BACTERIAL TRANSLOCATION IN HEPATOSPLENIC SCHISTOSOMIASIS PATIENTS SEEN AT THE UNIVERSITY TEACHING HOSPITAL IN LUSAKA, ZAMBIA

By

Edford Sinkala

The University of Zambia, School of Medicine, Department of Internal Medicine

A thesis submitted to the University of Zambia in fulfilment of the requirements of the degree (Doctor of Philosophy- PhD) in Gastroenterology

January 2017

Computer number**: 511600705**

Copyright

All rights reserved; no part of this thesis may be produced, stored in a retrieval system or transmitted in any form or by other means, electronic, mechanical, photocopy or recoding without prior consent of the author

.

Statement of Own Work

I, Edford Sinkala, confirm that the work presented in this thesis is my own. It has been done in accordance with the guidelines for the theses for the University of Zambia and it has not been submitted elsewhere for a degree at this or another University. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

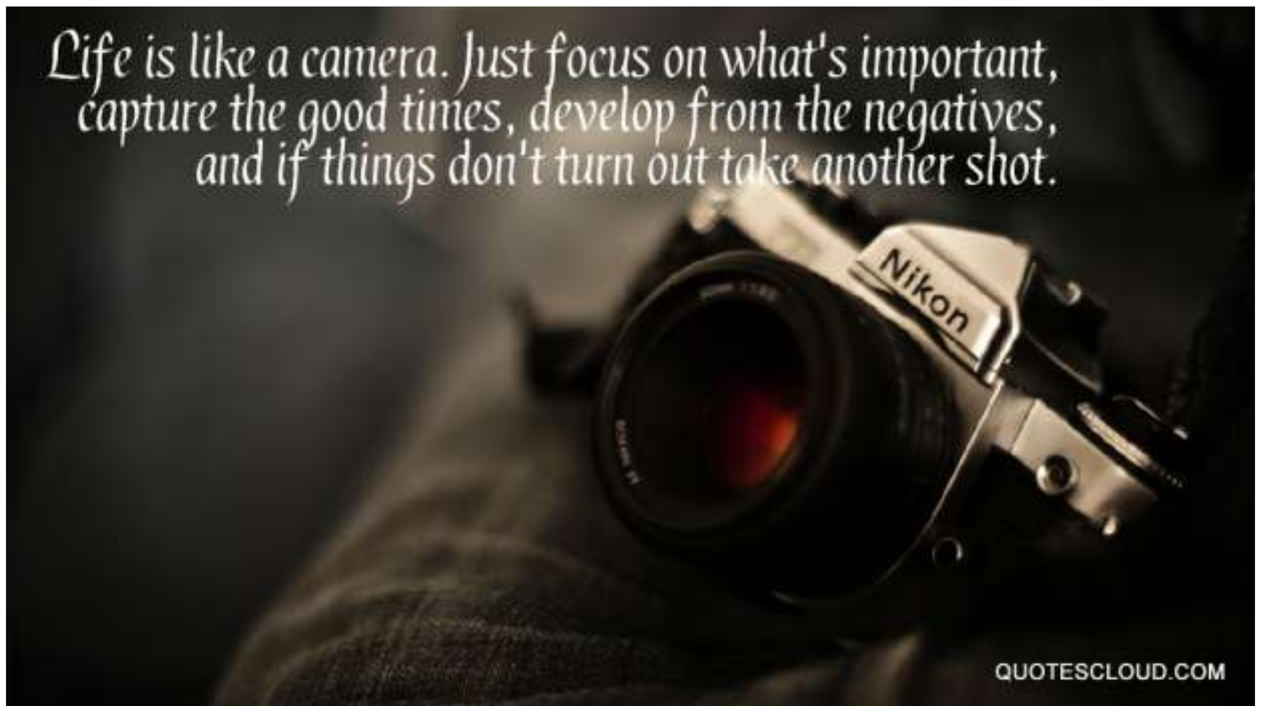
Signature: ………………………

Date:………………………………

Dedication

I wish to dedicate this thesis to my lovely wife **Mutinta** for her support and understanding my prolonged absence from home most of the time to pursue this PhD. I also dedicate it to my children; **Twapelwa, Lukundo, Lukomano and Taila** for their moral support. I love you all and God richly bless you.

Quote



Ziad k. Abdelnour

Certificate of completion of thesis

This thesis is submitted with the approval of the following supervisor:

Professor Paul Kelly MD FRCP

Blizard Institute

Barts & The London School of Medicine

Queen Mary University of London

4 Newark Street, London E1 2AT, UK

Tel: +44 20 7882 2643

and

Tropical Gastroenterology and Nutrition Group

Department of Internal Medicine

University of Zambia, School of Medicine

Nationalist Road, Lusaka, Zambia

Date:…………………………….

Signature:………………………

Certificate of Approval

This thesis of Edford Sinkala has been approved as fulfilment of the requirements for the award of Doctor of Philosophy in Gastroenterology by the University of Zambia.

……………………	………………….	………………
Examiner 1	Signature	Month/Date/Year
……………………	……………………	…………………..
Examiner 2	Signature	Month/Date/Year
…………………..	…………………….	…………………….
Examiner 3	Signature	Month/Date/Year

Assistant Dean-Post Graduate

Dr. Selestine Nzala

Department of Public Health

School of Medicine

University of Zambia

Date: ……………………..

Signature: ………………..

Abstract

Background: Cirrhosis is a dominant cause of portal hypertension globally but is overshadowed by schistosomiasis in parts of the tropics, including in Zambia where hepatosplenic schistosomiasis (HSS) sero-prevalence can reach 88% in some districts. Bacterial translocation (BT) drives portal hypertension in cirrhosis and is the main cause of mortality but it remains unexplored in HSS. Rifaximin, a minimally absorbable antibiotic may reduce BT in HSS.

Objectives: To determine whether bacterial translocation is associated with HSS in a case-control study and then using a therapeutic trial of rifaximin.

Methods: A case-control study (70 cases, 41 controls) was conducted at the University Teaching Hospital followed by a clinical trial of rifaximin. In the trial 186 patients with HSS were evaluated from January 2014 to January 2016. Eighty-five (85) fulfilled the criteria and were randomised to rifaximin with standard care, or standard care only, for 42 days. These patients were followed up for 180 days. Plasma lipopolysaccharide binding protein (LBP), polymerase chain reaction (PCR) for bacterial 16SrRNA and lipopolysaccharide (LPS) measured BT while hyaluronan (HA) & laminin measured fibrosis. Tumour necrosis factor receptor 1 (TNFR 1), soluble CD14 (sCD14), interleukin 1 β (IL1β), interleukin 6 (IL6), C-reactive protein (CRP) and soluble CD163 (sCD163) measured inflammation.

Results: Median (interquartile range) lipopolysaccharide binding protein was elevated in patients [44.3 ng/ml (35.7, 57.1)] compared to controls [30.7 ng/ml (30.4,35.5), $P < 0.0001$]. Hyaluronan was higher in patients [111.6 ng/ml (39.1,

240.3)] compared to controls [21.0 ng/ml (12.4, 37.6), $P < 0.0001$] and so was laminin [2.2 µg/ml (1.0,3.7)] compared to controls [0.9 µg/ml (0.7, 1.2), $P = 0.0015$]. Inflammatory markers, except C-reactive protein, were elevated in cases compared to controls.

In the clinical trial 16S rRNA reduced, baseline (median 129 copies/µl, IQR 23, 498) compared to 42 days of rifaximin; (median 71 copies/µl, IQR 36, 327; P=0.01) but not in the non-rifaximin group, baseline (median 50 copies/µl, IQR 19, 112) compared to day 42, (median 60 copies/µl, IQR 26, 123; P=0.45). The change in soluble CD14 over 42 days was lower (P=0.0006) in the rifaximin group (median rise 122 ng/ml, IQR-184, 783) than in the non-rifaximin group (median rise 832 ng/ml, IQR 530, 967). TNFR 1 concentrations decreased (P=0.0009) in the rifaximin group (median -39ng/ml IQR, -306, 563) but increased in the non-rifaximin group (median 166 ng/ml, IQR 3, 337). LPS, LBP and HA concentrations were not significantly affected after 42 days of rifaximin.

Conclusions: The elevated inflammatory and BT markers in cases compared to controls in the case control study suggest that BT may drive inflammation in HSS. Rifaximin led to a reduction of inflammatory markers which may implicate BT in the inflammation in HSS. Further work is needed to define the pathway of disease progression and to determine if rifaximin can give clinical benefit. The elevated markers of fibrosis in cases compared to controls suggest that they could be useful in diagnosis and monitoring of fibrosis in HSS.

Acknowledgements

I would like to thank everyone who contributed in one way or another to make this work possible. I want to thank sincerely my supervisor Professor Paul Kelly who encouraged me and contributed a lot to my mentorship. I also thank my second supervisor Professor Graham Foster for his input in this work. I want to thank the Head of Department of Medicine, Dr Nason Lambwe for the support. I want to thank doctors; Violet Kayamba, John Zainsenga and Bright Nsokolo for helping with endoscopies. I want to extend my appreciation to the endoscopy nurses; Temba Banda, Rose Banda and Joyce Sibwani. I would like to thank Professor Mathias Egger (Bern University) and Dr Michael Vinikoor (Centre for Infectious Disease Research in Zambia) for the loan of the FibroScan machine used in the study and this also includes Alice Siyunda who performed most of the scanning. I am grateful to Kanekwa Zyambo, Ellen Besa, Patrick Kaonga, John Chisoso, Rabson Zulu and Martin Bwaya for helping with some laboratory work. I am grateful to Michelo Simuyandi for conducting randomisation in the clinical trial. I want to thank the Wellcome Trust through the Southern Africa Consortium for Research Excellence (SACORE) for the sponsorship and Tropical Gastroenterology and Nutritional Group (TROPGAN) colleagues including other PhD scholars for the support. Last but not the least I want to extend my gratitude to the patients and other participants who made this work possible.

Table of contents

COPYRIGHT .... **ii**

STATEMENT OF OWN WORK .... **iii**

DEDICATION .... **iv**

QUOTE .... **v**

CERTIFICATE OF COMPLETION .... **vi**

CERTIFICATE OF APPROVAL .... **vii**

ABSTRACT .... **viii**

ACKNOWLEGEMENTS .... **x**

TABLE OF CONTENTS .... **xi**

LIST OF TABLES .... **xvi**

LIST OF FIGURES .... **xvii**

LIST OF ABBREVIATIONS .... **xix**

Chapter 1 Introduction 1

Chapter 2 Literature review 5

2.1 Hepatosplenic schistosomiasis 5

2.1.1 Epidemiology 7

2.1.2 Pathophysiology of hepatosplenic schistosomiasis 7

2.1.3 Diagnosis of hepatosplenic schistosomiasis 9

2.2 Bacterial translocation in cirrhosis 12

2.3 Bacterial translocation in hepatosplenic schistosomiasis 15

2.4 Serum markers of inflammation and fibrosis 18

2.4.1 Inflammatory cytokines 21
Chapter 3 Statement of the problem 24
3.1 Significance 24
3.2 Research Question 25
3.3 General objective 25
3.3.1 Specific objectives 25
Chapter 4 Methodology 26
4.1 Case control study 27
4.1.1 Study site 27
4.1.2 Inclusion criteria for cases 27
4.1.3 Exclusion criteria for cases 27
4.1.4 Inclusion criteria for controls 27
4.1.5 Exclusion criteria for controls 28
4.1.6 Sampling methods and sample size 28
4.1.7 Clinical and laboratory information 28
4.2 Randomised control trial 29
4.2.1 Study site 30
4.2.2 Inclusion criteria 30
4.2.3 Exclusion criteria 30
4.2.4 Sampling methods and sample size 30
4.2.5 Randomisation 31
4.2.6 Rifaximin 31
4.2.7 Clinical, laboratory and follow-up information 31

4.2.8 End points of 42-day follow-up 32
4.3 Extended follow up of randomised control trial participants 32
4.3.1 End points 33
4.4 Nested case control study of FibroScan 33
4.4.1 Study site 33
4.4.2 Inclusion and exclusion criteria 33
4.4.3 Sampling methods 33
4.4.4 Clinical and laboratory information 34
4.4.5 Transient elastography (FibroScan) 34
4.5 Laboratory assays for all the studies 35
4.5.1 Schistosomiasis serology microwell ELISA 36
4.5.2 Lipopolysaccharide (LPS) assay 36
4.5.3 16S rRNA gene 37
4.5.4 Human Lipopolysaccharide Binding Protein (Human LBP) 37
4.5.5 Hyaluronan Immunoassay 37
4.5.6 Laminin 37
4.5.7 C-reactive protein (CRP) 37
4.5.8 Interleukin 1 beta (IL-1β) 38
4.5.9 Interleukin 6 (IL-6) 38
4.5.10 Tumour Necrosis Factor Receptor 1 (TNFR1) 38
4.5.11 Soluble CD14 38
4.5.12 Soluble CD163 39
4.5.13 Sample analysis for all ELISA assays 39

4.5.14 Full blood count and chemistry 39

4.6 Abdominal ultrasound 39

4.7 Data analysis for all patients 40

4.8 Ethical consideration 40

Chapter 5 Study results 41

5.1 Results I: Case control study 41

5.1.1 Clinical characteristics 41

5.1.2 Parasitological findings 43

5.1.3 Endoscopic findings 43

5.1.4 Markers of translocation 47

5.1.5 Markers of fibrosis and inflammation 47

5.1.6 Correlations between fibrosis, inflammation and translocation Markers 48

5.1.7 Summary of the findings of the case control study 49

5.2 Results II: Clinical trial of rifaximin 50

5.2.1 Clinical trial follow-up for 42 days 51

5.2.2 Blood tests and pulse rate after 42 days of follow-up 53

5.2.3 Adverse events during the 42-day follow-up 61

5.2.4 Summary of the findings of 42-day follow-up in the clinical trial 62

5.3 Result III: Follow-up after day 42 to day 180 62

5.3.1 Clinical characteristics 62

5.3.2 Abdominal ultrasound findings 64

5.3.3 Full blood count 66

5.3.4 Adverse events during follow-up between day 42 and day 180 66

5.3.5 Summary of findings during extended (180 day) follow-up 67

5.4 Result IV: Nested case control study of FibroScan 68

5.4.1 Summary results of nested case control study of FibroScan 71

Chapter 6 Discussion 72

6.1.1 Conclusions and recommendations 76

6.2 Discussion of results II: 42 day randomised clinical trial with rifaximim 77

6.2.1 Conclusions and recommendations 80

6.3 Discussion of results III: The extended follow-up to day 180 80

6.3.1 Conclusions and recommendations 82

6.4 Discussion of results IV: Nested case control study with use of FibroScan 83

6.4.1 Conclusions and recommendations 84

6.5 Contribution of knowledge to science 86

6.6 Application of results in public health 86

References 87

APPENDICES 112

List of Tables

Table 2-1: Highlighting some differences between HSS and cirrhosis 17

Table 5-1: Demographic, clinical, laboratory and ultrasound data for patients and controls 42

Table 5-2: Baseline demographic and laboratory information 51

Table 5-3: Comparisons of pulse and full blood count between baseline and day 42 53

Table 5-4: Comparisons of clinical variables between two groups at day 180 62

Table 5-5: Demographic and laboratory data in a nested case control study 67

List of figures

Figure 2-1: Schematic presentation of pathogenesis of BT in cirrhosis 14

Figure 2-2: Clinical consequences of BT in cirrhosis 15

Figure 2-3: Schematic representation of fibrogenesis 19

Figure 4-1: Study design flow chart 26

Figure 4-2: FibroScan demonstration 34

Figure 5-1: Portal hypertensive gastropathy 43

Figure 5-2: Oesophageal varices 44

Figure 5-3: Gastric varices 45

Figure 5-4: Markers of bacterial translocation and fibrosis 46

Figure 5-5: Makers of inflammation (CRP, sCD14 and CD163) 46

Figure 5-6: Markers of inflammation (TNFR 1, IL-1β and IL-6) 47

Figure 5-7: Correlations of HA with TNFR 1 & correlations of LBP with TNFR 1, IL-1β and IL-6 48

Figure 5-8: Clinical trial flow- chart 49

Figure 5-9: 16S rRNA before and after 42 days 54

Figure 5-11: Soluble CD14 in rifaximin and non-rifaximin groups at baseline and after 42 days 56

Figure 5-12: Comparison of delta sCD14 in rifaximin and non-rifaximin Groups 56

Figure 5-13: TNFR 1 in rifaximin group and non-rifaximin group at baseline and after 42 days 57

Figure 5-14: Comparison of delta TNFR 1 in rifaximin and non-rifaximin groups 57
Figure 5-15: LPS in rifaximin group and non-rifaximin group at baseline and after 42 days 58
Figure 5-16: Comparison of delta LPS in rifaximin and non-rifaximin group 58
Figure 5-17: LBP in rifaximin group and non-rifaximin group at baseline and after 42 days 59
Figure 5-18: Comparison of change in LBP in rifaximin and non-rifaximin groups 59
Figure 5-19: Hyaluronan in rifaximin and non-rifaximin groups at baseline and after 42 days 60
Figure 5-20: Comparison of delta HA in rifaximin and non-rifaximin groups 60
Figure 5-21: Radial pulse rate in rifaximin and non-rifaximin groups 63
Figure 5-22: Delta values of radial pulse in rifaximin and non-rifaximin groups after 180 day 63
Figure 5-23: Portal vein diameter after 180 days of follow-up 64
Figure 5-24: Delta values of portal vein diameter in rifaximin and non-rifaximin groups after 180 days 64
Figure 5-25: Comparison of splenic size between baseline and day 180 65
Figure 5-26: Comparison of delta values of splenic size between rifaximin and non-rifaximin groups 65
Figure 5-27: Comparison of FibroScan scores in HSS patients and controls 68
Figure 5-28: Spearman's correlation of FibroScan score and hyaluronan 69

Figure 5-29: Spearman's correlation of FibroScan score and TNFR 1 70

LIST OF ABREVIATIONS

ALT	Alanine aminotransferase
APRI	AST to platelet ratio index
AST	Asparte aminotransferase
BMI	Body mass index
BT	Bacterial translocation
CA	California
CD	Cluster of Differentiation
CSE	Control standard endotoxin
CI	Confidence Interval
CTGF	Connective tissue growth factor
CRP	C- Reactive Protein
DKA	Diabetic ketoacidosis
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-linked immunosorbent assay
EndCAb	Endotoxin core antibodies
EGF	Epidermal growth factor
ECM	Extracellular matrix
GI	Gastro-Intestinal
HA	Hyaluronan

HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency Virus
HSC	Hepatic stallate cells
HSS	Hepatosplenic schistosomiasis
HVPG	Hepatic venous pressure gradient
LPS	Lipopolysaccharide
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
ICAM	Intracellular adhesive molecule
IFNGR	Interferon gamma receptor
INFɣ	Interferon gamma
IGF	Insulin -like growth factor
IL	Interleukin
IQR	Interquartile range
LAL	Limulus amabocyte lysate chromo
LBP	Lipopolysaccharide binding protein
LPS	Lipopolysaccharide
MCV	Mean cell volume
MIP	Macrophage inflammatory protein
MLNs	Mesenteric lymph nodes
MMP	Matrix metalloproteinases

MPV	Main portal vein
MRI	Magnetic resonance imaging
NOD	Nucleotide-binding oligomerisation domain –containing protein
PCR	Polymerase Chain Reaction
PDGF	Platelet derived growth factor
PINP	N-terminal pro-peptide collagen type I
PIIINP	N-terminal pro-peptide collagen type III
PLT	Platelet
PVCP	Pro-peptide of collagen type V
RBC	Red blood cell
RES	Reticuloendothelial system
RCT	Randomised control trial
SACORE	Southern Africa Consortium for Research Excellence
SBP	Spontaneous Bacterial Peritonitis
TGF-β1	Transforming growth factor beta 1
TIMPs	Tissue inhibitors of metalloproteinases
TLR	Toll-like receptor
TNF	Tumour necrosis factor
TNFR	Tumour necrosis factor receptor
TROPGAN	Tropical Gastroenterology and Nutritional Group
UK	United Kingdom
USA	United States of America
WCC	White cell count

CHAPTER 1: INTRODUCTION

The most significant cause of mortality and morbidity in patients with hepatosplenic schistosomiasis (HSS) is variceal bleeding as a result of portal hypertension (Kheir et al., 1999; Shaker et al., 2014; Chofle et al., 2014). Hepatosplenic portal hypertension is the most severe consequence of HSS in endemic areas (Pyrrho et al., 2002; Pyrrho et al., 2002). Portal hypertension may lead to formation of oesophageal varices, gastric varices or both. Factors such as size of varices, portal vein diameter, thrombocytopaenia, red color sign, portal gastropathy and fundal varices are some of the predictors of oesophageal variceal bleeding in patients with portal hypertension (Limquiaco et al., 2006; Martins et al., 2000). Schistosomiasis can present with an acute stage that may proceed unnoticed into a chronic form which may be noticed years later when it causes periportal fibrosis (Marinho et al., 2010; Wyszomirska et al., 2005). The main parasites responsible for HSS are *Schistosoma mansoni* and *Schistosoma japonicum* (Jia et al., 2011), but *Schistosoma intercalatum* and *Schistosoma mekongi* are also known to cause HSS. Worldwide, cirrhosis leads as a main cause of portal hypertension but in the tropics schistosomiasis is very common (Parise et al., 1992). Liver cirrhosis is a disease entity that is similar to schistosomal liver disease and causes portal hypertension with variceal formation. The major difference between HSS and cirrhosis is that in schistosomal liver disease, the function remains normal while in liver cirrhosis the function is impaired to some degree (Chang et al., 2006; Ross et al., 2002, Shaker et al., 2014). The main pathology in schistosomiasis related liver

disease is periportal fibrosis whereas fibrosis due to cirrhosis involves the liver parenchyma with apoptosis and necrosis of hepatocyte leading to fibrosis and cirrhosis (Kamal et al., 2004; Zhou et al., 2014).

Liver disease, especially in the chronic form, has an interaction with the gut microbiota (Giannelli et al., 2014). The bacteria in the gut have shown to play a significant role in progression of cirrhosis and contribute to portal hypertension through bacterial translocation (BT). Therefore the main cause of morbidity and mortality in cirrhosis is BT which by definition is the movement of bacteria from the intestine to the mesenteric lymph nodes and/or portal vein, then into the systemic circulation (Bellot et al., 2013, Wiest et al., 1999; Gou et al., 2006; Balzan et al., 2007). Bacteria may also seed into peritoneal fluid causing spontaneous bacterial peritonitis (SBP) (Ramachandran & Balasubramanian 2001). Changes in small bowel motility, bacterial overgrowth in small bowel and increased intestinal permeability have shown to increase BT and endotoxaemia in patients with cirrhosis (Bellot et al., 2013). Therefore use of antibiotics in cirrhosis to prevent BT is widely promoted (Fukui 2015, Rasaratnam et al., 2003; Nusrat et al., 2014; Chavez-Tapia et al., 2011). There is less evidence to show involvement of BT in schistosomiasis-related portal hypertension and it is unclear whether it significantly contributes to portal hypertension in these patients. A short report from Western Kenya suggested an increased endotoxaemia in patients exposed to *S. mansoni* (Onguru et al., 2011). Another study suggested that BT may play a role in post-operative infectious complications in patients with schistosomal portal hypertension (Ferraz et al.,

2005). There is need to establish the evidence of BT in HSS and this may be confirmed by giving an antibiotic (rifaximin) orally to these patients to sterilize the gut. Rifaximin is a non-absorbable broad spectrum antibiotic (Bass et al., 2010) which is bacteriocidal and is active against Gram negative and Gram positive bacteria including anaerobes (Dabo et al., 2009; Lutz et al., 2014). Rifaximin can therefore be used as a measure of the burden of BT. If markers of translocation fall on treatment compared to the baseline that would be strong evidence of bacterial translocation from the gut.

In my thesis, I set out to test the hypothesis that BT is a part of the pathophysiology of HSS, first by doing a case control study and then by carried out a phase II clinical trial using rifaximin to detect any effect on inflammatory and fibrotic markers in these patients.

The choice of end points for the clinical trial was not straightforward. Markers of translocation such as lipopolysaccharide (LPS), lipopolysaccharide binding protein (LBP), bacterial DNA, soluble cluster of differentiation 14 (sCD14) and endotoxin core antibodies (EndoCAb), (Koutsounas, 2015; Marchetti et al., 2013) are evidence of BT. However, to detect evidence of benefit in terms of liver pathology is much more difficult as the gold standard for assessment of portal hypertension is hepatic portal venous pressure gradient which can only be measured using very invasive techniques in very sophisticated clinical laboratories (Snowdon et al., 2012). Non-invasive markers of fibrosis are much easier to measure, but a subject of intense debate (Sebastiani et al., 2009; Baranova et al., 2011; Lombardi et al., 2015). Liver biopsy, which is a gold

standard in diagnosing fibrosis, is invasive and prone to sampling error (Ahmed et al., 2009; Olveda et al., 2014). Moreover, liver biopsy is not readily available in most medical centres in Africa. Hyaluronan (HA), a fibrotic marker, is promising in this aspect and has shown to be positively correlated with portal hypertension in patients with schistosomiasis in rural Brazil (Marinho et al., 2010).

The role of inflammatory markers such as cytokines in chronic HSS can also be evaluated. One study evaluated cytokines such as interleukin (IL)-13, tumour necrosis factor (TNF)-β, interferon (IFN)-γ and TNF-α but found no significant differences between patients with HSS, those infected with hepatitis C virus and those who were co-infected. However, TNF-α was found to be a predictor of both mild and severe fibrosis in these patients (Clarice et al., 2010). Another study looking at periportal fibrosis in individuals infected with schistosomiasis and viral hepatitis showed significantly elevated IL-1β, IL-6 and IL-10 in patients with hepatitis B, C viral infections and schistosomal liver disease (Abdel et al., 2005).

Liver stiffness can be assessed using transient elastography (FibroScan), providing another non-invasive assessment of stage of liver disease. This has been widely used in assessing liver stiffness and has shown to be a useful non-invasive tool in assessing and diagnosing liver fibrosis and cirrhosis and even in predicting variceal bleeding (Jung et al., 2012; Myers et al., 2010). It has been shown to improve diagnosis of liver cirrhosis compared to other routine screening tests as it is able to detect early cirrhotic changes (Göbel et al., 2015).

Kyung et al demonstrated that It is also useful in monitoring treatment response in chronic active hepatitis B infection (Kyung et al., 2010). However, the use of FibroScan in schistosomal portal hypertension remains unexplored and it is not clear if it can be used to discriminate between cirrhosis and HSS in schistosomiasis endemic areas. Portal hypertension due to schistosomiasis in patients attending the gastroenterology clinic at the University Teaching Hospital in Lusaka, Zambia is increasing and therefore I set out to evaluate markers of fibrosis, inflammation, bacterial translocation and determine FibroScan score in these patients.

CHAPTER 2: LITERATURE REVIEW

2.1 Hepatosplenic schistosomiasis

Human schistosomiasis is caused by a number of schistosomes that can affect the liver but the common causes of HSS are *S. mansoni* and *S. japonicum.* Other species like *S. intercalatum, S. mekongi, S. malayens or S. haematobium* can also affect the liver but *S. haematobium* is primarily associated with genitourinary disease (Gryseels et al., 2006). Social economic factors account for the high prevalence of schistosomiasis in the African set up as most people get exposed by repeated contact with infected water bodies through fishing as a way of earning a living while others acquire it through swimming, washing and drawing water for drinking (Kabatereine et al., 2004; Handzel et al., 2003; Mugono et al., 2014; El-Khoby et al., 2000; Payne et al., 2013; Pyrrho et al., 2002; Worku et al., 2014). Human schistosomiasis can be acute or chronic in its manifestation (Wyszomirska et al., 2005). A minority of patients with acute schistosomiasis may present with Katayama fever, which is a serum sickness-like syndrome and manifests with fever, headache, malaise, urticaria, diarrhoea, weight loss, mild hepatosplenomegaly and lymphadenopathy (Kibiki et al., 2004; Tzanetou et al., 2010; Janda et al., 2007; Gray et al., 2011; Silveira-Lemos et al., 2012). In *S. mansoni* infection the disease process to chronic hepatosplenic schistosomiasis may take 5-15 years (Da Silva et al., 2005) while for *S. japonicum* progression to chronicity is more rapid and severe with little or no interval between acute and chronic manifestations (Gryseeds et al., 2006). The progression to chronic HSS takes long and some authors have said the risk of

periportal fibrosis is higher if an individual has been exposed to schistosomiasis for more than 10 years (Mazigo et al., 2015) and there have been reports of infections with *S. mansoni* in children as young as 4 years old (Mugono et al., 2014). A community based clinico- epidemiological study conducted in one district in northern Ethiopia found cases of schistosomiasis infection due to *S. mansoni* even in very young children below the age of 10 years but none had periportal fibrosis which was only reported in those between 10-20 years and above (Abebe et al., 2014). This shows that the effect of chronic form of schistosomiasis can be seen in patients as early as 10 years of age. A small percentage (5-10%) of people infected with *S. mansoni* develops periportal fibrosis implying that among other factors (such as genetics) may play some role. Interferon gamma receptor 1 (IFNGR1) is an important gene locus predisposing to fibrosis due to schistosomiasis, and because of that there are suggestions that genes in the region of IL13- IL4 and tissue growth factor beta 1 (TGFβ1) might be linked (Blanton et al., 2005). Even the severity of HSS is dependent on genetic susceptibility of the individual together with other factors such as duration of infection, intensity of infection, nutritional status of the patient and co-infections such as malaria and viral hepatitis (Abath et al., 2006).

Schistosomiasis due to *S. mansoni* has been reported to affect other organs other than the liver in humans. Schistosomiasis is known to cause pulmonary hypertension. Ferreira and others noted about 11% occurrence of pulmonary hypertension in HSS although there was no correlation of hepatic fibrosis and pulmonary hypertension (Ferreira et al., 2009; Cheever et al., 2002). Others

have described cerebral schistosomiasis as a result of *S. japonicum* and *S. mansoni* (Li et al., 2011; Manzella et al., 2012). Schistosomiasis is also known to cause glomerulopathy (Otoni et al., 2014).

2.1.1 Epidemiology

Schistosomiasis remains a global problem which is more prevalent in developing and poor countries (Engels et al., 2002 ; Gazzinelli et al., 2012). An estimated 200 million people worldwide are affected by schistosomiasis and about 120 million are symptomatic with 20 million having severe form of disease of which most of these patients are found in Africa (Chitsulo et al., 2000). According to Elbaz et al *S. mansoni* infections are prevalent in South America, Africa and Middle East. *S. japonicum* infections are frequent in China, East Asia and the Philippines and *S. haematobium* is mainly found in Africa and Middle East. *S. mekongi* and *S. malayensis* are prevalent in South East Asia while *S. intercalatum* is mainly found in Africa (Elbaz & Esmat 2013). Parts of Zambia are hyper-endemic, with prevalence of *S. mansoni* being 77 % in some areas (Chipeta et al., 2009). In western Zambia, Payne and others in a report found high prevalence of schistosomal related portal hypertension with higher levels of sero-prevalence of 88% (Payne et al., 2013) while Mutengo and others in the same area found 42% prevalence of *S. mansoni* on stool samples using Kato-Katz method but also found 26% with portal fibrosis (Mutengo et al., 2014). They also observed that adults were more likely to have portal fibrosis radiologically than children.

2.1.2 Pathophysiology of hepatosplenic schistosomiasis

Worms and eggs account for the pathophysiology of the disease. The worms lay eggs which are either sequestrated in the intestinal submucosa or carried to the liver and therefore elicit an inflammatory process (Tzanetou et al., 2010). The eggs are deposited in the intestine and migrate to the liver via the portal circulation. Eggs in the hepatic sinusoids elicit a granulomatous inflammation that may lead to periportal fibrosis leading to increased portal hypertension (Wynn et al., 2004). *S. mansoni* is an intravascular organism which in known to reside in the hepatic and mesenteric veins where an adult female worm is able to lay about 300 eggs per day (Andrade 2009). Although the granulomatous lesions produced by *S. mansoni* cause harm by giving rise to periportal fibrosis, they are at the same time of some importance as they are able to elicit immune reaction for host protection. Some antigen produced by the eggs of *S mansoni* are neutralised by the immune system thereby protecting some affected tissues from permanent damage (Wynn et al., 2004; Herbert et al., 2008). The main pathway drive for fibrosis is a cytokine response involving tumour necrosis factor alpha (TNF α) and interleukin 4 (IL-4) cytokines among others (Yu et al., 2012; Wilson et al., 2008). The eggs in the sinusoids of the liver will eventually die and then the granulomata resolve leaving the fibrotic plaques which leads to periportal fibrosis, rendering the liver firm and difficult to perfuse. This then causes increased portal hypertension and portal systemic venous shunting giving rise to ascites and varices (Pearce & MacDonald 2002).

The most important set of clinical data for predicting bleeding from varices in HSS are gastropathy, red spots on the varix, portal vein diameter and variceal size (Martins et al., 2000) while pregnancy in cirrhosis also predisposes to variceal bleeding (Aggarwal et al., 2014). We know that hepatic venous pressure gradient (HVPG) is the gold standard for measuring portal hypertension in cirrhosis and the normal HVPG is 1-5 mmHg while for varices to form, >10 mmHg of HVPG is needed. For variceal bleeding to happen more than 12 mmHg of HVPG is required (Aggarwal et al., 2014; Addley et al., 2012). The studies to evaluate hepatic wedge pressure in HSS are scanty.

The intensity of the *S. mansoni* infection in people affected matters as it affects the extent to which periportal fibrosis, enlargement of the spleen and of the left lobe of the liver occur (Mazigo et al., 2015). Even if there are similarities between *S. mansoni* and *S. japonicum,* granuloma formation demonstrated by different animal models shows that there are differences in the granulomata elicited by these parasites. *S. japonicum* eggs tend to be laid in clusters leading to formation of large lesions and compared with *S. mansoni*. *S. japonicum* produces much more eggs resulting in magnified pathology (Warren et al., 1975). *S. mansoni* eggs are deposited along the large portal veins of the hilum of the liver, whereas eggs of *Schistosoma japonicum* are deposited in the peripheral small veins, hence periportal fibrosis is peripherally distributed in patients infected with *S. japonicum* (Strauss et al., 2004). Schistosomiasis can affect the colon. In the colonic form of schistosomiasis, the deposited eggs in

the colonic wall incite an exudative granulomatous response resulting in formation of inflammatory polyps, fibrosis, wall thickening and stenosis [68].

2.1.3 Diagnosis of hepatosplenic schistosomiasis

Identification of eggs in the stool or rectal biopsy provides a definitive diagnosis for schistosomiasis (Gray et al., 2011; Hoare et al., 2005; Barsoum et al., 2013). When intestinal schistosomiasis is suspected with history of rectal bleeding, it is important to consider doing rectal biopsy and in some instances also doing colonoscopy (Tzanetou et al., 2010). Diagnosis of both acute and chronic schistosomiasis can be relied upon the detection of antibodies against parasite antigens (Ross et al., 2002). Smith and others in a study involving 4 medical international parasitology laboratories found that using egg microscopy as 'gold standard' and *S. mansoni* cercarial transformation fluid had a sensitivity of 100% and specificity of 62.5% while soluble egg antigen ELISA showed sensitivity of 100% and specificity of 59.5% (Smith et al., 2012). In some chronic cases with advanced disease of HSS, the yield of eggs in stool examination is rare and so serology becomes important to make a diagnosis (Goljan et al., 2007).

Imaging findings using liver ultrasound may help in making the diagnosis of schistosomiasis. In schistosomal liver disease, periportal fibrosis appears as echogenic bands surrounding the portal veins. Although liver parenchyma is spared, the surface of the liver may develop pseudonodules especially in advanced disease due to fibrosis and retraction (Pinto-silva et al., 2010). Ultrasonography used with clinical examination can be useful in detecting and quantifying hepatosplenic disease (Olveda et al., 2008; Silva et al., 2011;

Carlton et al., 2010; Prata et al., 2010), but inexperienced ultrasonographers often find it difficult to distinguish between Symmer's fibrosis in HSS and cirrhosis. Ultrasonography has been compared with liver biopsy and showed that it is significant in diagnosing liver pathology due to schistosomiasis (Marinho et al., 2010). Granulomas are very important in the pathogenesis of schistosomias and ultrasound is unable to visualise them due to their small sizes (Cerri et al., 1984). Ultrasound is reliable in making a diagnosis of liver fibrosis and also handy in the differential diagnosis with other liver diseases such as cirrhosis, steatosis and liver abscess. The other differentiating feature on ultrasound between cirrhosis and schistosomiasis is that portal and splenic veins are more frequently enlarged (Giovanni et al., 1984). The main abnormal ultrasonographic findings in HSS include; periportal fibrosis, left lobe hypertrophy of the liver, small right lobe of the liver, gall bladder wall thickening and splenomegaly (David et al., 2008). In cirrhosis, common findings include irregular edges, coarse parenchyma and relative enlargement of the caudate lobe although enlargement of caudate lobe is not specific to cirrhosis as it can occur in HSS (Moon et al., 2013; Zheng et al., 2003; Lee et al., 2010; Sérgio et al., 2008). In some patients despite advanced fibrosis the spleen is quite normal and thrombosis is not a common finding despite the splenic and portal veins being frequently enlarged (Giovanni et al, 1984). Magnetic Resonance Imaging (MRI) is another useful investigation in diagnosing schistosomiasis. It can be used to discriminate between cirrhosis and schistosomiasis and also useful as several characteristics such as peripheral periportal fibrosis and presence of

siderotic nodules in the spleen on MRI are more frequent findings in hepatosplenic patients than in viral or alcohol induced cirrhosis (Sérgio et al., 2008). The combination of ultrasound and clinical evaluation in patients with hepatosplenic schistosomiasis with emphasis on periportal thickening and palpable splenomegaly, aids in diagnosis of hepatosplenic schistosomiasis (Cota et al., 2006; Marinho et al., 2006). Marinho et al, (2006) reports that quite a substantial number of patients with periportal fibrosis as seen on ultrasound were shown to have fat infiltration of the periportal tracts by use of MRI. They therefore advised that the wisest approach to make a diagnosis is to combine ultrasound, clinical examination and MRI, if MRI is readily available (Marinho et al., 2006).

Transient elastography (FibroScan) is promising to be a non-invasive tool for assessing liver fibrosis. Studies involving the FibroScan in patients with chronic hepatitis B, C and non-alcoholic fatty liver disease have shown that FibroScan could be more superior in assessing liver fibrosis than the aspartate aminotransferase-to-platelet ratio index (APRI) score but not yet validated to replace liver biopsy (Myers et al., 2010, Liu et al., 2011). There are very scanty data on its use in schistosomal liver diseases and it is not clear whether it can be employed to differentiate between cirrhosis and HSS in areas were schistosomiasis is endemic. It is also not clear whether it can be used to monitor disease progression in HSS. Although FibroScan is a useful tool, it has its pitfalls. The scores may be overestimated in situations such as obesity, liver

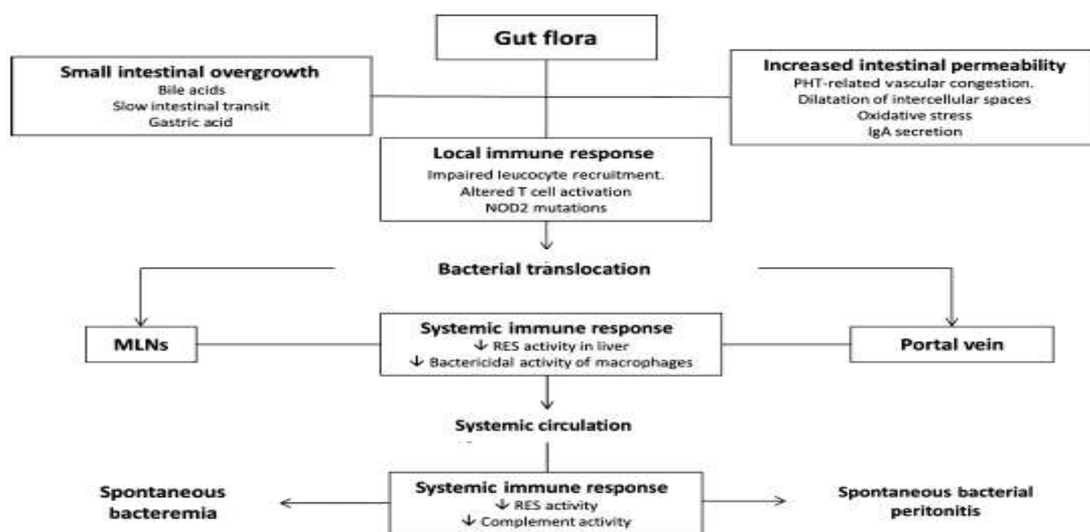
inflammation, non-fasting state, cholestasis, liver congestion, alcohol and also requires experience of the operator (Tapper et al., 2015).

2.2 Bacterial translocation in cirrhosis

Mortality in cirrhosis is multifactorial but a major cause of morbidity and mortality is bacterial translocation (BT) which usually leads to spontaneous bacterial peritonitis (SBP) (Pinzone et al., 2012). Mortality in SBP can reach 40% and in these patients it is associated with advanced liver disease causing septic shock and multi organ failure (Desai et al., 2012). SBP occurrence in cirrhosis is not dependent on the aetiology of the liver disease (Thanopoulou et al., 2002). Sometimes patients with SBP may be asymptomatic or may just present with subtle renal impairment which is a common cause of mortality with the common organisms implicated being the gram negative bacteria (Thanopoulou et al., 2002). Common presentations with fever and abdominal pains are present in about 45% while those of child C liver cirrhosis are more likely to present with more pronounced liver decompensation with encephalopathy (Thanopoulou et al., 2002; D'Amico et al., 2006). Ascitic fluid in patients suspected to have SBP is important in making the diagnosis. Polymorphonuclear cells of 250cells/mm^3 or more in ascitic fluid can be used to diagnose SBP with or without positive ascitic culture (Desai et al., 2012). Endotoxaemia in cirrhotic patients has been largely attributed to bacterial translocation which has been reported to increase portal hypertension (Vlachogiannakos et al., 2009). Factors that contribute to bacterial translocation include the relative immunosuppression associated with impaired liver function, increased growth of gram-negative aerobic bacilli in the

jejunum, changes in the intestinal barrier, increased permeability and factors that lead to reduction in the blood flow locally (Pinzone et al., 2012; Garcia-Tsao et al., 2004). Translocation of enteric bacteria to mesenteric lymph nodes is increased in patients with advanced cirrhosis especially those in Child –Pugh class C. The process of translocation is known to activate the immune response resulting in cytokine production, although this may be inadequate to prevent infection (Pinzone et al., 2012). SBP in patients with cirrhosis is one of the predisposing factors to hepatic encephalopathy. Prophylactic antibiotics decrease the risk of SBP in cirrhotic patients with gastrointestinal haemorrhage and those with prior episodes of SBP (Chavez-Tapia et al., 2011). Gut decontamination with norfloxacin has shown to reduce hepatic venous gradient in cirrhotic patients (Rasaratnam et al., 2003), which suggests that bacterial translocation contributes to disease progression. Rifaximin, a minimally absorbable antibiotic, has been of use in both treatment and prevention of hepatic encephalopathy in cirrhotic patients. It is a derivative of rifamycin and exhibits broad spectrum antimicrobial activity against both aerobic and anaerobic gram-positive including gram-negative organisms in the gastrointestinal tract (Bass et al., 2010; Flamm et al., 2011; Kimer et al., 2014). It has also shown to be useful in treating hepatic encephalopathy in cirrhotic patients on long term basis (Puxeddu et al., 1995) and has shown to reduce significantly the risk of hospitalisation (Bass et al., 2010). In one study, rifaximin showed a reduction in hepatic venous pressure gradient in alcohol-related decompensated cirrhotic patients by intestinal decontamination. This was

achieved by significantly reducing plasma endotoxin levels (Vlachogiannakos et al., 2009). Endotoxins and cytokines may increase portal pressure by stimulating endothelin release by the spleen and the gut in cirrhosis and this contributes to increased vascular resistance hence increasing portal pressure (Nagasue et al., 2000; Thalheimer et al., 2005). The ability of rifaximin to change selectively the microenvironment of the intestine could potentially be useful in preventing bacterial translocation and reducing endotoxin levels in cirrhotic patients hence ameliorating liver hemodynamic in these patients (Vlachogiannkos et al., 2009; Qiang et al., 2012).



Key

MLNs- mesenteric lymph nodes

RES- reticuloendothelial system

IgA – immunoglobulin A

NOD –nucleotide-binding oligomerisation domain- containing protein

Figure 2-1: Schematic presentation of pathogenesis of BT in cirrhosis (Bellot et al., 2013)

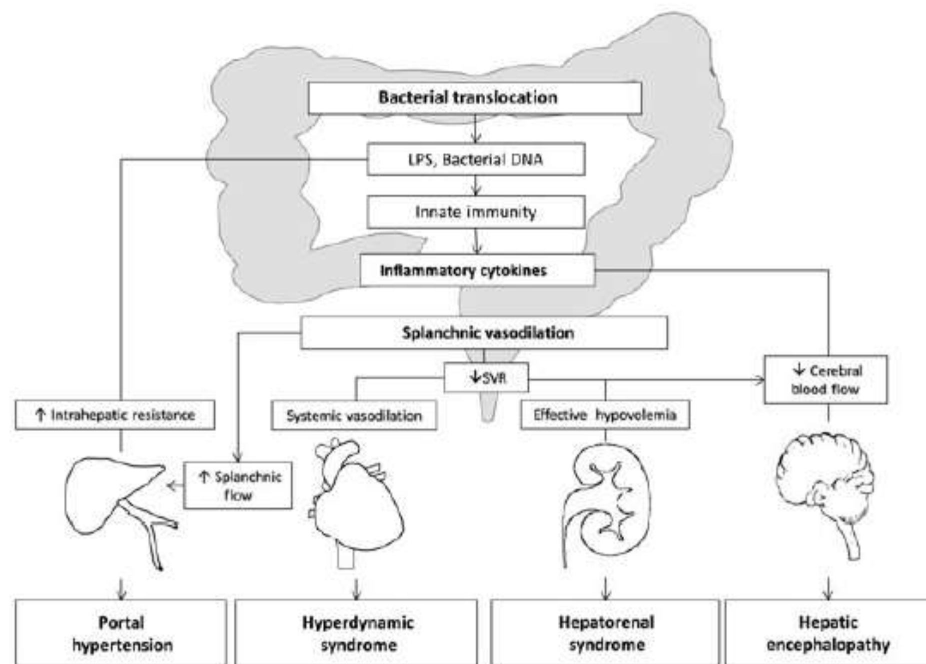


Figure: 2-2 Clinical consequences of BT in cirrhosis

2.3 Bacterial translocation in hepatosplenic schistosomiasis

The role of bacterial translocation in HSS has not been well studied. It is not clear whether bacterial translocation has any influence on portal hypertension as it is the case with cirrhotic portal hypertension (Bellot et al., 2010; Vlachogiannakos et al., 2009). Ferraz et al studied bacterial translocation in HSS but this was confined to postoperative (i.e. splenectomy and gastric devascularisation) patients. They concluded that bacterial translocation may play a role in the development of postoperative infectious complications. They also suggested that aerobic bacteria may play a role in development of sepsis as post-operative complication in these patients, suggesting a possibility of

bacterial translocation being a factor in HSS (Ferraz et al., 2005). A short report from Western Kenya showed that endotoxaemia and Toll- Like Receptor 2 were associated with human schistosomiasis (Onguru et al., 2011). Schistosomiasis has been associated with relative immunodeficiency (Ferraz et al., 2005) and this may explain the possibility of bacterial translocation occurring in these patients. There is a possibility that bowel oedema, with increased permeability due to portal hypertension may also contribute to bacterial translocation.

Hepatosplenic schistosomiasis differs from cirrhosis in that liver parenchymal function is well preserved in hepatosplenic schistosomiasis but both disease entities can cause portal hypertension which is their hall mark (Ross et al., 2002; Strauss et al., 2002; Barsoum et al., 2013). Histologically the fibrosis in hepatic schistosomal liver disease is around the portal area and sparing the parenchyma while that of cirrhosis starts from portal tracts and hepatic veins spreading out and forming bridges in between the vessels distorting the arctecture (Strauss et al., 2002). However the two disorders can be difficult to distinguish clinically as some cases of schistosomal liver disease in very advanced stages have presented with low serum albumin levels, wasting and ascites, even coma (Andrade 2004) though this is very rare in our experience in our University Teaching Hospital. Schistosomiasis may be accompanied by chronic viral hepatitis. It is debatable on the outcome of schistosomal liver disease co-infected with hepatitis B and C, although it appears that hepatitis B virus exacerbates hepatic pathology in patients who have schistosomiasis (Gasim et al., 2015). One study from Egypt showed that patients with

schistosomal liver disease who were co-infected with hepatitis C virus had poor response to antiviral treatment but hepatitis C positive serology did not affect fibrosis in these patients (Abdel-Rahman et al., 2013). Others have also observed the poor response to antiviral therapy in schistosomiasis patients co-infected with hepatitis C virus (HCV) but noted that liver fibrosis worsens in patients with schistosomal liver disease who are co-infected with HCV (Osada & Kanazawa 2011). More work is needed to find out on the interaction between HSS and chronic viral hepatitis

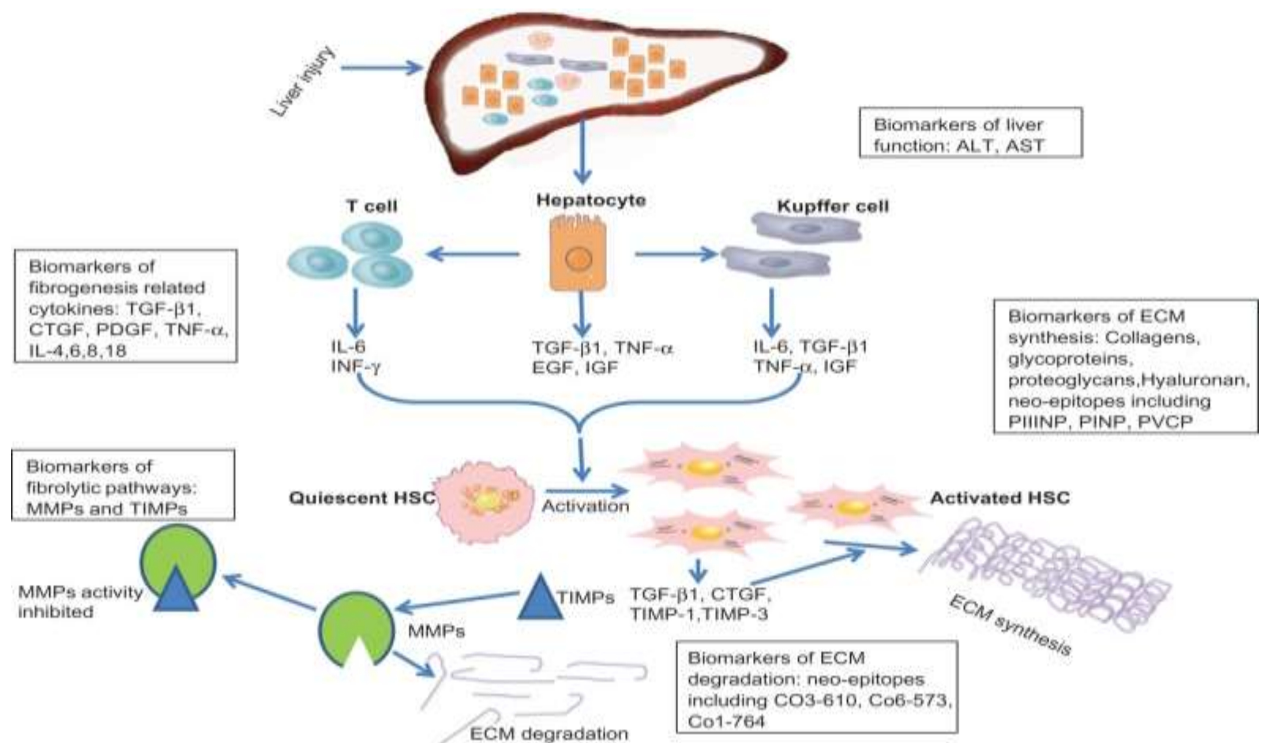
Table 2-1: Highlighting some differences between HSS and cirrhosis

Hepatosplenic schistosomiasis	**Cirrhosis**	**Reference**
Periportal fibrosis	Liver parenchymal fibrosis	Kamal et al., 2004; Zhou et al., 2014
Usually normal liver function tests	Usually deranged liver function tests	Reboucas et al., 1975; Shaker et al., 2014
Hepatic coma very rare	Hepatic coma is very common	Reboucas et al., 1975
Generally absent stigmata of chronic liver disease such as jaundice, spider nevi, palmar erythema, testicular atrophy and gynecomastia	Generally, stigmata of chronic liver disease are present	Reboucas et al., 1975; Shaker et al., 2014
More pronounced splenic siderotic nodules	Less pronounced siderotic splenic nodules	Sergio et al., 2008
Pronounced liver fissure widening	Less pronounced liver fissure widening	Sergio et al., 2008
Jaundice is very rare	Jaundice is common	Reboucas et al., 1975
Ascites is rare	Ascites occurs commonly	Reboucas et al., 1975
Abdominal wall collaterals are uncommon	Abdominal wall collaterals are common	Reboucas et al., 1975

2.4 Serum markers of inflammation and fibrosis

Although liver biopsy is the gold standard in staging fibrosis, it is invasive and associated with potential side effects and sampling error (Ahmed et al., 2009; (Tannapfel et al., 2012). It is also contra-indicated in many patients, in whom

thrombocytopenia or ascites make it risky (El-Shabrawi et al., 2012). Serum markers of fibrosis need to be widely studied since they are non-invasive and could be clinically useful in stratifying the level of monitoring patients require. These markers can be useful in assessing liver fibrosis especially in patients with portal hypertension complicating ascites, coagulopathy and thrombocytopaenia where liver biopsy may be associated with complications (Leonard et al., 2010). Non-invasive biomarkers of liver disease are becoming more popular. These may be used to assess disease progression and response to treatment (Fallatah 2014). Liver fibrosis results from deposition of extracellular matrix (ECM) in the liver. This may follow any chronic insult to the liver with inflammation leading to elevated Inflammatory markers, of which some are known to be pro-fibrogenic such as transforming growth factor beta 1 (TGF-β1), connective tissue growth factor (CTGF), platelet-derived growth factor (PDGF), tumour necrosis factor alpha (TNF-α) and IL-4,6,8,18 (Liu et al., 2012) .



Key

CTGF- connective tissue growth factor; TGF-transforming growth factor; MMPs-matrix metalloproteinases; ECM – extracellular matrix; HSC-hepatic stellate cells; TIMPs- tissue inhibitors of metalloproteinases; PIIINP-N-terminal pro-peptide collagen type III; IGF- insulin- like growth factor; PINP- N- terminal pro-peptide collagen type I; TNF- tumour necrosis factor; PVCP- pro-peptide of collagen type V; IL-interleukin; PDGF- platelet-derived growth factor; ALT- alanine aminotransferase; AST-aspartate aminotransferase; EGF- epidermal growth factor

Figure 2-3: Schematic representation of fibrogenesis (Liu et al., 2012)

This schematic representation depicts hepatic fibrogenesis associated with biomarkers. Some of these markers may be a reflection of hepatic fibrosis. Molecules such as neo-epitopes are products of extracellular matrix (ECM)

degradation while pro-collagen reflects synthesis of ECM. Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) reflect fibrolysis of ECM. Elevated serum ALT and AST denotes hepatic inflammation or injury. Other serum markers shown above are cytokines that relate to hepatic fibrosis (Liu et al., 2012).

Some authors have also noted that tumour necrosis factor receptor 1 (TNF-R1) promotes hepatic fibrosis in animal models as deletion of TNFR gene reduces early fibrogenesis (Tarrats et al., 2011). Therefore, the markers of fibrosis are becoming important as many researchers think they could play a critical role in diagnosing and monitoring liver fibrosis. Markers of fibrosis are classified as direct and indirect markers. The direct ones are those known to reflect the deposition and removal of the hepatic extracellular matrix material. These are glycoproteins such as hyaluronate, laminin and YKL-40 and collagens like procollagen III, N-peptide, type IV collagen, collagenases and their inhibitors like metalloproteases and tissue inhibitory metelloprotease-1 [121]. Those referred to as indirect markers of fibrosis are those that indicate changes in hepatic function and they can be measured routinely in blood. These are prothrombin index, platelet count and ratio of AST to ALT. The combination of direct and indirect markers can be used in diagnosis (Castera, 2012).

Hyaluronic acid as a marker of fibrosis has shown to be of use in fibrosis due to schistosomiasis but may need studies that will correlate with histology in order to determine its level of significance in diagnosis of schistosomal liver disease (Marinho et al., 2010). Other authors noted that hyaluronic acid and soluble

intercellular adhesion molecule (Sicam-1) could be used to differentiate severe from mild hepatic fibrosis (Ismail et al., 2012). Multiple fibrotic markers in evaluating patients with schistosomal liver disease would be more prudent. Wyszomirska and others demonstrated that laminin and collagen type IV are elevated in patient with HSS due to *S. mansoni* (Wyszomirska et al., 2005).

2.4.1 Inflammatory cytokines

Cytokines are important as inflammatory markers and also initiators of fibrosis. A study from Brazil looked at cytokines such as IL- 13, TNF- β, TNF- α, IFN gamma in patients with HSS, those infected with hepatitis C virus only and those co-infected but found no significant difference. However it was noted that TNF α was a good predictor of fibrosis in these patients (Clarice et al., 2010). Ahmed and others also found that alpha-2-macroglobulin, haptoglobin, apolipoprotein A 1 in addition to AST platelet ratio index (APRI) and modified APRI were important in predicting hepatic fibrosis (Ahmed et al., 2009). Although HSS is a chronic disease, it has been associated with elevated serum cytokines especially in patients co-infected with viral hepatitis. To evaluate this, a study in Egypt focussing on schistosomal liver disease co-infected with viral hepatitis revealed elevated IL-1α, IL-6 and IL-10 (Abdel et al., 2005).

Chemokines and cytokines seem to be critical in the pathogenesis of HSS. Macrophage inflammatory protein (MIP)-1, TNF-α and IL-13 seem to be associated with parasite burden and may predict schistosomal liver disease. IL-5 also has shown to be associated with HSS (De Souza et al., 2012; Wilson et al., 2008) while IL-10 appears to down regulate the pathogenesis of

schistosomal liver disease (Abdel et al., 2005). Others have also reported that during acute schistosomiasis infection IL-10 and TGF-β are protective against severe form of hepatic injury (Herbert et al., 2008). The immune response to *Schistosoma mansoni* infection goes through phases. In acute phase of the infection the immune response is mainly of T_H1 and there is an overlap at 6 weeks with T_H 2 and thereafter T_H1 response goes down and T_H 2 peaks at about week 8 and gradually comes down but remains above T_H1 as the infection enters chronicity (Pearce & MacDonald 2002). Some of the T_H1 schistosomal associated cytokines are IL-4, IL- 5 and IL-13 while the T_H2 associated ones are IFN-γ, IL-2 and IL-12p70 (Bourke et al., 2013). Some authors have suggested that T_H 2 cytokines are responsible for male biased prevalence of fibrosis in patients with *S. japonicum* (Coutinho et al., 2007). Soluble Tumour necrosis factor receptor- II (sTNFR-II) and soluble intracellular adhesive molecule- 1(sICAM-1) could be useful markers in making diagnosis of acute disease and monitoring of liver inflammation in patients with *S. japonicum* (Ellis et al., 2008). Mwatha and others also reported that soluble TNF receptors, sICAM -1 and IFN-γ have been associated with HSS due to *S. mansoni* (Mwatha et al., 1998). Other markers of inflammation such as soluble CD163 have been described in cirrhotic portal hypertension. This marker is due to the activation of Kupffer cells which are fixed hepatic macrophages and this inflammatory marker seems to contribute to portal hypertension in cirrhotic patients (Gronbaek et al.,2012). It also appears that sCD163 is not just acute reactant molecule but a specific macrophage marker and also may be

associated with markers of bacterial translocation in cirrhotic patients (Holland-Fischer et al., 2011).

Portal hypertension due to cirrhosis is known to be driven by infections resulting from BT and there is advocacy for antibiotic use in these patients. This is not the case with HSS as BT is not well documented. HSS is chronic disease and is endemic in some parts of Zambia with much morbidity attributed to portal hypertension (Chipeta et al., 2009, Payne et al., 2013; Mutengo et al., 2014). There is scarcity of data to justify use of antibiotics in HSS as very little is known about BT driving portal hypertension in HSS.

CHAPTER 3: STATEMENT OF THE PROBLEM

Given that infections associated with portal hypertension are associated with significant morbidity, and given the potential benefits from readily available antibiotics as in patients with cirrhosis, there is a pressing need for studies of bacterial translocation in HSS to determine whether antibiotics for prophylaxis and treatment should be widely used. In order to determine if bacterial translocation is present, I intend to measure lipopolysaccharide (LPS), lipopolysaccharide binding protein (LBP) and bacterial DNA in peripheral blood. However, these may be insensitive markers of translocation in HSS because it is not unlikely that the Kupffer cell compartment is intact which would filter out much molecular evidence of translocation. I therefore intend also to observe the response of inflammatory markers such as C – Reactive Protein (CRP), tumour necrosis factor receptor 1 (TNFR1), interleukin 1 beta (IL-1β), soluble cluster of differentiation 14 (sCD14), soluble cluster of differentiation 163 (sCD163) and interleukin 6 (IL-6) to rifaximin. Rifaximin is beneficial in cirrhosis in preventing bacterial translocation but I am not sure if using it can demonstrate bacterial translocation in hepatosplenic schistosomiasis.

3.1 Significance

Hepatosplenic schistosomiasis is prevalent in the tropics and Zambia is no exception. Variceal bleeding due to portal hypertension is the commonest cause of morbidity and mortality (Payne et al., 2013). Antibiotics are not routinely used in HSS as it is not clear whether bacterial translocation occurs in these patients

as it is the case with cirrhosis. So finding evidence of bacterial translocation may mandate the use of prophylactic antibiotics in HSS to reduce the substantial morbidity and mortality from HSS especially in hyperendemic areas such as Western Province in Zambia (Mutengo et al., 2014).

3.2 Research Question

Does bacterial translocation play a role in schistosomal portal hypertension?

3.3 General objective

To determine whether bacterial translocation is associated with HSS in a case-control study and then using a therapeutic trial of rifaximin.

3.3.1 Specific objectives

1. To search for molecular evidence of bacterial translocation in patients with HSS.
2. To determine whether HSS is associated with elevated plasma inflammatory and fibrotic markers.
3. To determine whether use of rifaximin is associated with changes in markers of inflammation, fibrosis and bacterial translocation.
4. To determine whether HSS is associated with liver stiffness using FibroScan score.

CHAPTER 4: METHODOLOGY

Study design

This was a mixed study design consisting of a case control study followed by a phase II randomised clinical trial, then extended follow up of the trial, and within the clinical trial another nested case control study was undertaken to assess liver stiffness in the patients using FibroScan (Fig. 4-1).

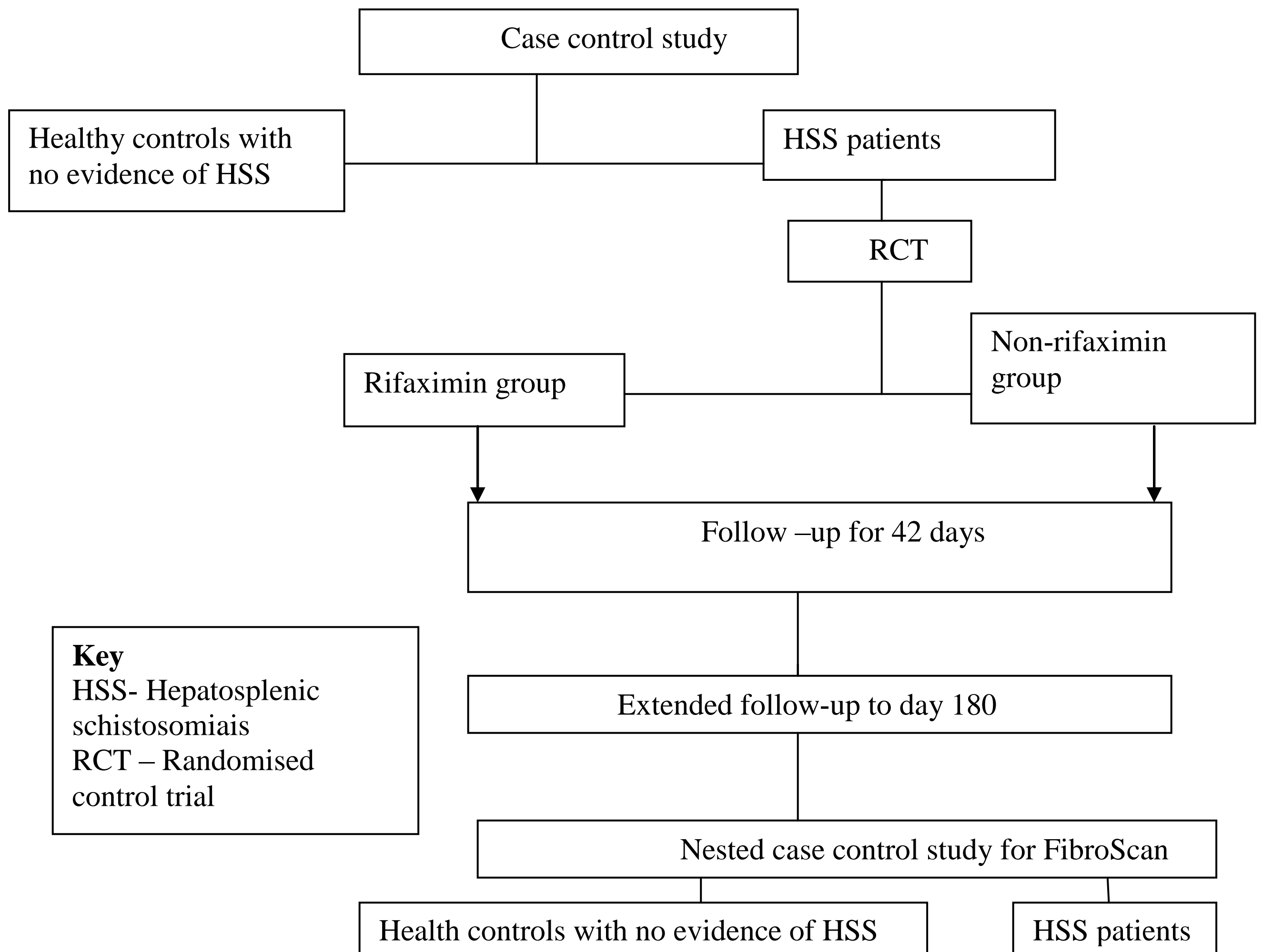


Fig 4-1: Study design flow chart

4.1 Case control study

The case control study was designed to ascertain whether there was evidence of bacterial translocation in HSS and to evaluate inflammation and fibrosis in these patients.

4.1.1 Study site

Patients with variceal bleeding or hypersplenism related to schistosomiasis were recruited from gastrointestinal (GI) clinic / endoscopy unit and medical wards at the University Teaching Hospital in Lusaka, Zambia between September 2012 and June 2014. Controls were recruited from the endoscopy unit alongside the cases during the same period.

4.1.2 Inclusion criteria for cases

1. Haematemesis and/or splenomegaly
2. Oesophageal and or gastric varices
3. Positive serology for schistosomiasis
4. Ability to give consent

4.1.3 Exclusion criteria for cases

1. Less than 18 years old
2. Inability to give consent
3. Cirrhosis
4. Sero- positive for HIV, HBV or HCV

4.1.4 Inclusion criteria for controls

1. Normal gastroscopy
2. 18 years and above

3. Able to give consent

4.1.5 Exclusion criteria for controls

1. History of haematemesis or rectal bleeding
2. Inability to give consent
3. Sero-positive for HIV, HBV or HCV

Patients with portal hypertension who were sero-positive for HIV were excluded from the study because HIV is known to be associated with bacterial translocation (Arjona et al.,2011) and so could be a confounder. Hepatitis B & C viral infections cause inflammation of the liver and so were excluded because they are potential confounders. Patients with cirrhosis of any cause were also excluded. Cirrhosis is the main cause of portal hypertension worldwide and therefore in this study it could have been a confounder.

4.1.6 Sampling methods and sample size

Sequential patients were recruited and of the 113 patients with HSS evaluated 70 fulfilled the criteria and were enrolled. Forty-one (41) controls alongside the cases were enrolled. This was not a matched case control study.

4.1.7 Clinical and laboratory information

The case definition for HSS was portal hypertension with oesophageal and or gastric varices including evidence of periportal fibrosis on abdominal ultrasound and a positive serology for schistosomiasis. Controls were individuals who came for endoscopy with non-specific abdominal complaints. They were healthy looking individuals with no complaints of gastrointestinal (GI) bleeding and had

no evidence of HSS or portal hypertension. They had normal upper endoscopy and normal abdominal ultrasound. The questionnaire was administered to the participants for clinical evaluation focusing on capturing basic demographic data, past medical history and social history. A thorough physical examination was performed on all the patients and controls. Abdominal ultrasound was done on both cases and controls. Blood samples (about 10ml from each patient or control) were drawn to check for full blood count, biochemistry, markers of translocation, and markers of inflammation and fibrosis. In this case control study, to measure bacterial translocation plasma lipopolysaccharide binding protein (LBP) was used. Quantitative polymerase chain reaction (PCR) for the bacterial 16S rRNA gene was used to measure bacterial DNA which also acted as an indicator of bacterial translocation. Plasma sCD14, sCD163, TNFR 1, IL1β, IL 6 and CRP were measured as markers of inflammation. To quantify liver fibrosis, plasma hyaluronan and laminin were measured.

4.2 Randomised control trial (RCT)

The clinical trial was designed as a phase II randomised control trial (RCT). This was an open label trial but the laboratory personnel were blinded to treatment allocation since the primary outcome was laboratory based. The RCT was designed to evaluate the impact of rifaximin on markers of bacterial translocation (BT), inflammation and fibrosis. Rifaximin is a non-absorbable antibiotic which has shown to reduce microbial translocation and inflammatory markers in patients with HIV infection (El-Shabrawi et al., 2012). It also has been used effectively in treatment and secondary prevention of hepatic

encephalopathy in patients with cirrhosis (Bass et al., 2010; Kimer et al., 2014; Puxeddu et al., 1995) and also key in reducing portal pressure in these patients by intestinal decontamination thereby reducing plasma endotoxins (Vlachogiannakos et al., 2009). It is a potent broad spectrum antibiotic which is effective against gram- negative, gram- positive, aerobic and anaerobic enteric bacteria (Flamm 2011; Koo et al., 2012). Therefore, the use of rifaximin in this study was to reveal evidence of bacterial translocation and not to investigate its efficacy. Also this study was not intended to evaluate the safety of the drug as the drug is safe and used routinely in clinical practice (Bajaj et al., 2015).

4.2.1 Study site

Patients with schistosomal related portal hypertension were recruited from gastrointestinal (GI) clinic / endoscopy unit and medical wards at the University Teaching Hospital in Lusaka, Zambia between June 2014 and January 2016.

4.2.2 Inclusion criteria

1. Haematemesis and/or splenomegaly
2. Oesophageal and or gastric varices
3. Positive serology for schistosomiasis
4. Ability to give consent

4.2.3 Exclusion criteria

1. Less than 18 years old
2. Inability to give consent
3. Cirrhosis
4. Sero- positive for HIV, HBV or HCV

4.2.4 Sampling methods and sample size

Assuming 95% confidence interval (CI), with a 30% reduction in bacterial translocation in HSS, a sample size of 40 subjects in each arm was sufficient with power of 80% to show a significant difference. This was calculated using Fleiss statistical methods in openEpi version 2. Sequential patients with HSS were recruited from June 2014 to January 2016 and of the 186 patients who had portal hypertension, 85 fulfilled the criteria.

4.2.5 Randomisation

Eight five (85) patients who fulfilled the criteria were randomised into rifaximin & standard care or standard care only. The rifaximin group consisted of 44 patients while non rifaximin group had 41 patients. Randomization was carried out using a list of codes prepared by an independent advisor with random number generator. The treatment allocation was decided by referring to the code list. Blinding was not possible as funds were insufficient to pay for a matching placebo.

4.2.6 Rifaximin

Rifaximin used in this study was donated by Norgine Pharmaceuticals, United Kingdom. Norgine Pharmaceuticals donated the investigational medicinal product but had no role in data acquisition, analysis, or interpretation.

4.2.7 Clinical, laboratory and follow-up information

The patients who were randomised into rifaximin and non-rifaximin groups were followed up for 42 days. These patients with HSS were evaluated on initial recruitment and after 42 days by administering a questionnaire to capture

clinical data and some demographic information. A thorough physical examination was done on all patients on recruitment and on day 42. Abdominal ultrasound was also performed during recruitment. Blood samples about 10ml were drawn on the initial visit and day 42 to check for markers of inflammation, fibrosis and translocation. Blood was also used to check for renal and liver function tests including full blood count. In this clinical trial, LBP and LPS were markers of bacterial translocation while sCD14, TNFR 1 and IL1β measured inflammation. Hyaluronan was used to measure liver fibrosis.

The intervention group received rifaximin 600mg twice per day orally for 42 days in addition to the standard care. The non-intervention group received standard care only which included praziquantel 40mg per kg body weight given in two divided doses orally for a day and beta blockers mainly propranolol 40mg three times per day, escalating the dosage upwards as tolerated and aiming for resting pulse of less than 60 beats per minute. Patients received haematinics as needed whether they were on rifaximin or not.

4.2.8 End points of 42 day follow -up

- Changes in markers of bacterial translocation, inflammation and fibrosis
- Variceal bleeding episodes
- Clinical effect of beta blockade (change in resting pulse rate)
- Mortality

4.3 Extended follow -up of RCT participants

After 42 days of follow-up, the participants were followed up to day 180**.** The participants were now on just standard care without any interventional drug.

This follow- up was for clinical evaluation. The final evaluation of the participants was done on day 180 by administering a questionnaire to captures clinical data and they also underwent a thorough physical examination. Abdominal ultrasound was also done on day 180 to evaluate the splenic size, portal vein diameter and to check for ascites. About 10ml of blood was drawn to assess liver and kidney function as well as full blood count.

4.3.1 End points

- Variceal bleeding episodes
- Response to beta blockers
- Change in portal vein diameter
- Splenic regression
- Mortality

4.4 Nested case control study of FibroScan

To ascertain liver stiffness in patients with HSS a nested case control study was undertaken within the clinical trial.

4.4.1 Study site

The nested case control study took place within the clinical trial at the same hospital. This took place after the clinical trial had commenced because this is the time when the FibroScan machine became available due to a loan of the instrument from Professor Mathias Egger and Dr Michael Vinikoor.

4.4.2 Inclusion and exclusion criteria

The cases were the same people as in the clinical trial. Some controls were recalled from the first case control study while others were recruited as new controls. The controls were healthy looking individual with non-specific abdominal pains with no history of haematemesis or rectal bleeding and had normal gastroscopy. These were also sero-negative for HBV, HCV and HIV.

4.4.3 Sampling methods

Forty eighty sequential patients with HSS underwent transient elastography (FibroScan). A comparative group (controls) of 21 individuals was chosen from the endoscopy unit during the same period. FibroScan was also done on the controls.

4.4.4 Clinical and laboratory information

A questionnaire was administered to capture demographic data, medical history and social history. They also underwent a thorough physical examination. Blood from cases and controls was drawn for full blood count and renal & liver function tests. In the cases the inflammatory markers measured were TNFR1 and sCD14 while HA was measured as a marker of fibrosis. LBP was measured as the index of bacterial translocation. There were no enough assays to measure markers of fibrosis, inflammation and bacterial translocation controls.

4.4.5 Transient elastography (FibroScan)

This was carried out on 48 patients with schistosomiasis related portal hypertension and 21 controls.

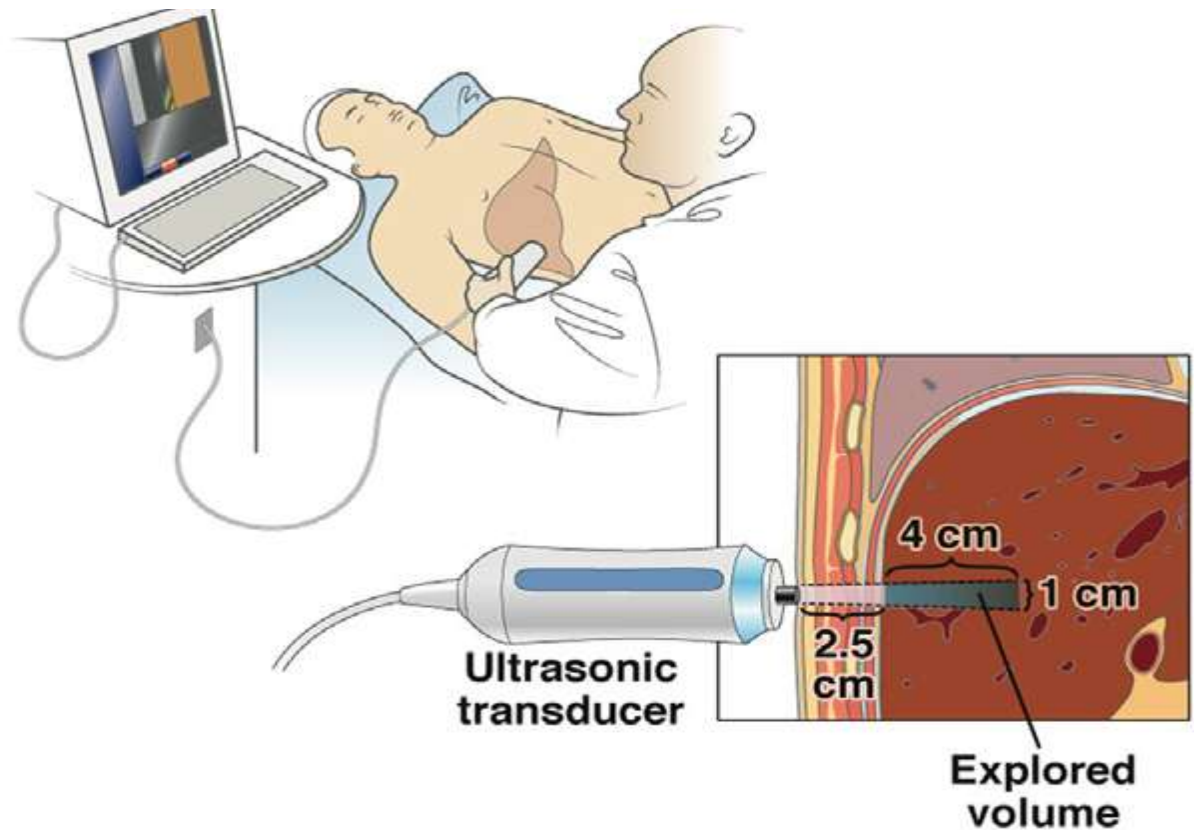


Figure 4-2: FibroScan demonstration

The figure above is a demonstration of how to measure liver stiffness on the right lobe of the liver in the intercoastal space using a FibroScan (Tapper et al., 2015). The transducer tip was placed on the lateral aspect of the liver on right side. Ten measurements were taken on each patient and stiffness was taken to be valid if the success rate was at least 60% and the median was taken as the representative measurement expressed in kiloPascals (kPa) (Mueller & Sandrin 2010; Kircheis et al., 2012). The FibroScan machine (model number: 402) used in this study was manufactured by Echosens, Paris, France in 2013.

4.5 Laboratory assays for all the studies

Approximately 10 ml blood was drawn from each individual at any given time in all the above studies. Part of this blood was put in EDTA bottle and taken for full

blood count using a Sysmex 800i analyser (Koke, Japan). The rest of the blood sample was centrifuged at 3000rpm at 4°C for 5 minutes. The plasma was aliquoted into triplicates and stored at -80°C.

4.5.1 Schistosomiasis serology microwell ELISA

This was a qualitative assay to determine antibodies in humans primarily IgG to schistosomal species using ELISA technique. This particular assay did not detect different species and was done using a microwell ELISA (SCIMEDX Corporation, Denville, New Jersey, USA). The plasma for this particular assay was diluted up to 40 times using dilution buffer provided in the kit.The cut-off used was greater or equal to 0.2 OD units.

4.5.2 Lipopolysaccharide (LPS) assay

Limulus amabocyte lysate chromo (LAL) was an ELISA assay used to detect LPS in the plasama samples. This was manufactured by Associates of Cape Cod Incorparated, 124 Bernard Saint Jean Drive, Falmouth, MA 02536, USA. Control Standard Endotoxin (CSE) was reconstituted with 8.5ml reagent water to obtain a 1000EU/ml concentration. This was left overnight at 2-8 degrees Celsius. 1000 EU/ml CSE was further diluted to working concentrations of 5.12EU/ml, 2.56EU/ml, 1.28 EU/ml, 0.64EU/ml, 0.32EU/ml, 0.16EU/ml, 0.08EU/ml and 0.04EU/ml. These constituted the standard curve values. Samples were thawed out. 1µl of the sample was added to 999µl of LAL reagent water. LAL pyrochrome was reconstituted with 3.2 ml pyrochrome reconstitution buffer. 50µl of sample was plated onto an endotoxin-free ELISA plate. 50µl of pyrochrome was then added to each well containing sample. The plate was

incubated for 32 minutes at 37 degrees celsius. 50% acetic acid was used to stop the reaction with 50µl. The plate was read at 405 nm.

4.5.3 16S rRNA gene

DNA was extracted using plasma Qiagen DNA extraction kit according to the manufacturer's instructions. Quantitative PCR for the 16S rRNA gene was done to check for bacterial DNA as an indication for bacterial translocation.

4.5.4 Human Lipopolysaccharide Binding Protein (Human LBP)

This protein was used as a surrogate for bacterial translocation (Stehle et al., 2012; Koutsounas 2015; Gou et al., 2006; Arjona et al., 2011; López et al., 2015). It was measured by an ELISA assay kit: Human LBP DuoSet (R&D Systems, Minneapolis, Minnesota, USA). The plasma from patients for this assay was diluted 2 times using dilution buffer.

4.5.5 Hyaluronan Immunoassay

This was measured by a Quantikine ELISA kit (R&D Systems, Abingdon, UK). The plasma sample was diluted 80 times using the dilution buffer provided.

4.5.6 Laminin

This assay was determined by Quantimatrix human laminin ELISA (MILLIPORE, Temecula, CA, USA). The instructions were followed according to the manufacture of the kit and the plasma sample was diluted 60 times using the dilution buffer provided in the kit.

4.5.7 C-reactive protein (CRP)

This is an acute inflammatory reactant produced mainly in the liver (Montecucco & Mach 2008; Zimmermann et al., 2014). It was determined by ELISA

Quantikine kit (R&D Systems, Abingdon, UK). The plasma samples were diluted 2000 times using a dilution buffer.

4.5.8 Interleukin 1 beta (IL-1β)

This is an inflammatory molecule produced mainly by the activated macrophages and is linked to innate immune response (Charles et al., 2008). It was measured by an ELISA Quantikine kit (R&D Systems, Abingdon, UK). The plasma was used neat (no dilution).

4.5.9 Interleukin 6 (IL-6)

This is an inflammatory cytokine and is produced by various cells in response to stimuli but mainly by monocytes & macrophages and has been reported to have both pro and anti-inflammatory properties. It is also associated with chronic liver disease (Giannitrapani 2013; Scheller et al., 2011). It was measured by an ELISA Quantikine kit (R&D Systems, Abingdon, UK). The plasma dilution factor was 5 using dilution buffer.

4.5.10 Tumour Necrosis Factor Receptor 1 (TNFR1)

This is constitutively expressed in a number of tissues and is known to respond to TNF alpha which is produced not only by macrophages but other cells like fibroblast, lymphoid cells, mast cells, endothelial cells and neuronal cells during an inflammatory process [142]. Once TNF-alpha binds to the receptor on the cell membrane soluble receptor is shed into the environment, so that TNF receptor 1 (also known as p55) is a stable reflection of the activation state of the TNF pathway. An ELISA Quantikine kit of R&D Systems of Abingdon, United Kingdom was used to assess TNFR1. The plasma samples were diluted 10

times using the dilution buffer provided in the kit.

4.5.11 Soluble CD14 (sCD14)

This marker rises in blood following monocyte and macrophage activation and it is also known to mediate bacterial LPS action (Ogawa et al., 2013; López et al., 2015). This molecule was determined by an ELISA Quantikine kit (R&D Systems Abingdon, UK). The plasma was diluted 400 times and the other steps were done as per instructions from the manufacturer.

4.5.12 Soluble CD163

This marker is increased in inflammatory diseases especially those with increased activity of macrophages including Kupffer cells (Etzerodt & Moestrup 2013). It was measured by an ELISA Quantikine kit (R&D Systems Abingdon, UK).

4.5.13 Sample analysis for all ELISA assays

All samples for all the assays above were run in duplicates. The means of the optic densities were then used to determine the concentration using a standard curve generated. The analysis was performed using Gen5 1.10 software on the Biotek EL 800 ELISA plate reader.

4.5.14 Full blood count and Chemistry

Full blood count was done on 5ml of the blood sample collected in the EDTA bottle using a Sysmex 800i analyser (Kobe, Japan). The biochemistry which included liver function tests and renal function tests were done using an ABX Pentra 400 machine manufactured in France.

4.6 Abdominal ultrasound

This was done on all patients who were recruited including controls. To do this a digital ultrasonic scanner (model P09, 2012, manufactured in Shenzen, China) was used. The abdominal ultrasound was done to check for periportal fibrosis, appearance of liver arctecture and ascites. Measurements for the portal vein (at the portal hepatis) and splenic size were also taken.

4.7 Data analysis for all the studies

Data were entered in Microsoft Excel and imported into Stata version 12.1 (Stata Corp College Station, Texas) and GraphPad PRISM 6.01 (GraphPad Software, San Diego, California, United States of America) for analysis. Most of the data were not normally distributed and so for description, median and inter-quartile ranges were used. To compare blood tests between controls and patients I used Wilcoxon rank-sum test and to compare blood tests between the cases I also used Wilcoxon rank-sum test. To compare measurements from baseline to day 42 and also from baseline to day 180 within the group I used Wilcoxon matched - pairs sign-rank test. For correlations I used Spearman's rank test. In the clinical trial intention to treat was used when analysing data. A p-value of less than 0.05 was considered significant.

4.8 Ethical consideration

Ethical review and approval was sought on 21st August 2012 (Ref: 006-07-12) from the University of Zambia Biomedical Research Committee and the Zambia Medicines Regulatory Authority (20th December 2013, (clinical trial number CT046/13)). Informed consent from participants and confidentiality was ensured

with names anonymised using codes and all records were kept under lock and key with only investigators having access to them. Standard care for HSS patients continued even if patients declined to be enrolled in the study. Informed consent was obtained from all participants in writing and the information sheet was explained in the language that participants understood. The benefits to the participants in this study were that patients were followed up and managed on a more regular and short intervals. Adverse event reports were submitted to the Research Ethics Committee and the Zambia Medicine Regulatory Authority.

CHAPTER 5: STUDY RESULTS

5.1 Results I: Case control study

In the case control study the aim was to search for molecular evidence of bacterial translocation and also to determine whether HSS is associated with elevated inflammatory and fibrotic markers in the blood. Of the 113 patients with varices who were evaluated from September 2012 to June 2014, 70 were recruited. The other 43 did not fulfil the criteria. Thirty-six (36) had negative serology for schistosomiasis, 2 were sero-positive for HIV and 3 were sero-positive for HBV. Forty-one (41) controls were recruited over the same period.

5.1.1 Clinical characteristics

Numbers of men and women were similar in patients and controls (Table 5-1). The commonest reason for referral for the patients was haematemesis (Table 5-1). History of previous exposure to water bodies through swimming or farming or fishing or drawing water was given by all patients except one (Table 5-1). Marked pancytopenia was found in patients compared to controls (Table 5-1). ALT values among cases and controls were similar, but AST was higher in cases (Table 5-1). Splenomegaly was noted in cases but not in controls.

Table 5-1: Demographic, clinical, laboratory and ultrasound data for patients and controls

Characteristic	Patients n=70		Controls n=41		*P*
Age in years	40 (30, 51)		32 (25, 38)		0.01
Gender, n & %	Male	36 (51%)	Male	20 (49%)	0.76
	Female	34 (49%)	Female	21 (51%)	
Exposure to water bodies; swimming/farming/fishing/ drawing water, n &%	Yes	69 (99%)	Yes	20 (49%)	<0.0001
	No	1 (1%)	No	21 (51%)	
Current alcohol intake, n & %	Yes	7 (10%)	Yes	8 (20%)	0.25
	No	63 (90%)	No	33 (80%)	
Past alcohol intake, n & %	Yes	21 (30%)	Yes	15 (37%)	0.53
	No	49 (70%)	No	26 (63%)	
History of jaundice	Yes	11 (16%)	Yes	2 (5%)	0.13
	No	59 (84%)	No	39 (95%)	
History of haematemesis	Yes	59 (84%)			
	No	11 (16%)			
Alanine aminotransferase (U/L)	25 (16,33)		20 (12,29)		0.09
Aspartate aminotransferase (U/L)	41 (33,55)		26 (21,31)		<0.0001
Albumin (g/l)	38 (34,40)		45 (42,47)		<0.0001
Haemoglobin (g/dl)	9 (6,11)		14 (13,16)		<0.0001
Platelet count (10^9/L)	52 (34,99)		189 (143,230)		<0.0001
White cell count (10^9/L	3 (2,4)		4 (4,7)		<0.0001
Main portal vein diameter (mm)	13 (12,15)		8 (8,9)		<0.0001
Splenic size (cm)	18 (16,19)		10 (9,10)		<0.0001

All continuous variables are represented as median with the interquartile range in parenthesis.

5.1.2 Parasitological findings

Of the 46 patients who submitted stool for parasitology 34 (73.9%) had no organism isolated, 4 (8.7%) had *Schistosoma* eggs, 2 (4.3%) had hook worms, 1 (2.2%) had ascariasis, 1 (2.2%) had *Enterobius vermicularis* and 4 (8.7%) had other organisms.

5.1.3 Endoscopic findings

In addition to oesophageal varices (Fig 5-2), 10 (14%) patients had gastric varices (Fig. 5-3) and 17 (24%) had gastropathy (Fig. 5-1).

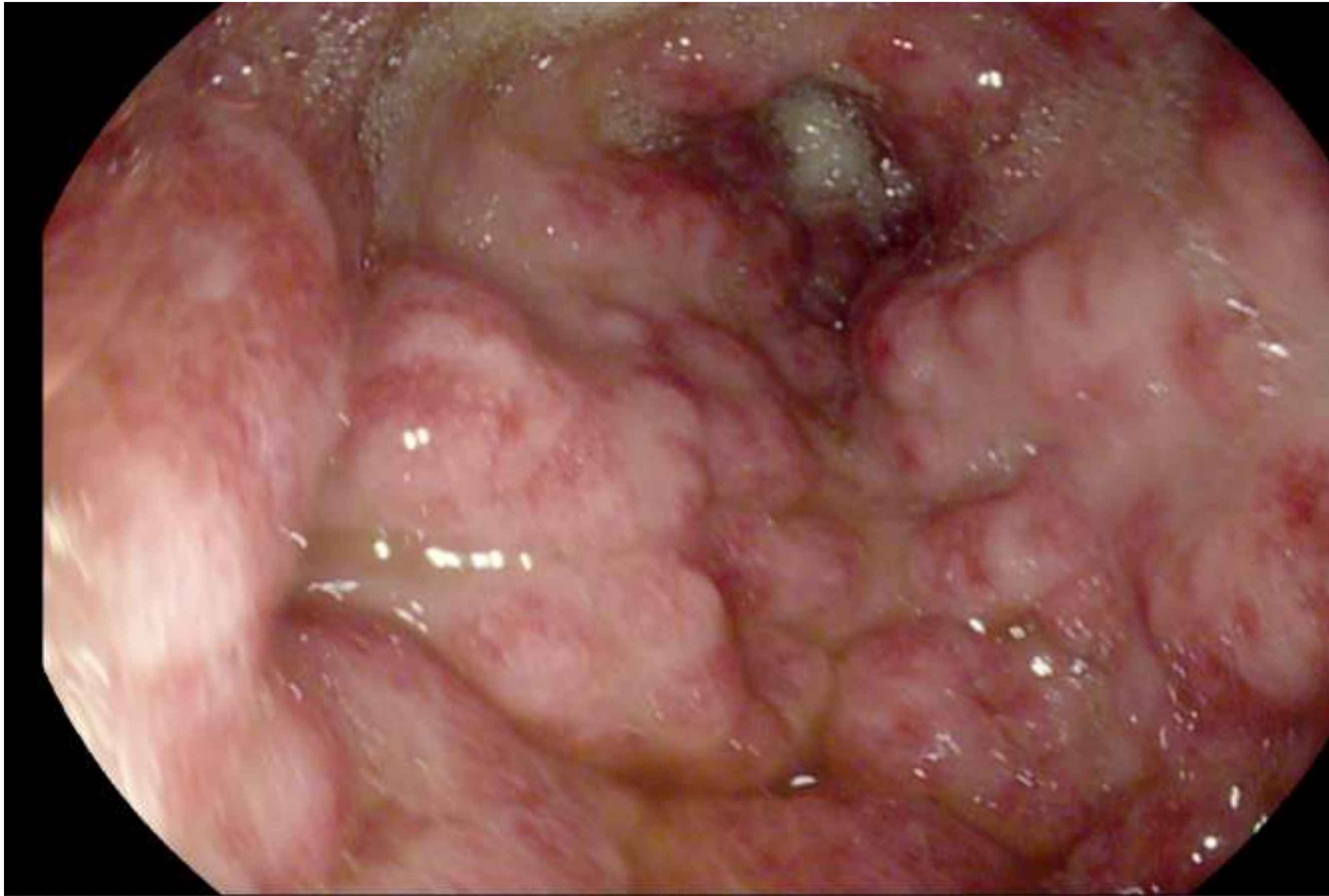


Figure 5-1: Portal hypertensive gastropathy

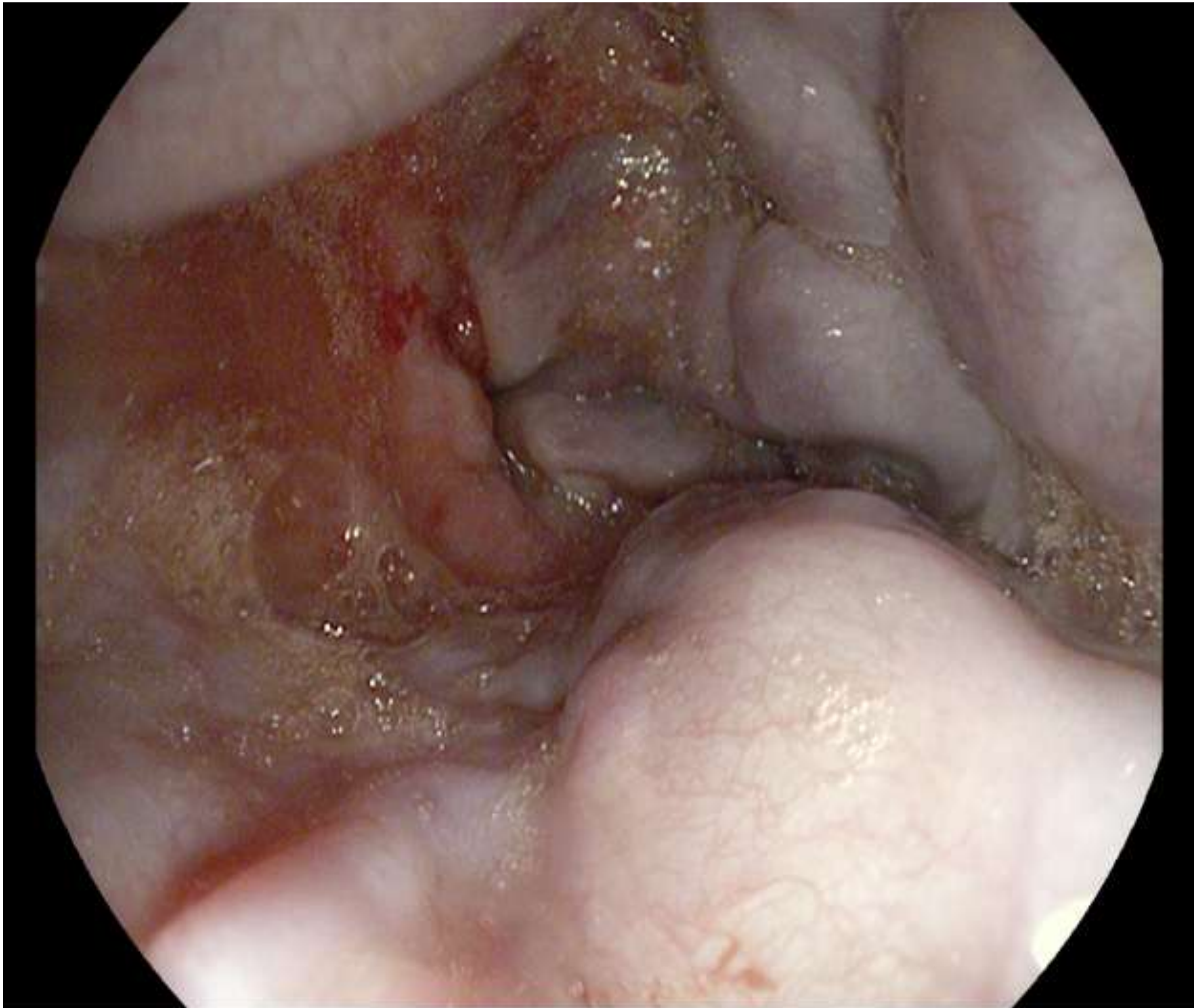


Figure 5-2: Oesophageal varices

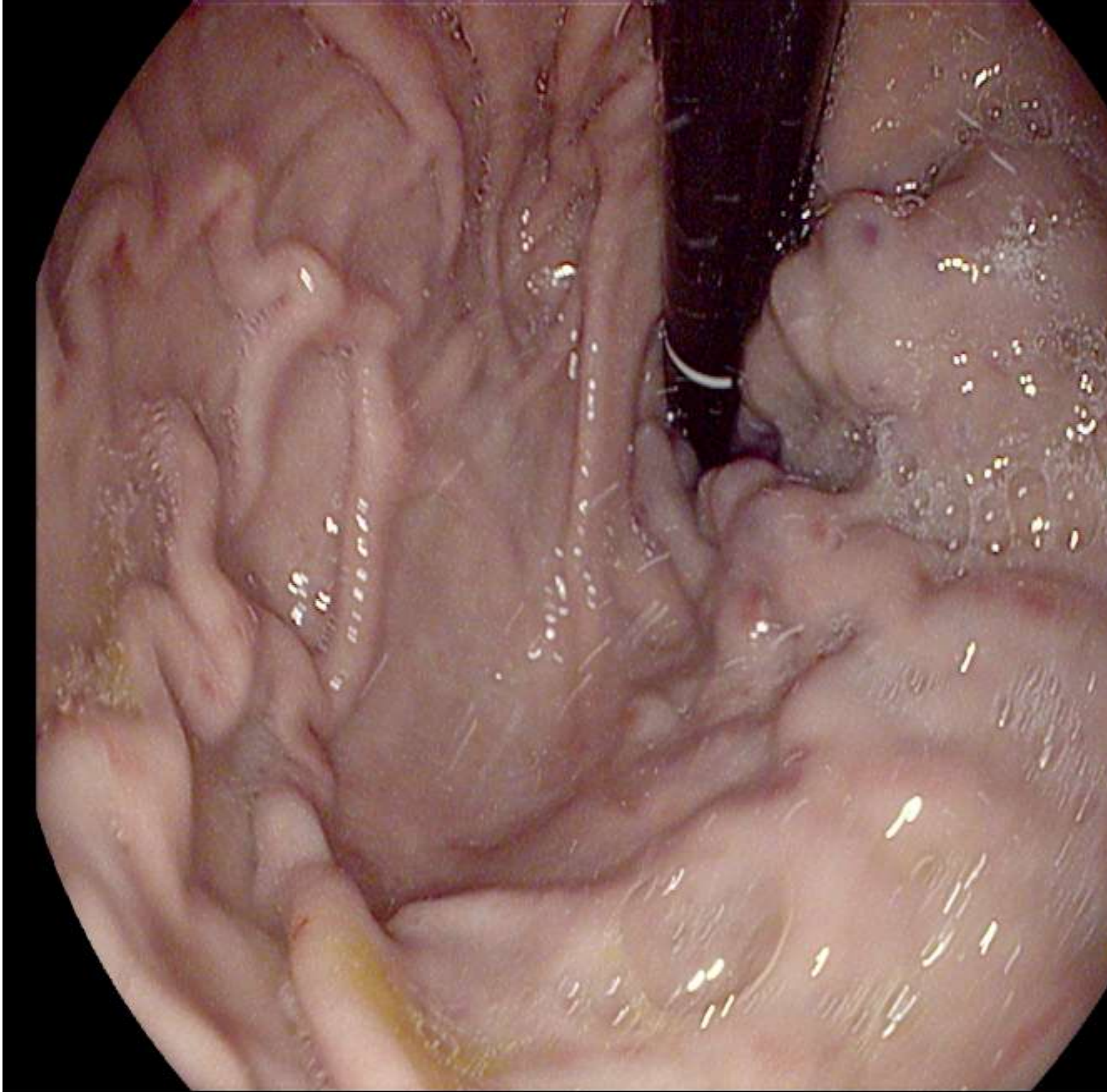


Figure 5-3: Gastric varices

5.1.4 Markers of translocation

LBP values were significantly elevated (P<0.0001) in patients compared to controls (Fig.5-4A). Bacterial PCR was positive in 19 (27%) of patients and 5 (13%) of controls (P=0.09).

5.1.5 Markers of fibrosis and inflammation

The patients showed elevated hyaluronan (HA) and laminin values in comparison with the controls (Fig. 5-4B & C).The inflammatory markers with the exception of CRP were higher in patients compared to controls (Fig. 5-5).

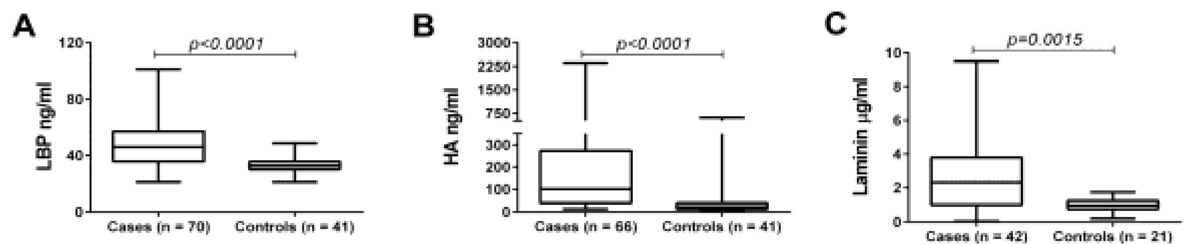


Figure 5-4: Markers of bacterial translocation (lipopolysaccharide binding protein, panel A) and fibrosis (hyarulonan & laminin, panel B & C) were significantly higher in cases than in controls.

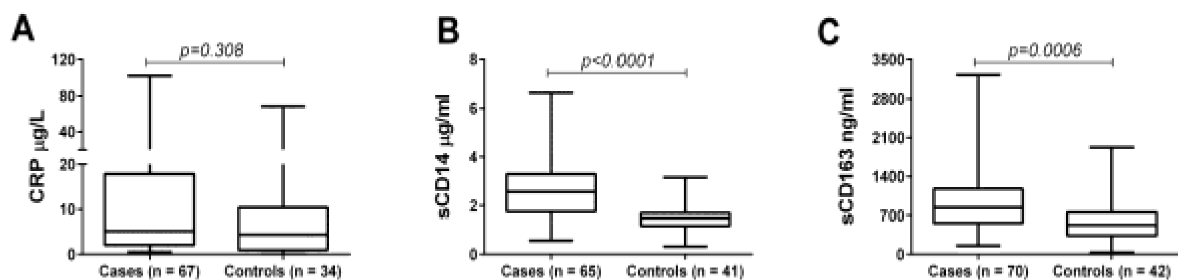


Figure 5- 5: C-reactive protein (CRP, panel A) did not differ between patients and controls, but sCD14 (panel B) and CD163 (panel C) were higher in cases.

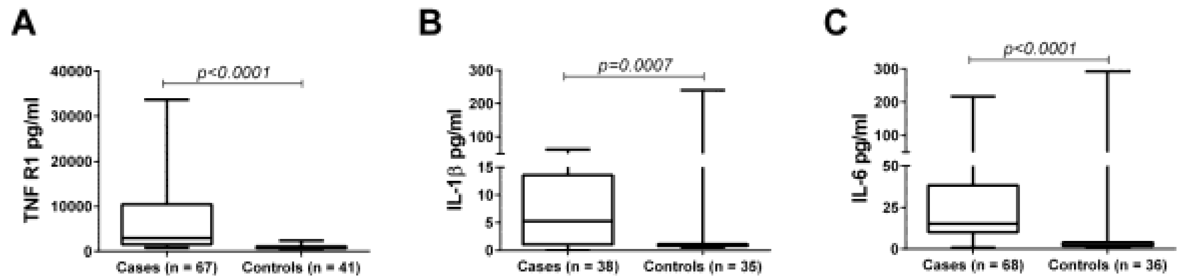


Figure 5-6: TNFR 1, IL-1 β and IL-6 concentrations were higher in cases than controls

5.1.6 Correlations between fibrosis, inflammation and translocation

Hyaluronan was directly correlated with TNF R1 (Fig.5- 7A), while LBP was positively correlated with TNF R1 (Fig.5- 7B), IL-1β and IL-6 (Fig.5- 7C & D). Other inflammatory markers such as sCD14, CRP and sCD163 did not show any significant correlation with fibrotic markers (hyaluronan & laminin) and there was also no significant correlation of LBP with these inflammatory or fibrotic markers. There was no significant correlation of portal vein diameter and the splenic size.

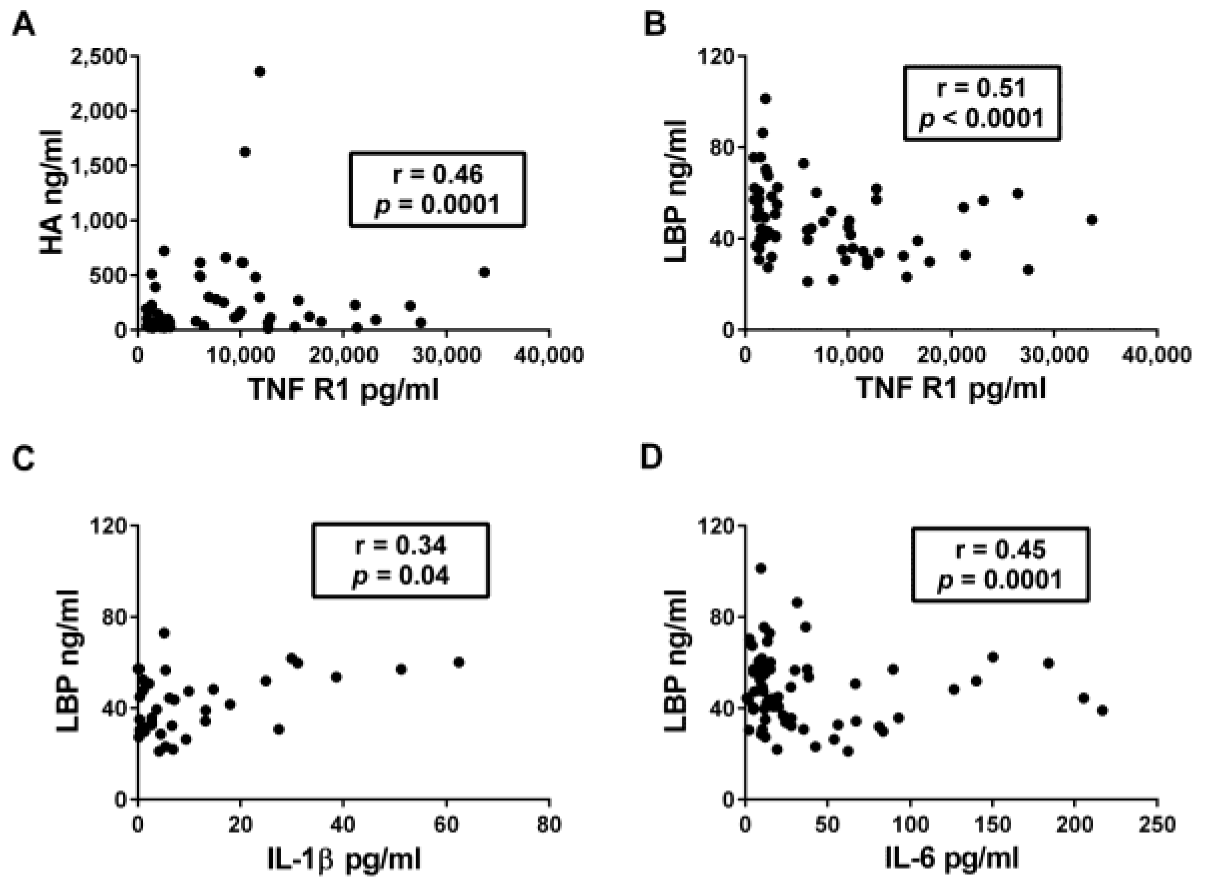


Figure 5-7: There were positive correlations of fibrotic marker (hyaluronan) with TNFR1 (panel A) while LBP showed positive correlations with TNFR1, IL-1β and IL-6 (panel B-D)

5.1. 7 Summary of the findings of the case control study

LBP a surrogate marker of BT was noted to be significantly elevated in HSS as compared to controls and the markers of inflammation were raised in cases as compared to controls except CRP. Fibrotic markers were significantly higher in HSS as compared to controls. This study also revealed positive correlations of LBP and inflammatory markers while HA showed a positive correlation with an

inflammatory marker (TNFR1) which has been described as pro-fibrotic in animal studies.

5.2 Results II: Clinical trial of rifaximin

Thirty-eight (38) patients from the case control study and 47 additional patients were recruited into the randomised control trial (RCT).

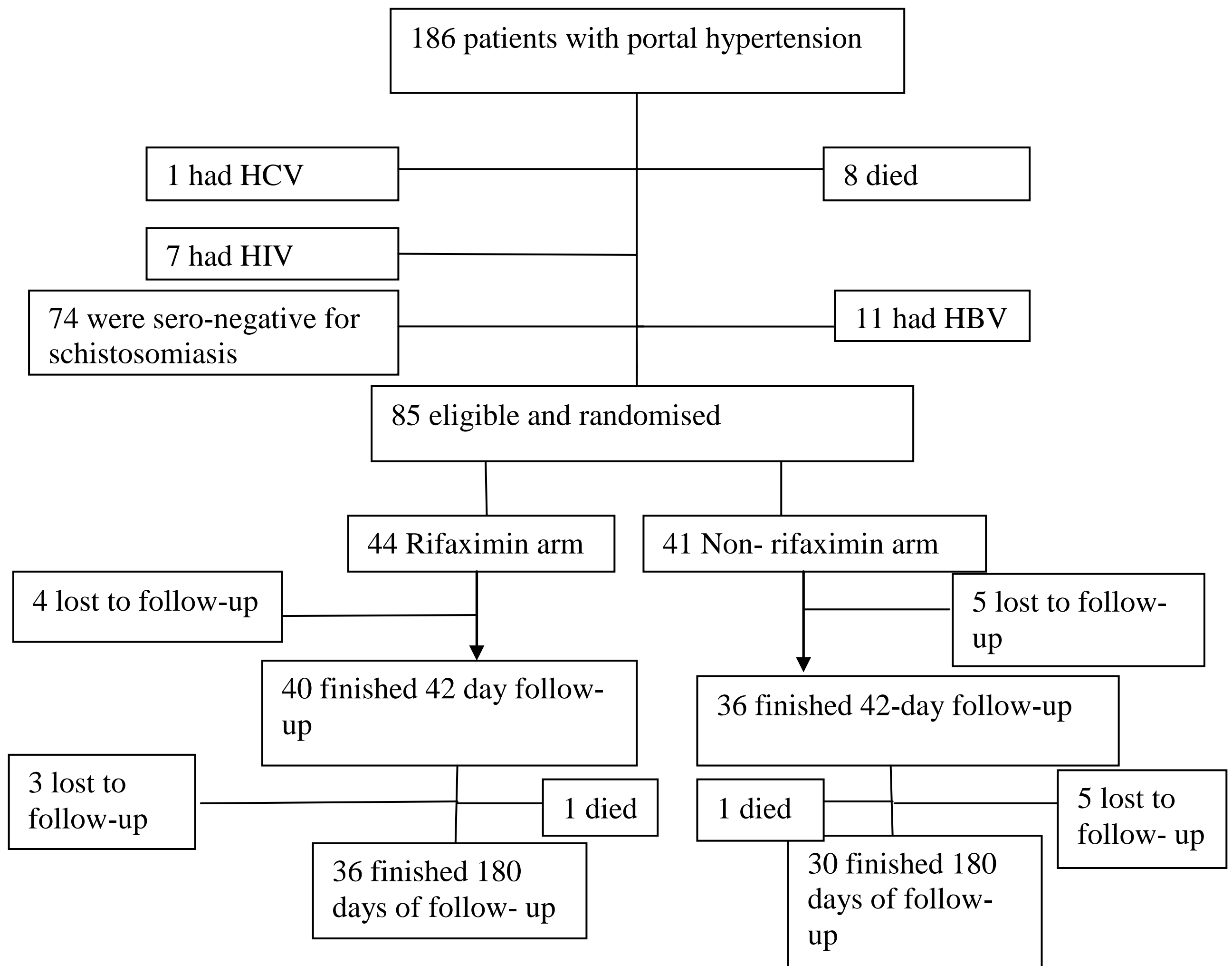


Figure 5-8: Clinical trial flow-chart

5.2.1 Clinical trial follow-up for 42 days

Of 186 patients who were approached and evaluated for recruitment, 101 were not eligible (Fig.5-8). The rifaximin and non-rifaximin groups did not differ much as the demographic information and some laboratory data in these two groups were quite comparable at baseline (Table 5-2

Table 5-2: Baseline demographic and laboratory information

Variable	Rifaximin group		Non-rifaximin group	
Age (years)	42 (30, 52)		38 (31,43)	
Gender	Females	22	Females	15
	Males	18	Males	25
BMI (kg/m^2)	21.6 (19.9, 23.2)		21.5 (20.3, 23.8)	
No of cases with History of haematemesis	Yes	25	Yes	25
	No	15	No	15
Pulse rate (beats/min)	76 (66, 82)		70 (64, 76)	
Haemoglobin (g/dl)	7.5 (6.2, 11.3)		9.2 (6.6, 11.0)	
White cell count (x10^9/l)	2.7 (1.6, 3.5)		1.8 (1.6, 3.0)	
Platelet count (x 10^9/l)	49 (31, 78)		44 (24, 64)	
MCV (fl)	80 (67, 87)		79 (70, 83)	
MPV (mm)	12.7 (11.2, 13.9)		12.1(10.8, 13.6)	
16S rRNA copies/µl	129(23, 499)		51 (19, 112)	
LPS (ng/ml)	74 (38, 157)		154 (45, 649)	
LBP (ng/ml)	27 (23, 30)		43 (38, 50)	
TNFR1 (ng/ml)	1443 (1232, 1911)		1495 (1238, 1657)	
sCD14 (ng/ml)	2402 (1930, 2798)		1487 (1299, 1964)	
Hyaluronan (ng/ml)	125 (57, 189)		102 (71, 176)	

All parameters are represented as median with interquartile range in parenthesis.

Key

BMI – body mass index

TNFR1- tumour necrosis factor receptor 1

MCV- mean cell volume

sCD14- soluble cluster of differentiation

MPV – main portal vein diameter

LPS – lipopolysaccharide

LBP- lipopolysacharide binding protein

BMI – body mass index

TNFR1- Tumour necrosis factor receptor 1

MCV- mean cell volume

sCD14- soluble cluster of differentiation

MPV – main portal vein diameter

LPS – lipopolysaccharide

LBP- lipopolysacharide binding protein

5.2.2 Blood tests and pulse rate after 42 days of follow-up

The full blood count picture after 42 days of follow up did not change much except the increase in haemoglobin in the two groups (Table 5-3). Pulse rate fell, presumably an effect of beta blockade, in both groups (Table 5-3).

Table: 5-3. Comparisons of pulse and full blood count between baseline and day 42

Variable	Rifaximin baseline	Rifaximin day 42	*P*	Non-rifaximin baseline	Non-rifaximin day 42	*P*
Pulse (b/min)	76 (66, 82)	65 (60, 72)	0.01	70 (64, 76)	66 (62, 70)	0.07
Hb (g/dl)	7.5 (6.2, 11.3)	11.5 (9.0, 13.0)	0.0006	9.2 (6.6, 11.0)	10.3 (8.50, 12.40)	0.03
MCV (fl)	80.0 (67.4, 87.4)	76.0 (66.5, 86.0)	0.09	79.0 (70.2, 82.7	79.4 (68.1, 91.9)	0.82
WCC ($x10^9$/l)	2.7 (1.6, 3.5)	2.0 (1.6, 2.5)	0.04	1.8 (1.6, 3.0)	2.2 (1.6, 3.5)	0.67
RBC ($x10^{12}$/l)	3.4 (2.7, 4.3)	4.5 (4.0, 4.9)	0.0003	3.6 (3.0, 4.4)	4.3 (3.6, 5.2)	0.06
PLT ($x10^9$/l)	49.0 (30.5, 77.5)	42.0 (28.0, 72.0)	0.61	44.0 (24.0, 64.0)	38.0 (25.0, 52.0)	0.74

Key;

Hb- haemoglobin

MCV- mean cell volume

WCC- white cell count

RBC- red blood cell count

PLT- platelet count

The 16S rRNA the direct marker of BT reduced in the rifaximin group compared to non-rifaximin group after 42 days (Figures 5-9, 5-10). TNFR1 and sCD14, the markers of inflammation considered in the clinical trial reduced after 42 days of rifaximin (Figures 5-11, 5-12, 5-13, 5-14). IL1 β concentrations were below the threshold level of detection and are not discussed further. There was no significant change in the plasma concentrations of LPS, LBP and HA after 42 days of rifaximin (Figures 5-15, 5-16, 5-17, 5-18, 5-19, 5-20).

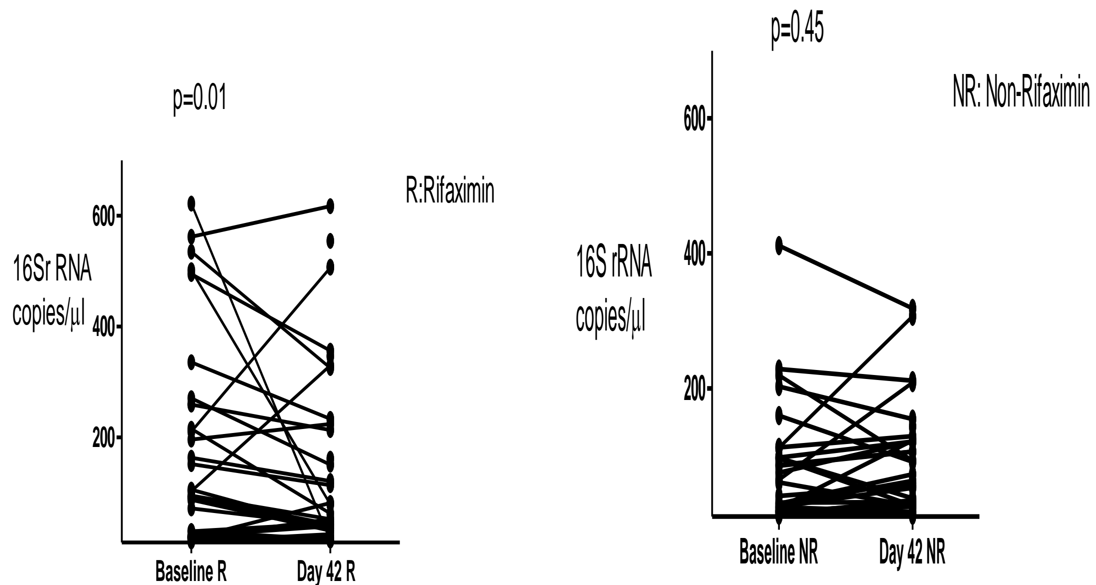


Figure 5-9: 16S rRNA copies fell in the rifaximin group while in the non-rifaximin group they remained the same over the 42 -day period

Figure 5-10: When expressed as change of (log) 16S rRNA copies, the difference between the two groups is clear

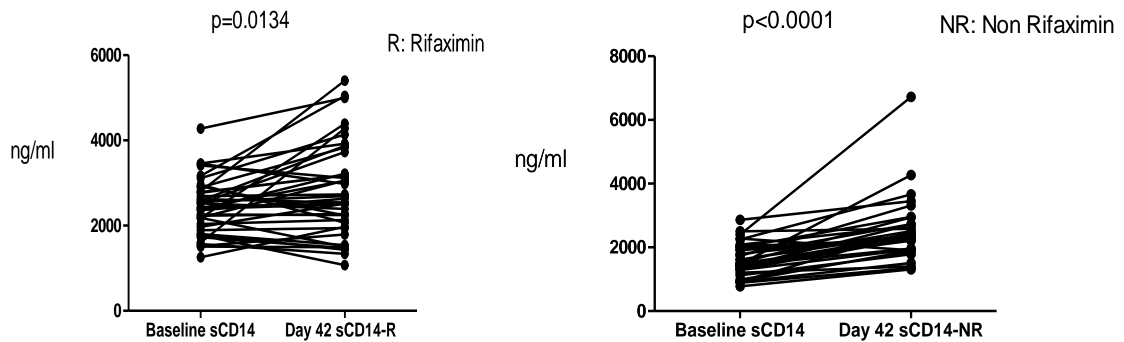


Figure 5-11: Soluble CD14 (sCD14) concentrations fell in the rifaximin group over 42 days, while in the non-rifaximin group they went up

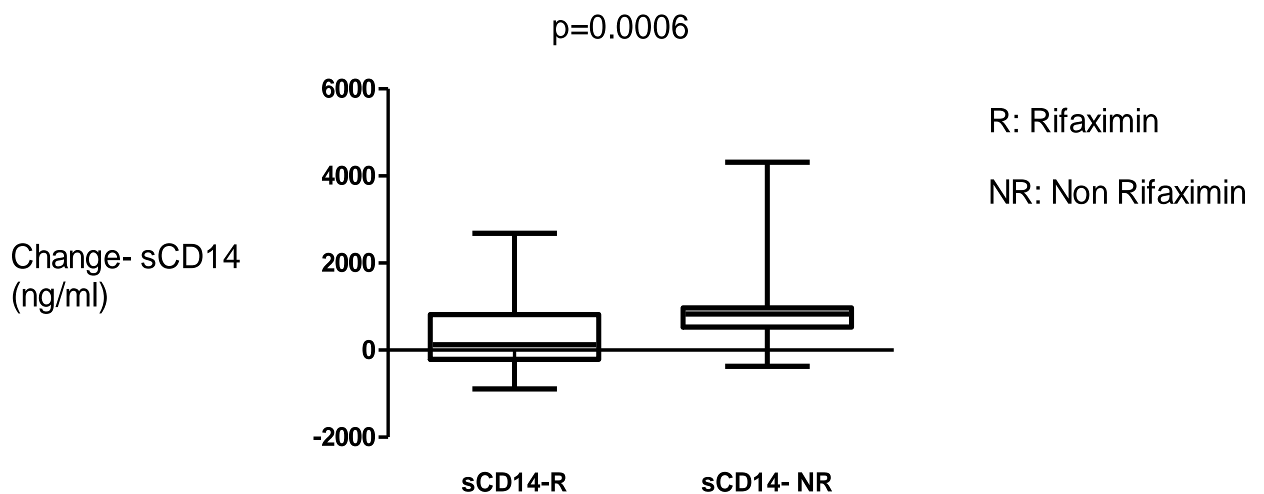


Figure 5-12: When expressed as change in sCD14, the difference between the rifaximin group and the non-rifaximin group is clear.

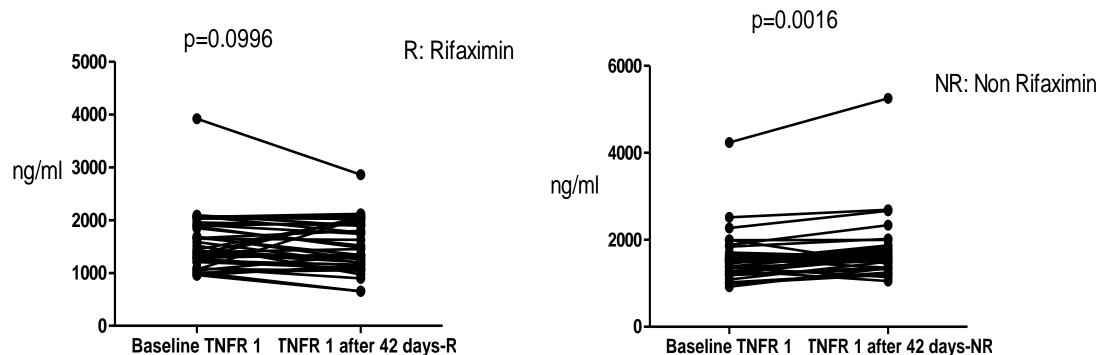


Figure 5-13: Tumour necrosis factor receptor 1 (TNFR1) concentrations fell (but non-significantly) in the rifaximin group over 42 days, while in the non-rifaximin group they went up

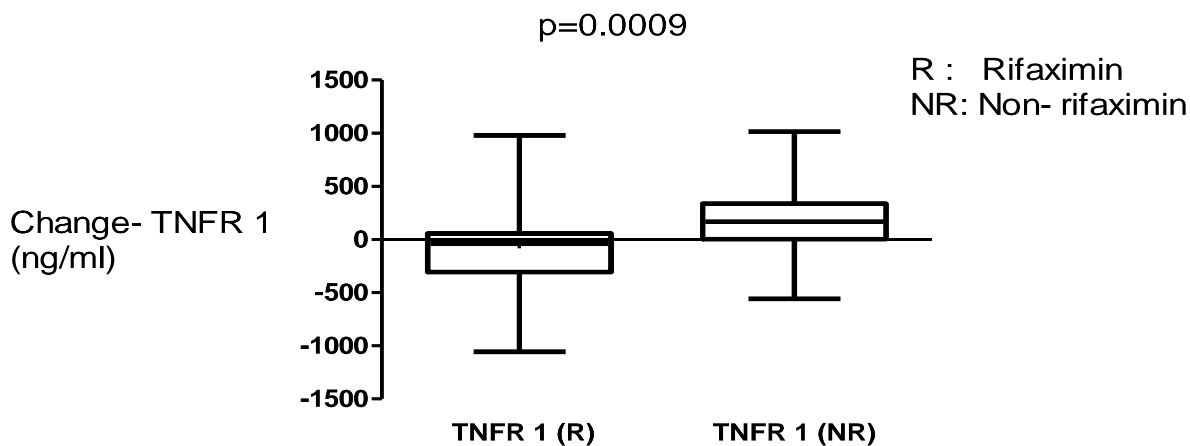


Figure 5-14: When expressed as change in TNFR1 the difference between the rifaximin group and the non- rifaximin group is clear

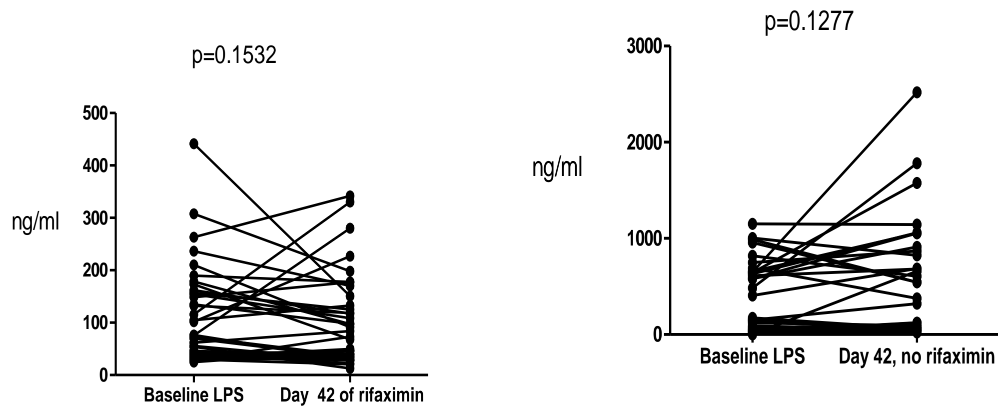


Figure 5-15: Lipopolysaccharide (LPS) concentrations did not show a significant change over 42 days in either the rifaximin or non- rifaximin groups

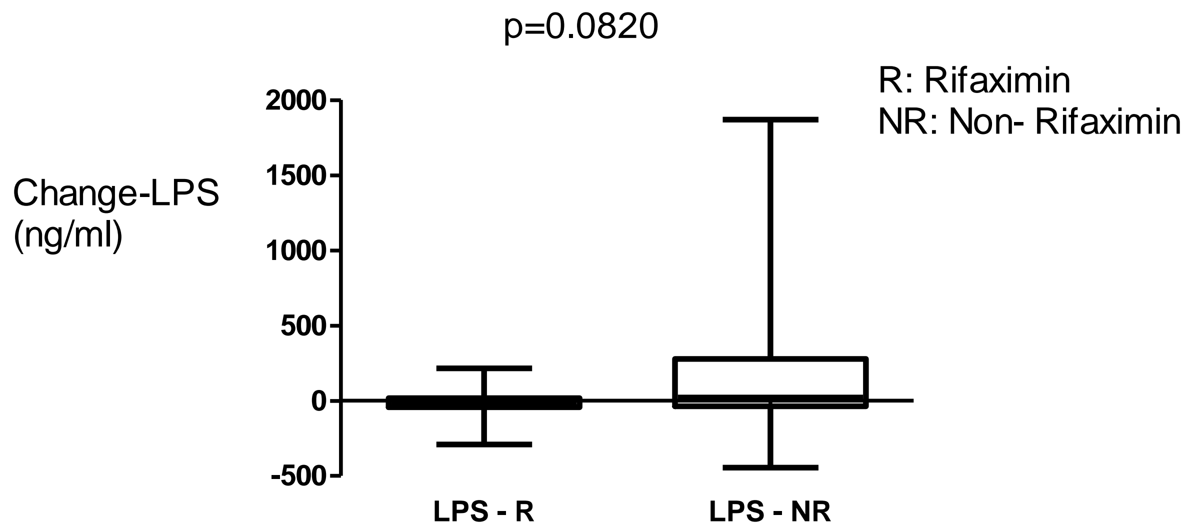


Figure 5-16: There was no significant change in lipopolysaccharide (LPS) concentrations in either group over 42 days

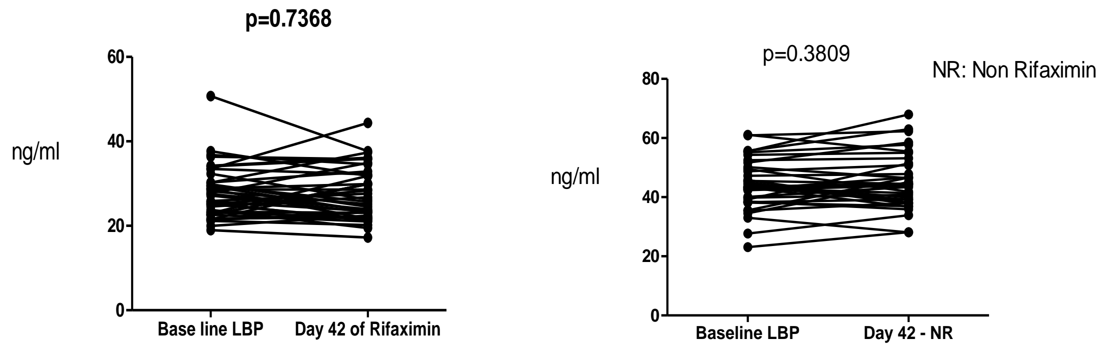


Figure 5-17: There was no significant change in lipopolysaccharide binding protein (LBP) concentrations in either group over 42 days

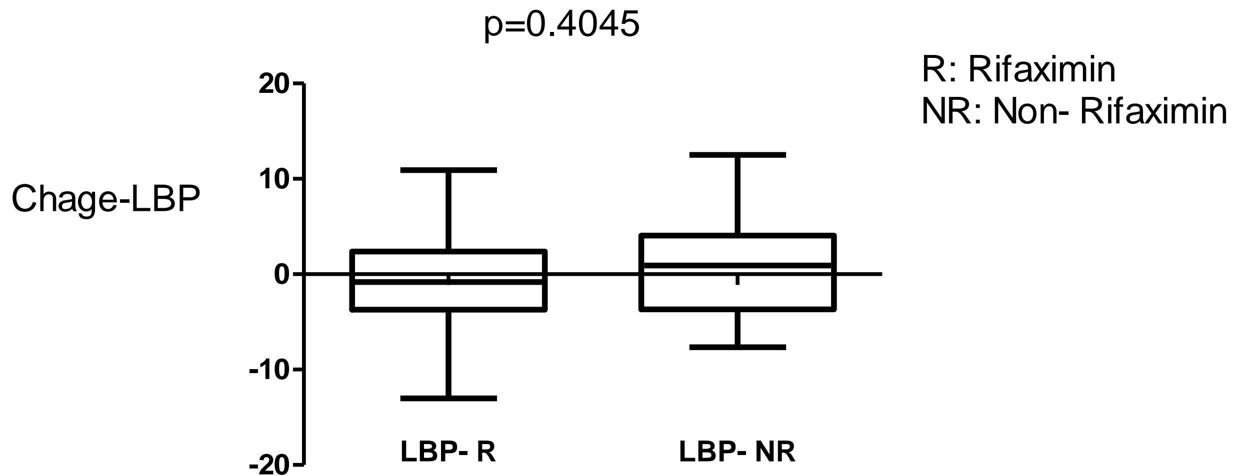


Figure 5-18: There was no significant change in LBP after 42 days in either rifaximin or non -rifaximin groups

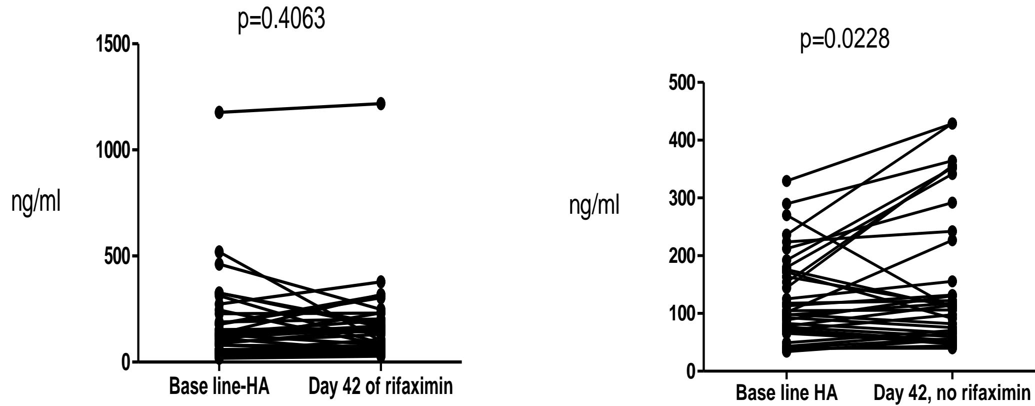


Figure 5-19: Hyaluronan (HA) concentrations did not change in the rifaximin group but rose after 42 days in the non-rifaximin group

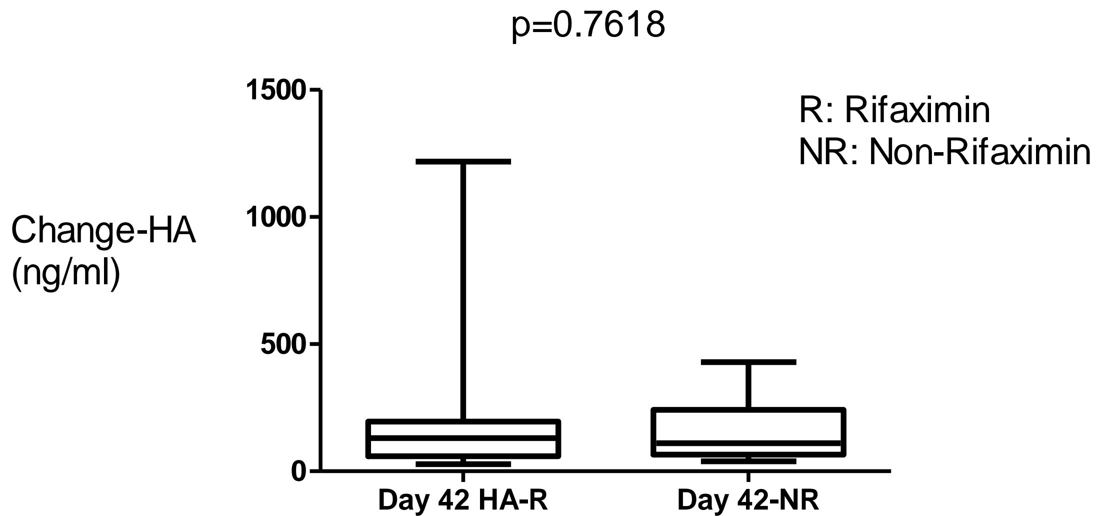


Figure 5-20: The change in HA after 42 days in rifaximin and non-rifaximin groups was not significantly different

5.2.3 Adverse events during 42-day follow-up

There were no major adverse events or mortality recorded in the 42-day period of follow –up except for one patient in the rifaximin group who reported history of

rectal bleeding on one occasion during this period. Another patient reported abdominal pain and discomfort one week after taking rifaximin but this resolved within the few days without any intervention.

5.2.4 Summary of the findings of 42-day follow-up in the clinical trial

After 42 days in the clinical trial sCD14 concentrations went down in the rifaximin group while in the non-rifaximin group the concentrations went up. The other inflammatory marker (TNFR1) measured in this clinical trial had similar trend as sCD14 after 42 days. There was no significant change of LPS after 42 days.

5.3 Result III: Follow up after day 42 to day 180

5.3.1 Clinical characteristics

All patients after 42 days in the trial were followed up where possible on standard care up to day 180. Follow up was achieved to 180 days in 36 patients in the rifaximin group and 30 patients in the non-rifaximin group. By the end of this period, several episodes of haematemesis had been reported (Table 5-4). Non-compliance to propranolol was noted more in the rifaximin group compared to the non-rifaximin group, while the radial pulse rate was comparable in both groups (Table 5-4). However, there was a significant reduction in radial pulse rate in the rifaximin group between baseline and day 180 while the reduction was not significant in the non-rifaximin group but delta values were similar between rifaximin and non- rifaximin groups (Figures 5-21 & 5-22). A small number had ascites, 6 (17%) in the rifaximin group at day 180 compared to 9 (23%) at baseline (p=0.5150). In the non- rifaximin group, 8 (28%) had ascites

after day 180 compared to 7 (21%) at baseline (p=0.5012).

Table 5-4: Comparisons of clinical variables between two groups at day 180

Variable	Rifaximin group n=36	Non-Rifaximin group n=30	*P*
Number of patients with history of haematemesis after 180 days of follow-up	3	4	0.54
Number of patients not compliant to propranolol	8	5	0.60
Pulse (beats/min)	65 (60,70)	64 (58,69)	0.43
Portal vein diameter (mm)	10 (9, 13)	11 (8, 13)	0.97
Splenic size (cm)	17 (15,20)	18 (17,19)	0.34
White cell count ($x10^9/l$)	1.9 (1.4,2.5)	2 (1, 3)	0.56
Red cell count ($x10^{12}/l$)	4.4 (3.9, 5.2)	4.4 (3.8, 4.9)	0.34
Platelet count ($x10^9/l$)	34 (22, 80)	40 (16, 53)	0.60
Haemoglobin (g/dl)	12 (11, 14)	10 (8, 13)	0.03

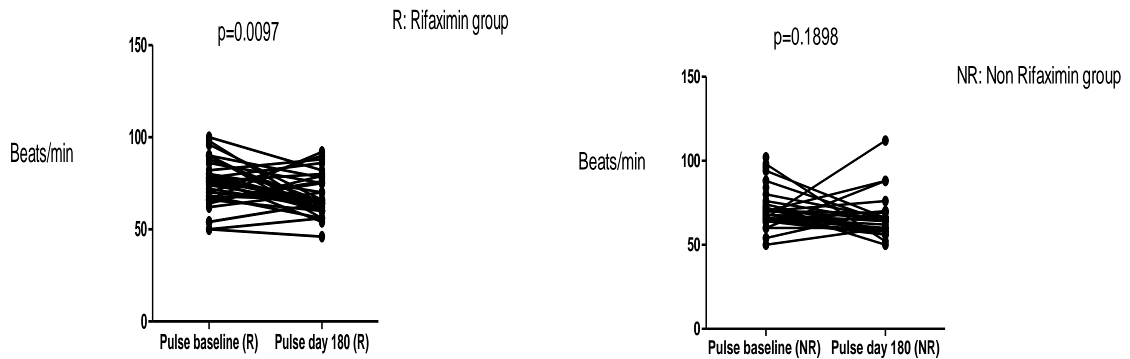


Figure 5-21: There was a significant reduction of radial pulse rate on day 180 in rifaximin group compared to non-rifaximin group

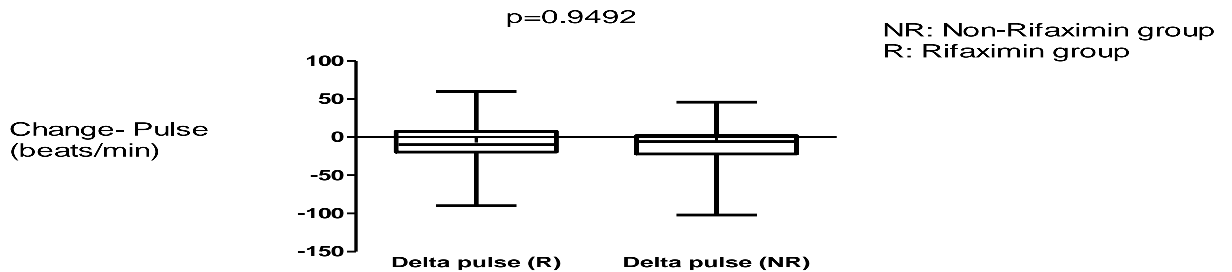


Figure 5-22: When expressed as change, pulse rate between the rifaximin and non-rifaximin groups after 180 days was similar

5.3.2 Abdominal ultrasound findings

Abdominal ultrasound was done on day 180 in both groups. This evaluated the portal vein diameter and the size of the spleen. The findings were comparable in both groups (Table 5-4). However, comparing baseline portal vein diameter and portal vein diameter on day 180 revealed a significant reduction in both groups (Fig. 5-21). The measurements of the spleen size at baseline and day 180 were

similar and this is further confirmed by the delta values (Figures 5-25, 5-26).

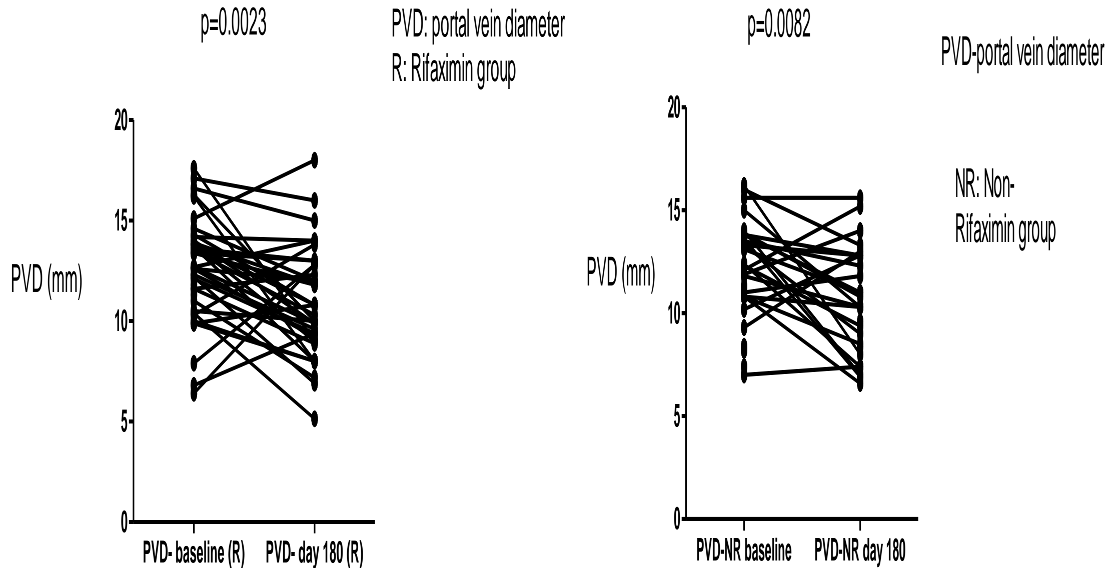


Figure 5-23: Portal vein diameter reduced after 180 days of follow-up in both groups

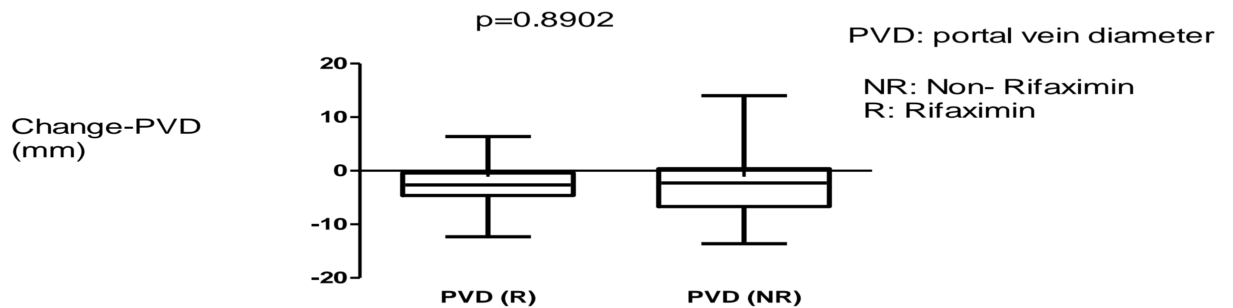


Figure 5-24: The change in portal vein diameter values between rifaximin and non-rifaximin groups after 180 days was similar

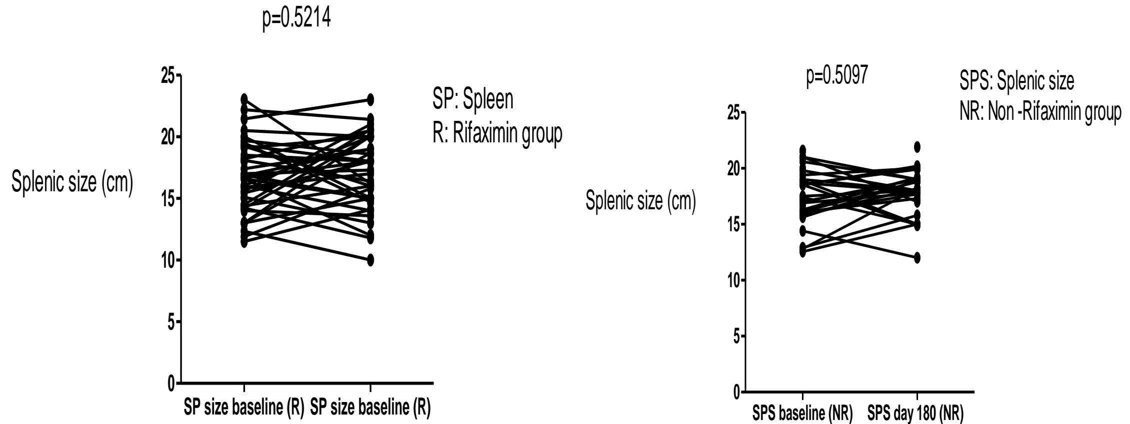


Figure 5-25: The splenic size between baseline and day 180 remained similar

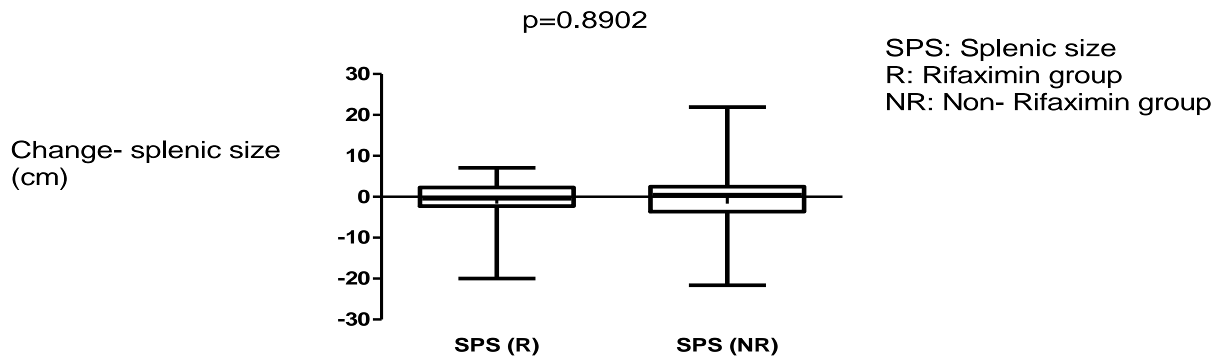


Figure 5-26: The change in values of splenic size between rifaximin group and non-rifaximin group was similar

5.3.3 Full blood count

The indices of full blood count in both groups showed lower and comparable values at day 180 except haemoglobin which was significantly higher in rifaximin group than in non-rifaximin group (Table 5-4).

5.3.4 Adverse events during follow-up between day 42 and day 180

Two severe adverse events were recorded during this time period (between 42

and 180 days). The first event was a patient in the rifaximin group who died 10 weeks after the last day of taking rifaximin. She died from acute diabetic complications of diabetic ketoacidosis complicating sepsis and renal failure. The second patient died in the 5thmonth of follow up. He was in the non-rifaximin group and died of massive variceal bleeding leading to hypovolaemic shock. Other patients reported history of haematemesis between day 42 and day 180 (Table 5-2). One patient from each group presented with jaundice by day 180.

5.3.5 Summary of findings during extended (180 day) follow- up

During the extended follow-up between day 42 and day 180 two mortalities occurred. These were not related to the trial drug. There were more episodes of variceal bleeding during this period compared to 42-day follow-up. At the end of 180 days there was reduction of portal vein diameter from baseline but the splenic size remained the same. The response to beta blockers was quite good.

5.4 Result IV: Nested case control study of FibroScan

This was designed to evaluate liver stiffness in patients with hepatosplenic schistosomiasis. It was carried out after the clinical trial had commenced because the FibroScan instrument (which is very costly) was not available at the beginning and a protocol amendment had to be sought to permit its use in this trial.

Demographic, ultrasound and full blood count data were not comparable between cases and controls except the body mass index (Table 5-5).

Table: 5-5. Basic demographic and laboratory data in a nested case control study

<table>
<tr><th></th><th colspan="2">Cases</th><th colspan="2">Controls</th><th>P value</th></tr>
<tr><td>Age (years)</td><td colspan="2">40 (31,36)</td><td colspan="2">32 (27, 35)</td><td>0.01</td></tr>
<tr><td rowspan="2">Gender</td><td>Females</td><td>25</td><td>Females</td><td>12</td><td rowspan="2">1.00</td></tr>
<tr><td>Males</td><td>22</td><td>Males</td><td>10</td></tr>
<tr><td>BMI (kg/m^2)</td><td colspan="2">22 (21, 25)</td><td colspan="2">23 (21, 26)</td><td>0.39</td></tr>
<tr><td>Spleen size (cm)</td><td colspan="2">17 (15, 18)</td><td colspan="2">10 (8, 11)</td><td>0.0001</td></tr>
<tr><td>Main portal vein (mm)</td><td colspan="2">12 (10, 14)</td><td colspan="2">8 (6, 8)</td><td>0.0001</td></tr>
<tr><td>WCC (x10^9/l)</td><td colspan="2">2.4 (1.6, 3.4)</td><td colspan="2">4.6 (3.8, 5.9)</td><td>0.0001</td></tr>
<tr><td>RBC (x10^{12}/l)</td><td colspan="2">3.4 (2.8, 4.4)</td><td colspan="2">4.7 (4.4, 5.4)</td><td>0.0001</td></tr>
<tr><td>Haemoglobin (g/dl)</td><td colspan="2">8 (6, 11)</td><td colspan="2">14 (12, 15)</td><td>0.0001</td></tr>
<tr><td>Platelet (x10^9/l)</td><td colspan="2">49 (27, 77)</td><td colspan="2">188 (172, 295)</td><td>0.0001</td></tr>
</table>

Key

BMI – body mass index

WCC- white cell count

RBC- red blood cell count

Transient elastography (FibroScan) was significantly pronounced in cases compared to controls (fig. 5-27).

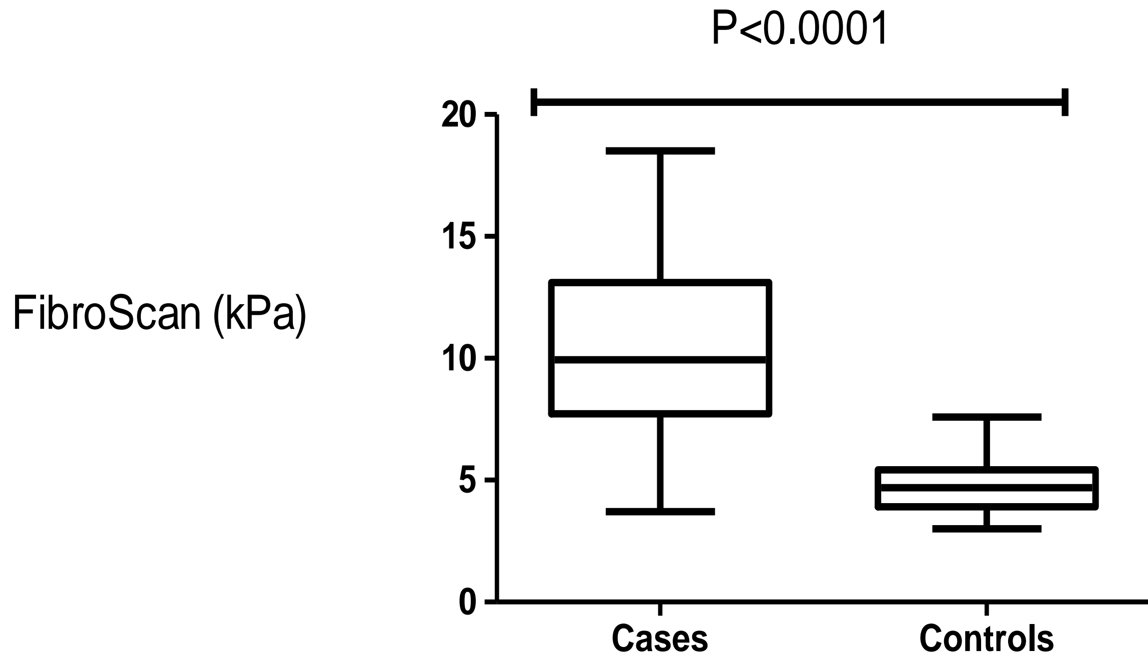


Figure 5-27: FibroScan score of HSS patients was higher than in controls

There were positive correlations of FibroScan scores and hyaluronan and tumour necrosis factor receptor 1 (Figures 5-26, 5-27). There were no significant correlations between FibroScan score and other parameters.

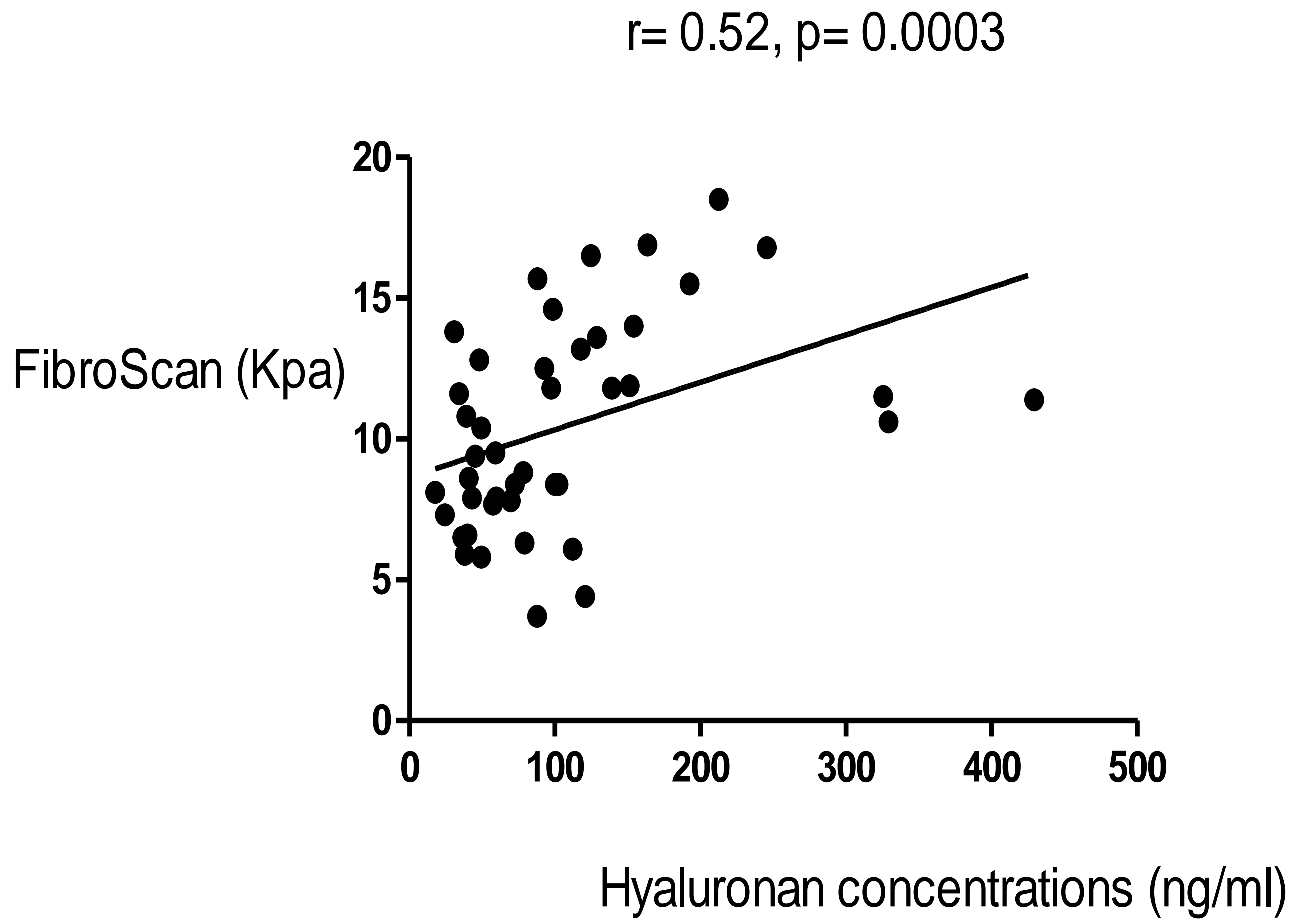


Figure 5-28: There was significant spearman's correlation of FibroScan score and HA concentrations in cases

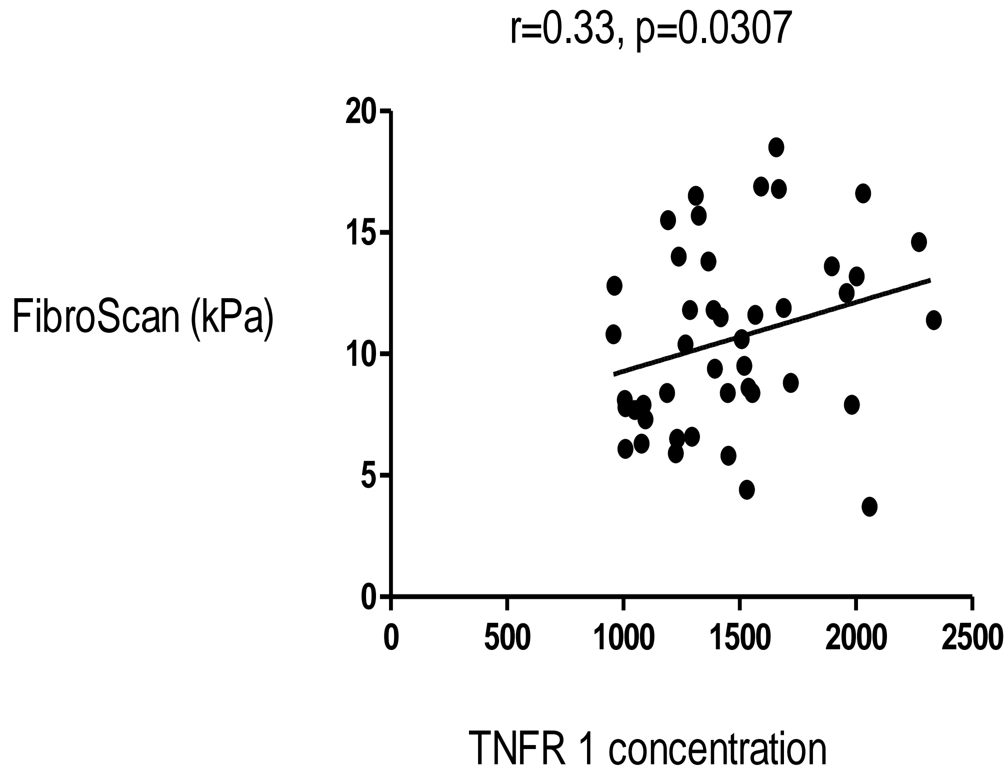


Figure 5-29: There was a positive correlation of FibroScan score and TNFR1 concentrations in cases

5.4.1 Summary results of nested case control study of FibroScan

This study shows that patients with HSS have higher FibroScan scores compared with controls. There was also a positive and significant correlation of the liver stiffness (measured as FibroScan score) with markers of fibrosis and inflammation.

CHAPTER 6: DISCUSSION

6.1 Discussion of results I: Case control study

The significantly elevated LBP levels in patients compared to controls suggest that BT occurs in patients with HSS. We know that BT is an important driver of disease process in cirrhosis leading to increased portal hypertension and consequently variceal bleeding (Vlachogiannakos et al., 2009) and treatment with antibiotics is now standard of care in patients with cirrhosis (Chavez-Tapia et al., 2011; Desai et al., 2012). These data prompted me to postulate that BT could also be a driver in schistosomal related portal hypertension, and may drive systemic inflammation. Several observations support this hypothesis.

TNFR1, IL-1β and IL-6 showed significant positive correlation with LBP. This positive correlation of systemic inflammatory markers and LBP a surrogate marker of BT suggests that BT induces the production of these inflammatory markers, but is consistent with there being some other process which drives both. Soluble CD14 is known to be required for transduction of toll-like receptor 4 (TLR4) signaling after lipid binding by bacterial lipopolysaccharide (Takeshita et al., 2000), and this has encouraged some workers to use sCD14 as a marker of translocation (Marchetti et al., 2013). The elevation of sCD14 concentrations compared with controls provides further support for the occurrence of BT in HSS. Holland et al have shown that sCD163 a marker of Kupffer cell activation is associated with portal hypertension in cirrhosis (Holland et al., 2011). Others have noted that sCD163 is elevated in chronic liver disease due to hepatitis B virus (Wang et al., 2015) while others think that it could be used as a good

predictor of mortality in acute liver failure (Holger et al., 2007) but none of the patients in this study presented with acute liver failure. The elevation of sCD163 concentrations compared with controls also suggests that there is activation of Kupffer cells in the sinusoids of the liver as a result of BT, although further work will be required to confirm this. TNFR1 is a systemic inflammatory marker and in this study it shows that it may be among the drivers of fibrosis in HSS as it showed a positive correlation with hyaluronan. Tarrats and others reported that TNFR1 plays a role in liver fibrosis in animal models (Tarrats et al., 2011).

In addition to biomarkers of the host response to translocation, I also measured bacterial components directly. We know that the direct measure of BT is bacterial DNA and LPS while LBP is an indirect marker (Koutsounas, 2015). Bacterial DNA, measured by real time PCR, was positive only in just over one third of patients, despite the elevated LBP and the elevated inflammatory markers. The low number of positive amplifications could be attributed to a short half-life of bacterial DNA (Bellot et al., 2013) or due to the extraction method which was used QIAmp DNA blood mini Kit (QIAGEN) whose efficiency is not 100% (Zucol et al., 2006). The detection limit of the PCR was determined at ≥ 10 copies/μl of 16S rRNA so it is possible that lower levels of translocation may be undetectable by this method.

Patients with schistosomal liver disease generally have normal liver parenchyma (Ross et al., 2002; Andrade, 2004) and for patients to present with complications such as portal hypertension takes many years. Most of the patients even in this study reported repeated exposure to streams and rivers

during childhood. This confirms that HSS runs a chronic course. Since the patients in this study were recruited as outpatients and had no clinical evidence of infection, the elevated inflammatory markers could be due to the natural progression of the disease, and not some intercurrent infection, as CRP did not differ between patients and controls. We know that CRP is an acute reactant marker of inflammation produced in the liver.

Periportal fibrosis is an important recognised feature of HSS and so many researchers have embarked on studying fibrotic markers. Laminin and hyaluronan, the two fibrotic markers measured in this study were markedly elevated in patients compared to the controls. These data are consistent with findings from Brazil (Marinho et al., 2010; Wyszomirska et al., 2005) and there is some evidence that α_2 macroglobulin is a good predictor of liver fibrosis (Ahmed et al., 2009). The consistency of these data suggests that these markers can be used to diagnose and monitor liver fibrosis in HSS.

Schistosomal liver disease is the major cause of variceal bleeding in Zambia and the majority of the patients were referred to the hospital for haematemesis (84%) while the rest were referred due to anaemia and unexplained splenomegaly. It is therefore important for clinicians to evaluate anemic patients for possible HSS by taking good social and economic history. Nearly all the patients gave past history of exposure to various water bodies which could explain acquisition of schistosomiasis infection. Very few patients gave a past history of jaundice and I found no icterus on physical examination. This is in agreement with other reports that jaundice is not a common feature of HSS and

there liver parenchyma is usually unaffected in these patients (Ross et al., 2002; Andrade, 2004). The normal alanine aminotransferase (ALT) values found in these patients further confirm that liver parenchyma is not affected by HSS. The raised aspartate aminotransferase (AST) compared with controls in this study may be attributed to different sources other than the liver. We know that AST is not very specific to the liver. Ascites is not reported as a common feature in HSS (Ibrahim et al., 2010; Carruthers, 1978), but this study found that nearly two thirds of patients had ascites and this was confirmed by ultrasound, perhaps indicating the advanced nature of their disease. This is consistent with our clinical experience with HSS.

The dilated portal vein on abdominal ultrasound found in these patients compared to the normal controls agrees with what other authors have stated. They have said that the leading sonographic sign in portal hypertension due to schistosomiasis is the dilated portal vein (Vocke et al., 1998). They however said that portal vein diameter is directly proportional to age and height in a normal setting. Among the endoscopic findings in HSS varices are the hall mark of portal hypertension in HSS. A small number of patients (24%) in this study also had gastropathy. Other authors have also found that gastropathy in HSS is not as common as it is in cirrhosis (Shavel et al., 2002). The current study however did not include any patients with cirrhosis.

The significant anaemia in these patients could be due to intermittent variceal bleeding or due to hypersplenism caused by splenomegaly. The hypersplenism would also explain the leukopenia and significant thrombocytopenia observed in

these patients. Of the patients who submitted stool for parasitology only 4 (8.7%) samples were positive for schistosomiasis. This small percentage could be due to low or no shedding of eggs as a result of the chronicity of the disease, or it is possible that many patients might have been treated for schistosomiasis before they presented for clinical evaluation. Treatment, sadly, does not reverse fibrotic changes once established, so fibrosis remains even after all traces of schistosoma have gone.

It is a limitation of this case control study that some patients did not return to submit stool samples and rectal biopsy was not done in many patients. Due to resource constraints, I was only able to complete limited assays for laminin and IL1β. However, even in this small study there was a significant difference between patients and controls.

6.1.1 Conclusions and recommendations

In this case control study, I found evidence that BT occurs in HSS, as shown by the elevation of circulating LBP concentrations compared with healthy controls. This appears to result in systemic inflammation, reflected in elevated levels of the inflammatory markers. The elevated levels of fibrotic markers suggest that they could be useful in monitoring periportal fibrosis. A randomised control trial was indicated to confirm that BT occurs in HSS, using antibiotics in a manner analogous to their use in cirrhosis.

6.2 Discussion of results II: 42 day randomised clinical trial with rifaximin

In this open-label clinical trial, the reduction in 16S rRNA and inflammatory markers after 42 days of intestinal decontamination with rifaximin provide evidence that BT may have a clinical association with HSS related portal hypertension. We know that BT is associated with cirrhosis and this drives disease process by increasing portal hypertension (Gou et al., 2006; Thalheimer et al., 2005; Bellot et al., 2010; Koutsounas, 2015). HSS is the leading cause of morbidity and mortality in patients with schistosomiasis (Kheir et al., 1999; Shaker et al., 2014) and it is likely that there could be similar mechanisms driving portal hypertension as in cirrhosis.

As rifaximin is a minimally absorbable antibiotic administered orally, the downward trend of plasma LPS and the significant reduction of systemic inflammatory markers after 42 days may be attributed to an effect of rifaximin on the process of bacterial translocation. The pronounced reduction of systemic inflammatory markers, 16S rRNA copies and the minimal reduction of LPS could mean that gram positive bacteria contribute to BT as LPS is a constituent of gram negative bacteria only. Among the inflammatory markers that were reduced after 42 days of rifaximin was soluble CD14 which is a marker which other workers have associated with BT (Marchetti et al., 2013). LPS affects secretion of sCD14 from macrophages including Kupffer cells (Ogawa et al., 2013). Toll- like receptor 4 (TLR 4) is the human receptor for LPS and therefore for signaling, LPS requires TLR4 and sCD14 (Berbée et al., 2010; Brenner et al., 2014). TNFR1, another systemic inflammatory marker, fell following 42 days

of rifaximin. This again suggests that reduction of BT led to the reduction of TNFR1. LBP did not change much after 42 days of rifaximin in this clinical trial, which is somewhat at odds with the effect we saw in the case-control study (Sinkala et al., 2015). We know that LBP is a surrogate marker of BT while LPS and 16S rRNA are direct markers (Stehle et al., 2012; Koutsounas, 2015). Other researchers have shown that LBP may also be elevated in patients with gram positive sepsis, the elderly and those who are obese (Myc et al., 1997; Stehle et al., 2012; Gonzalez-Quintela et al., 2013). In this clinical trial, no patient was found to be obese and there was no extremity of age as the median age was about 40 years. No patient presented with clinical sepsis during the 42 day follow up. The lack of effect of rifaximin on LBP may have a similar explanation to those for the lack of effect on LPS.

In cirrhosis a study done by Albillos and others showed increased concentrations of LBP and other inflammatory cytokines including TNFR1 in blood and these reduced after norflaxacin treatment (Agustı´n Albillos et al., 2003). Possible explanations include that norfloxacin is an absorbable antibiotic while rifaximin is not, or that norfloxacin has additional effects on macrophages. Tarrats and others have associated TNFR1 with liver fibrosis in animal models (Tarrats et al., 2011) while others have associated TNFR1 with schistosomiasis infection (Ellis et al., 2008).

The only fibrotic marker measured in patients in the clinical trial was HA which was not affected by rifaximin. I think that the duration of rifaximin was too short to have any impact on HA. It is likely that for periportal fibrosis to become

clinically important it takes many years. Sinkala et al found a positive correlation of HA with TNFR1 (Sinkala et al., 2015). HA is an important molecule which is associated with hepatic fibrosis and is useful in the diagnosis of fibrosis in HSS (Marinho et al., 2010). In an animal model of cirrhosis, fibronectin, another fibrotic marker, was reduced following intestinal decontamination with rifaximin (Qiang et al., 2012). It is not clear whether this could translate to humans, but my data suggest not in the short term.

Most of the patients had pancytopenia which is probably explained by hypersplenism as most of the patients had massive splenomegaly. There was profound thrombocytopenia in both groups but patients did not present with bleeding during follow up. Arther and others have described platelet counts of 100 $x10^9$/l or less as being associated with upper digestive bleeding even in asymptomatic schistosomiasis related portal hypertensive patients (Arthur & Leite, 2013). There were no patients who had bleeding in my cohort despite the median platelet count of 40 $x10^9$/l. This may have been due to a short follow up time, or some as yet unexplained biological factors.

There were no major adverse events associated with rifaximin during the 42 day follow up period. One patient complained of abdominal discomfort for about 5 days but did not discontinue taking the drug. Another patient reported an episode of rectal bleeding. The rectal bleeding may have been from haemorrhoids which can complicate portal hypertension as peri-anal vessels also constitute a port-systemic anastomosis. The reason for the low variceal re-bleeding rate during the 42 day follow up could be due to adherence to

propranolol as confirmed by good beta blockade at the end of 42-day period in both groups compared to baseline. This emphasises the importance of beta blockers in preventing variceal bleeding in schistosomal related portal hypertension (Funakoshi et al., 2010). The minor adverse events experienced in this clinical trial agree with what other authors have said about the safety of rifaximin (Bajaj et al., 2015; Vlachogiannakos., 2009). Rifaximin is a safe drug and is therefore widely used in treatment of hepatic encephalopathy in patients with cirrhosis.

It is a limitation of this study that it was an open label clinical trial, which is prone to bias, although the laboratory personnel were blinded. Confirmation of compliance to rifaximin in these patients was not possible as rifaximin blood levels could not be measured. As it is a non-absorbable drug the colour of urine does not indicate recent intake as it does for rifampicin. The effect of BT on clinical end points may also have been underestimated in this clinical trial because the patients were stable and seen as outpatients. They did not have acute variceal bleeds on recruitment and would be predicted to have lower re-bleeding rates on follow-up.

6.2.1 Conclusion and recommendations

These data suggest that rifaximin led to a reduction of systemic inflammatory cytokines and biomarkers. This suggests that BT may be involved in HSS and warrants the further study of antibiotics in patients with HSS related portal hypertension. It would be of interest to study patients recruited immediately after

acute variceal bleeds, with clinical end points, perhaps as a multi-center placebo control trial on a larger scale.

6.3 Discussion of results III: The extended follow- up to day 180

After 42 days of follow –up, the patients were then followed and monitored up to day 180. During this period two severe adverse events occurred. One patient in the rifaximin group died 10 weeks after the last day of taking rifaximin. This adverse event seems not to be related to the trial drug as this patient was diabetic and died from diabetic ketoacidosis (DKA), in addition to sepsis with renal dysfunction. Any rifaximin in the gut would have been excreted long before this adverse event occurred. The other patient in the non-rifaximin group died in the fifth month of follow-up due to massive variceal bleeding. In many cases of re-bleeding, we find that the patient was not compliant to taking propranolol, which is part of the standard care of the patients with schistosomal portal hypertension.

By the end of 180-day follow- up period there were 7 patients who reported upper GI bleeding. This could have been due to variceal bleeding as a result of poor compliance to propranolol which is consistent with our clinical experience. Generally, the response to beta blockers at the end of 180 days was encouraging and this might have contributed to the smaller number of patients with variceal bleeding. This is supported by the significant reduction of portal vein diameter on day 180 compared with baseline. This reduction could be of clinical value in monitoring patients with schistosomal portal hypertension, as a predictor of variceal bleeding, and to use it as a surrogate for compliance to

beta blockers. The person who measured the portal vein diameter at day 180 was the same person who did even at baseline but was blinded to the baseline measurements to avoid bias.

Ascites is not a common feature of HSS (Ibrahim et al., 2010; Carruthers 1978) and it appears the few cases that had ascites on inception still had it even at the end of 6 months. Although propranolol can affect the haemodynamics of portal hypertension in cirrhosis by reducing the portal pressure it has not been effective in the treatment of ascites (Levitt & Levitt 2012).

The splenic size remained the same at baseline and day 180. Although it occurs from time to time, the reduction of splenomegaly is not a common occurrence in patients with portal hypertension. Luiz and others have reported that splenectomy is of clinical importance in patients with HSS. They said it leads to an increase in platelet count, improves haemostatic & liver function and reduces portal vein diameter and overall leading to reduced portal pressure (Luiz et al., 2015). In this study, none of the patients underwent splenectomy. The full blood count picture did not differ much between baseline and day 180 apart from a marginal improvement of the haemoglobin levels after 180 days. The improvement of haemoglobin could be attributed to the lower rates of variceal bleeding experienced at the end of the study which could probably be due to good beta blockade. The other indices of the full blood count that remained low and could be attributed to hypersplenism.

At the end of the 180 -day follow -up the two patients who presented with jaundice were not encephalopathic. It is known that jaundice is not a common

feature of HSS and liver parenchyma is usually normal (Andrade 2004) but in very rare circumstances jaundice can occur in very advanced and decompensated HSS (Reboucas 1975). Probably these two cases had advanced forms of HSS.

6.3.1 Conclusions and recommendations

The reduction of portal vein diameter values measured at baseline and after 180 days appears to confirm the effectiveness of beta blockers which are the mainstay of our management. It would be useful to establish, in a prospective cohort study, if measurement of portal vein diameter by ultrasound can be used as a surrogate for beta blocker compliance. It would also be useful to establish whether portal vein diameter can be used to predict episodes of variceal bleeding in patients with HSS.

6.4 Discussion of results IV: Nested case control study with use of FibroScan

The importance of FibroScan in diagnosing liver disease has evolved over time and has been shown to be an important tool in the diagnosis of cirrhosis and there is great interest in using it instead of liver biopsy (Chang et al., 2016). Currently, the gold standard to diagnose liver diseases is liver biopsy. This however is an invasive procedure and is associated with risk of bleeding, injury to surrounding structures and introduction of infection (Leonard et al., 2010) although mortality risk is as low as 0.03% (Nedredal et al., 2011). Many studies of transient elastogrophy (FibroScan) have been published in cirrhosis but are scanty in HSS related portal hypertension, and the clinical role of FibroScan in HSS remains unexplored. The elevated FibroScan score in HSS, which is a

measure of liver stiffness shows that this could be an important non- invasive method in assessing liver stiffness in these patients. We know that HSS is associated with normal liver cell function. However, it was noted that in this study the FibroScan scores were higher in cases than normal individuals. Even though the scores were higher in HSS, they were below that of the reported cirrhotic range worldwide (Kircheis et al., 2012; Göbel et al., 2015). This shows that FibroScan may be a useful tool to discriminate cirrhosis from HSS especially in HSS endemic areas. The combination of ordinary liver ultrasound with transient elastography in suspected cases of schistosomal portal hypertension would be of great use in these patients.

Hyaluronan, a marker liver fibrosis was elevated in the cases and showed a strong positive correlation with the FibroScan score. This may partly explain the raised FibroScan score in HSS considering that fibrosis in these patients only occurs in periportal veins while sparing the liver parenchyma. In this nested case control study, all patients had periportal fibrosis on liver ultrasound. This strong correlation of FibroScan score and HA in HSS also strengthens the need to use non -invasive markers in diagnosing fibrosis in HSS. TNFR1 which is a systemic inflammatory cytokine was also positively correlated with FibroScan score. This shows that inflammation may be a driver for the fibrosis seen in HSS. A study in animal models also found that TNFR1 in a pro-fibrotic marker in liver disease (Tarrats et al., 2011). This therefore strengthens the need to come up with a combination of non- invasive markers and tools in assessing and diagnosing HSS.

FibroScan is good tool for assessing hepatic fibrosis but is not without shortcomings. The scores tend to be influenced by obesity, acute hepatitis, cholestasis and performer experience (Chang et al., 2016; Tapper et al., 2015). In this nested case control study, no patient had obesity as the median body mass Index in cases was 22 kg/m^2 and there was no much evidence of liver inflammation as liver enzymes were within normal range while no patient had jaundice clinically. The FibroScan was performed by an experienced person who had performed over a thousand tests. Therefore, the FibroScan scores noted in patients with HSS may reflect the actual stiffness of the liver. The massive splenomegaly noted in cases compared to controls may even explain pancytopenic picture seen in cases.

It is a limitation of this nested case control study that liver biopsies were not done and there were no cirrhotic patients as positive controls.

6.4.1 Conclusions and recommendations

The elevated FibroScan score compared with normal individuals suggests that HSS patients despite the liver parenchyma being normal have increased liver stiffness. Therefore, FibroScan may be useful in discriminating between schistosomal and cirrhosis related portal hypertension in schistosomiasis endemic areas since the average FibroScan score is lower than cirrhotic range. There is a need to do further clinical studies to evaluate the response of liver stiffness to beta blockers and intestinal decontamination in HSS.

6.5 Contribution of knowledge to science

According to my knowledge this is a novel finding which seems to implicate inflammation in HSS to BT as revealed in the case control study and the clinical trial of rifaximin. The nested case control study of FibroScan also seems to reveal that liver stiffness in HSS patients is abnormal despite liver parenchyma being normal in these patients.

6.6 Application of results in public health

These results may help design therapeutic antibiotic interventions in HSS as it is the case with cirrhosis. The observance of the reduction of portal vein diameter after 180 days of propranolol in HSS patients may necessitate use of abdominal ultrasound which is readily available and cheap in monitoring response, compliance to beta blockers and also predicting variceal bleeding in HSS especially in resource constraint places where invasive and sophisticated portal hypertension measurement facilities are not readily available. The elevated FibroScan score in HSS which measures liver stiffness may be used to discriminate between schistosomal and cirrhosis related portal hypertension in HSS endemic areas since the average score is lower than that of cirrhotic range.

References

1. Kheir MM, Eltoum I a., Saad AM, Ali MM, Baraka OZ, Ali Homeida MM. Mortality due to schistosomiasis mansoni: A field study in Sudan. *Am J Trop Med Hyg*. 1999;60(2):307-310.
2. Shaker Y, Samy N, Ashour E. Review Article Hepatobiliary Schistosomiasis. *J Clin Transl Hepatol*. 2014;2:212-216. doi:10.14218/JCTH.2014.00018.
3. Chofle A a, Jaka H, Koy M, Smart LR, Kabangila R, Ewings FM, Mazigo HD, Johnson WD, Fitzgerald DW, Peck RN, Downs J a. Oesophageal varices, schistosomiasis, and mortality among patients admitted with haematemesis in Mwanza, Tanzania: a prospective cohort study. *BMC Infect Dis*. 2014;14(1):303. doi:10.1186/1471-2334-14-303.
4. Pyrrho S, Ramos JA, Neto RM, Lenzi HL, Takiya CM. Dexamethasone , a Drug for Attenuation of Schistosoma mansoni Infection Morbidity. *Antimicrob Agents Chemother*. 2002;46(11):3490-3498. doi:10.1128/AAC.46.11.3490.
5. Limquiaco J, Nolasco ER, Lourdes M, Gloria VI, Domingo EO, Banez P, Zano FM, Atienza MA, Sy PP, Labio E, Comia AC, De MAA, Pilapil J, Feir S, Cabanayan E, Prodigalidad PA. Clinical predictors of bleeding from esophageal varices : a retrospective study. *Phil J Gastroenterol*. 2006;2:103-111.
6. Martins RD, Szejnfeld J, Lima FG, Ferrari AP. Endoscopic,

Ultrasonographic, and US-Doppler Parameters as Indicators of Variceal Bleeding in Patients with Schistosomiasis. *Dig Dis Sci*. 2000;45(5):1013-1018.

7. Wyszomirska RM, Nishimura NF, Almeida JRS, Yamaka A, Soares EC. High serum laminin and type IV collagen levels in schistosomiasis Mansoni. *Arq Gastroenterol*. 2005;42(4):221-225.

8. Marinho CC, Bretas T, Voieta I, Queiroz LC, Ruiz-guevara R, Teixeira AL, Antunes CM, Prata A, Lambertucci JR. Serum hyaluronan and collagen IV as non-invasive markers of liver fibrosis in patients from an endemic area for schistosomiasis mansoni : a field-based study in Brazil. *Mem Inst Oswaldo Cruz, Rio Janeiro*. 2010;105(July):471-478.

9. Jia T-W, Utzinger J, Deng Y, Yang K, Li Y-Y, Zhu J-H, King CH, Zhou X-N. Quantifying Quality of Life and Disability of Patients with Advanced Schistosomiasis Japonica. *PLoS Negl Trop Dis*. 2011;5(2):e966. doi:10.1371/journal.pntd.0000966.

10. Chang D, Ramalho LNZ, Ramalho FS, Martinelli ALC, Zucoloto S. Hepatic stellate cells in human schistosomiasis mansoni: A comparative immunohistochemical study with liver cirrhosis. *Acta Trop*. 2006;97(3):318-323. doi:10.1016/j.actatropica.2005.12.006.

11. Ross AG., Bartley PP, Sleigh AC, Olds RG, Li Y, Gail WM, McManus DP. Schistosomiasis. *N Engl J Med*. 2002;346(16):1212-1220.

12. Kamal SM, Graham CS, He Q, Bianchi L, Tawil A a, Rasenack JW, Khalifa K a, Massoud MM, Koziel MJ. Kinetics of intrahepatic hepatitis C

virus (HCV)-specific CD4+ T cell responses in HCV and Schistosoma mansoni coinfection: relation to progression of liver fibrosis. *J Infect Dis*. 2004;189(7):1140-1150. doi:10.1086/382278.

13. Zhou W, Zhang Q, Qiao L. Pathogenesis of liver cirrhosis. *World J Gastroenterol*. 2014;20(23):7312-7324. doi:10.3748/wjg.v20.i23.7312.

14. Giannelli V, Gregorio V Di, Iebba V, Giusto M, Schippa S, Merli M, Giannelli V, Gregorio V Di, Giusto M. Microbiota and the gut-liver axis : Bacterial translocation , inflammation and infection in cirrhosis. 2014;20(45):16795-16810. doi:10.3748/wjg.v20.i45.16795.

15. Bellot P, Francés R, Such J. Pathological bacterial translocation in cirrhosis: Pathophysiology, diagnosis and clinical implications. *Liver Int*. 2013;33(1):31-39. doi:10.1111/liv.12021.

16. Wiest R, Das S, Cadelina G, Garcia-Tsao G, Milstien S, Groszmann RJ. Bacterial translocation in cirrhotic rats stimulates eNOS-derived NO production and impairs mesenteric vascular contractility. *J Clin Invest*. 1999;104(9):1223-1233. doi:10.1172/JCI7458.

17. Gou Y-Z, Lian J-Q, Nie Q-H. Bacterial translocation and its consequences in patients with liver cirrhosis. *Zhonghua Gan Zang Bing Za Zhi*. 2006;14(10):796-797.

18. Balzan S, De Almeida Quadros C, De Cleva R, Zilberstein B, Cecconello I. Bacterial translocation: overview of mechanisms and clinical impact. *J Gastroenterol Hepatol*. 2007;22(4):464-471. doi:10.1111/j.1440-1746.2007.04933.x.

19. Ramachandran A, Balasubramanian K a. Intestinal dysfunction in liver cirrhosis: Its role in spontaneous bacterial peritonitis. *J Gastroenterol Hepatol*. 2001;16(6):607-612. doi:10.1046/j.1440-1746.2001.02444.x.

20. Fukui H. Gut-liver axis in liver cirrhosis: How to manage leaky gut and endotoxemia. *World J Hepatol*. 2015;7(3):425-442. doi:10.4254/wjh.v7.i3.425.

21. Rasaratnam B, Kaye D, Jennings G, Dudley F, Chin-Dusting J. The effect of selective intestinal decontamination on the hyperdynamic circulatory state in cirrhosis. A randomized trial. *Ann Intern Med*. 2003;139(3):186-193. http://www.ncbi.nlm.nih.gov/pubmed/12899586.

22. Nusrat S, Khan MS, Fazili J, Madhoun MF. Cirrhosis and its complications: Evidence based treatment. *World J Gastroenterol*. 2014;20(18):5442-5460. doi:10.3748/wjg.v20.i18.5442.

23. Chavez-Tapia NC, Barrientos-Gutierrez T, Tellez-Avila F, Soares-Weiser K, Mendez-Sanchez N, Gluud C, Uribe M. Meta-analysis: antibiotic prophylaxis for cirrhotic patients with upper gastrointestinal bleeding - an updated Cochrane review. *Aliment Pharmacol Ther*. 2011;34(5):509-518. doi:10.1111/j.1365-2036.2011.04746.x.

24. Onguru D, Liang Y, Griffith Q, Nikolajczyk B, Mwinzi P, Ganley-Leal L. Short report: Human schistosomiasis is associated with endotoxemia and toll-like receptor 2- and 4-bearing B cells. *Am J Trop Med Hyg*. 2011;84(2):321-324. doi:10.4269/ajtmh.2011.10-0397.

25. Ferraz AAB, Campos JM, Júnior JGCDA, De Albuquerque AC, Ferraz

EM. Gut bacterial translocation and postoperative infections: a prospective study in schistosomotic patients. *Surg Infect (Larchmt)*. 2005;6(2):197-201. doi:10.1089/sur.2005.6.197.

26. Bass M. Nathan, Mullen D. Kelvin, Sanyal Arun, Poordad Fred, Neff Guy, Leevy B. Carroll, Sigal Samuel, Sheikh Y. Muhammad, Beavers Kimberly, Frederick Todd, Teperma Lewis, Hillebrand Donald, Huang Shirley MK. Rifaximin Treatment in Hepatic Encephalopathy. *N Eng J Med*. 2010;362(12):1071-1081.
27. Dabo Xu, Jun Gao, Merritt Gillilland III, Xiaoyin Wu, Il Song, John Y. Kao and C, Owyang. Rifaximin Alters Intestinal Bacteria and Prevents Stress-Induced Gut Inflammation and Visceral Hyperalgesia in Rats. *Gastroenterol 2014 Febr ; 146(2) 484–96.e4 doi101053/j.gastro201310026*. 2009;27(5):417-428. doi:10.1055/s-0029-1237430.
28. Lutz P, Parcina M, Bekeredjian-Ding I, Nischalke HD, Nattermann J, Sauerbruch T, Hoerauf A, Strassburg CP, Spengler U. Impact of rifaximin on the frequency and characteristics of spontaneous bacterial peritonitis in patients with liver cirrhosis and ascites. *PLoS One*. 2014;9(4):e93909. doi:10.1371/journal.pone.0093909.
29. Koutsounas I. Markers of bacterial translocation in end-stage liver disease. *World J Hepatol*. 2015;7(20):2264. doi:10.4254/wjh.v7.i20.2264.
30. Marchetti G, Tincati C, Silvestri G. Microbial translocation in the pathogenesis of HIV infection and AIDS. *Clin Microbiol Rev*. 2013;26(1):2-

18. doi:10.1128/CMR.00050-12.

31. Snowdon VK, Guha N, Fallowfield JA. Noninvasive evaluation of portal hypertension: emerging tools and techniques. *Int J Hepatol*. 2012;2012. doi:10.1155/2012/691089.

32. Sebastiani G. Non-invasive assessment of liver fibrosis in chronic liver diseases: Implementation in clinical practice and decisional algorithms. *World J Gastroenterol*. 2009;15(18):2190-2203. doi:http://dx.doi.org/10.3748/wjg.15.2190.

33. Baranova A, Lal P, Birerdinc A, Younossi ZM. Non-Invasive markers for hepatic fibrosis. *BMC Gastroenterol*. 2011;11(1):91. doi:10.1186/1471-230X-11-91.

34. Rosa Lombardi, Elena Buzzetti, Davide Roccarina EAT. Non-invasive assessment of liver fibrosis in patients with alcoholic liver disease. *World J Gastroenterol*. 2015;21(39):11044-11052. doi:10.3748/wjg.v21.i39.11044.

35. Ahmed L, Salama H, Ahmed R, Mahgoub AMA, Hamdy S, Abd S, Shafi A, Akel W Al, Hareedy A, Fathy W. Evaluation of Fibrosis Sero-Markers versus Liver Biopsy in Egyptian Patients with Hepatitis C and / or NASH and / or Schistosomiasis. *Parastologists United J*. 2009;2(1):67-76.

36. Olveda DU, Olveda RM, McManus DP, Cai P, Chau TNP, Lam AK, Li Y, Harn DA, Vinluan ML, Ross AGP. The chronic enteropathogenic disease schistosomiasis. *Int Soc Infect Dis*. 2014;28(2014):193-203. doi:10.1016/j.ijid.2014.07.009.

37. Clarice Neuenschwander Lins de Morais/+ B de MC, Wlademir Gomes de

Melo, Fábio Lopes de Melo EP de AL, Ana Lúcia Coutinho Domingues, Norma Jucá JRMM, George Tadeu Nunes Diniz SMLM. Correlation of biological serum markers with the degree of hepatic fibrosis and necroinflammatory activity in hepatitis C and schistosomiasis patients. *Mem Inst Oswaldo Cruz, Rio Janeiro*. 2010;105(4):460-466.

38. Abdel Atty M. Elgonimy, Suzan M.Farouk EAR. The pathogenesis of cytokines in preportal fibrosis of human infected with schistosomiasis and viral hepatitis. *Egypt J Hosp Med*. 2005;20:16-28.

39. Jung KS, Kim SU. Clinical applications of transient elastography. *Clin Mol Hepatol*. 2012;18(2):163-173. doi:http://dx.doi.org/10.3350/cmh.2012.18.2.163.

40. Myers RP, Elkashab M, Ma M, Crotty P, Pomier-layrargues G. Transient elastography for the noninvasive assessment of liver fibrosis : A multicentre Canadian study. *Can J Gastroenterol*. 2010;24(11):661-670.

41. Göbel T, Schadewaldt-tümmers J, Greiner L, Poremba C, Häussinger D, Göbel T, Schadewaldt-tümmers J, Erhardt A, Gastroenterologie K. Transient elastography improves detection of liver cirrhosis compared to routine screening tests. *World J Gastroenterol*. 2015;21(3):953-960. doi:10.3748/wjg.v21.i3.953.

42. Ja Kyung Kim DWM, Kwan Sik Lee and Yong-Han Paik3. Assessment of hepatic fibrosis by transient elastography in patients with chronic hepatitis B. *Pathol Int*. 2010;60(4):284-290. doi:10.1111/j.1440-1827.2010.02515.x.

43. Gryseels B, Polman K, Clerinx J, Kestens L. Human schistosomiasis.

Lancet. 2006;368(9541):1106-1118. doi:10.1016/S0140-6736(06)69440-3.

44. Kabatereine NB, Brooker S, Tukahebwa EM, Kazibwe F, Onapa AW. Epidemiology and geography of Schistosomo mansoni in Uganda: Implications for planning control. *Trop Med Int Heal*. 2004;9(3):372-380. doi:10.1046/j.1365-3156.2003.01176.x.
45. Thomas Handzel, Diana M. S. Karanja, David G. Addiss, Allen W. Hightower DHR, Daniel G. Colley, Julius Andove, Laurence Slutsker Awes. Geographic distribution of Schistosomiasis & Soil-Transmitted Helminth in Western Kenya: Implication for anthelminthic mass treatment. *Am J Trop Med Hyg*. 2003;69(3):318-323.
46. Mugono M, Konje E, Kuhn S, Mpogoro FJ, Morona D, Mazigo HD. Intestinal schistosomiasis and geohelminths of Ukara Island, North-Western Tanzania: prevalence, intensity of infection and associated risk factors among school children. *Parasit Vectors*. 2014;7(1):1-9. doi:10.1186/s13071-014-0612-5.
47. El-Khoby T, Galal N, Fenwick A, Barakat R, El-Hawey A, Nooman Z, Habib M, Abdel-Wahab F, Gabr NS, Hammam HM, Hussein MH, Mikhail NNH, Cline BL, Strickland GT. The epidemiology of schistosomiasis in Egypt: Summary findings in nine governorates. *Am J Trop Med Hyg*. 2000;62(2 SUPPL.):88-99.
48. Payne L, Turner-Moss E, Mutengo M, Asombang AW, Kelly P. Prevalence of schistosome antibodies with hepatosplenic signs and symptoms among

patients from Kaoma, Western Province, Zambia. *BMC Res Notes*. 2013;6(1):344. doi:10.1186/1756-0500-6-344.

49. Worku L, Damte D, Endris M, Tesfa H, Aemero M. Schistosoma mansoni infection and associated determinant factors among school children in Sanja Town, northwest Ethiopia. *J Parasitol Res*. 2014;2014. doi:10.1155/2014/792536.

50. Kibiki GS, Drenth JPH, Nagengast JMF. Hepatosplenic Schistosomiasis : A Review. *East Afr Med J*. 2004;81(9):480-485.

51. Tzanetou K, Astriti M, Delis V, Moustakas G, Choreftaki T, Papaliodi E, Sarri K, Adamis G. Intestinal schistosomiasis caused by both Schistosoma intercalatum and Schistosoma mansoni. *Travel Med Infect Dis*. 2010;8(3):184-189. doi:10.1016/j.tmaid.2010.04.003.

52. Janda JM, Abbott SL. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. *J Clin Microbiol*. 2007;45(9):2761-2764. doi:10.1128/JCM.01228-07.

53. Gray DJ, Ross AG, Li Y-S, McManus DP. Diagnosis and management of schistosomiasis. *BMJ*. 2011;342(May):d2651. doi:10.1136/bmj.d2651.

54. Silveira-Lemos D, Fernandes Costa-Silva M, Cardoso De Oliveira Silveira A, Azevedo Batista M, Alves Oliveira-Fraga L, Soares Silveira AM, Barbosa Alvarez MC, Martins-Filho OA, Gazzinelli G, Corrêa-Oliveira R, Teixeira-Carvalho A. Cytokine pattern of T lymphocytes in acute schistosomiasis mansoni patients following treated praziquantel therapy. *J Parasitol Res*. 2012;2013:1-13. doi:10.1155/2013/909134.

55. Da Silva LC, Chieffi PP, Carrilho FJ. Schistosomiasis mansoni -- clinical features. *Gastroenterol Hepatol*. 2005;28(1):30-39. doi:10.1157/13070382.

56. Mazigo HD, Dunne DW, Morona D, Lutufyo TE, Kinung'hi SM, Kaatano G, Nuwaha F. Periportal fibrosis, liver and spleen sizes among S. mansoni mono or co-infected individuals with human immunodeficiency virus-1 in fishing villages along Lake Victoria shores, North-Western, Tanzania. *Parasit Vectors*. 2015;8(1):1-13. doi:10.1186/s13071-015-0876-4.

57. Abebe N, Erko B, Medhin G, Berhe N. Clinico-epidemiological study of Schistosomiasis mansoni in Waja-Timuga, District of Alamata, northern Ethiopia. *Parasit Vectors*. 2014;7(1):158. doi:10.1186/1756-3305-7-158.

58. Blanton RE, Salam EA, Ehsan A, King CH, Goddard KA. Schistosomal hepatic fibrosis and the interferon gamma receptor: a linkage analysis using single-nucleotide polymorphic markers. *Eur J Hum Genet*. 2005;13(5):660-668. doi:10.1038/sj.ejhg.5201388.

59. Abath FGC, Morais CNL, Montenegro CEL, Wynn A, Montenegro SML. Immunopathogenic mechanisms in schistosomiasis : what can be learnt from human studies ? *TRENDS Parasitol*. 2006;22(2):85-91. doi:10.1016/j.pt.2005.12.004.

60. Ferreira RCS, Domingues LC, Bandeira P, Markman Filho B, Albuqerque Filho ES, Correiade de Araújo a CC, Batista LJB, Markman M, Campelo a RL. Prevalence of pulmonary hypertension in patients with schistosomal liver fibrosis. *Ann Trop Med Parasitol*. 2009;103(2):129-143.

doi:10.1179/136485909X398168.

61. Cheever AW, Lenzi JA, Lenzi HL. Experimental Models of Schistosoma mansoni Infection. *Mem Inst Oswaldo Cruz, Rio Janeiro*. 2002;97(October):917-940.

62. Li Y, Ross AG, Hou X, Lou Z, McManus DP. Oriental Schistosomiasis with Neurological Complications: Case report. *Ann Clin Microbiol Antimicrob*. 2011;10(1):5. doi:10.1186/1476-0711-10-5.

63. Manzella A, Borba-Filho P, Brandt CT, Oliveira K. Brain magnetic resonance imaging findings in young patients with hepatosplenic schistosomiasis mansoni without overt symptoms. *Am J Trop Med Hyg*. 2012;86(6):982-987. doi:10.4269/ajtmh.2012.11-0419.

64. Otoni A, Teixeira AL, Voieta I, Antunes CM, Costa Melo VL, Drummond SC, Rodrigues VL, Lambertucci JR. Chemokine profile in the sera and urine of patients with schistosomal glomerulopathy. *Am J Trop Med Hyg*. 2014;90(1):48-53. doi:10.4269/ajtmh.13-0270.

65. Engels D, Chitsulo L, Montresor A, Salvioli L. The global epidemiological situation of shistosomiasis and new approaches to control and research. *Acta Trop*. 2002;82:139-146.

66. Gazzinelli A, Correa-Oliveira R, Yang G-J, Boatin B a., Kloos H. A Research Agenda for Helminth Diseases of Humans: Social Ecology, Environmental Determinants, and Health Systems. *PLoS Negl Trop Dis*. 2012;6(4):e1603. doi:10.1371/journal.pntd.0001603.

67. Chitsulo L, Chitsulo L, Engels D, Engels D, Montresor a, Montresor a,

Savioli L, Savioli L. The global status of schistosomiasis and its control. *Acta Trop*. 2000;77(1):41-51. doi:10.1016/S0001-706X(00)00122-4.

68. Elbaz T, Esmat G. Hepatic and Intestinal Schistosomiasis: Review. *J Adv Res*. 2013;4(5):445-452. doi:10.1016/j.jare.2012.12.001.

69. Chipeta J, Mwansa J, Kachimba JS. Schistosomiasis Disease Burden in Zambian Children : Time for affirmative action is now. *Med J Zambia*. 2009;36(1):1-5.

70. Mutengo MM, Mwansa JCL, Mduluza T, Sianongo S, Chipeta J. High Schistosoma mansoni Disease Burden in a Rural District of Western Zambia. *am j Trop Med Hyg*. 2014;91(5):965-972. doi:10.4269/ajtmh.13-0612.

71. Wynn T a, Thompson RW, Cheever AW, Mentink-Kane MM. Immunopathogenesis of schistosomiasis. *Immunol Rev*. 2004;201(1):156-167. http://www.ncbi.nlm.nih.gov/pubmed/19292768.

72. ANDRADE ZA. Schistosomiasis and liver fibrosis. *Parasite Immunol*. 2009;31(November 2008):656-663. doi:10.1111/j.1365-3024.2009.01157.x.

73. Herbert DR, Orekov T, Perkins C, Finkelman FD. IL-10 and TGF-beta redundantly protect against severe liver injury and mortality during acute schistosomiasis. *J Immunol*. 2008;181(10):7214-7220. doi:181/10/7214 [pii].

74. Yu L, Sun X, Yang F, Yang J, Shen J, Wu Z. Inflammatory cytokines IFN-γ, IL-4, IL-13 and TNF-a alterations in schistosomiasis: A meta-analysis.

Parasitol Res. 2012;110(4):1547-1552. doi:10.1007/s00436-011-2661-4.

75. Wilson S, Jones FM, Mwatha JK, Kimani G, Booth M, Kariuki HC, Vennervald BJ, Ouma JH, Muchiri E, Dunne DW. Hepatosplenomegaly is associated with low regulatory and Th2 responses to schistosome antigens in childhood schistosomiasis and malaria coinfection. *Infect Immun*. 2008;76(5):2212-2218. doi:10.1128/IAI.01433-07.

76. Pearce EJ, MacDonald AS. The immunobiology of schistosomiasis. *Nat Rev Immunol*. 2002;2(7):499-511. doi:10.1038/nri843.

77. Aggarwal N, Negi N, Aggarwal A, Bodh V, Dhiman RK. Pregnancy with portal hypertension. *J Clin Exp Hepatol*. 2014;4(2):163-171. doi:10.1016/j.jceh.2014.05.014.

78. Addley J, Tham TC, Cash WJ, Yoshida S. Use of portal pressure studies in the management of variceal haemorrhage. *World J Gastrointest Endosc*. 2012;16(47):281-289. doi:10.4253/wjge.v4.i7.281.

79. Warren KS, Boros DL, Hang LM, Mahmoud AA. The Schistosoma japonicum egg granuloma. *Am J Pathol*. 1975;80(2):279-294. http://www.ncbi.nlm.nih.gov/pubmed/1163631.

80. Hoare M, Gelson WTH, Davies SE, Curran M, Alexander GJM. Hepatic and intestinal schistosomiasis after orthotopic liver transplant. *Liver Transplant*. 2005;11(12):1603-1607. doi:10.1002/lt.20622.

81. Barsoum RS, Esmat G, El-Baz T. Human schistosomiasis: clinical perspective: review. *J Adv Res*. 2013;4(5):433-444. doi:10.1016/j.jare.2013.01.005.

82. Smith H, Doenhoff M, Aitken C, Bailey W, Ji M, Dawson E, Gilis H, Spence G, Alexander C, van Gool T. Comparison of Schistosoma mansoni Soluble Cercarial Antigens and Soluble Egg Antigens for Serodiagnosing Schistosome Infections. *PLoS Negl Trop Dis*. 2012;6(9):1-14. doi:10.1371/journal.pntd.0001815.

83. Goljan J, Nahorski WL, Felczak-Korzybska I, Wroczyńska A, Myjak P, Kotłowski A. Difficulties in the diagnosis of schistosomiasis in patients of the Department of Tropical and Parasitic Diseases of the Medical University of Gdańsk. *Int Marit Health*. 2007;58(1-4):157-165.

84. Pinto-silva RA, Queiroz LC De, Azeredo LM, Cristina L, Lambertucci JR. Ultrasound in schistosomiasis mansoni. *men Inst Oswaldo Cruz, Rio janeiro*. 2010;105(4):479-484.

85. David U. Olveda, Remigio M. Olveda, Alfred K. Lam, Thao N.P. Chau YL, Angelo Don Gisparil II and AGPR. Utility of Diagnostic Imaging in the Diagnosis and Management of Schistosomiasis. *Clin Microbiol*. 2008;15(10):1203-1214. doi:10.1016/j.drugalcdep.2008.02.002.A.

86. Silva CC, Domingues AL, Lopes EP, Morais CN, Santos RB, Luna CF, Nader HB, Martins JR. Schistosomiasis mansoni: ultrasound-evaluated hepatic fibrosis and serum concentrations of hyaluronic acid. *Ann Trop Med Parasitol*. 2011;105(3):233-239. doi:10.1179/136485911X12987676649629.

87. Carlton EJ, Hsiang M, Zhang Y, Johnson S, Hubbard A, Spear RC. The impact of Schistosoma japonicum infection and treatment on ultrasound-

detectable morbidity: a five-year cohort study in Southwest China. *PLoS Negl Trop Dis*. 2010;4(5):1-9. doi:10.1371/journal.pntd.0000685.

88. Prata A, Ruiz-guevara R, Mauricio C, Antunes DF, Marinho CC, Queiroz LC, Voieta I, Lambertucci JR. Comparison between clinical and ultrasonographic findings in cases of periportal fibrosis in an endemic area for schistosomiasis mansoni in Brazil Comparação entre achados clínicos e ultrassonográficos no diagnóstico da fibrose periportal em uma área endê. *Rev Soc Bras Med Trop*. 2010;43(2):129-134.

89. Cerri Giovanni, Alves Venancio. Hepatosplenic Schistosomiasis Mansoni : Ultrasound Manifestations. *Radiology*. 1984;153:777-780.

90. Moon KM, Kim G, Baik SK, Choi E, Kim MY, Kim HA, Cho MY, Shin SY, Kim JM, Park HJ, Kwon SO, Eom YW. Ultrasonographic scoring system score versus liver stiffness measurement in prediction of cirrhosis. *Clin Mol Hepatol*. 2013;19(4):389-398. doi:10.3350/cmh.2013.19.4.389.

91. Zheng RQ, Wang QH, Lu MD, Xie SB, Ren J, Su ZZ, Cai YK, Yao JL. Liver fibrosis in chronic viral hepatitis: an ultrasonographic study. *World J Gastroenterol*. 2003;9(11):2484-2489.

92. Lee HS, Kim JK, Cheong JY, Han EJ, An SY, Song JH, Jung YJ, Jeon SC, Jung MW, Jang EJ, Cho SW. Prediction of compensated liver cirrhosis by ultrasonography and routine blood tests in patients with chronic viral hepatitis. *Korean J Hepatol*. 2010;16(4):369-375. doi:10.3350/kjhep.2010.16.4.369.

93. Sérgio A, Bezerra DA, Ippolito GD, Leopoldino DD, Batista GR, Jesus G

De, Filho L, Ahmed M, Asa B, Ippolito DG, Rp C. Differentiating Cirrhosis and Chronic Hepatosplenic Schistosomiasis Using MRI. 2008;(March):201-207. doi:10.2214/AJR.07.2639.

94. Cota UF. Ultrasound and clinical investigation of hepatosplenic schistosomiasis : evaluation of splenomegaly and liver fibrosis four years after mass chemotherapy with oxamniquine. *Am J Trop Med Hyg*. 2006;74(1):103-107.

95. Marinho CC, Voieta I, Azeredo LM, Nishi MP, Batista TS, Carolina A, Pereira F, Serufo JC, Queiroz LC De, Ruiz-guevara R, Antunes CM, Prata A, Lambertucci JR. Clinical versus ultrasound examination in the evaluation of hepatosplenic schistosomiasis mansoni in endemic areas. 2006;101(3):317-321.

96. Liu C-H, Liang C-C, Huang K-W, Liu C-J, Chen S-I, Lin J-W, Hung P-H, Tsai H-B, Lai M-Y, Chen P-J, Chen J-H, Chen D-S, Kao J-H. Transient Elastography to Assess Hepatic Fibrosis in Hemodialysis Chronic Hepatitis C Patients. *Clin J Am Soc Nephrol*. 2011;6(5):1057-1065. doi:10.2215/CJN.04320510.

97. Tapper EB, Castera L, Afdhal NH. FibroScan (Vibration-Controlled Transient Elastography): Where Does It Stand in the United States Practice. *Clin Gastroenterol Hepatol*. 2015;13:27-36. doi:10.1016/j.cgh.2014.04.039.

98. Pinzone MR, Celesia BM, Di Rosa M, Cacopardo B, Nunnari G. Microbial translocation in chronic liver diseases. *Int J Microbiol*. 2012;2012:1-12.

doi:10.1155/2012/694629.

99. Desai AP, Reau N, Reddy KG, Te HS, Mohanty S, Satoskar R, Devoss A, Jensen D. Persistent spontaneous bacterial peritonitis: a common complication in patients with spontaneous bacterial peritonitis and a high score in the model for end-stage liver disease. *Therap Adv Gastroenterol*. 2012;5(5):275-283. doi:10.1177/1756283X11417037.

100. Thanopoulou AC, Koskinas JS, Hadziyannis SJ. Spontaneous bacterial peritonitis (SBP): clinical, laboratory, and prognostic features. *Eur J Intern Med*. 2002;13(3):194-198. doi:10.1016/S0953-6205(02)00027-4.

101. D'Amico G, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: A systematic review of 118 studies. *J Hepatol*. 2006;44(1):217-231. doi:10.1016/j.jhep.2005.10.013.

102. J.Vlachogiannakos, A.S.Saveriadis N, . Viazis, I. Theodoropoulos k. F, Manolakopoulos S, À. Intestinal decontamination improves liver haemodynamics in patients with alcohol-related decompensated cirrhosis. *Aliment Pharmacol Ther*. 2009;29:992-999. doi:10.1111/j.1365-2036.2009.03958.x.

103. Flamm SL. Rifaximin treatment for reduction of risk of overt hepatic encephalopathy recurrence. *Adv Gastroenterol,*. 2011;4(3):199-206. doi:10.1177/1756283X11401774.

104. Kimer N, Krag a, Møller S, Bendtsen F, Gluud LL. Systematic review with meta-analysis: the effects of rifaximin in hepatic encephalopathy. *Aliment Pharmacol Ther*. 2014;40(2):123-132. doi:10.1111/apt.12803.

105. Puxeddu A, Quartini M, Massimetti A, Ferrieri A. Rifaximin in the treatment of chronic hepatic encephalopathy. *Curr Med Res Opin*. 1995;13(5):274-281. doi:10.1185/03007999509111552.

106. Nagasue N, Dhar DK, Yamanoi A, Emi Y, Udagawa J, Yamamoto a, Tachibana M, Kubota H, Kohno H, Harada T. Production and release of endothelin-1 from the gut and spleen in portal hypertension due to cirrhosis. *Hepatology*. 2000;31(5):1107-1114. doi:10.1053/he.2000.6596.

107. Thalheimer U, Triantos CK, Samonakis DN, Patch D, Burroughs a K. Infection, coagulation, and variceal bleeding in cirrhosis. *Gut*. 2005;54(4):556-563. doi:10.1136/gut.2004.048181.

108. Qiang Zhu, Li Zou, Kumaravelu Jagavelu Douglas A. Simonetto, Robert C.Huebert, Zhi-Dong Jiang, Herbert L. DuPont and VHS. Intestinal decontamination inhibits TLR4 dependent fibronectin mediated crosstalk between stellate cells and endothelial cells in liver fibrosis in mice. *J Hepatol*. 2012;56(4):893–899. doi:10.1016/j.micinf.2011.07.011.Innate.

109. Bellot P, García-Pagán JC, Francés R, Abraldes JG, Navasa M, Pérez-Mateo M, Such J, Bosch J. Bacterial DNA translocation is associated with systemic circulatory abnormalities and intrahepatic endothelial dysfunction in patients with cirrhosis. *Hepatology*. 2010;52(6):2044-2052. doi:10.1002/hep.23918.

110. Strauss E. Hepatosplenic schistosomiasis : a model for the study of portal hypertension. *Ann Hepatol*. 2002;1(1):6-11.

111. Andrade Z A. Schistosomal hepatopathy. *Mem Inst Oswaldo Cruz*.

2004;99(5 Suppl 1):51-57. doi:/S0074-02762004000900009.

112. Gasim, Gasim I, Bella Abdelhaleem AI. Schistosomiasis , hepatitis B and hepatitis C co-infection. *Vilorogy J*. 2015;12(19):4-9. doi:10.1186/s12985-015-0251-2.

113. Abdel-Rahman M, El-Sayed M, Raziky M El, Elsharkawy A, El-Akel W, Ghoneim H, Khattab H, Esmat G. Coinfection with hepatitis C virus and schistosomiasis: Fibrosis and treatment response. *World J Gastroenterol*. 2013;19(17):2691-2696. doi:10.3748/wjg.v19.i17.2691.

114. Osada Y, Kanazawa T. Schistosome: Its benefit and harm in patients suffering from concomitant diseases. *J Biomed Biotechnol*. 2011;2011(Table 1). doi:10.1155/2011/264173.

115. Tannapfel A, Dienes H-P, Lohse AW. The indications for liver biopsy. *Dtsch Arztebl Int*. 2012;109:477-483. doi:10.3238/arztebl.2012.0477.

116. El-Shabrawi MH, El-Karaksy HM, Okahsa SH, Kamal NM, El-Batran G, Badr KA. Outpatient Blind Percutaneous Liver Biopsy in Infants and Children: Is it Safe? *Saudi J Gastroenterol*. 2012;18(1):26-33. doi:10.4103/1319-3767.91735.

117. Leonard B. Seeff, Gregory T. Everson, Timothy R. Morgan TM, Curto, MSW, William M. Lee, Marc G. Ghany MLS, Robert J. Fontana, Adrian M. Di Bisceglie HLB, Dienstag JL. Complication Rate of Percutaneous Liver Biopsies among Persons with Advanced Chronic Liver Disease in the HALT-C Trial. *Clin Gastroenterol Hepatol*. 2010;8(10):877-883. doi:10.1021/ja8019214.Optimization.

118. Fallatah HI. Noninvasive Biomarkers of Liver Fibrosis: An Overview. *Adv Hepatol*. 2014;2014(8):1-15. doi:10.1155/2014/357287.

119. Liu T, Wang X, Karsdal M a., Leeming DJ, Genovese F. Molecular serum markers of liver fibrosis. *Biomark Insights*. 2012;7:105-117. doi:10.4137/BMI.S10009.

120. Tarrats N, Moles A, Morales A, García-Ruiz C, Fernández-Checa JC, Marí M. Critical role of tumor necrosis factor receptor 1, but not 2, in hepatic stellate cell proliferation, extracellular matrix remodeling, and liver fibrogenesis. *Hepatology*. 2011;54(1):319-327. doi:10.1002/hep.24388.

121. Castera L. Noninvasive methods to assess liver disease in patients with hepatitis B or C. *Gastroenterology*. 2012;142(6):1293-1302.e4. doi:10.1053/j.gastro.2012.02.017.

122. Ismail Khadiga Almed, Almed Abd-EL-Ghany E abdel FN. Serum Hyaluronic Acid (HA) and Soluble Intercellular Adhesion Molecule-1(Sicam-1) as Non-Invasive Markers of Liver Fibrosis in Viral Hepatitis, Schistosomiasis mansoni and Co-infected Patients. *J Bacteriol Parasitol*. 2012;3(8):1-7. doi:10.4172/2155-9597.1000155.

123. De Souza RDP, Cardoso LS, Lopes GTV, Almeida MCF, Oliveira RR, Alcântara LM, Carvalho EM, Araujo MI. Cytokine and chemokine profile in individuals with different degrees of periportal fibrosis due to Schistosoma mansoni infection. *J Parasitol Res*. 2012;2012. doi:10.1155/2012/394981.

124. Bourke CD, Nausch N, Rujeni N, Appleby LJ, Mitchell KM, Midzi N, Mutapi F. Integrated Analysis of Innate , Th1 , Th2 , Th17 , and

Regulatory Cytokines Identi fi es Changes in Immune Polarisation Following Treatment of Human Schistosomiasis. *J Infect Dis*. 2013;208:159-169. doi:10.1093/infdis/jis524.

125. Coutinho HM, Acosta LP, Wu HW, Mcgarvey ST, Su L, Langdon GC, Jiz MA, Jarilla B, Olveda RM, Friedman JF, Kurtis JD. Th2 Cytokines Are Associated with Persistent Hepatic Fibrosis in Human Schistosoma japonicum Infection. *JID*. 2007;195:288-295.

126. Ellis MK, Li Y, Hou X, Chen H, McManus DP. sTNFR-II and sICAM-1 are associated with acute disease and hepatic inflammation in schistosomiasis japonica. *Int J Parasitol*. 2008;38(6):717-723. doi:10.1016/j.ijpara.2007.09.013.

127. Mwatha JK, Kimani G, Kamau T, Gabriel G, Ouma JH, Mumo J, Fulford AJC, Jones FM, Butterworth AE, Roberts MB, Dunne DW. High Levels of TNF, Soluble TNF Receptors, Soluble ICAM-1, and IFN- γ , but Low Levels of IL-5, Are Associated with Hepatosplenic Disease in Human Schistosomiasis Mansoni. *J Immunol*. 1998;(160):1992–1999.

128. Grønbæk H, Sandahl TD, Mortensen C, Vilstrup H, Møller HJ, Møller S. Alimentary Pharmacology and Therapeutics Soluble CD163 , a marker of Kupffer cell activation , is related to portal hypertension in patients with liver cirrhosis. 2012;(May):173-180. doi:10.1111/j.1365-2036.2012.05134.x.

129. Peter Holland-Fischer, Henning Grønbæk TDS, Søren K Moestrup, Oliviero Riggio, Lorenzo Ridola NKA, Holger Jon Møller HV. Kupffer cells

are activated in cirrhotic portal hypertension and not normalised by TIPS. *Hepatology*. 2011;60:1389-1393. doi:10.1136/gut.2010.234542.

130. de Oca Arjona MM, Marquez M, Soto MJ, Rodriguez-Ramos C, Terron A, Vergara A, Arizcorreta A, Fernandez-Gutierrez C, Giron-González JA. Bacterial Translocation in HIV-Infected Patients With HCV Cirrhosis: Implication in Hemodynamic Alterations and Mortality. *J Acquir Immune Defic Syndr*. 2011;56(5):420-427. doi:10.1097/QAI.0b013e31820ef408.

131. Koo, Dr. Sabounchi, Dr. Huang, Dr. DuPont, Koo, Dr. Sabounchi, Dr. Huang, Dr. DuPont. Rifaximin Therapy of Irritable Bowel Syndrome. *Clin Med Insights Gastroenterol*. 2012:31. doi:10.4137/CGast.S7382.

132. Bajaj JS, Barrett a C, Bortey E, Paterson C, Forbes WP. Prolonged remission from hepatic encephalopathy with rifaximin: results of a placebo crossover analysis. *Aliment Pharmacol Ther*. 2015;41(1):39-45. doi:10.1111/apt.12993.

133. Mueller S, Sandrin L. Liver stiffness: a novel parameter for the diagnosis of liver disease. *Hepatic Med Evid Res*. 2010;2:49-67. doi:10.2147/HMER.S7394.

134. Kircheis G, Sagir A, Vogt C, Dahl S Vom, Kubitz R, Häussinger D. Evaluation of acoustic radiation force impulse imaging for determination of liver stiffness using transient elastography as a reference. *World J Gastroenterol*. 2012;18(10):1077-1084. doi:10.3748/wjg.v18.i10.1077.

135. Stehle JR, Leng X, Kitzman DW, Nicklas BJ, Kritchevsky SB, High KP. Lipopolysaccharide-Binding Protein, a Surrogate Marker of Microbial

Translocation, Is Associated With Physical Function in Healthy Older Adults. *Journals Gerontol Ser A Biol Sci Med Sci*. 2012;67(11):1212-1218. doi:10.1093/gerona/gls178.

136. López M, Figueras F, Coll O, Goncé A, Hernández S, Loncá M, Vila J, Gratacós E, Palacio M. Inflammatory Markers Related to Microbial Translocation Among HIV-Infected Pregnant Women: A Risk Factor of Preterm Delivery. *J Infect Dis*. 2015:jiv416. doi:10.1093/infdis/jiv416.

137. Montecucco F, Mach F. New evidences for C-reactive protein (CRP) deposits in the arterial intima as a cardiovascular risk factor. *Clin Interv Aging*. 2008;3(2):341-349. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2546477&tool=pmcentrez&rendertype=abstract.

138. Zimmermann O, Li K, Zaczkiewicz M, Graf M, Liu Z, Torzewski J. C-Reactive Protein in Human Atherogenesis: Facts and Fiction. *Mediators Inflamm*. 2014;2014(2):1-6. doi:10.1155/2014/561428.

139. Charles A. Dinarello, Anna Simon and Van der M. Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. *Nat Rev Drug Discov*. 2008;42(2):157-162. doi:10.1037/a0030561.Striving.

140. Giannitrapani L. Genetic association of interleukin-6 polymorphism (-174 G/C) with chronic liver diseases and hepatocellular carcinoma. *World J Gastroenterol*. 2013;19(16):2449. doi:10.3748/wjg.v19.i16.2449.

141. Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochim Biophys*

Acta - Mol Cell Res. 2011;1813(5):878-888. doi:10.1016/j.bbamcr.2011.01.034.

142. Bremer E. Targeting of the tumor necrosis factor receptor superfamily for cancer immunotherapy. *ISRN Oncol*. 2013;2013:371854. doi:10.1155/2013/371854.

143. Ogawa Y, Imajo K, Yoneda M, Kessoku T, Tomeno W, Shinohara Y, Kato S, Mawatari H, Nozaki Y, Fujita K, Kirikoshi H, Maeda S, Saito S, Wada K, Nakajima A. Soluble CD14 Levels Reflect Liver Inflammation in Patients with Nonalcoholic Steatohepatitis. *PLoS One*. 2013;8(6):e65211. doi:10.1371/journal.pone.0065211.

144. Etzerodt A, Moestrup SK. CD163 and Inflammation: Biological, Diagnostic, and Therapeutic Aspects. *Antioxid Redox Signal*. 2013;18(17):2352-2363. doi:10.1089/ars.2012.4834.

145. Takeshita S, Nakatani K, Tsujimoto H, Kawamura Y, Kawase H, Sekine I. Increased levels of circulating soluble CD14 in Kawasaki disease. *Clin Exp Immunol*. 2000;119(2):376-381. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1905502&tool=pmcentrez&rendertype=abstract.

146. Wang J, Yu Y, Yang Y, Wu SS, Zhu HH, Liu YN, Liu WX, Hu Y, Wu W. Expression of serum sCD163 in patients with liver diseases and inflammatory disorders. *Int J Clin Exp Pathol*. 2015;8(7):8419-8425.

147. Holger Jon Møller, Henning Grønbæk, Frank V. Schiødt P, Holland-Fischer, Michael Schilsky, Santiago Munoz TH, Lee and WM. Soluble

CD163 from activated macrophages predicts mortality in acute liver failure. *J Hepatol*. 2007;47(5):671-676.

148. Zucol F, Ammann RA, Berger C, Aebi C, Altwegg M, Niggli FK, Zucol F, Ammann RA, Berger C, Aebi C, Altwegg M, Niggli FK, Nadal D. Real-Time Quantitative Broad-Range PCR Assay for Detection of the 16S rRNA Gene Followed by Sequencing for Species Identification Real-Time Quantitative Broad-Range PCR Assay for Detection of the 16S rRNA Gene Followed by Sequencing for Species Identifica. *J Clin Microbiol*. 2006;44(8):2750-2759. doi:10.1128/JCM.00112-06.

149. Ibrahim SZ, Arbab BM, Suleiman SH, Abdelwahab O, Hassan MA. The natural history of bleeding oesophageal varices in patients with schistosomal portal hypertension. *Khartoum Med J*. 2010;3(1):385-392.

150. Carruthers H. Bilharzial portal fibrosis : portal hypertension. *Ann R Coll Surg Engl*. 1978;60:49-52.

151. Vocke A-K, Kardorff R, Ehrich JH. Sonographic measurements of the portal vein and its intrahepatic branches in children. *Eur J Ultrasound*. 1998;7(2):121-127. doi:10.1016/S0929-8266(98)00016-0.

152. Berbée JFP, Coomans CP, Westerterp M, Romijn JA, Havekes LM, Rensen PCN. Apolipoprotein CI enhances the biological response to LPS via the CD14/TLR4 pathway by LPS-binding elements in both its N- and C-terminal helix. *J Lipid Res*. 2010;51(7):1943-1952. doi:10.1194/jlr.M006809.

153. Brenner SA, Zacheja S, Sch M, Bischoff SC. Soluble CD14 is essential for

lipopolysaccharide-dependent activation of human intestinal mast cells from macroscopically normal as well as Crohn's disease tissue. *Immunology*. 2014;143(2):174-183. doi:10.1111/imm.12299.

154. Sinkala E, Kapulu MC, Besa E, Zyambo K, Chisoso NJ, Foster GR, Kelly P. Hepatosplenic schistosomiasis is characterised by high blood markers of translocation, inflammation and fibrosis. *Liver Int*. 2015;(4):n/a-n/a. doi:10.1111/liv.12891.

155. Myc A, Buck J, Gonin J, Reynolds B, Hammerling U, Emanuel D. The level of lipopolysaccharide-binding protein is significantly increased in plasma in patients with the systemic inflammatory response syndrome. *Clin Diagn Lab Immunol*. 1997;4(2):113-116. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=170487&tool=pmcentrez&rendertype=abstract.

156. Gonzalez-Quintela A, Alonso M, Campos J, Vizcaino L, Loidi L, Gude F. Determinants of Serum Concentrations of Lipopolysaccharide-Binding Protein (LBP) in the Adult Population: The Role of Obesity. *PLoS One*. 2013;8(1):1-8. doi:10.1371/journal.pone.0054600.

157. Agustı´n Albillos, Antonio de la Hera, M´ onica Gonz´alez, Jose-Luis Moya, Jose-Luis Calleja, Jorge Monserrat, Alvarez-Mon LR-A and M. Increased Lipopolysaccharide Binding Protein in Cirrhotic Patients With Marked Immune and Hemodynamic Derangement. *Hepatology*. 2003;37(1):209-216. doi:10.1053/jhep.2003.50038.

158. Arthur L, Leite C. Relationship between splenomegaly and hematologic

findings in patients with hepatosplenic schistosomiasis. *Rev Bras Hematol Hemoter*. 2013;35(5):DOI: 10.5581/1516-8484.20130098. doi:10.5581/1516-8484.20130098.

159. Funakoshi N, Ségalas-largey F, Duny Y, Oberti F, Valats J, Bismuth M, Daurès J, Blanc P, Funakoshi N, Ségalas-largey F. Benefit of combination β -blocker and endoscopic treatment to prevent variceal rebleeding : A meta-analysis. *World J Gastroenterol*. 2010;16(47):5982-5992. doi:10.3748/wjg.v16.i47.5982.

160. Levitt DG, Levitt MD. Quantitative modeling of the physiology of ascites in portal hypertension. *BMC Gastroenterol*. 2012;12(1):26. doi:10.1186/1471-230X-12-26.

161. Rebouças G. Clinical aspects of hepatosplenic schistosomiasis: a contrast with cirrhosis. *Yale J Biol Med*. 1975;48(5):369-376.

162. Nedredal GI, Yin M, McKenzie T, Lillegard J, Luebke-Wheeler J, Talwalkar J, Ehman R, Nyberg SL. Portal hypertension correlates with plenic stiffness as measured with magnetic resonance elastography. *J Magn Reson Imaging*. 2011;34(1):79-87. doi:10.1002/jmri.22610.PORTAL.

163. Chang PE, Goh GB, Ngu JH, Tan HK, Tan CK. Clinical applications , limitations and future role of transient elastography in the management of liver disease. *World J Gastrointest Pharmacol Ther*. 2016;7(1):91-106. doi:10.4292/wjgpt.v7.i1.91.