombopoietin resulting in lower platelet count in these patients. This finding is in line with that of Yoshida, et al., who reported that a decreasing tendency in platelet counts is observed in most chronic liver diseases etiologies, with thrombocytopenia mostly associated with hepatitis B and/or C (Yoshida et al., 2022). Also, Yang et al., demonstrated that the etiology of thrombocytopenia in liver disease including chronic HBV may be attributed to splenomegaly, hypersplenism, portal hypertension and decreased thrombopoietin production and that platelet count is closely associated with the severity of liver injury in patients with chronic hepatitis B virus infection (Yang et al., 2020). The platelet count may indicate the severity of liver injury and fibrosis. Accordingly, previous studies observed that worsening of fibrosis and increasing portal pressure are associated with the reduced production of thrombopoietin by hepatocytes and increased platelet sequestration within the spleen (Wang et al., 2016).

In this study, serum albumin was significantly lower in patients LF compared to CHBV and controls, this may suggest some level of malnutrition and impaired liver ability to produce albumin in these patients. The observed hypoalbuminemia is similar to that of a previous study, which considered that serum albumin level below 3.5 g/dL is a known predictor of chronic liver disease and decreased survival (Takahashi et al., 2023).

The significantly higher levels of GPI, CAR and APRI score in subjects with LF compared to CHBV and controls may suggest gradual decrease in ALB, PLT and increase in CRP, GLO, AST in subjects with LF due to the inability of the compromised liver to produce adequate amount ALB and PLT and/or disappearance of ALB due to immune response, coupled with an enhanced production of CRP, GLO by immune response and AST from damaged hepatocytes. This observation is similar to those of previous studies that observed an increased CAR was significantly associated with worse outcomes in HBV-decompensated cirrhosis patients (Rungta et al., 2021, Li et al., 2018, Çelikkol 2022).

Also, the area under the receiver operator characteristic curve (AUC) for GPI was 0.923. Using a potential cutoff value of 1.66, the sensitivity, specificity, positive, negative predictive values and overall efficiency of GPI were 93.3 %, 24.7 %, PPV=79 %, NPV=91.8 % and overall efficiency = 84 % respectively in predicting significant fibrosis. These findings are similar to that of a previous study which

validated GPI in Turkish HBV patients using a cutoff value, sensitivity, specificity, positive, negative predictive values of 1.5, 75.2, 62.8, 62 and 75 respectively for the prediction of significant fibrosis (Parikh et al., 2017, Coskun et al., 2015). The APRI with an AUC of values of ≤ 0.3 rules out significant fibrosis, a value of ≥ 0.5 rules in significant fibrosis with a sensitivity, specificity, PPV and NPV of 100 %, 1.2 % PPV=99.8 %, NPV=100 % and overall efficiency (OE) of 99 % respectively for the prediction of significant fibrosis. This finding is in accordance with that of a previous study that ruled out 95.4 % of F3/F4 of liver fibrosis and rule in any grade of liver fibrosis in CHB patients by 90.78 % (Moosavy et al., 2023). Using a potential cutoff value of 0.81, the sensitivity, specificity, positive, negative predictive values and overall efficiency of CAR were 86.7 %, 20.0 %, PPV=81 %, NPV=86 % and OE = 83 % respectively, in predicting significant fibrosis. At this cutoff value about 87 of subjects with fibrosis will be correctly classified as positive and about 20 without fibrosis will be incorrectly classified as positive. This finding is in line with the observations of an earlier study which demonstrated CAR to have a high diagnostic value and performs the best among multiple combinations of inflammatory biomarkers (Shi et al., 2023).

A favorable test model should have high sensitivity and high specificity characteristics with an AUROC of ≥0.900. In this study, GPI, CAR and APRI had AUROC of 0.923, 0.940 and 1.00. These models demonstrated good test instruments in predicting fibrosis. Liver biopsy remains the "gold standard" for assessing and evaluating hepatic fibrosis. However, it has limitations, such as invasiveness high cost, associated risk for complications and sampling variability. Liver biopsy medical utility has lately been challenged by the advent of novel noninvasive procedures, including noninvasive models of predicting fibrosis, imaging techniques and serum markers of hepatic fibrosis. However, the availability and cost of the equipment remains a concern in resource-limited establishment. Serum ALB, PLT, AST, LPS, CRP, are simple laboratory test scores that change with the severity of liver injury and are useful in deducing noninvasive diagnostic and predictive models for assessing significant fibrosis in patients with chronic hepatitis B virus infection.

Limitations: This study has its limitations. The current study is limited by the small sample size. The limited number of subjects within the various stages of liver fibrosis F0, F1, F2, F3, F4, did not permit detailed comparison between patients in these categories and should have affected the levels of significant, although the sample size formula justified the use of the sample size to represent the population of study.

CONCLUSION

This study has shown that dysbiosis of the gut microbiome and LPS mediated immune activation may underlie the pathogenesis of liver injury in subjects with chronic hepatitis B virus. These models, GPI, CAR and APRI are good test instruments in predicting significant fibrosis in subjects with chronic hepatitis B virus and their use may represent simple and low-cost options for assessing liver fibrosis in patients where FibroScan, transient elastography or liver biopsy is not accessible

Ethical consideration: This study was carried out in accordance with the ethical principles for Medical Research involving human subjects as outlined in the Helsinki Declaration in 1975 and subsequent revisions. The study protocol was approved by the Health Research Ethics Committee, University of Calabar Teaching Hospital, Calabar, Cross River State (Approval number: UCTH/HREC/33/VOL.111/040).

Informed consent: Written informed consents were obtained from each of the study participants. The confidentiality of patient's information was preserved at all steps. The rights to withdraw from participation in the study at any point in time were respected.

Authors' Contributions

R E E, ACNA, UAF, NAN and RUB initiated the work, performed methodology, investigation, and formal data collection, processing and analysis. CCT, MCN, ARE, IME and O U E, wrote and prepared the manuscript and Interpretation of Results. R E E and A C N A conceived the study, supervised the study and approved the final Version to be published.

REFERENCES

Atas, D. B., Sahin, G. K., Şengül, Ş., Kaya, B., Paydaş, S., Alibaz-Oner, F., Direskeneli, H., Tuglular, S., and Asicioglu, E. 2023. C-Reactive Protein to Albumin Ratio is Associated with Disease Activity in Anti-Neutrophil Cytoplasmic Antibody Associated Vasculitis. Mediterranean Journal of Rheumatology, 34(1), 71–77.

R. E. EWORO, U. A. FABIAN, N. A. NTAMU, C. C.THOMAS, U. O. EGOM, M. C. NSONWU,
R. U. BASAKE, A. R. ESSIEN, I. M. EKAM-UKERE, A. C. NSONWU-ANYANWU

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Bayram, A. A., Al-Dahmoshi, H. O. M., Al-Khafaji, N. S. K., Al Mammori, R. T. O., Al-Shimmery, A. H. S., and Saki, M. 2021. Study of the D-dimer, C-reactive protein, and autoantibodies markers among HBV infected patients in Babylon province, Iraq. BioMedicine, 11(4): 67–72.

Çelikkol, A., Güzel, E. Ç., Doğan, M., Erdal, B., and Yilmaz, A. 2022. C-Reactive Protein-to-Albumin Ratio as a Prognostic Inflammatory Marker in COVID-19. Journal of Laboratory Physicians, 14(1): 74–83.

Chaiwut, R., and Kasinrerk, W. 2022. Very low concentration of lipopolysaccharide can induce the production of various cytokines and chemokines in human primary monocytes. BMC Research Notes, 15(1): 42.

Ciesielska, A., Matyjek, M., and Kwiatkowska, K. 2021. TLR4 and CD14 trafficking and its influence on LPS-induced pro-inflammatory signaling. Cellular and Molecular Life Sciences: CMLS, 78(4): 1233–1261.

Coskun, B. D., Altinkaya, E., Sevinc, E., Ozen, M., Karaman, H., Karaman, A., and Poyrazoglu, O. 2015. The diagnostic value of a globulin/platelet model for evaluating liver fibrosis in chronic hepatitis B patients. Revista espanola de enfermedades digestivas, 107(12): 740–744.

Ding, J. H., Jin, Z., Yang, X. X., Lou, J., Shan, W. X., Hu, Y. X., Du, Q., Liao, Q. S., Xie, R., and Xu, J. Y. 2020. Role of gut microbiota via the gut-liver-brain axis in digestive diseases. World Journal of Gastroenterology, 26(40): 6141–6162.

Doumas, B. T., Watson, W. A., Biggs, H.G. 1971. Albumin standards and the measurement of serum albumin with bromcresol green. Clinica Chimica Acta; International Journal of Clinical Chemistry, 31(1):87–96.

Fakharian, F., Thirugnanam, S., Welsh, D. A., Kim, W. K., Rappaport, J., Bittinger, K., and Rout, N. 2023. The Role of Gut Dysbiosis in the Loss of Intestinal Immune Cell Functions and Viral Pathogenesis. Microorganisms, 11(7): 1849.

Fux, A. C., Casonato Melo, C., Michelini, S., Swartzwelter, B. J., Neusch, A., Italiani, P., and Himly, M. 2023. Heterogeneity of Lipopolysaccharide as Source of Variability in Bioassays and LPS-Binding Proteins as Remedy. International Journal of Molecular Sciences, 24(9): 8395.

Han, W., Huang, C., Ji, Y., Zhou, L., Chen, J., and Hou, J. 2021. Alterations in the Gut Microbiota and Hepatitis-B-Virus Infection in Southern Chinese Patients With Coexisting Non-Alcoholic Fatty Liver Disease and Type-2 Diabetes Mellitus. Frontiers in Medicine, 8, 805029.

Hao, S., Wang, Y., Gao, G., and Li, Z. 2017. Hepatitis B Virus Upregulates the Expression of C-reactive Protein Both in vivo and in vitro. Annals of Clinical and Laboratory Science, 47(4): 432–435.

Hou, K., Wu, Z. X., Chen, X. Y., Wang, J. Q., Zhang, D., Xiao, C., Zhu, D., Koya, J. B., Wei, L., Li, J., and Chen, Z. S. 2022. Microbiota in health and diseases. Signal Transduction and Targeted Therapy, 7(1): 135.

Huang, S. S., Xie, D. M., Cai, Y. J., Wu, J. M., Chen, R. C., Wang, X. D., Song, M., Zheng, M. H., Wang, Y. Q., Lin, Z., and Shi, K. Q. 2017. C-reactive protein-to-albumin ratio is a predictor of hepatitis B virus related decompensated cirrhosis: time-dependent receiver operating characteristics and decision curve analysis. European Journal of Gastroenterology and Hepatology, 29(4): 472–480.

Jang, J. H., Hong, S., and Ryu, J. A. 2022. Prognostic Value of C-Reactive Protein and Albumin in Neurocritically Ill Patients with Acute Stroke. Journal of Clinical Medicine, 11(17): 5067.

Kartjito, M. S., Yosia, M., Wasito, E., Soloan, G., Agussalim, A. F., and Basrowi, R. W. 2023. Defining the Relationship of Gut Microbiota, Immunity, and Cognition in Early Life-A Narrative Review. Nutrients, 15(12): 2642.

Katkat, F., Kalyoncuoglu, M., Ozcan, S., Tugrul, S., Abanus, H., Ince, O., Balli, M., Sahin, I., and Okuyan, E. 2022. C-Reactive Protein to Albumin Ratio as A Novel Inflammatory-Based Marker for 30-Day Mortality in Patients Undergoing Transcatheter Aortic Valve Replacement. Brazilian Journal of Cardiovascular Surgery, 37(3): 292–300.

Kaya, A., Barutcu, S., and Gulsen, M. T. 2023. Evaluation of fibrosis with noninvasive biochemical tests in chronic viral hepatitis B. Hepatology Forum, 4(1): 25–29.

Khanam, A.; Chua, J.V.; Kottilil, S. 2021. Immunopathology of Chronic Hepatitis B Infection: Role of Innate and Adaptive Immune Response in Disease Progression. International Journal of Molecular Sciences, 22, 5497.

Li, Q., Lu, C., Li, W., Huang, Y., and Chen, L. 2018. Globulin-platelet model predicts significant fibrosis and cirrhosis in CHB patients with high HBV DNA and mildly elevated alanine transaminase levels. Clinical and Experimental Medicine, 18(1): 71–78.

Li, Y. G., Yu, Z. J., Li, A., and Ren, Z. G. 2022. Gut microbiota alteration and modulation in hepatitis B virus-related fibrosis and complications: Molecular mechanisms and therapeutic inventions. World Journal of Gastroenterology, 28(28): 3555–3572.

Li, Y. G., Yu, Z. J., Li, A., and Ren, Z. G. 2022. Gut microbiota alteration and modulation in hepatitis B virus-related fibrosis and complications: Molecular mechanisms and t herapeutic inventions. World Journal of Gastroenterology, 28(28): 3555–3572.

Li, Y. N., Kang, N. L., Jiang, J. J., Zhu, Y. Y., Liu, Y. R., Zeng, D. W., and Wang, F. 2022. Gut microbiota of hepatitis B virus-infected patients in the immune-tolerant and immune-active phases and their implications in metabolite changes. World Journal of Gastroenterology, 28(35): 5188–5202.

Liu, S., and Yang, X. 2023. Intestinal flora plays a role in the progression of hepatitis-cirrhosis-liver cancer. Frontiers in Cellular and Infection Microbiology, 13, 1140126.

Lopez-Scarim, J., Nambiar, S. M., and Billerbeck, E. 2023. Studying T Cell Responses to Hepatotropic Viruses in the Liver Microenvironment. Vaccines, 11(3): 681.

Lozenov, S., Krastev, B., Nikolaev, G., Peshevska-Sekulovska, M., Peruhova, M., and Velikova, T. 2023. Gut Microbiome Composition and Its Metabolites Are a Key Regulating Factor for Malignant Transformation, Metastasis and Antitumor Immunity. International Journal of Molecular Sciences, 24(6): 5978.

Lu, H., Cao, W., Zhang, L., Yang, L., Bi, X., Lin, Y., Deng, W., Jiang, T., Sun, F., Zeng, Z., Lu, Y., Zhang, L., Liu, R., Gao, Y., Wu, S., Hao, H., Chen, X., Hu, L., Xu, M., Xiong, Q., … Xie, Y. 2023. Effects of hepatitis B virus infection and strategies for preventing mother-to-child transmission on maternal and fetal T-cell immunity. Frontiers in Immunology, 14, 1122048.

Maciel-Fiuza, M. F., Muller, G. C., Campos, D. M. S., do Socorro Silva Costa, P., Peruzzo, J., Bonamigo, R. R., Veit, T., and Vianna, F. S. L. 2023. Role of gut microbiota in infectious and inflammatory diseases. Frontiers in Microbiology, 14, 1098386.

Mazziotta, C., Tognon, M., Martini, F., Torreggiani, E., and Rotondo, J. C. 2023. Probiotics Mechanism of Action on Immune Cells and Beneficial Effects on Human Health. Cells, 12(1): 184.

Moosavy, S. H., Eftekhar, E., Davoodian, P., Nejatizadeh, A., Shadman, M., Zare, S., and Nazarnezhad, M. A. 2023. AST/ALT ratio, APRI, and FIB-4 compared to FibroScan for the assessment of liver fibrosis in patients with chronic hepatitis B in Bandar Abbas, Hormozgan, Iran. BMC Gastroenterology, 23(1): 145

Neag, M. A., Mitre, A. O., Catinean, A., and Buzoianu, A. D. 2021. Overview of the microbiota in the gut-liver axis in viral B and C hepatitis. World Journal of Gastroenterology, 27(43): 7446–7461.

Nevola, R., Beccia, D., Rosato, V., Ruocco, R., Mastrocinque, D., Villani, A., Perillo, P., Imbriani, S., Delle Femine, A., Criscuolo, L., Alfano, M., La Montagna, M., Russo, A., Marfella, R., Cozzolino, D., Sasso, F. C., Rinaldi, L., Marrone, A., Adinolfi, L. E., and Claar, E. 2023. HBV Infection and Host Interactions: The Role in Viral Persistence and Oncogenesis. International journal of Molecular Sciences, 24(8), 7651.

Oikonomou, T., Goulis, I., Kiapidou, S., Tagkou, N., Akriviadis, E., Papatheodoridis, G., and Cholongitas, E. 2020. The significance of C-reactive protein to albumin ratio in patients with decompensated cirrhosis. Annals of gastroenterology, 33(6): 667–674.

Parikh, P., Ryan, J. D., and Tsochatzis, E. A. 2017. Fibrosis assessment in patients with chronic hepatitis B virus (HBV) infection. Annals of Translational Medicine, 5(3): 40.

Pepys, M. B. 1981. C-reactive protein fifty years on. Lancet (London, England), 1(8221): 653–657.

Rajpoot, S., Wary, K. K., Ibbott, R., Liu, D., Saqib, U., Thurston, T. L. M., and Baig, M. S. 2021. TIRAP in the Mechanism of Inflammation. Frontiers in Immunology, 12, 697588.

Rastogi, S., Mohanty, S., Sharma, S., and Tripathi, P. 2022. Possible role of gut microbes and host's immune response in gut-lung homeostasis. Frontiers in Immunology, 13, 954339.

Reitman, S., Frankel S. 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology, 28(1): 56–63.

Rungta, S., Kumari, S., Verma, K., Akhtar, G., Deep, A., Sr, and Swaroop, S. 2021. A Comparative Analysis of the APRI, FIB4, and FibroScan Score in Evaluating the Severity of Chronic Liver Disease in Chronic Hepatitis B Patients in India. Cureus, 13(11): e19342.

Shen, Y., Wu, S. D., Chen, Y., Li, X. Y., Zhu, Q., Nakayama, K., Zhang, W. Q., Weng, C. Z., Zhang, J., Wang, H. K., Wu, J., and Jiang, W. 2023. Alterations in gut microbiome and metabolomics in chronic hepatitis B infection-associated liver disease and their impact on peripheral immune response. Gut Microbes, 15(1): 2155018.

Shen, Y., Wu, S. D., Chen, Y., Li, X. Y., Zhu, Q., Nakayama, K., Zhang, W. Q., Weng, C. Z., Zhang, J., Wang, H. K., Wu, J., and Jiang, W. 2023. Alterations in gut microbiome and metabolomics in chronic hepatitis B infection-associated liver disease and their impact on peripheral immune response. Gut Microbes, 15(1): 2155018.

Shi, W., Jiang, Y., Tian, H., Wang, Y., Zhang, Y., Yu, T., and Li, T. 2023. C-Reactive Protein-to-Albumin Ratio (CAR) and C-Reactive Protein-to-Lymphocyte Ratio (CLR) are Valuable Inflammatory Biomarker Combination for the Accurate Prediction of Periprosthetic Joint Infection. Infection and Drug Resistance, 16, 477–486.

Shi, W., Jiang, Y., Tian, H., Wang, Y., Zhang, Y., Yu, T., and Li, T. 2023. C-Reactive Protein-to-Albumin Ratio (CAR) and C-Reactive Protein-to-Lymphocyte Ratio (CLR) are Valuable Inflammatory Biomarker Combination for the Accurate Prediction of Periprosthetic Joint Infection. Infection and Drug Resistance, 16, 477–486.

Song, Z., Xie, Q., Zhou, Y., Song, S., Gao, Z., Lan, Y., Wu, Z., Cai, H., Yu, D., Liu, C., Liang, J., Xie, B., and Sun, S. (2023). Effect of Artificial Liver Support Systems on Gut Microbiota in Patients with HBV-Related Acute-on-Chronic Liver Failure. Pathogens (Basel, Switzerland), 12(9): 1094.

Spanu, D., Pretta, A., Lai, E., Persano, M., Donisi, C., Mariani, S., Dubois, M., Migliari, M., Saba, G., Ziranu, P., Pusceddu, V., Puzzoni, M., Astara, G., and Scartozzi, M. 2022. Hepatocellular carcinoma and microbiota: Implications for clinical management and treatment. World Journal of Hepatology, 14(7): 1319–1332.

State, N. 2021. CRP and the Prognosis of Patients with Cirrhosis. Maedica, 16(3): 353–361.

Takahashi, H., Kawanaka, M., Fujii, H., Iwaki, M., Hayashi, H., Toyoda, H., Oeda, S., Hyogo, H., Morishita, A., Munekage, K., Kawata, K., Tsutsumi, T., Sawada, K., Maeshiro, T., Tobita, H., Yoshida, Y., Naito, M., Araki, A., Arakaki, S., Kawaguchi, T., 2014. Japan Study Group Of Nonalcoholic Fatty Liver Disease Jsg-Nafld 2023.
Association of Serum Albumin Levels and Long-Term Prognosis in Patients with Biopsy-Confirmed Nonalcoholic Fatty Liver Disease. Nutrients, 15(9):

Thapa, H. B., Kohl, P., Zingl, F. G., Fleischhacker, D., Wolinski, H., Kufer, T. A., and Schild, S. 2023. Characterization of the Inflammatory Response Evoked by Bacterial Membrane Vesicles in Intestinal Cells Reveals an RIPK2-Dependent Activation by Enterotoxigenic Escherichia coli Vesicles. Microbiology Spectrum, 11(4): e0111523.

Tietz, NW., ed. Clinical guide to laboratory tests, 3rd, ed. Philadelphia, Pa, W.B Sounders, 1995, 518-122

Vajro, P., Paolella, G., and Fasano, A. 2013. Microbiota and gut-liver axis: their influences on obesity and obesity-related liver disease. Journal of Pediatric Gastroenterology and Nutrition, 56(5): 461–468.

Walter, A. J., van de Klundert, M. A., and Jung, S. 2022. Many Ways to Communicate-Crosstalk between the HBV-Infected Cell and Its Environment. Pathogens (Basel, Switzerland), 12(1): 29.

Wang, C. J., Wu, J. P., Zhou, W. Q., Mao, W. L., and Huang, H. B. 2019. The C-Reactive Protein/Albumin Ratio as a Predictor of Mortality in Patients with HBV-Related Decompensated Cirrhosis. Clinical Laboratory, 65(8), 10.

Wang, L., Wang, S., Zhang, Q., He, C., Fu, C., and Wei, Q. 2022. The role of the gut microbiota in health and cardiovascular diseases. Molecular Biomedicine, 3(1): 30

Wang, R. Q., Zhang, Q. S., Zhao, S. X., Niu, X. M., Du, J. H., Du, H. J., and Nan, Y. M. 2016. Gamma-glutamyl transpeptidase to platelet ratio index is a good noninvasive biomarker for predicting liver fibrosis in Chinese chronic hepatitis B patients. The Journal of International Medical Research, 44(6): 1302–1313.

Xi, Y., Li, Y., Ying, S., Yan, J., and Shi, Z. 2023. Bacterial lipopolysaccharide with different administration routes affects intestinal mucosal morphological, immunological, and microbial barrier functions in goslings. Poultry Science, 102(5): 102599.

Yan, F., Zhang, Q., Shi, K., Zhang, Y., Zhu, B., Bi, Y. and Wang, X. 2023. Gut microbiota dysbiosis with hepatitis B virus liver disease and association with immune response. Frontiers in Cellular and Infection Microbiology, 13, 1152987.

Yang, M., Yang, Y., He, Q., Zhu, P., Liu, M., Xu, J., and Zhao, M. 2021. Intestinal Microbiota-A Promising Target for Antiviral Therapy?. Frontiers in Immunology, 12, 676232.

Yang, X., Mai, H., Zhou, J., Li, Z., Wang, Q., Lan, L., Lu, F., Yang, X., Guo, B., Ye, L., Cui, P., Liang, H., and Huang, J. 2023. Alterations of the gut microbiota associated with the occurrence and progression of viral hepatitis. Frontiers in Cellular and Infection Microbiology, 13, 1119875.

Yang, Y. T., Wang, L. L., Yan, L. T., Zhang, L. T., Zhou, W., Chen, Q. F., Chen, Y., Zheng, S. J., Duan, Z. P., and Li, J. F. 2020. Platelet count is closely associated with the severity of liver injury in patients with chronic hepatitis B virus infection: A cross-sectional study. Experimental and Therapeutic Medicine, 20(1): 243–250.

Yoshida, M., Tateishi, R., Hiroi, S., Fujiwara, M., Kitanishi, Y., Iwasaki, K., Takeshima, T., and Igarashi, A. 2022. Changes in Platelet Counts and Thrombocytopenia Risk in Patients with Chronic Liver Disease with Different Etiologies Using Real-World Japanese Data. Advances in therapy, 39(2): 992–1003.

Zavalaga-Zegarra, H. J., Palomino-Gutierrez, J. J., Ulloque-Badaracco, J. R., Mosquera-Rojas, M. D., Hernandez-Bustamante, E. A., Alarcon-Braga, E. A., Benites-Zapata, V. A., Herrera-Añazco, P., and Hernandez, A. V. 2022. C-Reactive Protein-to-Albumin Ratio and Clinical Outcomes in COVID-19 Patients: A Systematic Review and Meta-Analysis. Tropical Medicine and Infectious Disease, 7(8): 186.

Zeng, Y., Chen, S., Fu, Y., Wu, W., Chen, T., Chen, J., Yang, B., and Ou, Q. 2020. Gut microbiota dysbiosis in patients with hepatitis B virus-induced chronic liver disease covering chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. Journal of Viral Hepatitis, 27(2): 143–155.

Zhou, W., Luo, J., Xie, X., Yang, S., Zhu, D., Huang, H., Yang, D., and Liu, J. 2022. Gut Microbiota Dysbiosis Strengthens Kupffer Cell-mediated Hepatitis B Virus Persistence through Inducing Endotoxemia in Mice. Journal of Clinical and Translational Hepatology, 10(1): 17–25.

Zhu, S., Waili, Y., Qi, X., Chen, Y., Lou, Y., and Chen, B. 2017. Serum C-reactive protein predicts early mortality in hospitalized patients with HBV-related decompensated cirrhosis. Medicine, 96(4): e5988.