# HIV INFECTION-ASSOCIATED LEAKY GUT: A POTENTIAL RISK FACTOR FOR PERNICIOUS ANAEMIA IN HIV PATIENTS

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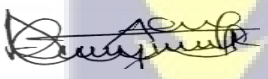
## DECLARATION

I, Daniel Amakye do at this moment declare that except for references to the literature and works of other researchers which have been duly cited, the work in this dissertation is the result of my original work conducted at the Department of Biochemistry, Cell and Molecular Biology, West African Centre for Cell Biology of Infectious Pathogens (WACCBIP), University of Ghana. The work was carried out under the close supervision of Prof Osbourne Quaye and Dr Emmanuel Ayitey Tagoe. I further declare that this dissertation has not been submitted for awarding any degree in this institution or elsewhere.

SIGNATURE  DATE: 26th November, 2024

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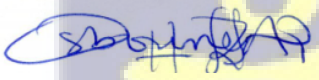
SIGNATURE:  DATE: 26th November, 2024

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## DEDICATION

I wish to dedicate this work of mine to my Father in Heaven and my parents, Mr and Mrs Amakye for being my rock.

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I am very grateful to all individuals and groups who contributed to making this study successful. To the Gracious God for the life, grace, and wisdom he gifted me with to complete this journey. I am humbled with gratitude to my supervisors, Prof Osbourne Quaye and Dr Emmanuel Ayitey Tagoe, for their immense support and guidance throughout this study. To all the staff of the Department of Biochemistry, Cell and Molecular Biology and that of the West African Centre for Cell Biology and Infectious Pathogens (WACCBIP). I also say a big thank you to DAAD for their support, and finally, members of the Quaye lab, I say thank you for your diverse contributions to this project.

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## LIST OF ABBREVIATIONS AND ACRONYMS

AIDS- Acquired Immune Deficiency Disease
anti-β2 GPI- Anti-β 2 -glycoprotein I
ART- Antiretroviral Therapy
bp- Base Pair
cAMP- Adenosine Monophosphate
CCR5- C-C Chemokine Receptor Type 5
CD4+- Cluster of Differentiation 4+
CDC- Centre for Disease Control
CNS- Central Nervous System
CXCR4- C-X-C chemokine receptor type 4
DNA- Deoxy Ribonucleic Acid
ELISA- Enzyme Linked Immunosorbent Assay
FDA- Food and Drugs Authority
GALT- Gut-Associated Lymphoid Tissue
GLP-1R- Glucagon-like Peptide-1 Receptor
GLUT-4- Glucose transporter type 4
GPR- G Protein-Coupled Receptor
gRNA- Genomin Ribonucleic Acid
HAART- Highly Active Antiretroviral Therapy
HAND- HIV-Associated Neurocognitive Disorders
HDL- High-Density Lipoprotein
HIV- Human Immunodeficiency Virus
HLA- Human Leukocyte Antigen
HRP- Horse Radish Peroxidase
IF- Intrinsic Factor
IFN- Interferon
IgG- Immunoglobulin G
IKK- Inhibitor of Nuclear Factor-κB Kinase
JNK- c-Jun N-terminal kinase
LDL- Low-Density Lipoprotein
LPS- Lipopolysaccharide
LTR- Long terminal repeats
MxA- Myxovirus Resistance Protein 1
NCD- Non-Communicable Diseases
NMDAR- N-methyl-D-Aspartate Receptor
NNRTI- Non Nucleoside Reverse Transcriptase Inhibitor
NRTI- Nucleoside Reverse Transcriptase Inhibitor
ORF- Open Reading Frame
PCR- Polymerase Chain Reaction
PEP- Post-Exposure Prophylaxis
PI- Protease Inhibitors
PLWH- People Living with HIV

PrEP- ..... Pre-Exposure Prophylaxis
RNA- ..... Ribonucleic Acid
SCFA- ..... Small Chain Fatty Acids
TAE- ..... Tris-Acetate EDTA
TLR4- ..... Toll-like Receptor 4
TNF- ..... Tumour Necrosis Factor Alpha
WHO- ..... World Health Organisation

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## ABSTRACT

Background: The Human immunodeficiency virus has circulated in the human population for over 25 years. According to the World Health Organization, as of the end of the year 2022, approximately 39 million people were living with HIV infection. This increase in the number of PLWH is attributed to the use of antiretrovirals. ARTs moved HIV infection from a deadly condition into a chronic and manageable one, accompanied by non-AIDS comorbidities. Associated autoimmune complications like pernicious anaemia have been reported in PLWH. Leaky gut, a complication of HIV infection leads to the translocation of gastric content into circulation priming the immune system to respond. The translocation of self-products like intrinsic factor leads to the production of autoantibodies that destroy them.

Method: The study was a case-control study with 55 each of HIV seropositive and seronegative. Haematological profiling focusing on red blood cell count, haemoglobin concentration and mean cell volume was used to detect suspected pernicious anaemia (PA) cases. LPS was measured using ELISA to detect leaky gut and translocation. Levels of intrinsic factor autoantibodies was measured in the serum using ELISA.

Results: There was a higher prevalence of pernicious anaemia in the HIV cohort as compared to the HIV seronegative, > 40% against 1.8% respectively. The study showed a pronounced case of leaky gut and translocation in the PLWH as compared with the seronegative group with concentration of LPS in their serum being significantly higher ($p < 0.0001$). PLWH who had pernicious anaemia also had a higher concentration of serum LPS than those who had HIV but did not have pernicious anaemia ($p < 0.001$). Levels of LPS had a direct relation with concentration of autoantibodies with PLWH and having pernicious anaemia having higher serum concentrations than those without PA which was also higher than HIV seronegatives ($p< 0.0001$).

Conclusion: Increased serum concentration of LPS which signifies leaky gut was observed in PLWH and suffering from pernicious anaemia. The translocation of intrinsic factor into circulation during the leaky gut led to the production of autoantibodies against intrinsic factor. This resulted in the destruction and hence unavailability of intrinsic factor for the absorption of vitamin B12.

# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1 Background

Humans have been struggling with diseases for centuries. Over the years, we have encountered both infectious and non-communicable diseases. Communicable diseases have microorganisms as their etiological agents (Cloeckaert and Guzmán-Verri, 2023). Usually, these conditions can be transferred from one person (infected) to another (uninfected) (Ellwanger et al., 2021). These agents belong to one of five groups: bacteria, viruses, fungi, helminths, and protozoa (Shukla et al., 2024). The global burden of diseases caused by these agents has seen a 46-percentage reduction, from 1.2 billion in 1990 to below 650 million in 2019 (WHO, 2022b). Africa has been the central hub of these infectious agents, claiming millions of lives (Margareth, 2017; Nii-Trebi, 2017; Schubert et al., 2021). Over the years, however, there has been a change in statistics in most of the African countries, including Ghana. The disease burden associated with infectious agents has fallen, and non-communicable diseases top the charts (Boakye et al., 2023; Moyo-Chilufya et al., 2023; Pham et al., 2022). As of 2019, seven of the ten leading causes of death were non-communicable diseases (Bigna and Noubiap, 2019). Researchers and scientists have tried to explain this new narrative, bringing about several hypotheses. Among these, the advancement in medication and adaptation of the immune system against infectious agents most explains why the burden due to infectious agents has been reduced (Baker et al., 2022). Researchers have proposed that the advancement in medication and the interaction of the immune system with these agents could account for the rise in non-communicable diseases(Ogoina and Onyemelukwe, 2009). There have been several reports of the involvement of bacteria, fungi, viruses, protozoa, and helminths in diseases like diabetes, cardiovascular diseases, cancer, etc (Coates et al., 2020; Furman et al.,

2019; Garcia-Bonete et al., 2023; Sohail et al., 2022).

The Human immunodeficiency virus has circulated in the human population for over 25 years (Fajardo-Ortiz et al., 2017). Since the virus made a jump from primates to the human population, it has caused about 86 million infections (Payagala and Pozniak, 2024). According to the World Health Organization, as of the end of the year 2022, approximately 39 million people were living with HIV infection. This increase in the number of PLWH is attributed to the use of antiretrovirals (Abubakari et al., 2023). The classes of antiretrovirals used against HIV, protease inhibitors, nucleoside reverse transcriptase inhibitors, integrase strand transfer inhibitors, non-nucleoside reverse transcriptase inhibitors, fusion inhibitors, CCR5 inhibitors, and post attachment inhibitors function by suppressing the viral loads (Aquaro et al., 2020). HIV-infected individuals who are put on therapy early are protected from the more severe form of the disease, AIDS (Suanzes et al., 2023). ARTs work by interfering with the replication of the virus. This leads to a suppressed viral count and the reduction in the destruction of CD4+ cells (Yang et al., 2020). The improvement in the life expectancy of people living with HIV, however, did not guarantee a sustained quality of life. The advent of effective ARTs moved HIV infection from a deadly condition into a chronic and manageable one (Cummins, 2022). PLWH tend to have a persistent immune activation due to the low viraemia (Pasternak and Berkhout, 2023). This sustained immune activation and the related inflammation contribute to non-AIDS complications in HIV patients receiving ART (Mazzuti et al., 2023).

There have been reports of people living with HIV developing chronic conditions, including diabetes, hypertension, and some cancers (Tegene et al., 2023). Associated autoimmune complications (Pernicious anaemia, rheumatoid arthritis, Graves disease, autoimmune hepatitis, systemic lupus erythematosus) that arise from chronic immune activation and inflammation

have been reported with increasing occurrence in these PLWH (Pellicano et al., 2019). These chronic diseases are more common in people living with HIV (PLWH) than people without HIV infection (Yang et al., 2019). This has challenged scientists and clinicians worldwide about effectively managing PLWH.

A complex of tight junction proteins maintains the structure of the epithelium that lines the intestine (Paradis et al., 2021). This prevents the movement of microbes, toxins and other molecules from the lumen of the gut into the systemic circulation (Buckley and Turner, 2018). The mechanism underlying immune activation in HIV patients on ART has been attributed to gut dysplasia, loss of structural integrity of the intestinal mucosa and a resulting permeability of the mucosal barrier (leaky gut) (Fakharian et al., 2023). The leaky theory suggests that injuries to the gut lining can result in the intestines becoming permeable, provided the source of the injury is persistent (Vanuytsel et al., 2021). During HIV infection, there is a persistent insult to the gut lining since the gut serves as home to white blood cells (so-called gut-associated lymphoid tissue), including CD4+ cells, the primary cell target for HIV (Moretti et al., 2023). Furthermore, ARTs do not eliminate HIV. The suppressed viral loads turn to elicit a sustained inflammation reaction that compromises the integrity of the gut lining (Ouyang et al., 2023). The HIV-induced gut permeability, in turn allows for the increased translocation of microbial antigens, which couples with other mechanisms to bring about a perpetual state of heightened immune activation (Mak et al., 2021). Increased microbial translocation, immune activation and inflammation have thus been reported in HIV- infected individuals, and leaky gut has been proposed to be the source of non-AIDS complications during HIV infection (Merlini et al., 2021).

Autoimmune diseases occur when the immune system is stimulated to produce antibodies that

attack the person's cells and tissue components. A fast-growing number of autoimmune diseases, including pernicious anaemia, are recognised to involve alterations in intestinal permeability related to changes in tight junction competency (Alzahrani et al., 2019a). Pernicious anaemia is an autoimmune disease of multifactorial aetiologies, characterised by autoimmune chronic atrophic gastritis, vitamin B12 deficiency, and the presence of intrinsic factor and parietal cell antibodies (Esposito et al., 2022). Vitamin B12 is essential for the production of blood cells and the myelination of nerves (Mathew et al., 2024). Low serum B12 levels have been associated with increased neurologic abnormalities and more rapid HIV disease progression (Kavitha et al., 2022). In pregnant women, low levels of vitamin B12 may lead to spontaneous abortion (Behere et al., 2021). Low serum B12 levels have been reported to be more common in HIV- positive subjects compared with healthy HIV-negative subjects (Kalejaiye et al., 2021). Absorption of vitamin B12 takes place in the gut with the aid of a carbohydrate produced by the parietal cells, an intrinsic factor (Allen et al., 2018). Without intrinsic factors, vitamin B12 absorption is compromised, leading to low serum vitamin and, consequently, unavailability of vitamin B12 for erythropoiesis. A leaky gut, which is more pronounced in HIV, results in the translocation of gastric content into the gut (Ashuro et al., 2020). Translocation of self-products like intrinsic factors into circulation may result in the production of antibodies against intrinsic factors, leading to the destruction and unavailability of intrinsic factors for vitamin B12 absorption.

### 1.2 Problem Statement

Even though great progress has been made in providing people living with HIV a normal life expectancy, individuals on antiretroviral therapy tend to develop non-AIDS morbidity and mortality (Trickey et al., 2024). There has been an alarming rise in non-communicable diseases

like cardiovascular diseases, metabolic diseases, neurological diseases (HAND) and autoimmune diseases in the population of PLWH (Chang et al., 2022). These conditions are thought to arise as a result of the persistent immune activation and sustained inflammatory response elicited as a result of the low viraemia (Zicari et al., 2019). Autoimmune diseases in HIV are thought to arise due to the translocation of gut microbes and other gastric contents into the circulation and inducing the persistent production of autoantibodies (Williams et al., 2016). Anaemia is a condition of public health concern affecting children, menstruating adolescent girls and women, pregnant and postpartum women (Ashraf et al., 2024). In PLWH, this condition is of particular concern as an estimated 63-95% of the population experience it (Geleta et al., 2021). Anaemia in HIV influences the progression of the disease, interferes with the management of the condition and increases their mortality risk (Cao et al., 2022). Pernicious anaemia is a rare autoimmune disease with a frequency of about 0.1 % in the general population (Shrestha et al., 2022). However, in HIV patients, especially in virally suppressed individuals, numbers as high as 51.9%, 55.8% and 25.8% have been reported in China, Nepal and Johannesburg, respectively (Harding et al., 2020). Persistent low levels of vitamin B12 in HIV patients have been associated with neuropsychiatric conditions like dementia, depression, demyelinating myelopathy and the occurrence of oral lesions (Puspasari et al., 2018). Proper management of HIV-associated pernicious anaemia is crucial for improving immune status, attaining near-normal life expectancy and enhancing the quality of life. In Ghana, the number of individuals with HIV suffering from pernicious anaemia has not been accurately reported, and the exact mechanism through which pernicious anaemia occurs in HIV patients is not known. This makes effective management of these patients a problem.

### 1.3 Significance of Study

The study will highlight the need to manage HIV-infected patients beyond the administration of ARTs holistically. Anaemia is a multifactorial condition leading to decreased production of red blood cells and low haemoglobin condition (Chaparro and Suchdev, 2019). Even though all the factors lead to the same outcome, properly elucidating the exact cause of the anaemia and which of the types of anaemia is most prevalent in HIV will help in the effective management of PLWH. This study seeks to establish the cause of pernicious anaemia in PLWH. Establishing the occurrence of leaky gut and the subsequent production of autoantibodies that inactivate intrinsic factors and parietal cells in PLWH will provide information on how these patients develop pernicious anaemia. An elucidated mechanism will direct the management of these patients and possibly guide the production and use of autoimmune antibody therapy to improve the quality of life of said patients.

### 1.4 Aim

To Investigate HIV Infection-Associated Leaky Gut as a Risk Factor of Pernicious Anaemia in Patients on Antiretroviral Therapy.

### 1.5 Specific objectives

- To assess gut barrier damage among HIV-infected patients using Lipopolysaccharides ELISA kits.
- To associate leaky gut with immuno-driven Vitamin B12 derangement in HIV-infected patients using Intrinsic factor autoantibodies ELISA kits.
- To determine clinical staging and haemato-biochemical status in HIV-infected patients.

# CHAPTER TWO

# 2.0 LITERATURE REVIEW

## 2.1 Infectious Agents and Their Roles in Non-Communicable Diseases.

Non-communicable diseases (NCDs) or chronic diseases are diseases characterised by slow progression and a longer duration of the condition (Milic et al., 2020). Data from the World Health Organisation show that NCDs are the most common causes of mortality worldwide, accounting for over 70% of total mortality each year (Boakye et al., 2023). A wide range of conditions fall under the umbrella of non-communicable diseases; however, the leading causes of death are cancers, diabetes, cardiovascular diseases and pulmonary diseases (Bigna and Noubiap, 2019). Currently, the NCD burden is on the rise in developing countries and the related mortality and morbidity have doubled (Ndumwa et al., 2023). Non-communicable diseases are non-infectious. They arise from a variety of factors, which can be classified into genetic, self-management, environmental, social demographic, and other medical conditions (Budreviciute et al., 2020). Even though NCDs are non-infectious, over the years, there has been increasing evidence suggesting the involvement of infectious agents in their pathogenesis. The gut microbiome, *H. pylori*, Hepatoviruses and several other microbes have been implicated in conditions like cancer, diabetes, and cardiovascular diseases.

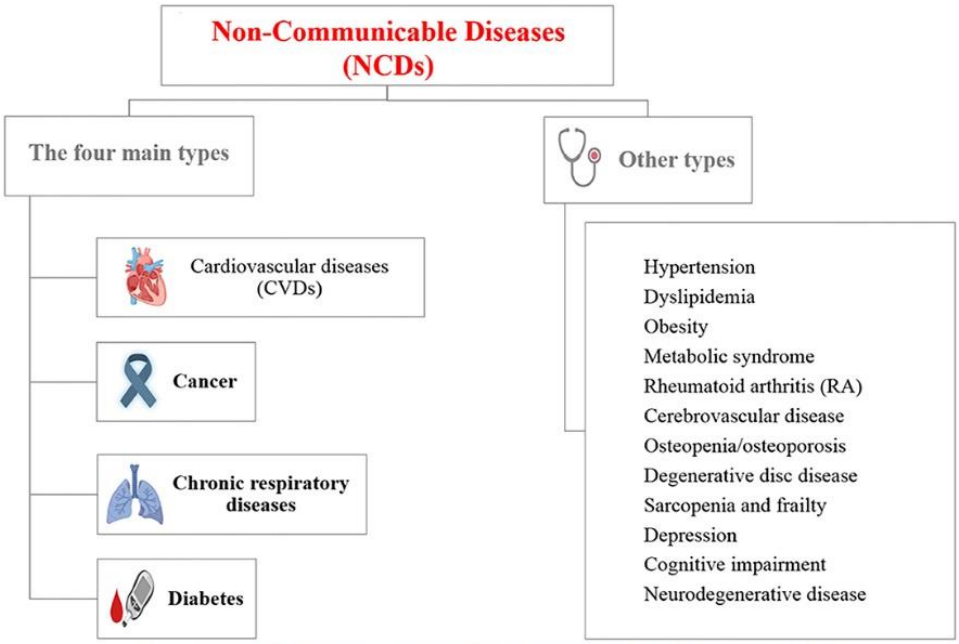


**Figure 2.1**: Main Groups of Non-Communicable Diseases (Budreviciute et al., 2020)

### 2.1.1 Infectious Agents and Diabetes

Diabetes continues to attract attention as one of the leading causes of morbidity. Since the year 2000, there has been a 70% increase in the cases of diabetes (Saeedi et al., 2019). As of the end of the year 2021, diabetes was the 8$^{th}$ leading cause of death worldwide (Ong et al., 2023). Diabetes is a chronic metabolic disorder characterised by the body's inability to utilise glucose effectively (Ong et al., 2023). This results in an increase in blood glucose, especially after meals. The pancreatic beta cells are responsible for producing insulin, a pancreatic hormone that regulates the uptake of glucose from the blood into cells for energy production (Dludla et al., 2023). The inability of these beta cells to produce the hormone or the failure of cells to respond to insulin leads to Type 1 and Type 2 diabetes mellitus, respectively (Tan et al., 2019).

Over the years, several microorganisms have been tied to diabetes. There is, however, the question of whether these microorganisms cause diabetes or having diabetes provided the environment for these microbes to thrive. Hyperglycaemia is an essential manifestation of diabetes (Galicia-Garcia

et al., 2020). It provides a suitable environment for microbes by causing immune dysfunction (Eckold et al., 2021). Hyperglycaemia hinders the functions of neutrophils, disrupts humoral immunity, and suppresses antioxidant activity (Dowey et al., 2021). The suppressed immune system results in infections that are difficult to treat. Bacterial and viral infections like *Streptococcus pneumonia*, *Chlamydophila pneumonia*, *Haemophilus influenza*, *Influenza A*, and *Hepatitis B* are among the infections exacerbated by diabetes (Brunetti et al., 2021; Cilloniz and Torres, 2024; X. yu Liu and Zhou, 2023; Verket et al., 2023). The exact mechanism through which hyperglycaemia brings about the exacerbated infections is not established, but alterations in i) energy supply to microbes, ii) inflammatory response, iii) pH and the viscosity of blood leading to a change in immune cells microenvironment and iv) oxidative stress because of metabolic activity of microbes have been proposed (Bigna and Noubiap, 2019).

Current research establishes the role microorganisms play in causing diabetes. The gut microbiota is of particular interest when it comes to diabetes, as there is growing evidence of an association between the gut flora and diabetes (Cunningham et al., 2021). The gut microbiota refers to the microbial community that resides in the gut, especially in the pockets of the large intestines (Bigna and Noubiap, 2019). These microbes, importantly bacteria, play a role in the digestion of food and control of the immune system (Hou et al., 2022). Alteration of the gut microbiota and people having a less diverse microbiota have been associated with metabolic diseases (Hrncir, 2022). The microflora of healthy individuals is populated by six phyla: *Proteobacteria*, *Fusobacteria*, *Bacteroidetes*, *Fermicutes*, *Actinobacteria* and *Verrucomicrobia* (Stojanov et al., 2020). Research into the role of the microbiome in diabetes highlights immune responses and metabolic processes as possible mechanisms (Zhang et al., 2021).

In a recent large-scale metagenomic analysis of human faecal samples of insulin-resistant diabetic patients and non-diabetic patients, *Faecalibacterium prausnitzii* and *Roseburia* were found to be lower in insulin-resistant patients (Leylabadlo et al., 2020). These bacteria are members of the butyrate-producing bacteria family in the gut. Butyrate is a straight-chain alkyl carboxylic acid known for its role in energy metabolism and intestinal homeostasis (Hodgkinson et al., 2023). Butyrate protects against obesity and diabetes by protecting β-cells in the pancreas from destruction or directly regulating the signal for insulin secretion through GPR-mediated signalling (Mayorga-Ramos et al., 2022). The protective role is achieved by reducing mitochondrial and oxidative stress in β-cells, ensuring survival. The role butyrate plays in insulin secretion signalling is still under debate. However, recent findings show that butyrate regulates cAMP signalling for the secretion of insulin through the GLP-1R receptors (Coppola et al., 2021). In an in-vivo study, treatment with sodium-butyrate indirectly enhanced the secretion of insulin by repressing functional genes in rat islets (Bridgeman et al., 2020). A leading source of butyrate in the gut is butyrate-producing bacteria like *F. prausnitzii* and *Roseburia*. These bacteria in the gut produce butyrate in one of two ways: a direct process where dietary fibre is fermented to produce butyrate by the bacteria (Singh et al., 2023). Butyrate is also produced indirectly through the action of *Bifidobacteria* (Van-Wehle and Vital, 2024). Gut dysbiosis, which can arise from dietary insufficiency and medications, results in the loss of these butyrate-producing bacteria making individuals prone to diabetes (Kullberg et al., 2024). In an experiment involving faecal bacteria transplant, insulin-resistant patients who were transplanted with microbiome from insulin-sensitive individuals showed a significant increase in their sensitivity to insulin (Wu et al., 2023).

Another mechanism through which microorganisms have been tied to the pathogenesis of diabetes is through immune stimulation (Sohail et al., 2022). Bacteria and bacteria components are very

potent immune stimulators (Zheng et al., 2020). Bacteria lipopolysaccharide is one of such immune stimulators. The presence of LPS in circulation leads to the production of inflammatory cytokines through adipocytes and immune cells, resulting in low-grade inflammation (Li et al., 2020). The toll-like receptor 4 is a transmembrane protein encoded by the TLR4 gene with a longstanding role in the recognition of LPS (Kim et al., 2023). Activation of TLR4 elicits a cascade that transcribes and produces inflammatory cytokines like TNF-α. With chronic inflammation being a key feature of diabetes and insulin resistance, TLR4 stimulation has been recognised for its possible role in the pathogenesis of diabetes (Yehualashet, 2020). TLR4 is thought to cause diabetes through its overexpression in insulin target sites. The expression of this receptor in target sites for insulin results in the activation of proinflammatory kinases like IKK, JNK and p38 (Sears and Kim, 2010). These kinases phosphorylate insulin receptor substrates, inhibiting their stimulation and directly interfering with the signal transduction of insulin. TLR4 also reduce insulin sensitivity in target cells by increasing the transcription of proinflammatory genes, increasing chemokines, cytokines, and reactive oxygen species that inhibit the transduction of insulin signals (Khalid et al., 2021).

### 2.1.2 Infectious Agents and Cardiovascular Diseases

As the leading cause of death globally, cardiovascular diseases (CVD) are of great public health concern (Vaduganathan et al., 2022). Cardiovascular diseases are a group of disorders affecting the heart and blood vessels (Frąk et al., 2022). They include but are not limited to coronary heart disease, cerebrovascular disease, deep vein thrombosis and pulmonary embolism. In the year 2019, cardiovascular diseases accounted for 32 per cent of all deaths, an estimated 17.9 million people (Roth et al., 2020). Symptoms demonstrated by people who suffer from cardiovascular diseases include pain in the centre of the chest, arms and left shoulder, unconsciousness, numbness on one

side of the face, arm or leg in strokes, shortness of breath, irregular heartbeats, and fatigue (NIH, 2023). Even though there are genetic and congenital heart diseases, risk factors attributed to heart diseases are mainly behavioural (Bays et al., 2022). The most common risk factors of CVDs are high blood pressure, high circulating levels of low-density lipoprotein cholesterol and diabetes (Ghodeshwar et al., 2023). These arise because of unhealthy diet and metabolic disorders. Microorganisms, especially the gut microbiome, play a crucial role in food metabolism (Wu et al., 2021). Alteration of the microbiome results in changes in metabolites that contribute to the development of cardiovascular diseases (Liu et al., 2022). Other microorganisms, through their metabolic activities, generate metabolites that affect signalling pathways, hindering the effective metabolism of some food constituents (Zhang et al., 2023).

Coronary heart disease is a condition where arteries cannot effectively deliver blood to the heart for circulation (Ralapanawa and Sivakanesan, 2021). This occurs when the lumen of blood vessels gets blocked by accumulated cholesterol, known as plaques. The continuous accumulation of plaques in the vessels leads to partial or total blockage of the blood vessels (Z. He et al., 2023). Coronary heart disease is the most common cardiovascular disease. In the year 2021, it killed about 375,476 people (Vaduganathan et al., 2022). Pathophysiological similarities between chronic infections and atherosclerosis triggered several studies into the association between infectious agents and coronary heart diseases (Pothineni et al., 2017). As early as 1997, *Helicobacter pylori* and *Chlamydia pneumonia* were acting as triggers for the inflammation in atherosclerosis (Ciarambino et al., 2024; B. Li et al., 2020). Other bacteria and viruses, including *Streptococcus sp*, *Prevotella intermedia*, *Enterobacter hormaechei*, *Cytomegalovirus* and *Hepatitis C,* have been associated with atherosclerosis (Chhibber-Goel et al., 2016; Jie et al., 2017; Li et al., 2020; Solis et al., 2021; Zhu and Liu, 2020). Over the years, ELISA, cell culture, immunohistochemical

techniques, and variations of PCR have been employed to address the controversies surrounding the roles these microbes play in coronary heart diseases (Fong, 2009).

Infections have long been reported to be involved in the initiation, progression, and rupture of atherosclerotic plaques (Khan et al., 2014). Bacteria are thought to contribute via indirect and direct mechanisms (Shen et al., 2021). The direct effects are injury to the endothelium, local inflammation, proliferation of smooth muscle and dysfunction of the endothelium through circulating endotoxins (Y. Zhu et al., 2020). The indirect effects include producing cross-reactive antibodies, fostering proinflammation, oxidation of LDL, inducing hypercoagulability and oxidative modification.

*H. pylori* is a gastric bacterium that is known to be an important cause of persistent gastro-digestive ulcers (Öztekin et al., 2021). However, recent evidence points to a role of the bacterium in extra gastroduodenal conditions, including heart diseases(Santos et al., 2020). The main mechanism through which *H. pylori* causes heart diseases is through inflammatory and immunological responses provoked by the virulent Cag A strain of the bacterium (Jamkhande et al., 2016). *H. pylori* infection-related heart disease is a multifactorial process (Li et al., 2024). Several mechanisms and processes utilised by *H. Pylori*, as summarised in Fig 2.2, contribute to heart diseases.

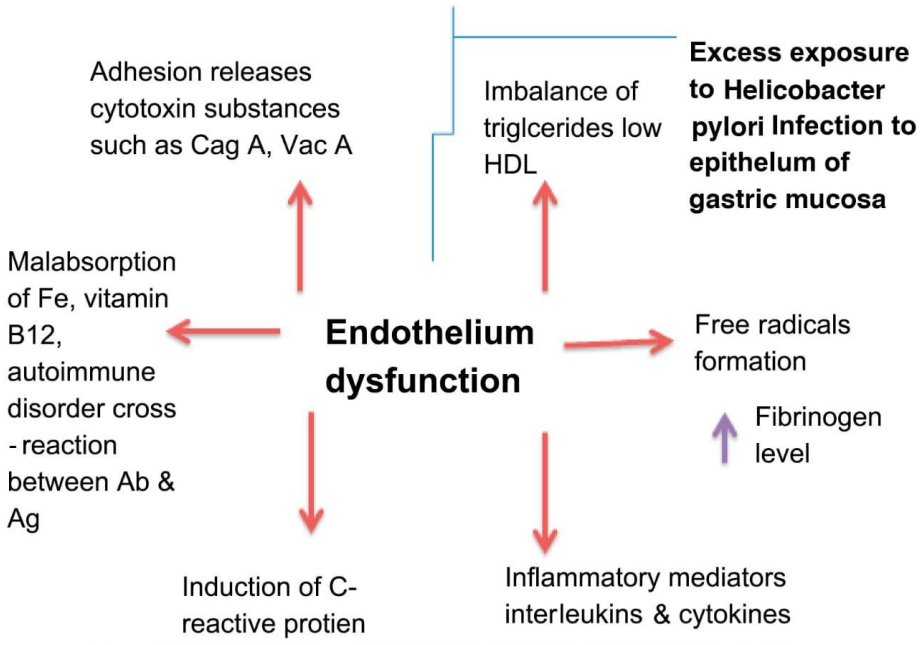


**Figure 2.2:** Different mechanisms by which H. pylori contributes to cardiovascular diseases (Jamkhande et al., 2016)

*H. pylori* is a common human infection (Kayali et al., 2018). Repeated exposures to the bacteria lead to chronic stimulation of the immune system, contributing to heart diseases (Tali et al., 2022). *H. pylori*-induced inflammatory response leads to altered lipid profiles, stimulates the release of c-reactive protein, and increases the levels of fibrinogen alongside proinflammatory cytokines (Temesgen et al., 2022). Fibrinogen is a plasma protein which plays a key role in the coagulation of blood (Butera and Hogg, 2020). When activated, it holds together platelets and other components to form a homeostatic plug that stops blood loss. In an unwarranted elevated state, it increases the viscosity of blood and causes aggregation of platelets (Sloop et al., 2020). This contributes to the narrowing of blood vessels and consequently causes coronary heart disease (Alexy et al., 2022). Dyslipidemia is a condition characterised by abnormal levels of cholesterol, especially an elevated level of LDL and a reduced concentration of HDL (Dybiec et al., 2023). HDL and LDL are lipoprotein cholesterols involved in the transport of lipids to the liver for metabolism and to cells, respectively, hence the names “good and bad” cholesterol (Mc Auley and

Morgan, 2022). *H. pylori*-induced dyslipidemia results in the accumulation of cholesterol in blood vessels because of elevated LDL (Hashim et al., 2022). This leads to the formation of plaques and atherosclerosis.

The residents of the guts are another group of microbes that have gained recognition for their possible role in cardiovascular diseases (Belli et al., 2023). Unusual changes in the human gut microbiome have been associated with the risk of cardiovascular diseases. The microbiome and the metabolites they produce greatly affect cardiovascular health (Rahman et al., 2023). The gut microbiome is altered in diversity and number in various disease states (Huang et al., 2022). This leads to changes in metabolites with a concomitant effect on physiological processes (S. Kim et al., 2024). According to a study by Emoto et al. (2016), patients with coronary artery disease had decreased levels of members of the phylum *Bacteroides*. Several pro-inflammatory microbes like *Klebsiella*, *Enterobacter*, *Escherichia coli*, *Collinsella* and *Streptococcus* have been identified to populate the gut of atherosclerotic patients (Yoo et al., 2022). Small-chain fatty acids (SCFAs) are a subgroup of fatty acids that are generated by the metabolic activity of the gut microbiota (Xiong et al., 2022). They are the main source of nutrition for cells of the colon and are produced when nondigestible polysaccharides are partially fermented by some bacteria residing in the gut (Martin-Gallausiaux et al., 2021). SCFAs are mainly of three types, acetate (C2), propionate (C3) and butyrate (C4), and they play an important role in colon health (He et al., 2020). They improve the health of the gut through several local effects ranging from maintaining the integrity of the intestinal barrier, production of mucus and offering protection against inflammation (Blaak et al., 2020). Recent evidence suggests that SCFAs are involved in the moderation of blood pressure (Fusco et al., 2023). Changes in the concentration of SCFAs have been reported in several cardiovascular diseases with butyrate lower in hypertensive individuals (Ostrowska et al., 2024).

The expression and activation of some G protein-coupled receptors are regulated by SCFAs (Carretta et al., 2021). These receptors are highly expressed by cells in the cardiovascular system and play an important role in regulating the functions of the system (Li et al., 2023). Analysis of the gut microbiome reported a decreased level of bacteria with fermenting abilities in cardiovascular diseases (Hou et al., 2022). This results in a decrease in the concentration of SCFAs and a resultant lack of protective functions.

## 2.2 HIV

### 2.2.1 Prevalence and Risk Factors of HIV Infection

The human immunodeficiency virus is a virus of public health concern (Nikolopoulos and Tsantes, 2022). The two groups of the virus, HIV 1 and 2 are distributed between specific geographic locations (Williams et al., 2023). HIV 1 has a worldwide distribution causing most of the reported HIV cases while HIV-2 is mainly responsible for infections in West Africa (Chilaka and Konje, 2021). Some parts of West Africa are affected by both HIV-1 and 2. Since its discovery in the year 1959, about 85.6 million people have contracted the infection out of which 40.5 million people died (Joint United Nations Programme on HIV/AIDS (UNAIDS), 2024). As of the end of the year 2022, about 39 million people were living with the infection worldwide and 630 thousand people died of HIV-related illnesses worldwide (WHO, 2023b). About 400 thousand of this number was from the African continent. Even though HIV poses a global health burden, reports by the CDC show that the highest percentage of the infection (>60%) is present in Sub-Saharan Africa (Moyo et al., 2023). Africa is the most infected with an estimated 1 in 25 adults (15-49) living with the infection. The overall prevalence of HIV in adults worldwide is 1.2 per cent worldwide but in sub-Saharan Africa, it is estimated at 9 per cent (UNAIDS, 2024). The Pacific, Caribbean, Asia and Latin America are also significantly impacted. Infection with HIV between the years 2010 and

2020 increased by 21% in Latin America, 72% in Central Asia and Eastern Europe and 22% in North Africa and the Middle East.

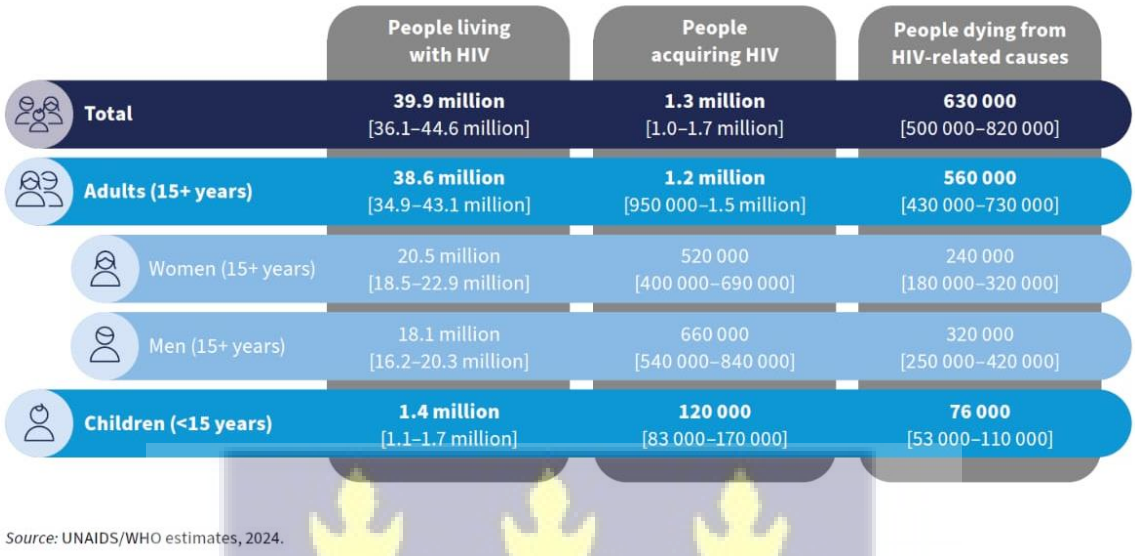


**Figure 2.3**: Summary of global HIV epidemic, 2023

According to the UNAIDS country factsheet, HIV has a prevalence of 1.53% in the Ghanaian adult population (UNAIDS, 2021). According to the report, about 334,095 people live with the infection, of which 5.3% (17,550) are children.

Certain members of the population are considered more at risk for infection with the virus. HIV disproportionately affect sex workers along with their clients, homosexuals, transgenders, and people who inject drugs (He et al., 2020). This at-risk group accounts for 65% of the global HIV toll, 80% of new infections that occur outside Sub-Saharan Africa (UNAIDS, 2024) and 55% of those that occur in Sub-Saharan Africa (Moyo et al., 2023). In North America and Western Europe, homosexuality accounts for about two-thirds of newly recorded infections.

### 2.2.2 Biology of HIV

The human immunodeficiency virus is a small (approximately 120nm) enveloped RNA virus that infects humans and primates (van Heuvel et al., 2022). The HIV virion has a genetic material surrounded by a membrane (McLaren and Fellay, 2021). The membrane of the virus houses envelope proteins, including gp120 and gp41, alongside several host cell proteins ( Li et al., 2021). The capsid is composed of about 1000 hexameric cone-shaped proteins encapsulating the positive sense, single-strand gRNA (Hudait and Voth, 2023).

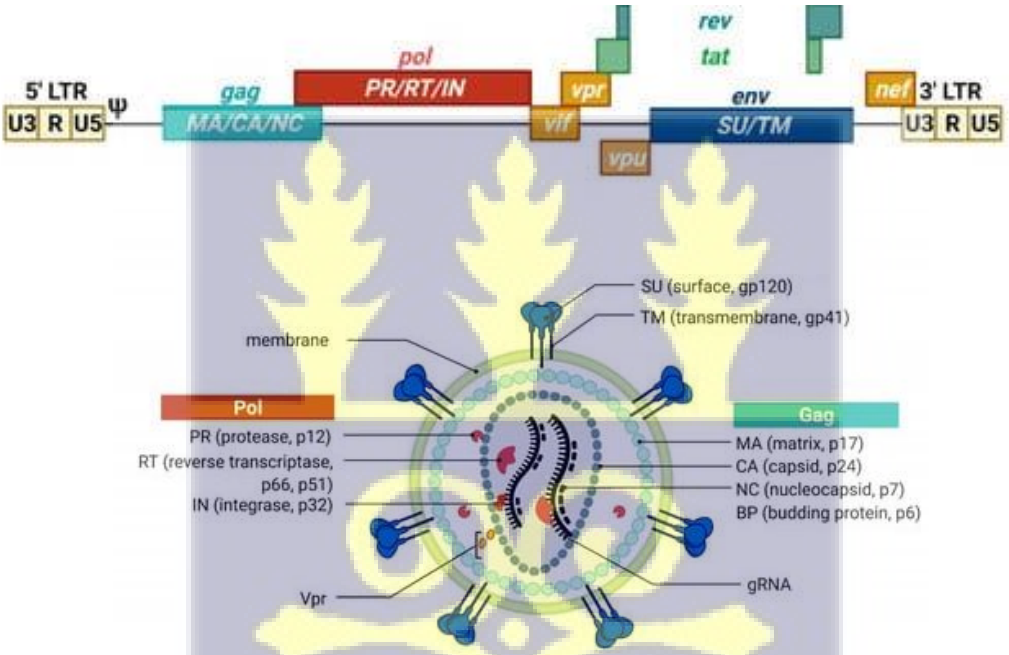


**Figure 2.4**: Structures of the HIV Genome and Mature Virion (van Heuvel et al., 2022)

The human immunodeficiency virus has a 9 kb genomic RNA with which it synthesises 15 critical proteins needed for its replicative process and assembly into new virions (van Heuvel et al., 2022). The genome contains nine open reading frames (ORFs), with some viral genes overlapping (Roesmann et al., 2024). This fosters the transcription of several proteins within a limited capacity. Long terminal repeats (LTRs) containing the promoter for integration, gene expression, and

integration flank the genome (Kalinichenko et al., 2022). HIV has three structural genes: the *gag* gene, the *pol* gene, and the *env* gene (Lewitus et al., 2024). The *gag* gene is translated to produce the core proteins of the virus (Rossi et al., 2021). During maturation of the virion, matrix, nucleocapsid and capsid proteins are produced from the precursor protein p55-Gag upon processing by viral protease (Novikova et al., 2019). The second structural gene (*pol* gene) encodes the viral enzymes reverse transcriptase, integrase and protease after processing of the precursor protein by viral protease (Yu et al., 2020). The *env* gene encodes the transmembrane unit and surface unit envelope glycoproteins, gp41 and gp120 respectively (Caillat et al., 2021). The structural genes are regulated by *tat* and *rev* (Lata et al., 2015). *Tat* is an activator of transcription (Clark et al., 2017), and *rev* functions as a nuclear export factor (Behrens et al., 2017).

### 2.2.3 Replication and Life Cycle of HIV

The life cycle of the virus can be grouped into two stages: the early stage and the late stage (Rossi et al., 2021) . The early stage of replication begins with the attachment of the virus to the host cell and ends with the integration of the viral genome into that of the host (Freed, 2019). The late stage involves all the processes that happen after integration until the new virion emerges from the host cell.

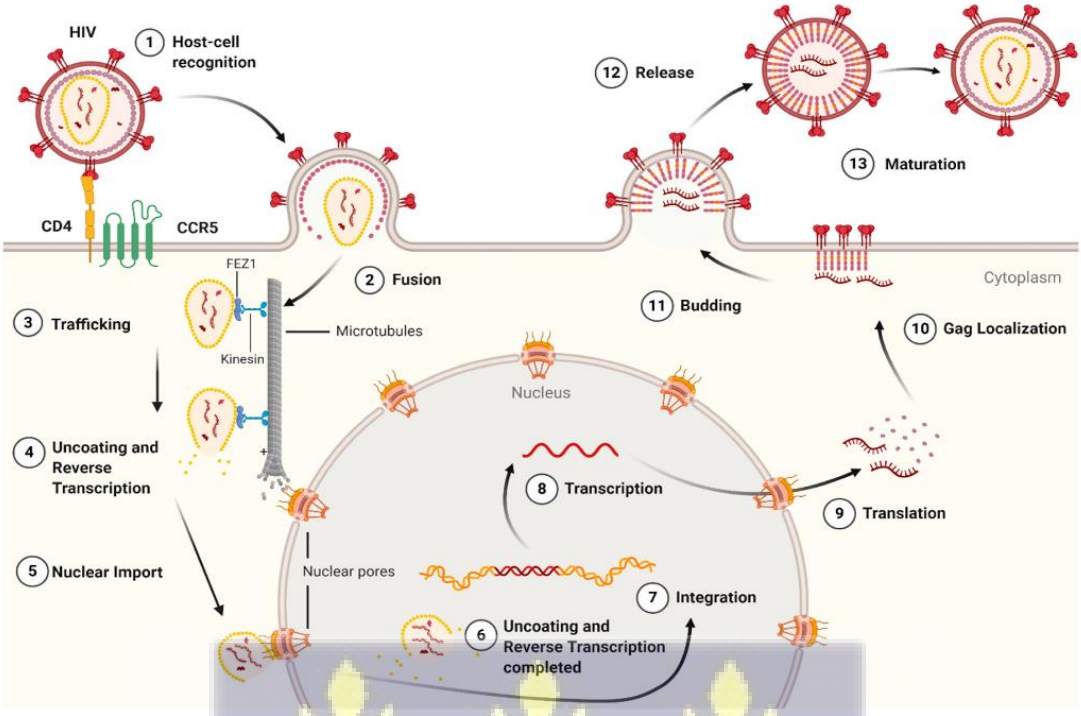


**Fig 2.5**: Replication and Life Cycle of HIV (Rossi et al., 2021)

HIV initiates its life cycle through the interaction of the Env glycoprotein complex of the virion with the receptors and co-receptors, CD4 and CXCR4/CCR5, respectively, of the host cell (Zhao et al., 2022). This interaction starts a series of conformational changes that lead to the fusion and release of the viral core into the host cell's cytoplasm. The release of the viral core (the capsid protein along with the enzymes) into the host cell's cytoplasm is followed by reverse transcription (Toccafondi et al., 2021). The reverse-transcription complex (viral core) is imported to the nucleus, where it becomes the pre-integration complex (Rossi et al., 2021). Following import to the nucleus, the capsid uncoats to release the viral RNA and enzymes (Burdick et al., 2020). The reverse transcriptase enzyme converts the viral RNA into DNA, which is integrated into the host DNA by the integrase enzyme (Singh and Das, 2022). Reverse transcription begins in the cytoplasm but ends in the nucleus (Chameettachal et al., 2023). Transcription and translation of the viral genes after integration are achieved using the host machinery and transported out of the nucleus

(Rampersad and Tennant, 2018). Gag localisation follows the export of the transcribed genes (Mailler et al., 2016). Immature virions bud out of the host cell coated by a membrane derived from the host cell (Masenga et al., 2023). Intact Gag polyproteins further coat this membrane. The virion matures through the cleavage of precursor proteins into functional proteins by the protease enzyme (Kleinpeter and Freed, 2020). The maturation is completed when the capsid forms and assembles into the fullerene cone (Zila et al., 2021).

### 2.2.4 Transmission, Pathogenesis and Symptoms

The virus's transmission mode is through exchanging fluids from the body of people living with the virus (Wilkins, 2020). These fluids include breast milk, blood, vaginal secretions, and semen. HIV can also be transmitted from mother to child through pregnancy and breastfeeding (Nlend, 2022). There have been misconceptions about virus transmission through daily activities like hugging, sharing personal objects and kissing (Agu et al., 2020). PLWH who are on antiretrovirals have undetectable viral loads and such, do not transmit the virus to their sexual partners (Bai et al., 2020).

In humans, HIV infects CD4+ cells, which play a crucial role in a person's immune health (Govindaraj et al., 2023). The replicative action of the virus leads to the destruction of these cells, rendering the person immunocompromised and susceptible to several opportunistic infections (Vijayan et al., 2017). HIV can infect people of all origins, both immune-competent and immune-compromised. However, the living conditions of some groups make them more vulnerable to contracting the infection (UNAIDS, 2024). Women, migrants, young people, long-distance drivers, and men in uniform are said to be among the risk groups for HIV.

Acquired immune deficiency syndrome, the primary manifestation of HIV, arises because of the virus' replication (Gupta and Saxena, 2021). After the successful entry of the cell, the virus uses

the cell's machinery to synthesise new viral components (Rampersad and Tennant, 2018). These are packaged into new infective virions that emerge from the cell, destroying it in the process. CD4+ cells are members of the immunoglobulin family, functioning as a bridge between the innate and adaptive immune system (Chatzileontiadou et al., 2021). It is involved in the activation of B-lymphocytes, cells of the innate immune system, killer T cells and other nonimmune cells (Künzli and Masopust, 2023). CD4+ cells also perform immunosuppressive activities (Künzli and Masopust, 2023). The continued destruction of these cells leads to a decrease in the number of functional CD4+ cells, rendering the host immunocompromised (Cao et al., 2021).

The symptoms presented by the virus vary based on the stage of infection (WHO, 2024b). The virus is highly transmissible in the early stages of infection (Cachay, 2024). In this stage, however, little to no symptoms show and hence, people are unaware of the infection until later stages (Nikolopoulos and Tsantes, 2022). In very few cases, general symptoms like fever, rash, headache, and sore throat are present. As the infection progresses, there is swelling of lymph nodes, diarrhoea, cough, fever, and loss of weight accompanied by a weakened immune system, rendering the host susceptible to cryptococcal meningitis, cancers such as lymphomas and Kaposi's sarcoma, tuberculosis, and severe bacterial infections (Mayo Clinic, 2024). It also exacerbates existing infections like Hepatitis B and C and mpox.

### 2.2.5 Management of HIV

Several interventions have been developed for HIV, but these exist to manage the condition. Current management is through the use of an antiretroviral regimen (Threats et al., 2021). ARTs involve the combinations of two or more antiretroviral classes; this suppresses the viral loads, thereby preventing the development of AIDS, provided treatment begins at an earlier stage (Moreno et al., 2019). Currently, there are five main classes of antiretrovirals based on their mode

of action (Page and Taylor, 2022). These interfere with the virus' mode of replication, thereby suppressing the viral loads. Highly active antiretroviral therapy is currently the primary method to prevent HIV-associated immune deterioration (Shin et al., 2021). The therapy may be coupled with prophylaxis for some associated opportunistic infections (Eggleton and Nagalli, 2024).

Being on an ART regimen means taking a combination of three drugs from at least two of the drug classes (Badowski et al., 2020). The classes of antiretrovirals currently in use are:

- Nucleoside reverse transcriptase inhibitors (NRTIs) (Amblard et al., 2022): These drugs work by inhibiting the action of reverse transcriptase, an enzyme that is used by the virus to replicate its genetic material (Kausar et al., 2021). Examples of NRTIs include emtricitabine (Emtriva), tenofovir disoproxil fumarate (Viread), abacavir (Ziagen), zidovudine (Retrovir) and lamivudine (Epivir) (Patel and Zulfiqar, 2023).
- Non-nucleoside reverse transcriptase inhibitors (Vanangamudi et al., 2020) (NNRTIs): This class of drugs works by binding and inhibiting reverse transcriptase's activity, thereby preventing it from multiplying. Unlike NRTIs, NNRTIs are non-competitive inhibitors that interact with reverse transcriptase away from its active site (DeStefano, 2019). This interaction induces a conformational change in the enzyme that reduces its activity. Examples include doravirine (Pifeltro), rilpivirine (Edurant), efavirenz (Sustiva) and etravirine (Intelence) (Vanangamudi et al., 2020).
- Protease inhibitors (PIs) are a class of antiretrovirals that inhibit the activity of the HIV protease enzyme (Marin et al., 2021). HIV uses its protease to cleave the large precursor proteins into small active proteins, which are packaged with the viral genome into active virions (Jacqueto et al., 2020). Examples of protease inhibitors are fosamprenavir (Lexiva),

atazanavir (Reyataz), ritonavir (Norvir), tipranavir (Aptivus) and darunavir (Prezista) (Atta et al., 2019).

- Integrase inhibitors interact with the integrase enzyme to inhibit its activity (Smith et al., 2021). The integrase enzyme is used by the virus to send its genetic material into that of the host cell during replication (Renzi et al., 2023). This class of drug is still in the development stage, but drugs like raltegravir and dolutegravir have been approved for use (A. V. Zhao et al., 2022).

- Entry inhibitors (Pattnaik and Chakraborty, 2020): This class of inhibitors inhibit the entry of host cells by the virus. They interfere with the interaction between the viral attachment proteins and host receptors (Orkin et al., 2022). This class is often administered when other treatments fail. Examples of such drugs include maraviroc and enfuvirtide (Cunha et al., 2021).

There currently exist two groups of prophylaxis taken to prevent HIV-related disease: pre-exposure prophylaxis (PrEP) (Özdener-Poyraz et al., 2020) and post-exposure prophylaxis (PEP) (Jin et al., 2022). PrEP is a regimen taken by individuals who have not yet been exposed to the virus to reduce their chances of getting infected (Shrestha and Copenhaver, 2018). This is usually indicated for people who are at a high risk of contracting the infection through injections or sex. There are currently three drugs, Truvada, Apretude and Descovy, approved by the FDA for use as PrEP (D'angelo et al., 2021; Landovitz et al., 2023). According to the CDC, when taken correctly, PrEP offers 99% protection from HIV contraction through sex and 74% through injection (CDC, 2022). PEP refers to taking medications after a suspected exposure to the virus (Ayieko et al., 2022). This is effective if the therapy is started within 72 days after possible exposure (WHO, 2008). Unlike PrEP, PEP is only taken in emergency situations and, as such, should not be used as a substitute

for PrEP. If taken at the correct dosage and for 28 days, it is effective in mitigating HIV infection. A single tablet of Truvada combined with two tablets of raltegravir is used for PEP (NHS, 2023).

### 2.3 HIV as a Chronic Condition

In the early years of the HIV pandemic, HIV caused AIDS, leading to the death of people infected with the virus (de Cock et al., 2021). AIDS leaves the host susceptible to infections with no form of defence and a prognosis of 2 years of life expectancy (Justiz Vaillant and Qurie, 2023). The narrative has changed over the years with the advent of antiretrovirals (Swinkels et al., 2024). Policies and measures put in place by the WHO have reduced the number of new infections and increased the number of infected undergoing treatment (WHO, 2023b). In the year 2022, an average of 1.3 million people acquired the infection, a 38% decrease since 2010. 630,000 people dying from the infection in 2022 represents a 50% drop in HIV-related deaths (WHO, 2022a). In America, out of the 3.8 million people living with HIV, 71% were on ART, with 65 per cent having suppressive viral loads (World Health Organization, 2022). 82% of the 25.6 million Africans living with HIV were on ART, and 76% had suppressed loads as of 2022. The successful implementation of protective measures and the increase in the number of people on ART contributes to the transformation of the infection by the virus into a chronic condition where AIDS-related complications are not the main problem (Boah et al., 2023). Clinicians now have the challenge of managing a chronic disease instead of an acute life-threatening condition. The persistence of the infection and the increase in the number of non-communicable disease cases in HIV-infected individuals highlights the possibility of the virus involvement in non-AIDS comorbidities (Casper et al., 2017).

### 2.3.1 Neurological conditions

Human immunodeficiency virus is a neuroinvasive, neurovirulent, neurotrophic pathogen (Sheybani et al., 2021). It has been shown to cause neurological degeneration directly and indirectly (Irollo et al., 2021). Before the advent of antiretroviral therapies, approximately 10% of HIV-infected presented with neurological disorders, of which about half developed neurological complications as the infection progressed (Sheybani et al., 2021). Neurological conditions associated with HIV persisted after the introduction of antiretrovirals (Uwishema et al., 2022). HIV-associated neurocognitive disorder (HAND) is the term used to describe all neurodegenerative conditions caused by HIV (Elendu et al., 2023). It is characterised by behavioural and cognitive deficits in people living with HIV. HAND encompasses the most severe HIV-associated dementia, mild HIV-associated neurocognitive disorders, and asymptomatic neurological impairment (Vastag et al., 2022). People living with HIV have demonstrated below-expectation performance on formal neurocognitive tests (Alford et al., 2021). The exact mechanism for HIV-induced neuronal damage is still unclear, but studies have reported direct and indirect mechanisms (Irollo et al., 2021). In the direct mechanism, the virus and its secreted proteins affect neurons through surface receptors found on neurons: low-density lipoprotein receptor-related protein, N-methyl-D-Aspartate Receptor (NMDAR) (Malik and A. Eugenin, 2016). Approximately 1-2 weeks after infections, there is a migration of HIV-infected monocytes into the CNS (Meyer et al., 2022). Through this “trojan horse” mechanism, the virus gains access to the CNS, establishes itself, and replicates by secreting proteins like HIV-Tat, HIV-gp120, TNF-α and some chemokines (Malik and Eugenin, 2016). The interaction between Tat and NMDAR induces calcium toxicity in neurons. Some reports propose that low viral loads persisting in the central nervous system drive neurodegeneration through toxic products from the virus and by chronic stimulation of the immune system (Balcom et al., 2019). Neuronal death that occurs as a

result of inflammatory responses mounted by both uninfected and infected brain cells against HIV describes the indirect mechanism through which HIV causes HAND (Jadhav and Nema, 2021). According to Irollo et al.(2021), HIV causes damage to the synaptodendritic junctions and destroys neuronal networks in the parts of the brain that control memory, learning and some executive functions.

### 2.3.2 Cardiovascular and metabolic diseases

People living with HIV have been reported to have an increased risk of developing cardiovascular diseases that range from myocardial infarction to heart failure (Ntsekhe and Baker, 2023). At the time when cardiovascular diseases in HIV patients were reported, the phenomenon was attributed to the use of antiretrovirals (Feinstein, 2021). Growing knowledge has shown that the primary drivers of this non-AIDS comorbidity are HIV-related viremia, inflammation, and immune dysregulation (Chichetto et al., 2020). People living with HIV are reported to have a 2-times higher risk of developing myocardial infection (Soares et al., 2023). By the year 2030, there is an estimation that 73% of people infected with the virus will be over 50 years and 78% of PLWH will have cardiovascular diseases (Wing, 2017). According to a systematic review by Ruamtawee et al. (2023) People living with HIV tend to develop carotid plaques more frequently than non-HIV-infected individuals.

Cardiovascular diseases are a group of disorders that affect the heart and blood vessels (Thiriet, 2018). Generally, atherosclerosis is the root cause of cardiovascular diseases (Kobiyama and Ley, 2018). Emerging evidence has indicated that HIV contributes to the development of atherosclerosis and, hence, other cardiovascular diseases (Kearns et al., 2017). Endothelial dysfunction, inflammation and oxidation factors, and epigenetic modifications lead to the development of atherosclerosis (Theofilis et al., 2021). The role that HIV plays in promoting these factors has been

under study to establish a link between HIV and cardiovascular diseases. A study by Teer et al.(2020) reported an essential interaction between macrophage/monocyte markers and the activation of cells of the adaptive immune system among HIV patients on second-line treatment. They also demonstrated how activation of the immune system caused an alteration of the lipid subclasses. Studies have reported an alteration in the monocyte subclass, with nonclassical macrophages expressing tissue factors, which are the most abundant in HIV patients (Cormican and Griffin, 2020). These macrophages are thought to be atherogenic and, by virtue of the tissue factor receptor, lead to the production of proinflammatory cytokines (Bobryshev et al., 2016). The suppressed viremia elicits a persistent immune response and an increase in the levels of inflammatory macrophages that produce inflammatory cytokines and reactive oxidative species (Wu and Simonetti, 2023). These cause lysis of myofibers and exacerbate injury to blood vessels (Andrés Juan et al., 2021).

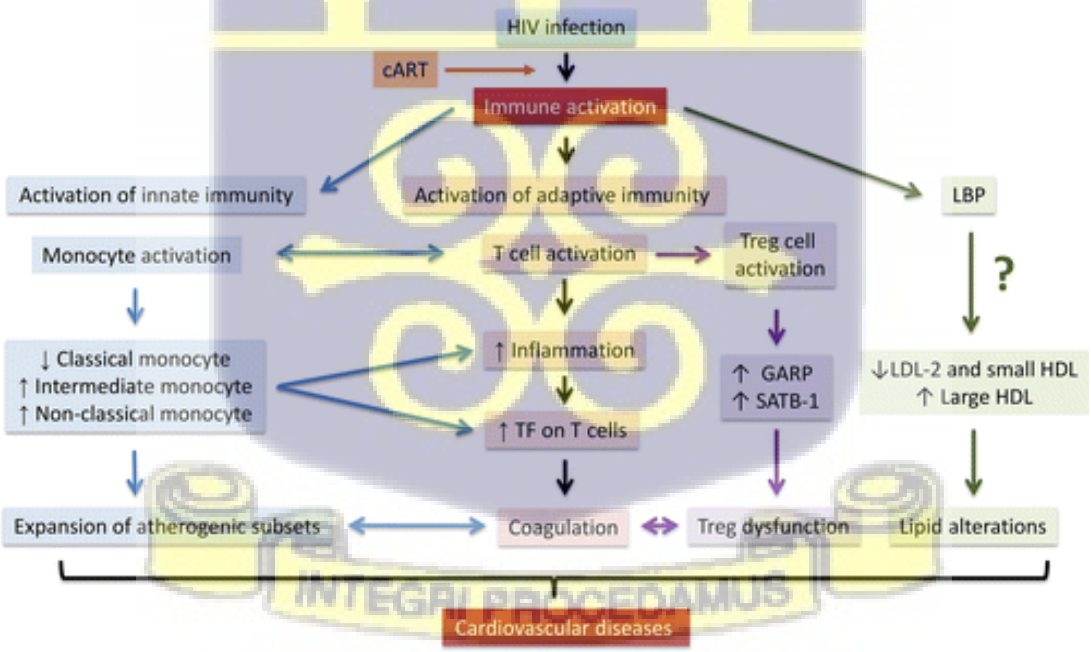


**Figure 2.6**: Schematic Diagram of the Pathogenesis of HIV-Associated CVDs (Teer et al., 2019)

Globally, 16.7% to 31.3% of HIV-positive patients present risks of developing a metabolic complication (Ergin et al., 2020a). A study in 2021 reports that at least 15% of PLWH have diabetes (Sudipa Sarkar, 2022). The risk of metabolic diseases in HIV patients has been well documented, and the condition is thought to arise because of antiviral usage, viral infection, and other immunological factors (Collins et al., 2019). Antiretrovirals are the standard treatment option for people infected with the virus (Collins et al., 2019). The metabolic side effects are avoided in most recommended antiretrovirals. However, modern antiretrovirals have been linked to obesity among PLWH (Sapuła et al., 2022). The adverse effects of the prolonged use of ART are thought to arise when the body mechanism is triggered, leading to metabolic syndrome (Thet and Siritientong, 2020). They are implicated both in changes in fat distribution and homeostasis of glucose. Studies concerning metabolic syndrome in HIV patients have reported lipodystrophy, insulin resistance and dyslipidaemia in patients who use regimens comprising nucleoside reverse transcriptase inhibitors and protease inhibitors (Pedro et al., 2018). ARTs in the body increase TNF-α, impairing metabolism and oxidation of lipids (Lv et al., 2021). Patients taking Zidovudine frequently presented symptoms of depletion of peripheral fat depletion (Koethe et al., 2020). Depletion of peripheral fat and altered lipid distribution causes hypertriglyceridemia and an increase in the circulation levels of low-density lipoproteins (Li et al., 2022). Hypertriglyceridemia has been reported to be associated with insulin resistance (Ma et al., 2020). ART use, especially protease inhibitors, has been reported to interrupt glucose homeostasis, with indinavir shown to inhibit the activity of GLUT-4, a glucose uptake receptor (Wang et al., 2020). Viremia-related mechanisms for metabolic diseases in HIV patients include an elevated inflammatory response and a dysregulated immune function (Lv et al., 2021). HIV downregulates anti-inflammatory genes and,

through ubiquitin-proteosome degradation, degrades anti-inflammatory products like tyrosine kinase RON. The persistence of this inflammatory environment results in increased thrombotic events and dyslipidaemia, processes implicated in cardiovascular and metabolic diseases (Hasheminasabgorji and Jha, 2021; Stark and Massberg, 2021).

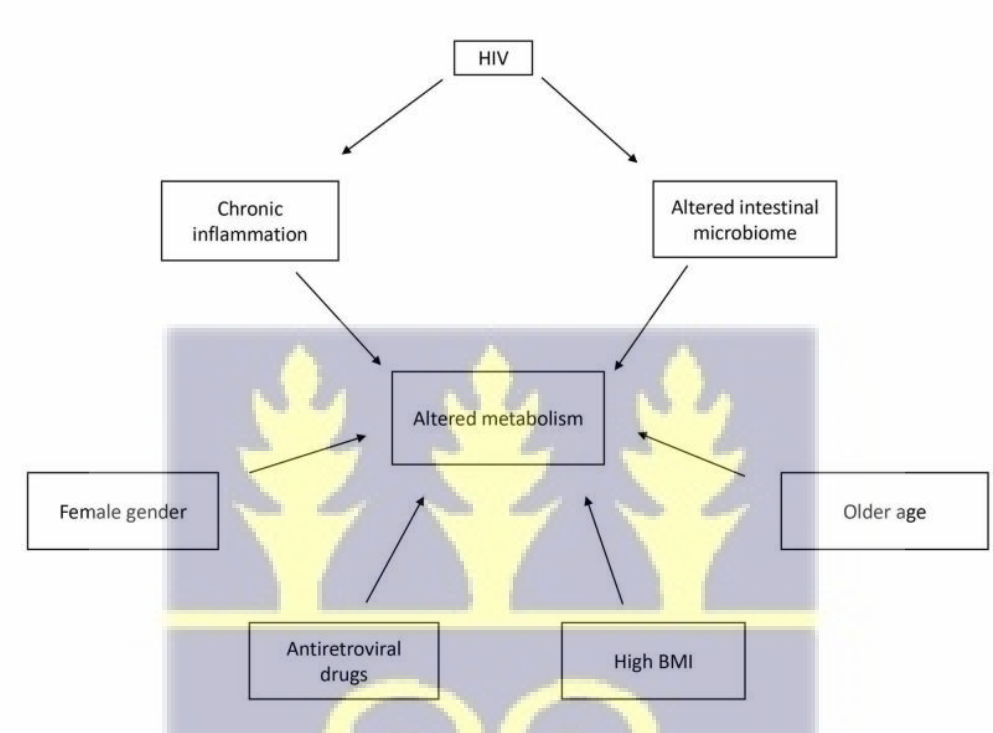


**Figure 2.7**: Factors contributing to altered metabolism in HIV (Ergin et al., 2020b)

### 2.3.3 Autoimmune Diseases

The topic of autoimmune diseases in HIV patients is a complex one, with people suggesting autoimmunity as a part of the body's natural response to the virus (Virot et al., 2017). Infection with the virus has been shown to affect the B cell population (Moir and Fauci, 2017). Immune complexes, hypergammaglobulinemia and an increased number of dividing B cells can be seen in HIV patients (Liechti et al., 2019; Lv et al., 2021; Zhao et al., 2021). These form hallmarks of several autoimmune diseases; the virus has therefore been associated with conditions like

rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis, etc (Belgaumkar et al., 2019; Hasan et al., 2022; Maharaj, 2022).

## 2.4 HIV and Autoimmune Diseases

### 2.4.1 The Gut Microbiota

The collection of microbes residing in and on the body constitutes the microbiota (Kennedy and Chang, 2020). The collective genomes of these microorganisms are the microbiome. These include the trillions of bacteria, fungi, and viruses that occupy the skin, mouth, stomach, and other mucosal surfaces (Dekaboruah et al., 2020). Their roles in human health and disease outcomes have become evident over the years (Ogunrinola et al., 2020). These microbes are usually non-pathogenic organisms but can cause opportunistic infections (Ogunrinola et al., 2020). The epithelium that lines the gut is a layer of interconnected columnar cells that are involved in absorbing nutrients (Kong et al., 2018). This mucosal surface is colonised by a population of diverse microorganisms called the gut microbiota (Kennedy and Chang, 2020). The gut colonisation by microbes occurs in distinct phases from about three months after birth, where the baby's microbiota begins to develop from the maternal microbiota (Moore and Townsend, 2019). Towards the end of the fifth year, the microbiota diversifies and gains the appearance of an adult microbiota (Moore and Townsend, 2019). These include mainly bacteria from the families *Bacteroidetes* and *Firmicutes* (Davenport et al., 2017).

The gut microbiota plays a vital role in digestion and maintaining homeostasis (Valdes et al., 2018). The microbiota provides the platform for the fermentation of non-digestible substrates (Davani-Davari et al., 2019). The end products of this fermentation are mainly short-chain fatty acids that serve as energy sources for some microorganisms in the gut and colonocytes, aid in glucose metabolism and induce apoptosis of cancer cells in the colon (Markowiak-Kopeć and Śliżewska,

2020). The gut microbiota is well-defined at the age of 5. However, some internal and external factors can lead to an altered microbiota where some overgrow and others get lost, a condition termed dysbiosis (Rinninella et al., 2019). Diet, abuse of antibiotics, stress and several other factors have been implicated in gut dysbiosis (Madison and Kiecolt-glaser, 2019). Gut dysbiosis has been reported in obesity, diabetes, cancer, and liver diseases (Breton et al., 2022; Ge et al., 2021; Nishikawa et al., 2021). It can also elicit a localised inflammation that compromises the integrity of the gastric epithelium, leading to the leakage of bacteria toxins in the bloodstream (leaky gut) (Hrncir, 2022).

### 2.4.2 HIV and its Role in Causing Leaky Gut

In HIV-mediated leaky gut syndrome, the virus destroys the integrity of the gut barrier in a way similar to inflammatory bowel diseases (Alzahrani et al., 2019b). The gut is an early target site for HIV since it is home to the majority of the lymphoid tissues in humans (Koay et al., 2018). The gut-associated lymphoid tissue, the largest lymphoid organ in the body, contains about 70% of CD4+ cells expressing the receptor CCR5 (Dillon et al., 2016). This important receptor for entry of the virus into the cells can be found in only 20% of peripheral blood CD4+ cells. This makes GALT the primary target and replication site for HIV (Thompson et al., 2017). The rapid destruction of epithelial and immune cells of the gut leads to an inflamed and leaky gut (Art et al., 2021). Several studies have highlighted possible mechanisms through which the virus causes a leaky gut. HIV has been shown to directly induce apoptosis in cells of the epithelial lining by targeting and draining the mitochondria during replication (Jabea Ekabe et al., 2022). Studies involving the use of Caco-2 cell lines showed that the virus' Tat protein inhibits the proliferation of intestinal epithelial cells hindering the repair of damaged tissue (Buccigrossi et al., 2011). In an in vitro assay, the HIV surface envelope protein gp120 impaired the integrity of the mucosal barrier

by reducing proteins that maintain tight junctions (Tugizov, 2016). This makes the epithelium permeable for microbial translocation.

Several biomarkers that demonstrate destruction and permeability of the gut epithelium are elevated in HIV patients (Ouyang et al., 2023). Alongside, microbial and microbial products that demonstrate the translocation of these microbes from the gut into circulation have been found in elevated concentrations in the blood of PLWH (Marchetti et al., 2013). Serum biomarkers like zonulin, intestinal fatty acid-binding protein and regenerating islet-derived protein 3α are validated biomarkers of gut damage reported to be elevated in HIV patients (Ouyang et al., 2023). sCD14, lipopolysaccharide and β-D-glucans have been used to prove microbial translocation in HIV patients.

### 2.4.3 Leaky Gut and Autoantibodies Production

The gut, lined by a single layer of columnar cells, is responsible for the absorption of nutrients into circulation (Basile et al., 2023). Aside from the absorptive role played, it also serves as a filtering system that controls which substances pass into circulation (Odenwald and Turner, 2017). Tight junction proteins aided by factors such as mucins, immunoglobulins, antimicrobial molecules, and cytokines maintain the integrity of the gut (Suzuki, 2020).

The leaky gut syndrome is a condition where the intestinal lining becomes permeable and permits the passage of bacteria, bacteria products, and toxins into the bloodstream (Lacy et al., 2024). The topic of leaky gut has become controversial, sparking several debates. Some schools of thought propose that the leaky gut is a symptom of an underlying condition, while others propose that it is a cause of diseases (Fasano, 2018). However, scientists have measured high levels of products from gut bacteria in the blood of patients with gastrointestinal diseases (Dupont et al., 2020). A leaky gut is caused by the gradual erosion of the intestinal lining due to a significant assault on the

lining (Camilleri, 2019b). Chronic alcohol abuse, drug abuse and radiation therapy cause significant damage to the gastric epithelium (Cleveland Clinic, 2022). Some common gastrointestinal symptoms include abdominal pain, bloating, sensitivities to food and indigestion.

Growing evidence highlights the role the gut microbiota and the integrity of the intestinal barrier play in autoimmune diseases (Christovich and Luo, 2022). In the leaky gut mediated model of autoimmune diseases, contents of the guts like resident and some pathogenic microbes, microbial products and other human products localised in the gut leak into circulation (Paray et al., 2020). These serve as antigens that illicit both local and systematic immune responses. A crucial immune response mechanism is the production of antibodies against invading antigens (Marshall et al., 2018). The trafficking of macromolecules across the gut barrier into circulation leads to immune dysregulation (Fine et al., 2020). This triggers a series of processes, including autoantibody production, that ultimately result in autoimmune diseases in susceptible groups (Kinashi and Hase, 2021). Kharrazian et al. (2023) reported elevated levels of biomarkers of intestinal permeability (zonulin and occludin) correlating with increased levels of 17 out of 24 autoantibodies targeted in their experiment.

### 2.4.4 Chronic HIV Infection and Autoimmune Diseases

Several autoimmune conditions have been associated with HIV, bringing about the question, is HIV a rare, neglected cause of autoimmune diseases? (Ruperto et al., 2022). Systemic lupus erythematosus, vasculitis, antiphospholipid syndrome, primary biliary cirrhosis, idiopathic thrombocytopenic purpura and most recently autoimmune hepatitis has been reported in HIV patients (Chaiteerakij et al., 2019; Ruperto et al., 2022; Sherman and Thomas, 2022; Vega and Espinoza, 2020; Belgaumkar et al., 2019). The role of HIV in causing autoimmune diseases is

cemented by the elevated levels of autoantibodies like anti-β2 GPI, anti-cardiolipin, and anti-small nuclear ribonucleoproteins in the circulation of HIV patients (Johnson and Jiang, 2023).

HIV infection associated leaky gut has been reported in the literature for years (Ashuro et al., 2020). Measurement of biomarkers associated with leaky gut, biopsy and immunohistochemistry of the gut has revealed HIV-associated damage to the lining of the gut lining(Schoultz and Keita, 2020). The gut, being home to the majority of the immune cells, which are the primary targets of the virus, makes the gut the primary infection site of the virus (Thompson et al., 2017). Rapid infection and destruction of the immune and epithelial cells of the gut destroy the lining of the gut (Art et al., 2021). The compromised integrity of the lining reduces its ability to regulate the movement of substances into the bloodstream (Gieryńska et al., 2022). A case-controlled study in the Chinese population by Zhang et al. (2023) they reported a significant change in the microbiota of HIV patients and an associated higher degree of microbial translocation in pre-AIDS and AIDS patients.

The use of ARTs results in a persistent low infection state of the human immunodeficiency virus (Koethe et al., 2020). In this state, the virus is non-replicative. However, this persistent chronic infection is a potent immune stimulator (Lv et al., 2021). During a leaky gut, microbial components and metabolic peptides translocate from the gut into circulation, triggering the immune system (Camilleri, 2019a). The continuous translocation leads to systemic immune activation and a chronic inflammation state (Zicari et al., 2019). Microbial translocation contributes to HIV infection-associated mortality and morbidity due to the chronic inflammation it induces and sustains (Zevin et al., 2016). A main microbial product frequently identified in the circulation of HIV patients is lipopolysaccharide (LPS), a component of the membrane of Gram-negative bacteria. LPS is a very potent stimulator of the immune system(Luo et al., 2022). Studies have

reported an association between LPS levels and cells, cytokines and genes of the immune system (Page et al., 2022). CD38+HLA-DR+CD8+ T and monocytes are highly activated in the presence of LPS (Page et al., 2022). Proinflammatory cytokines such as TNF-α, IFN and IL-6, along with genes that respond to interferon activation like MxA, are increased in the presence of LPS (Noori et al., 2020). It is, however, unclear how much of the immune response is directly related to microbial translocation because of the number of immune events that occur concurrently during HIV infection.

One essential function of the immune system is the production of antibodies (Nicholson, 2016). Antibodies are proteins produced by the immune system in response to a specific antigen (Jacofsky et al., 2020). Components of the gut that leak into circulation are potentially immunogenic and hence stimulate the immune system, resulting in the production of antibodies against them (Lo et al., 2021). Several research groups have reported autoantibodies in people infected with the virus (Vega and Espinoza, 2018; Vornicu et al., 2023). These autoantibodies are thought to be produced through mechanisms such as activation of polyclonal B cells, a dysregulated interaction between the two lymphocyte classes and molecular mimicry (Yu et al., 2023). Luo et al. (2019), reported production of autoantibody by promoting germinal centre response of B cells. The autoantibodies produced by translocation attack self-cells, resulting in the autoimmune diseases reported in HIV patients (Jacofsky et al., 2020).

### 2.5 Pernicious Anaemia In HIV

#### 2.5.1 Anaemia in HIV

The World Health Organization classifies anaemia as a public health problem (WHO, 2023a). This condition mainly affects children, women and girls in their menstrual cycle and pregnant women (Sun et al., 2021). Anaemia is a condition where red blood cells and, consequently, haemoglobin

reduce in number, with levels below 12 g/dL in women and 13 g/dL in men (Braat et al., 2024). This results in reduced perfusion of tissues and organs (Kusumawati and Lebak, 2024). The burden of anaemia is highest in low- and middle-income countries and, in severe cases, results in impairment of cognitive and motor functions (Safiri et al., 2021). There are several types of anaemia based on the presentation and the cause (Abaynew et al., 2023). Nutritional deficiencies, medications, haemolysis, infection, and some chronic diseases are causes of anaemia (Hussien et al., 2023).

Pernicious anaemia is a condition where the synthesis of red blood cells is hindered by the inability to effectively absorb and use vitamin B12 (Brittenham et al., 2023). It is a rare autoimmune disease occurring in about 1% of the general population and affecting 25 per 100000 persons aged 40 and above (Seage et al., 2024). Symptoms of the condition include stomach upset, fatigue, weakness, and tachycardia (Yocum et al., 2023). Blood film comment shows abnormally large red blood cells called megaloblasts (Hamid, 2024b). This abnormal shape marks the red blood cells for destruction by the spleen. When left undiagnosed and treated for an extended period, it causes neurological conditions (Ibraheem et al., 2023). Blood cells and nerve cells depend on vitamin B12 for proper functioning (Mathew et al., 2024).

A common complication of HIV infection is reduced cell count arising mainly from defective production by the bone marrow, increased destruction of blood cells and increased peripheral loss (Ralston, 2023). Anaemia is by far the most common cytopenia recorded in HIV patients on ARTs (Zayed et al., 2023). The prevalence of anaemia in HIV tends to vary among populations based on demographics and the stage of infection (Zayed et al., 2023). Infants, women, and people living in low- and middle-income countries record higher levels of anaemia among HIV patients (Silubonde et al., 2023). ARTs have increased the life expectancy of PLWH; complications like anaemia,

however, affect the quality of life of the group (Tamsukhin et al., 2023). The complications associated with anaemia in PLWH range from a decrement in the quality-of-life and functional states of patients to rapid progression of the disease and loss of life (Mandania, 2024). HAARTs are among the factors that contribute to anaemia in HIV patients (Wagnew et al., 2019). HIV patients on HAARTs develop medication-induced anaemia during their first week of medication (Wei et al., 2023). Medication is the most common cause of megaloblastic anaemia in HIV patients (Berhane et al., 2020). Studies also revealed nonresponsive erythropoietin production, suggesting insufficient erythropoietin as another cause of anaemia in HIV patients (Zewdu et al., 2024).

### 2.5.2 Absorption of Vitamin B12 and Pathogenesis of Pernicious Anaemia

Vitamin B12 or cobalamin is a water-soluble vitamin readily available in animal foods (Wolffenbuttel et al., 2024). It is used by the body to make DNA and keep nerve and blood cells healthy (Harikrishnan et al., 2024). For haematopoiesis, vitamin B12 serves as an important cofactor in the production of haem (Dunphy and Tang, 2023). It is also involved in the synthesis of DNA in the early stages of erythropoiesis. Vitamin B12 is obtained by the body through ingestion of vitamin B12-containing foods like fish, poultry, and eggs (Suter, 2020). Vitamin B12 in the diet is absorbed into the bloodstream aided by intrinsic factors (Mucha et al., 2024). Gastric intrinsic factor is a glycoprotein that is produced by cells of the stomach known as oxyntic or chief cells (dos Santos et al., 2024). They bind to cobalamin to facilitate its transport and absorption (Halczuk et al., 2023).

Pernicious anaemia arises due to the destruction of parietal cells and unavailable intrinsic factors (Halczuk et al., 2023). Parietal cells are gastric cells that produce an intrinsic factor, the glycoprotein that transports vitamin B12 from the intestines into circulation (McQuilken, 2024). Most cases of pernicious anaemia involve the destruction of parietal cells; in some cases,

autoantibodies are also produced against the intrinsic factor (Song et al., 2022). These autoantibodies attack and destroy intrinsic factors, leading to deficiency and unavailability of the vitamin for the body's use (Atisha-Fregoso et al., 2024). Vitamin B12 deficiency leads to the production of abnormal blood cells known as megaloblasts (Aher et al., 2024). The abnormal morphology of these cells marks them for rapid removal by the spleen (Hamid, 2024a). The persistent destruction of the cells by the spleen leads to the anaemic state.

### 2.5.3 HIV-Infection Associated Leaky Gut as a Risk Factor for Pernicious Anaemia

Pernicious anaemia is a rare autoimmune disease, however, common in PLWH, with about 35.45% of patients having serum vitamin B12 below 200 pg/ml (Berhane et al., 2020). Leaky gut in HIV has been proposed as the primary mechanism through which vitamin B12 deficiency occurs in HIV patients (Alzahrani et al., 2019a). The rapid destruction of immune and epithelial cells of the gut leads to the destruction of parietal cells and the development of a leaky gut. The compromised gastric epithelium affects the filtering ability of the epithelium, leading to the translocation of microbes, microbial products, and some other components of the gut into circulation (Ghosh et al., 2020). For pernicious anaemia that arises as a result of HIV-infection-associated leaky gut, we propose a passage of intrinsic factors from the gut into circulation. The translocation elicits a persistent immune response that attacks self-cells, including parietal cells and intrinsic factors. Destruction of the parietal cells and the intrinsic factors compromises the body's ability to absorb and use vitamin B12 leading to pernicious anaemia (Rizzo and Laganà, 2019).

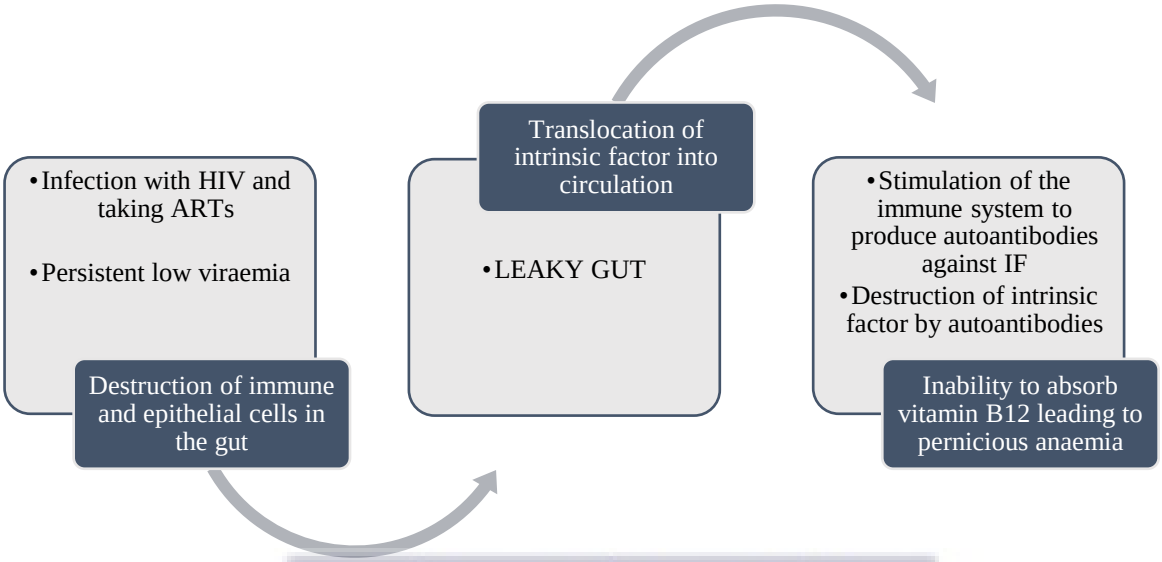


**Figure 2.8:** Proposed Mechanism for Pernicious Anaemia in PLWH

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Study Site

The study site was the fevers unit of the Korle-Bu Teaching Hospital (KBTH), Accra, Ghana (the largest referral hospital in Ghana). KBTH was used because, being the largest referral facility, it had the appropriate population to sample from. The Fevers Unit is under the Department of Medicine and Therapeutics of KBTH. The unit is responsible for registering and managing all patients diagnosed with HIV, primarily in KBTH, as well as referrals from other centres.

## 3.2 Study Setting

The lab work for this study was conducted at the virology and cancer lab of the West African Centre for Cell Biology of Infectious Pathogens (WACCBIP), University of Ghana. This is located at the University of Ghana's Biochemistry Department, Legon.

## 3.3 Study Design

A case-control study

## 3.4 Study population

The study was conducted with HIV patients on antiretrovirals and non-infected individuals.

### 3.4.1 Sample size

The minimum number of participants needed for the study was determined from the formula.

$N=\frac{Z^2P(1-P)}{e^2}$,

Where:

Z is the standard normal variate at a confidence interval of 95% = 1.96.

p is the prevalence = 0.017 (Boah et al., 2023)

e is the margin of error = 0.05.

N (Minimum number of participants) = =25

### 3.4.2 Cases

The case group consisted of 55 HIV seropositive patients on combined antiretroviral therapy. A structured questionnaire was administered to obtain the socio-demographic characteristics of the subjects, after which blood was drawn from each patient.

### 3.4.3 Control

55 HIV seronegative individuals will serve as control groups.

### 3.4.4 Selection criteria

#### 3.4.4.1 Inclusion criteria

- Seropositive HIV patients on antiretrovirals.
- Seropositive HIV patients taking supplements.
- HIV seronegative individuals.

#### 3.4.4.2 Exclusion criteria

- Patients with diagnosed stomach ulcer.
- Patients on broad-spectrum antibiotics.
- Patients with nutritional deficiencies.
- Pregnant women.

### 3.5 Sampling and Processing

Samples were selected randomly from archived blood samples from a previous study. The samples stored at -80 °C at the WACCBIP laboratory were taken from the -80 °C freezer, slowly thawed at room temperature, transferred into Eppendorf tubes and used for the study. Selected samples

were centrifuged to obtain different blood components, plasma was used for serological assays.

### 3.6 Participants Clinical Data

Socio-demographic and clinical data of participants were retrieved from a previous study database.

### 3.7 Laboratory analysis

#### 3.7.1 Procedure for HIV Test

##### 3.7.1.1 Screening for HIV in Serum

The serum samples were screened for the presence of HIV using JusChek rapid anti-HIV cassettes. This is a rapid test to qualitatively detect the presence of antibodies to HIV 1 and HIV 2 in whole blood, serum or plasma specimens. The test utilises latex conjugate and multiple recombinant HIV proteins to selectively detect antibodies to HIV 1 and 2 in serum or plasma.

##### 3.7.1.2 Principle of Operation

In this test procedure, anti-human IgG is immobilised in the test line region of the test. After the specimen is added to the specimen well of the device, it reacts with recombinant HIV proteins in the test. This mixture migrates chromatographically along the length of the cassette and interacts with the immobilised anti-human IgG. If the specimen contains HIV antibodies, a coloured line will appear in the test line region, indicating a positive result. If the specimen does not contain HIV antibodies, a coloured line will not appear in this region, indicating a negative result. To serve as a procedural control, a coloured line will always appear in the control line region, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

##### 3.7.1.3 Assay Procedure

A drop of serum was placed on the lower edge of the sample pad of the test cassette using a Pasteur pipette. Two drops of sample diluent were added to the upper edge of the sample pad afterwards, and the result was read after 15 minutes.

### 3.7.2 Procedure for Full Blood Count Using Sysmex Xp-300 Analyzer

Whole blood samples were collected from both study groups and placed into an EDTA container. Before analysis, the sample in the tube was mixed thoroughly by inverting the sample tube before placing it on a roller. The tube was then set to the sucking probe, after which the analyser was initiated. The sample was removed when the status display changed from aspirating to running. The complete blood component analysis comprising of Haemoglobin, Erythrocyte Count, White Blood Cell Count, Platelet Count, Haematocrit, Mean Platelet Volume, Mean Cell Volume, Mean Cell Haemoglobin and Mean Cell Haemoglobin Concentration was displayed in approximately 60 seconds after starting the analysis.

### 3.7.3 ELISA for Serum Lipopolysaccharide (LPS)

The levels of LPS in the sample were estimated using the Elisa technique. Mybiosource's LPS ELISA kit was used. The test kit employed the quantitative sandwich type of Elisa using 96 wells microtitre plate pre-coated with anti-LPS antibodies. Standard samples were included in every plate, and the standard curves were plotted as a guide.

#### 3.7.3.1 Principle

This assay employs the quantitative sandwich enzyme immunoassay technique. The antibody specific to LPS has been pre-coated onto a microplate. Standards and samples are pipetted into the wells, and any LPS present is bound by the immobilised antibody. After removing any unbound substances, a biotin-conjugated antibody specific for LPS is added to the wells. After washing, avidin-conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells, and colour develops in proportion to the amount of LPS bound in the initial step. The colour development is stopped, and the intensity of the colour is measured.

### 3.7.3.2 Procedure

All working reagents, standard solutions and samples were prepared following the manufacturer's instructions. All steps were performed at room temperature. A 100µl portion of the standards and sample were put in their designated wells. The plate was covered with the adhesive strip provided and incubated for 2 hours at 37°C. The seal was removed after 2 hours, and the liquid was removed from each well. A 100µl of Biotin-antibody (1x) was added to each well, and the plate was covered with a new adhesive strip and incubated for 1 hour at 37°C. After an hour, each well was aspirated and washed three times by filling each well with 200µl wash Buffer and letting it stand for 2 minutes. The wash buffer was removed entirely and blotted against clean paper towels. A 100µl of HRP-avidin (1x) was added to each well, and the microtiter plate was covered with a new adhesive strip. The plate was incubated for 1 hour at 37°C. After an hour, the liquid was aspirated, and the wells were washed five times. A 90µl portion of TMB Substrate was added to each well and incubated for 25 minutes at 37°C, protected from light. A 50µl stop solution was added to each well, gently tapping the plate to ensure thorough mixing. The optical density of each well was determined immediately using using LUX multimode microplate reader operating on the SkanIt™ software (Thermo Scientific™ Varioskan™, USA) set to a wavelength of 450 nm. The standard curve was plotted, and the concentration of zonulin in each of the samples was determined.

### 3.7.4 ELISA for Intrinsic Factor Autoantibodies

The levels of intrinsic factor autoantibodies in the sample were estimated using the Elisa technique. Mybiosource's intrinsic factor autoantibodies ELISA kit was used. The test kit employed the competitive type of Elisa using 96 wells microtitre plate pre-coated with anti-LPS antibodies. Standard samples were included in every plate, and the standard curves were plotted to serve as a guide.

### 3.7.4.1 Principle

IF-Ab ELISA kit applies the competitive enzyme immunoassay technique utilising an Intrinsic Factor antigen and an IF-Ab-HRP conjugate. The assay sample and buffer are incubated together with IF-Ab-HRP conjugate in a pre-coated plate for one hour. After the incubation period, the wells are decanted and washed five times. The wells are then incubated with a substrate for HRP enzyme. The product of the enzyme-substrate reaction forms a blue-coloured complex. Finally, a stop solution is added to stop the reaction, which will then turn the solution yellow. The intensity of the colour is measured spectrophotometrically at 450nm in a microplate reader. The intensity of the colour is inversely proportional to the IF-Ab concentration since IF-Ab from samples and IF-Ab-HRP conjugate compete for the Intrinsic Factor antigen binding site. Since the number of sites is limited, as more sites are occupied by IF-Ab from the sample, fewer sites are left to bind IF-Ab-HRP conjugate.

### 3.7.4.2 Procedure

A 100 uL portion of standards and samples were added to their designated wells in the antibody pre-coated Microtiter Plate. A 100 uL of PBS (pH 7.0) was added in the blank control well.) A 50 uL of Conjugate was added to each well except the blank control well. The constituents of the wells were mixed properly. The plate was covered and incubated for 1 hour at 37 °C. After an hour, the plate was removed, and the seal was taken off. The plate was washed five times with 300 µL of wash buffer, ensuring it soaked for a minute during each wash. The plate was inverted on paper towels to blot dry. A 50 uL portion of Substrate A followed by 50 uL Substrate B was added to each well, including a blank control well. The plate was covered and incubated for 15 minutes at 25 °C, avoiding sunlight. A 50 uL of stop solution was added to each well and mixed. The optical density of each well was determined immediately using a LUX multimode microplate

reader operating on the SkanIt™ software (Thermo Scientific™ Varioskan™, USA) set to a wavelength of 450 nm. The standard curve was plotted, and the concentration of intrinsic factor antibodies in each of the samples was determined.

### 3.8 Statistical analyses

Data was entered into an Excel spreadsheet and analysed using Statistical Package for Social Sciences (SPSS). Summaries of the descriptive data were presented as mean and standard deviation. A T-test was used to determine the significant difference between the parameters. One way ANOVA was used to compare the means of more than two groups. Quantitative data were presented as percentages and chi-square was used for comparison. All statistical tests will be analysed at a 95% confidence interval ($p$-value $< 0.05$).

### 3.9 Ethics consideration

Ethical approval was sought from the Ethics Committee of Basic and Applied Sciences (ECBAS), College of Basic and Applied Sciences, University of Ghana. The data used and obtained from this study was kept confidentially under lock and key. All information obtained was used for the purpose of this study, and only the researcher had access to the research materials.

### 3.10 Dissemination of results

The information obtained from this research work was presented to the Department of Biochemistry, Cell and Molecular Biology and the libraries of the School of Biological Sciences and the College of Basic and Applied Sciences, University of Ghana, Legon. The informative findings will also be presented at scientific and health-related conferences and published in peer-reviewed medical journals.

# CHAPTER FOUR

# RESULTS

## 4.1 Clinical parameters of the study population

Table 4.1 shows the clinical parameters of the study participants. There was no significant difference between the ages of the HIV seropositive participants and HIV seronegative participants, 46.42 ± 15.32 as compared with 45.45 ± 11.46, respectively. The blood pressures, both systolic and diastolic, of the HIV seropositive participants were significantly higher than their HIV seronegative participants counterparts ($p < 0.001$). In terms of the BMI, HIV seropositive participants had a significantly lower index compared with the HIV seronegative participants ($p < 0.01$)

**Table 4.1**: Clinical parameters of the study population

| Parameters | HIV Patients (N=55) | HIV Seronegative (N=55) | 95% CI (Mean Difference) | p-value |
|---|---|---|---|---|
| Age (years) | 46.42 ± 15.32 | 45.45 ± 11.46 | -4.07-5.99 | 0.7095 |
| BMI (kg/m$^2$) | 21.67 ± 1.71 | 23.23 ± 3.47 | -2.62-(-0.50) | <0.01** |
| SBP (mmHg) | 141.22± 13.03 | 126.29 ± 17.38 | 9.05-20.80 | <0.001*** |
| DBP (mmHg) | 86.18± 9.88 | 76.45 ± 16.14 | 5.27-14.19 | <0.001*** |

N = population size, data is expressed as mean ± standard deviation, and Student t-test was used to compare mean differences. SBP= systolic blood pressure, DBP = diastolic blood pressure, BMI= Body mass index (BMI) category; normal range (18.5 – 24.99), overweight (25.0 – 29.5), Obese class I (30 – 34.99), *p-value is statistically significant

## 4.2 Social demographic parameters of the study population

Table 4.2 shows the social demographic characteristics of the study participants. The table shows no significant association between the categories of age, gender and educational status with HIV status. However, there was a significant association between relationship status and HIV status, as indicated by a p-value less than 0.0006.

**Table 4.2**: Social demographic parameters of the study population.

| Parameters | HIV Patients (N=55) | HIV Seronegative (N=55) | Chi-Square (χ2) | p-value |
|---|---|---|---|---|
| *Age categories n (%)* | | | | |
| < 30 | 8 (14.5) | 4 (7.0) | | |
| 30 -45 | 19 (34.5) | 29 (53.0) | 4.137 | 0.1264 |
| > 45 | 28 (50.0) | 22 (40.0) | | |
| *Gender n (%)* | | | | |
| Female | 26 (47.0) | 23 (42.0) | 0.3312 | 0.5649 |
| Male | 29 (53.0) | 32 (58.0) | | |
| *Relationship Status n (%)* | | | | |
| Single | 19 (34.5) | 31 (56.4) | | |
| Married | 19 (34.5) | 22 (40.0) | 14.94 | 0.0006*** |
| Divorced/ Widowed | 17 (31.0) | 2 (3.6) | | |
| *Education Level n (%)* | | | | |
| Basic/None | 18 (32.7) | 11 (20.0) | | |
| Secondary | 25 (45.5) | 31 (56.4) | 2.373 | 0.3054 |
| Tertiary | 12 (21.8) | 13 (23.6) | | |

N = population size, n = size of subgroup. Data is expressed as frequency and percentage n (%). Chi-square (χ2) was used to test for association. NS = not statistically significant. *p-value is statistically significant at a 95% confidence interval.

### 4.3 Comparison of red blood cell counts among PLWH and non-infected participants.

Figure 4.1 compares the red blood cell counts in PLWH and non-infected participants of both genders. The red blood cell counts in PLWH were significantly lower than their non-infected counterparts in males and females ($p < 0.01$).

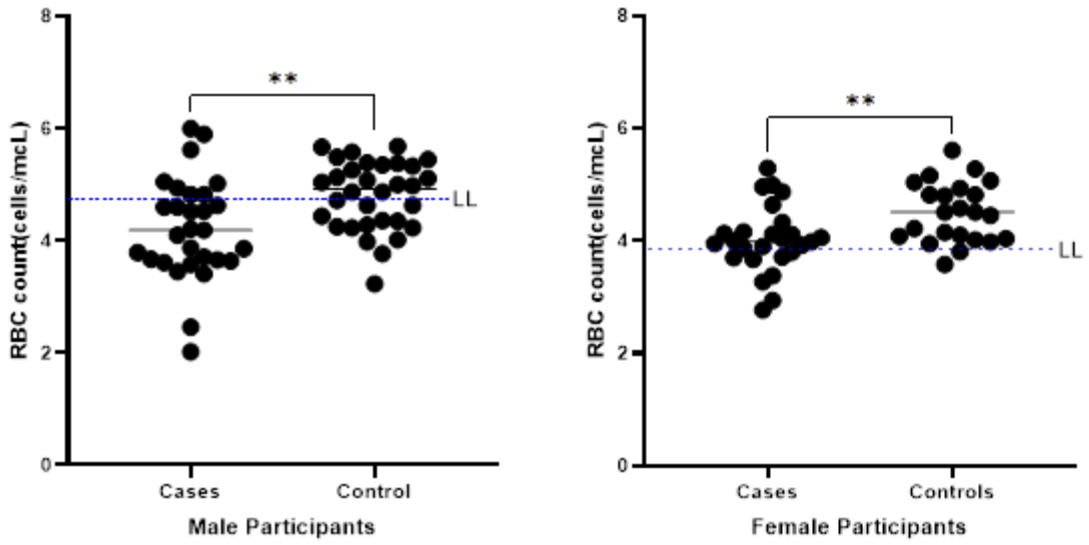


**Figure 4.1.** Red blood cell counts between PLWH and non-infected participants. (*LL= Lower limit of RBC range)

### 4.4 Comparison of haemoglobin concentrations among HIV-infected patients and non-infected participants.

Figure 4.2 compares the haemoglobin concentrations in HIV-infected patients and non-infected participants of both genders. The haemoglobin concentrations in PLWH were significantly lower than their non-infected counterpart for males ($p < 0.0001$). Even though Hb levels in PLWH were lower in the female participants, the difference between the two groups was not statistically significant.

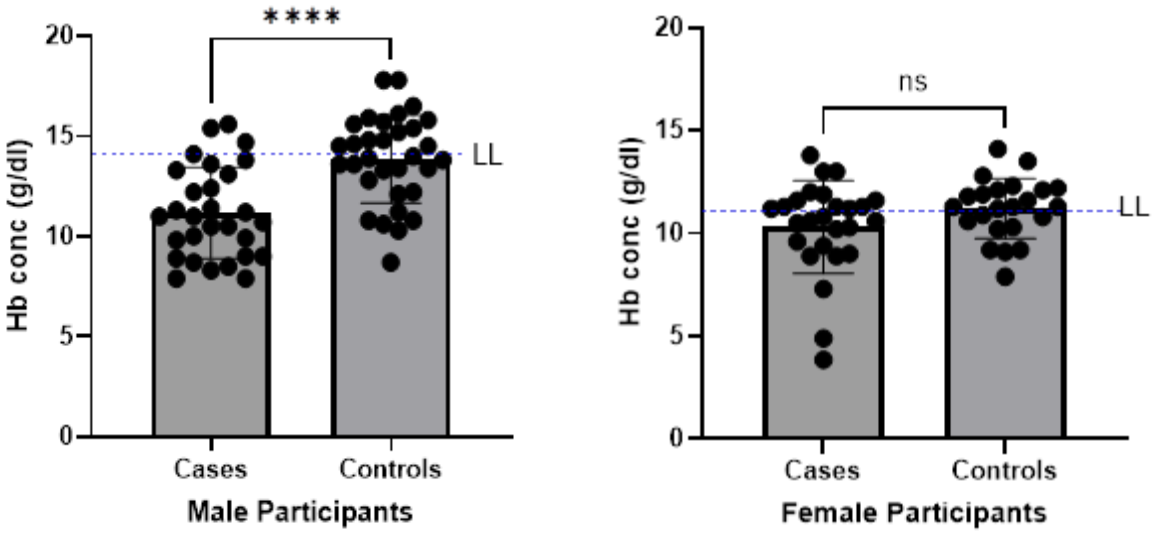


**Figure 4.2.** Comparison of haemoglobin concentration between PLWH and non-infected participants. (*LL= Lower limit of Hb range)

### 4.5 Comparison of mean cell volume among HIV-infected patients and non-infected participants.

Figure 4.3 compares the mean cell volume of HIV-infected participants with non-infected participants of both. The PLWH had significantly higher cell volumes than their non-infected counterpart, with a more significant proportion having cell volumes above the upper limit ($p < 0.0001$). Several controls had cell volumes below the lower limit for mean cell volume.

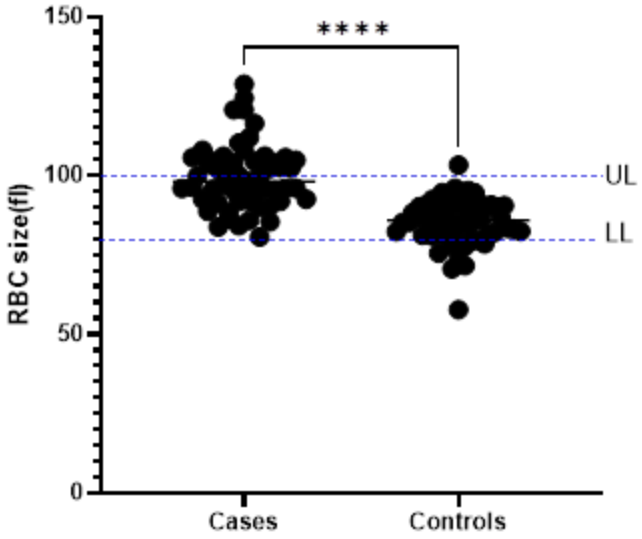


**Figure 4.3.** Comparison of mean cell volume between PLWH and non-infected participants. (*LL= Lower limit of RBC range, UL= Upper limit of RBC range)

### 4.6 Risk of developing pernicious anaemia with HIV infection.

Table 4.3 shows the risk of developing pernicious anaemia if you are living with HIV compared with people who do not have the HIV infection. The table shows an increased risk for developing pernicious anaemia with HIV infection reflected by a p-value less than 0.0001.

**Table 4.3**: Risk of developing pernicious anaemia with HIV infection.

| | **Pernicious Anaemia** | | | |
|---|---|---|---|---|
| Predictor | Yes n | No n | OR (CI) | P-value |
| HIV Infected | 24 | 31 | 34.06 | <0.0001**** |
| Non-HIV Infected | 1 | 44 | | |

Data is expressed as frequency n, and the odds ratio (OR) was used to test for the risk of developing pernicious anaemia association. *p-value is statistically significant at 95% confidence interval

### 4.7 Prevalence of pernicious anaemia among HIV-infected patients and non-infected participants.

Figure 4.4 compares the prevalence of pernicious anaemia among HIV-infected participants with non-infected participants of both. There was a higher prevalence of pernicious anaemia in PLWH compared with non-infected participants, 43.6% against 1.8%, respectively.

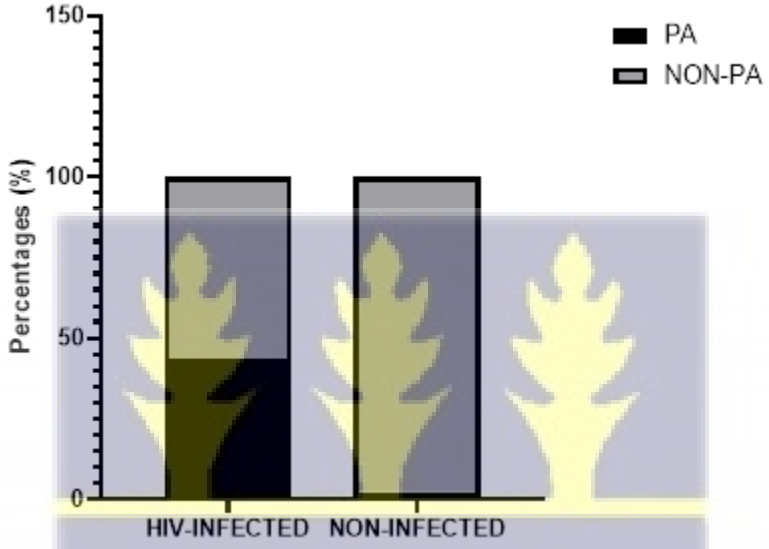


**Figure 4.4.** Comparison of the prevalence of pernicious anaemia between PLWH and non-infected participants.

### 4.8 Comparison of Serum Concentration of Lipopolysaccharide among HIV-infected patients and non-infected participants.

Figure 4.5 compares the circulating levels of lipopolysaccharide in the serum of the study groups. The PLWH had significantly higher concentrations of the antibodies than their non-infected counterpart ($p < 0.0001$).

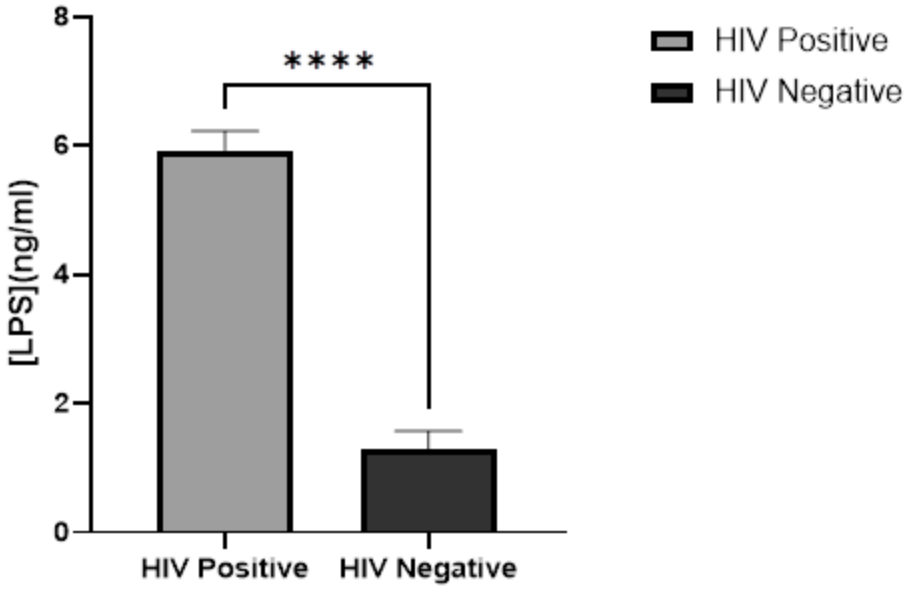


**Figure 4.5** Comparison of serum concentration of lipopolysaccharide between PLWH and non-infected participants.

**4.9 Comparison of Serum Concentration of Anti-Intrinsic Factor Antibodies among HIV-infected patients and non-infected participants.**

Figure 4.6 compares the circulating levels of anti-intrinsic factor antibodies in the serum of the study groups. The PLWH had significantly higher concentrations of the antibodies than their non-infected counterpart ($p < 0.0001$).

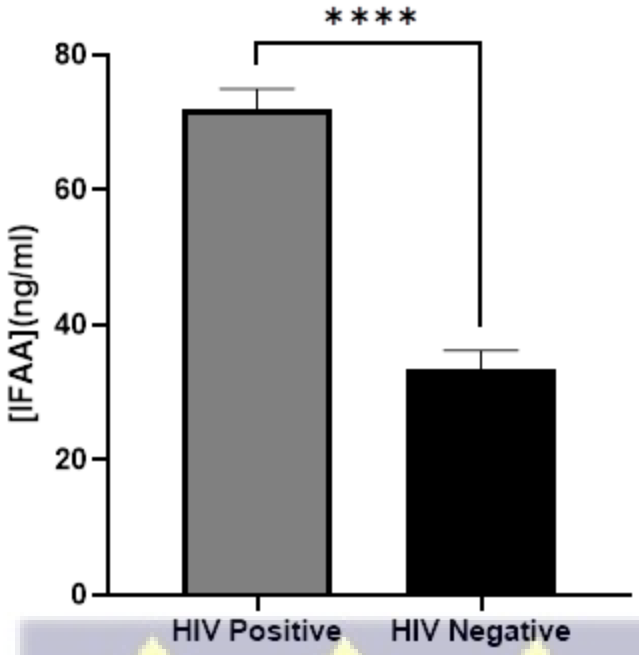


**Figure 4.6** Comparison of anti-intrinsic factor antibodies between PLWH and non-infected participants.

### 4.10 Clinical parameters of the sub population

Table 4.4 shows the clinical parameters of the study participants. There was no significant difference between the ages of HIV seropositive participants having pernicious anaemia, HIV seropositive participants without pernicious anaemia and HIV seronegative participants, 50.88±15.16 as compared with 42.42±14.03, 45.67±11.57 and 46.42 ± 15.32 respectively. There was no significant difference in the BMI of the groups, however, HIV seropositive participants without pernicious anaemia had an average BMI in the slightly overweight region, 29.94±2.99. The blood pressures, both systolic and diastolic, differed significantly among the groups with systolic pressure and diastolic pressure having p values <0.0001 and 0.0318 respectively. There was no statistically significant difference between how long the two groups of the HIV seropositive positive individuals have had the infection averaging 19.00±13.67 and 16.50±12.39 months. The

concentration of LPS (ng/ml) in the serum of the subgroups differed significantly, 6.843±2.154 as against 5.077±1.765 and 1.293±1.545, with a p value <0.0001. There was a statistically significant difference in the levels of anti-intrinsic factor antibodies (ng/ml) in the serum of the participants, 82.30±11.91, 62.43±23.66 and 33.38±15.74 for HIV infected with PA, HIV infected without PA and HIV seronegative respectively <0.0001.

**Table 4.4**: Clinical parameters of the sub population.

| Parameter | HIV Positive | | HIV Negative (n=30) | P-value |
|---|---|---|---|---|
| | HIV+/PA (n =24) | HIV/non-PA (n=26) | | |
| Age | 50.88±15.16 | 42.42± 14.03 | 45.67±11.57 | ns |
| BMI | 21.70±1.36 | 29.94±2.99 | 24.25±4.066 | ns |
| DOI | 19.00±13.67 | 16.50±12.39 | --- | ns |
| DBP | 86.08±9.930 | 85.88±10.86 | 78.00±16.11 | 0.0318 |
| SBP | 145.50±10.24 | 138.7±15.26 | 123.8±17.24 | <0.0001 |

n = size of subgroup, data is expressed as mean ± standard deviation, one way ANOVA and Student t-test were used to compare mean differences. SBP= systolic blood pressure, DBP = diastolic blood pressure, BMI= Body mass index (BMI) category; normal range (18.5 – 24.99), overweight (25.0 – 29.5), Obese class I (30 – 34.99), DOI= duration of infection, *p-value is statistically significant at $p < 0.05$

### 4.11 Comparison of Serum Concentration of Lipopolysaccharide Among Subgroups.

Figure 4.7 compares the circulating concentration of lipopolysaccharide in the serum of the subgroups. The HIV cohort that developed pernicious anaemia has significantly higher levels of LPS compared to those without HIV and the HIV seronegative population, with p-values < 0.01 and < 0.0001 respectively. Also, the HIV seropositive group without pernicious anaemia had statistically significantly higher LPS than the seronegative group (p< 0.0001).

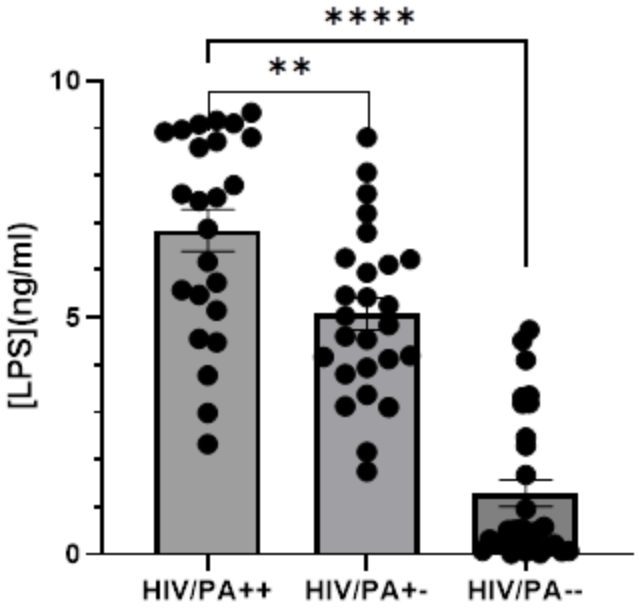


**Figure 4.7** Concentrations of lipopolysaccharide in the serum of HIV seropositive with pernicious anaemia, HIV seropositive without pernicious anaemia and HIV seronegative. (HIV/PA++= HIV seropositive with pernicious anaemia, HIV/PA+-= HIV seropositive without pernicious anaemia, HIV/PA--= HIV seronegative)

### 4.12 Comparison of Serum Concentration of Intrinsic Factor Autoantibodies Among Subgroups.

Figure 4.8 compares the circulating concentration of intrinsic factor autoantibodies in the serum of the subgroups. The HIV cohort that developed pernicious anaemia has significantly higher levels of IFAA compared to those without HIV and the HIV seronegative population, with p-values $< 0.001$ and $< 0.0001$ respectively. Also, the HIV seropositive group without pernicious anaemia had statistically significantly higher LPS than the seronegative group.

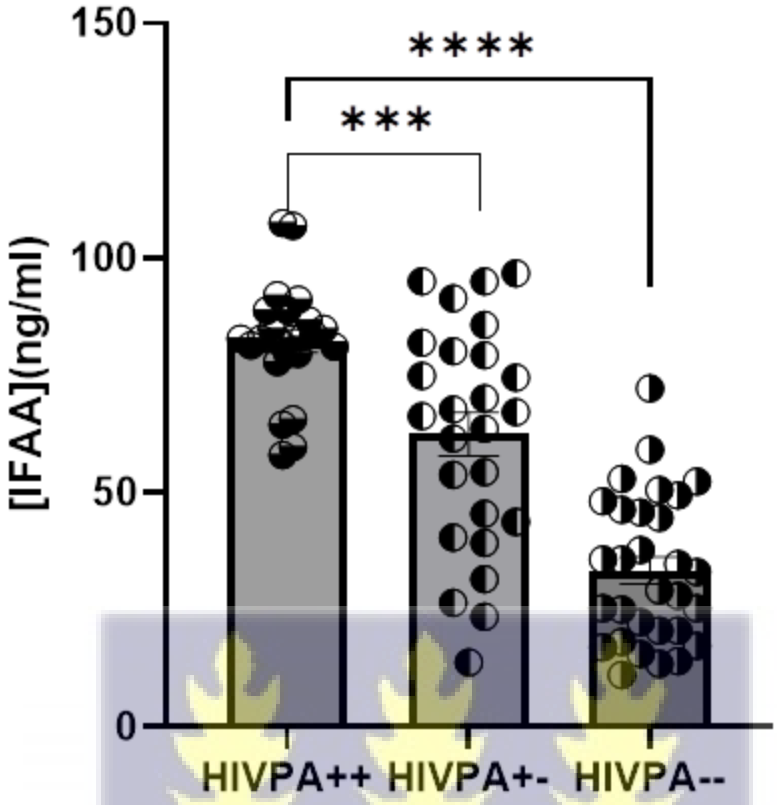


**Figure 4.8** Concentrations of IFAA in the serum of HIV seropositive with pernicious anaemia, HIV seropositive without pernicious anaemia and HIV seronegative. (HIV/PA++= HIV seropositive with pernicious anaemia, HIV/PA+-= HIV seropositive without pernicious anaemia, HIV/PA--= HIV seronegative)

### 4.13 Association Between Serum Concentration of Lipopolysaccharide, Intrinsic Factor Autoantibodies and Clinical Parameters

Table 4.4 measures the linear association between variables using the Pearson correlation. Age only showed a statistically significant association with systolic blood pressure. There was no significant association between BMI and any of the variables measured. Systolic blood pressure showed a statistically significant association with age, diastolic blood pressure, mean cell volume, serum concentration of lipopolysaccharide and serum concentrations of intrinsic factor autoantibodies with p-values <0.0001. Table 4.4 shows a statistically significant linear association between diastolic blood pressure and serum concentration of lipopolysaccharide and serum

intrinsic factor autoantibodies with p-values <0.0001. RBC count showed a positive significant association with hemoglobin concentration but negative associations with mean cell volume, serum concentration of lipopolysaccharide and serum intrinsic factor autoantibodies. Hemoglobin concentration negatively associated with mean cell volume, serum concentration of lipopolysaccharide and serum intrinsic factor autoantibodies with p-values 0.0358, 0.0045 and 0.0320 respectively.

**Table 4.5:** Correlation matrix of serum concentration of lipopolysaccharide, intrinsic factor autoantibodies and clinical parameters.

| Pearson Correlation | | Age | BMI | SBP | DBP | RBC | HGB | MCV | LPS | IFAA |
|---|---|---|---|---|---|---|---|---|---|---|
| Age | r | 1.000 | 0.095 | 0.229 | 0.091 | -0.005 | 0.037 | 0.146 | 0.028 | 0.099 |
| | p | | ns | 0.0414* | ns | ns | ns | ns | ns | ns |
| BMI | r | 0.095 | 1.000 | 0.042 | 0.073 | 0.110 | 0.013 | -0.032 | 0.032 | -0.167 |
| | p | ns | | ns | ns | ns | ns | ns | ns | ns |
| SBP | r | 0.229 | 0.042 | 1.000 | 0.661 | -0.073 | 0.058 | 0.471 | 0.542 | 0.488 |
| | p | 0.0414* | ns | | <0.0001**** | ns | ns | <0.0001**** | <0.0001**** | <0.0001**** |
| DBP | r | 0.091 | 0.073 | 0.661 | 1.000 | 0.083 | 0.083 | 0.209 | 0.360 | 0.306 |
| | p | ns | ns | <0.0001**** | | ns | ns | ns | <0.0010*** | 0.0057** |
| RBC | r | -0.005 | 0.110 | -0.073 | 0.083 | 1.000 | 0.242 | -0.272 | -0.311 | -0.137 |
| | p | ns | ns | ns | ns | | 0.0307* | 0.0147* | 0.0049** | ns |
| HGB | r | 0.037 | 0.013 | 0.058 | 0.083 | 0.242 | 1.000 | -0.235 | -0.311 | -0.240 |
| | p | ns | ns | ns | ns | 0.0307* | | 0.0358* | 0.0045** | 0.0320* |
| MCV | r | 0.146 | -0.032 | 0.471 | 0.209 | -0.272 | -0.235 | 1.000 | 0.724 | 0.652 |
| | p | ns | ns | <0.0001**** | ns | 0.0147* | 0.0358* | | <0.0001**** | <0.0001**** |
| [LPS] | r | 0.028 | 0.032 | 0.542 | 0.360 | -0.311 | -0.314 | 0.724 | 1.000 | 0.601 |
| | p | ns | ns | <0.0001**** | <0.0010*** | 0.0049** | 0.0045** | <0.0001**** | | <0.0001**** |
| [IFAA] | r | 0.099 | -0.167 | 0.488 | 0.306 | -0.137 | -0.240 | 0.652 | 0.601 | 1.000 |
| | p | ns | ns | <0.0001**** | 0.0057** | ns | 0.0320* | <0.0001**** | <0.0001**** | |

r = Pearson correlation coefficient, SBP= systolic blood pressure, DBP = diastolic blood pressure, BMI= Body mass index (BMI) category; normal range (18.5 – 24.99), overweight (25.0 – 29.5), Obese class I (30 – 34.99), RBC= red blood cell counts, HGB = haemoglobin, MCV= mean cell volume, [LPS]= serum concentration of lipopolysaccharide, [IFAA]= serum concentration of intrinsic factor autoantibodies, *p-value is statistically significant at $p < 0.05$

# CHAPTER 5

# DISCUSSION, CONCLUSION, LIMITATIONS AND RECOMMENDATIONS, FUTURE DIRECTIONS

## 5.1 Discussion

This study used 110 participants, which comprised 55 individuals who were seropositive for the human immunodeficiency virus and were taking antiretrovirals and 55 individuals who were seronegative for the HIV infection. The study compared the clinical parameters between the two groups and showed that the mean age for the HIV-infected was 46.42 ± 15.32. There was no significant difference between the ages of the two groups (Table 1). This similarity is because, in sampling for the controls, they were age-matched, giving comparable mean ages for the two groups. However, a mean age of 46 in the cases raises some questions. According to the Centre for Disease Control, in the year 2022, 60% of people infected with HIV in the USA were between the ages of 13 and 34 (CDC, 2024). Current reports by the Statistical Service of the United States of America show that the most infected ages are between 25 and 34 years old (U.S. Statistics, 2024). According to the World Health Organization and Ghana AIDS Commission, the at-risk age group is between the ages of 15 and 49 years (WHO, 2024a). A mean age of 46 suggests that most people infected with the virus get diagnosed late. This late diagnosis could be because people hesitate to get tested; they report to hospitals only after seeing symptoms and poor surveillance systems in the country. A study conducted in 2020 at the Korle-bu Teaching Hospital by Dako-Gyeke *et al.*( 2020) reported severe stigmatisation and discrimination among adolescent HIV patients. Even adolescents who contracted the infection through birth hesitated to visit the hospitals because of the general assumption that HIV infection is through promiscuity. The fear of being discriminated against keeps people from testing to know their status, and those who know their

status hesitate to visit the hospital for fear of people finding out. Late detection of the infection poses other effects aside from developing AIDS. As of 2023, the average age for having a first child in Ghana was 21 (World Population Review, 2024). This average childbearing age, compared with the age of diagnosis of HIV, increases the likelihood of maternal transmission, considering the poor attendance rate to antenatal services in the country. This incoherence poses a challenge to the CDC and WHO's goals to reduce HIV infections and AIDS-related deaths since infected individuals unaware of the status will transmit the virus and also not get access to the necessary medical intervention on time.

There is a worldwide increase in BMI, with a report of a steady rise in the prevalence of people who are overweight (BMI of 25–29 kg/m$^2$) and obese (BMI ≥30 kg/m$^2$) in both low-middle and high-income countries (Phelps et al., 2024). PLWH tends to interact complexly with their body weight. Before patients start taking ARTs, they develop low BMI due to HIV and other opportunistic infections (Carrillo-Larco et al., 2024). Following treatment, BMI increases in unison with CD4+ cell counts (Koethe et al., 2016). This study revealed a statistically significant difference in BMI between the two study groups, as shown in Table 1. This difference was, however, not biologically significant as the case group had an average BMI within the normal range. This observation mirrors the results produced by Kalinjuma *et al.*(2023). In their work, they saw a steady rise in the BMI of HIV patients within 6-12 months after beginning antiretroviral therapy. Some classes of antiretrovirals, like integrase inhibitors, have been found to stimulate weight gain. The observed weight gain has raised the concern of a possible epidemic of obesity, which was not reflected in this study. Other works that saw a similar trend proposed that compromised metabolism is the reason for the weight gain seen in PLWH on ARTs (Bailin et al., 2020; Lake et al., 2018).

This study also showed a significant difference in the two groups' systolic and diastolic blood pressures. As captured in Table 1, this difference was not biologically relevant, with PLWH having normal diastolic and slightly elevated systolic blood pressure. This is in contrast to the work of Niwaha *et al.*( 2021), who saw a decrease in systolic and diastolic blood pressure (–5.1 mmHg, 95% CI: –7.4 to –2.4) and (–4.0 mmHg, 95% CI: –5.6 to –2.5), respectively, in PLWH. Their result is, however, in contrast to the current reports of the possible role of HIV in fostering cardiovascular comorbidities (Fragkou et al., 2023; Hadavandsiri et al., 2023; Taiwo et al., 2023).

Except for the relationship status, there was no association between the social demographic parameters of the HIV participants and their status (Table 2). Over 30 per cent of the HIV cohort were divorced. In a study by Tenkorang (2014), where he assessed the association between marital status and HIV, he saw the highest distribution of HIV among the divorced/widowed group. He explained that this was concerning as most HIV campaigns are directed towards those who are single. He also reasoned that this high distribution could be due to the stigma of PLWH. Nutor *et al.*( 2024) reported high stigmatisation and discrimination among PLWH living in the Volta region of Ghana. Their survey reports that many people in relationships hide their HIV status out of fear of losing their loved ones.

A decrease in cell counts(cytopenia) is a significant manifestation of HIV. The reduction in blood cells, especially white blood cells, was very pronounced in PLWH until the advent of ARTs. ARTs prevent the rapid infection and destruction of CD4+ cells by HIV, protecting them from destruction. Over the years, there have been conflicting reports about what happens to other blood cells, i.e., red blood cells and platelets, with the mechanisms surrounding the decreased levels of these cells still in debate (Satpute et al., 2024; Mandania, 2024). This study compared the red blood cell count in PLWH with those without the infection. There was a statistically significant

difference between the cell counts of the two groups (Fig 4.1) ($p< 0.01$). Compared with the control group, PLWH in both genders had red blood cell counts lower than the outside the established limit, 4.2 to 5.4 million/mcL for females and 4.8 to 6.1 million/mcL for males. This is similar to the results of Malapati, Nadeem and Shah (2020), who saw a significantly reduced red blood cell count in PLWH upon comparing blood profiles. Based on a previous experiment which found a reduced concentration of immunoreactive erythropoietin in the serum of PLWH, they reasoned that the decreased red blood cell concentration resulted from impaired erythropoiesis. Other studies that reflected this result proposed that some factors circulating in PLWH could inhibit erythropoiesis.

Moreover, HIV is thought to directly affect haematopoiesis by infecting progenitor cells in the bone marrow. The same result was reproduced in PLWH in India, Malawi, and Mozambique (Bhardwaj et al., 2020; Ciccacci et al., 2020). Decreased RBC count in HIV is reported to be present before and after antiretroviral therapy. The persistence of decreased RBC count after HAART is thought to be associated with the medication, as seen with the drug Zidovudine.

Anaemia is a public health concern. In PLWH, this is especially worrying because it affects quality of life, increases the rate of disease progression and interferes with therapy. Decreased haemoglobin levels and red blood cell count characterise anaemia. Haemoglobin has been found to drop significantly during HIV infection. The drop in Hb is thought to arise through the rapid breakdown of red blood cells through autoimmune events, disseminated intravascular coagulation and ineffective production. Iron, folate and vitamin B12 deficiency results in the production of abnormal red blood cells. These cells are rapidly removed from circulation by the spleen. The destruction of these cells is accompanied by the catabolism of haemoglobin and a consequent fall in its concentration. Studies have found that, during HAART, haemoglobin rises in unison with

CD4+, bringing about the possibility of using haemoglobin as a prognostic marker. In this study, we found a more significant percentage of PLWH having lower than average haemoglobin concentration when compared to the control group. However, the difference between the two groups was more prominent in the male subjects than the females (Fig 4.2). Females are among the groups of individuals considered to be most at risk for anaemia. Biological processes such as menstruation and pregnancy reduce blood circulation and haemoglobin concentration. Therefore, females of childbearing age are encouraged to take supplements that enable blood and blood products to form. The loss of blood through biological processes in females accounts for the similarity in the haemoglobin concentrations of the two groups of females used in the study. In the males, those infected with HIV had statistically reduced haemoglobin concentrations compared to the control group. The graph shows a good number of infected males with average haemoglobin concentrations. This agrees with the work of Woldeamanuel and Wondimu (2018). In their work, he saw the haemoglobin levels rise during follow-ups after ART initiation. Obeagu ( 2016) found similar results in Umuahia, Nigeria.

There are several forms of anaemia based on the mechanism through which it manifests. Anaemia is classified based on the size of red blood cells into microcytic, normocytic and macrocytic. Red blood cells have an average size of 80-100 fL. This is known as the mean cell volume. Anaemia patients with a cell volume less than 80 fL are said to have microcytic anaemia. This type of anaemia is caused mainly by a deficiency of iron but could also arise from lead poisoning and thalassemia. Microcytic anaemia is the most common type in the average population, as reflected in this study (Fig 4.3). A cohort study conducted in Malaysia revealed iron deficiency to be the most common cause of anaemia and microcytosis as the most common manifestation (Abdullah et al., 2020). This result was also replicated by Ahankari *et al.* (2017) in India. Iron is a recyclable

nutrient available in several plants and animal products. However, iron deficiency is a global pandemic that has affected over a billion people worldwide. Iron deficiency alone accounts for about 50% of all anaemia cases. This is because, even though iron is recycled, several physiological and pathological processes lead to iron loss. The primary means through which iron is lost is through blood loss. Menstruation, worm infections, chronic bleeding and gastrointestinal disorders lead to loss of blood and iron. The scenario of microcytic anaemia being the most common anaemia in the population was not reflected in the people living with HIV.

As shown in Fig 4.3, none of the HIV-infected cohorts had a mean cell volume lower than 80fl. Recent studies have shown that people living with HIV had higher mean cell volumes after initiation of antiretroviral therapy (Chinwe Favour *et al.*, 2018; Aziz *et al.*, 2019; Damtie *et al.*, 2021; Martina and Chinemerem, 2022). It was interesting to note the number of people living with HIV that had mean cell volumes higher than the upper range limit, macrocytosis. Macrocytic anaemia is a rare form of anaemia occurring in less than 2% of the population. Macrocytosis is a manifestation of nutritional deficiencies, mainly vitamin B12 and folate. In this study, we found a higher prevalence of macrocytosis in PLWH than in non-infected participants ($p < 0.0001$). This result supports reports of the current rise in the cases of pernicious anaemia in PLWH, a condition caused by the unavailability of vitamin B12.

Following the high incidence of macrocytosis in our HIV-infected population, this study combined red blood cell counts, haemoglobin concentration and mean cell volume for use as a diagnostic marker for pernicious anaemia in the study groups. It was observed in the study that PLWH have a higher tendency to develop pernicious anaemia, reflected by a prevalence of 43.6 per cent as compared to 1.8 in the control group. The prevalence of pernicious anaemia obtained in this study demonstrates what has been reported, a general prevalence of 0.1% and 1.9% in people between

40 and 59 years (Seage et al., 2024). Esposito *et al.* (2022) also reported a low prevalence of pernicious anaemia in the general population. They reasoned that the low prevalence can be attributed to the availability of vitamin B12 in our everyday diet. This makes the condition mainly an autoimmune disorder and rare in the population, like most autoimmune conditions. This study, however, showed a high prevalence of pernicious anaemia in the HIV cohort. This is in agreement with current reports of an increase in non-AIDS comorbidities in PLWH. Even though there have been reports of cases of pernicious anaemia in PLWH, the prevalence in that cohort has not been firmly established, hindering the effective management of people living with HIV. Upon recognising the suspected prevalence of pernicious anaemia in PLWH, the study investigated the odds of developing pernicious anaemia if a person has HIV compared to one who does not. The result obtained from that analysis, as shown in Table 4.3, shows that PLWH have a higher chance of developing pernicious anaemia. This study highlights the need to manage PLWH holistically.

Lipopolysaccharide (LPS) is an immunogenic component of the cell membrane of Gram-negative bacteria. In this study, we compared the levels of LPS in the circulation of PLWH and HIV seronegative. Figure 4.5 shows about 3-fold higher concentration of LPS in PLWH ($P<0.0001$). The gut microbiome comprises a variety of bacteria mostly Gram-negative which play an important role in maintaining health and causing illnesses in humans. In the event of a compromise in the intestinal barrier, these bacteria and their components especially LPS leak into the circulation, a process known as translocation. In agreement with the results of this study, Isaguliants et al. (2021), Ramendra et al. (2019) and Routy et al. (2022) reported an increase in the levels of LPS in the circulation of HIV patients compared to the control group. Also, Cassol et al. (2010) in a longitudinal study to assess LPS as a marker for translocation, reported in an increase in the levels of endotoxin in the circulation of HIV patients over time. LPS is a potent immune

stimulator, interacting with Toll-like receptors on cells to produce pro-inflammatory cytokines and increase the costimulatory molecules on APCs. Chronic immune stimulation is widely reported in HIV patients on ARTs. As a marker of biomarker translocation, the high levels of LPS in the circulation of PLWH in this study also reflect the compromised integrity of the intestinal barrier in the circulation of PLWH. Nagavci and Szab (2024) also reported leaky gut in PLWH, they reasoned that the compromised intestinal barrier integrity is a result of the replicative action of the virus in the early stages of the infection and the chronic immune stimulation elicited by the low viraemia in PLWH.

The leaky gut-mediated mechanism of autoimmune diseases proposes that, during translocation, there is also the movement of self-products into circulation. This results in immune stimulation and the production of autoantibodies. To assess HIV-associated leaky gut as the mechanism for pernicious anaemia in HIV patients, we measured circulating levels of intrinsic factor autoantibodies in the two study groups. Figure 4.6 shows elevated levels of intrinsic factor autoantibodies compared to the control group. This result highlights the movement of intrinsic factors from the gut of PLWH into their circulation. This resulted in the production of autoantibodies that subsequently attack and destroy intrinsic factors thereby compromising the ability of PLWH to absorb and use vitamin B12.

In this study, we go on to stratify PLWH in those with pernicious anaemia and those without. Table 4.4 compares various clinical parameters among the three groups. It is observed that there was no significant difference in the ages of the subgroups. In the general population, pernicious anaemia affects people of older ages with a prevalence of about 1.9% (Andres and Serraj, 2012). In our study, the average age of the participants suffering from pernicious anaemia is 50 years, corresponding with what is reported in literature. There was no statistically significant difference

in the BMIs of the group even though PLWH without pernicious anaemia were slightly overweight. There have been reports of PLWH getting slightly overweight because of antiretrovirals (Guaraldi et al., 2023). The blood pressures, both systolic and diastolic varied significantly among the three groups. However, only the PLWH and having pernicious anaemia had biologically significant elevated systolic blood pressures averaging 145mmHg. In agreement with the results from this study, HIV has been reported to elevate systolic blood pressure (Denu et al., 2024). Table 4.4 also shows no significant difference in the duration of infections between the HIV subpopulations. This goes to show that, there is no specific timeline for developing pernicious anaemia after infection, necessitating the need to holistically manage PLWH right from the onset. Comparison of LPS and intrinsic factor autoantibodies, shown in Figure 4.7 and 4.8 respectively among the three subpopulations show statistically significant differences with PLWH and having pernicious anaemia having the highest serum concentrations of the analytes and the HIV seronegative having the lowest levels. Leaky gut in HIV is pronounced but the results from this study show a more pronounced translocation of gastric contents reflected by the LPS levels which lead to the production of a more pronounced immune response. However, the levels in the HIV patients without PA are more elevated than in the control group showing some degree of translocation and autoantibody production not captured in the control group. This mirrors the results produced by Liu et al. (2021) where they reported increased levels of LPS in the group suffering from autoimmune diseases.

Following the results from the initial analysis, a correlation analysis was performed to ascertain a linear relationship between the variables. The results from Table 4.5 show a significant positive correlation between systolic blood pressure and age. Systolic blood pressure has been found to increase in several populations with age (Bencivenga et al., 2022; Bilo et al., 2020). This is thought

to be a result of factors like vascular stiffness, increased peripheral resistance, hormonal changes and some comorbidities. However certain populations like the Tsimanes and Okinawans have been reported to have a decreasing or stable blood pressure as they age (Gurven et al., 2012; Poulain and Herm, 2024), mainly as a result of their genetic makeup and lifestyle. The correlation matrix also revealed a significant positive relation between systolic blood pressure and mean cell volume. A study conducted by Chen et al. (2024) reported an increase in mean cell volume as systolic blood pressure increases mirroring what was reported in this study. They reasoned that this could be a result of the influence blood pressure has on erythropoiesis. Also, oxidative stress arising from hypertensive states affects the membranes of red blood cells (Jewell et al., 2013). However, the relationship between the two is complex as some studies reported no association and others reported a negative relationship. The positive association between red blood cell count and haemoglobin in this study has been confirmed by Kishimoto et al. (2020). Red blood cells are the carriers of haemoglobin which transports oxygen to tissues therefore an increase in the cells increases the haemoglobin. There are however conditions where this situation does not hold, a change in plasma volume may dilute or concentrate haemoglobin and abnormal shapes of red blood cells tend to affect haemoglobin levels (Goodhead and MacMillan, 2017). This study reported a negative association between red blood cell counts and mean cell volume and by extension, a negative association between haemoglobin and mean cell volume. Maner et al., (2024) propose that large mean cell volumes arise from ineffective erythropoiesis (due to factors like folate and vitamin b12 deficiency), the abnormal size of these cells marks them for rapid destruction resulting in a decrease in cell counts and haemoglobin. This could also be a mechanism of regulation where mean cell volumes are decreased with increased cell counts in order to maintain blood viscosity.

The concentration of LPS in the serum of the subjects had a positive correlation with blood pressure and mean cell volume and negative correlations with red blood cell count and haemoglobin concentrations. LPS has been found to increase blood pressure through immune activation, endothelial dysfunction, activating vasoconstrictors and reducing vasodilators like prostacyclin (Wang et al., 2023; Yücel et al., 2017). The positive association between LPS and blood pressure has been corroborated in several studies (Grylls et al., 2021; Page et al., 2022). LPS has been reported to reduce red blood cell count and haemoglobin concentration as observed in this study through several mechanisms (Brauckmann et al., 2016). LPS has been found to both inhibit the production of red blood cells and increase the destruction of these cells through LPS-induced oxidative stress. Levels of serum intrinsic factor autoantibodies and LPS had a positive relation with mean cell volume. Large red blood cell sizes (megaloblasts) are a characteristic of pernicious anaemia. In the autoimmune mechanism of pernicious anaemia, autoantibodies against intrinsic factor and parietal cells lead to the inability to effectively absorb and use vitamin B12. This results in the production of abnormally large red blood cells as was seen in this study. The large red blood cell sizes mark these cells for destruction resulting in decreased red blood cell counts and haemoglobin concentration seen in PLWH and having pernicious anaemia.

**5.2 Conclusion**

This study investigated the role leaky gut plays in the pathogenesis of pernicious anaemia in people living with HIV. The results from this study showed a higher prevalence of pernicious anaemia in PLWH as compared to the general population. Analysis of biomarkers of leaky gut and intrinsic factor autoantibodies concentration in the serum showed an important relationship between the leaky gut and pernicious anaemia in PLWH. This study showed levels of LPS in the serum of PLWH to be significantly higher than the HIV-seronegative proving how chronic infection with the virus disrupts the integrity of the intestinal barrier bringing about the condition “leaky gut”.

The leaky gut leads to the movement of products from the gut into circulation. In this study, the increase in the concentration of intrinsic factor autoantibodies highlights the movement of self-proteins including intrinsic factor, the glycoprotein needed for the absorption of vitamin B12 in the intestines. The subsequent priming of the immune system to intrinsic factors led to the production of autoantibodies that target and destroy intrinsic factor.

The mechanism proposed in this study shows how important it is to manage HIV patients beyond viral suppression but also include strategies that target AIDS comorbidities. The autoimmune-mediated mechanism of pernicious anaemia in HIV requires the adjustment of current management strategies for pernicious anaemia in PLWH, specifically through antibody therapy or intravenous supplementation. Furthermore, addressing leaky gut by improving gut health with probiotics and targeted medicine will address the root cause of the increased serum LPS and IFAA concentrations decreasing systemic inflammation and HIV-associated autoimmune diseases like pernicious anaemia.

**5.3 Limitations and Recommendations**

1. The study was a one-time point study therefore the changes in levels of LPS and intrinsic factor autoantibodies and the development of pernicious anaemia over time could not be ascertained limiting the study to an associative relationship. A longitudinal study will provide insights into how the severity of leaky gut and intrinsic factor autoantibodies change with time and how these relate to pernicious anaemia providing a causative relationship instead of an associative one.
2. The use of archive samples limited our ability to account for dietary habits and other confounding factors.

3. The number of biomarkers tested in this study was limited. Markers of epithelial barrier destruction like zonulin and some inflammatory cytokines could have provided a more comprehensive image of the gut-immune axis in PLWH.
4. The samples were obtained from one setting, a multi-centre study will provide a more representative description of what happens in PLWH.

### 5.4 Future Directions

A multi-centre and longitudinal study should be conducted to monitor how leaky gut and associated immune response varies among diverse HIV groups. Also, interventions like probiotics, and modifications in diet could be explored to restore gut barrier integrity. Furthermore, a predictive model could be developed using several markers to aid in early diagnosis and consequently targeted management of pernicious anaemia and other HIV leaky gut-associated autoimmune diseases. Finally, Monoclonal antibodies directed at the intrinsic factor autoantibodies could be used in the management of PLWH.



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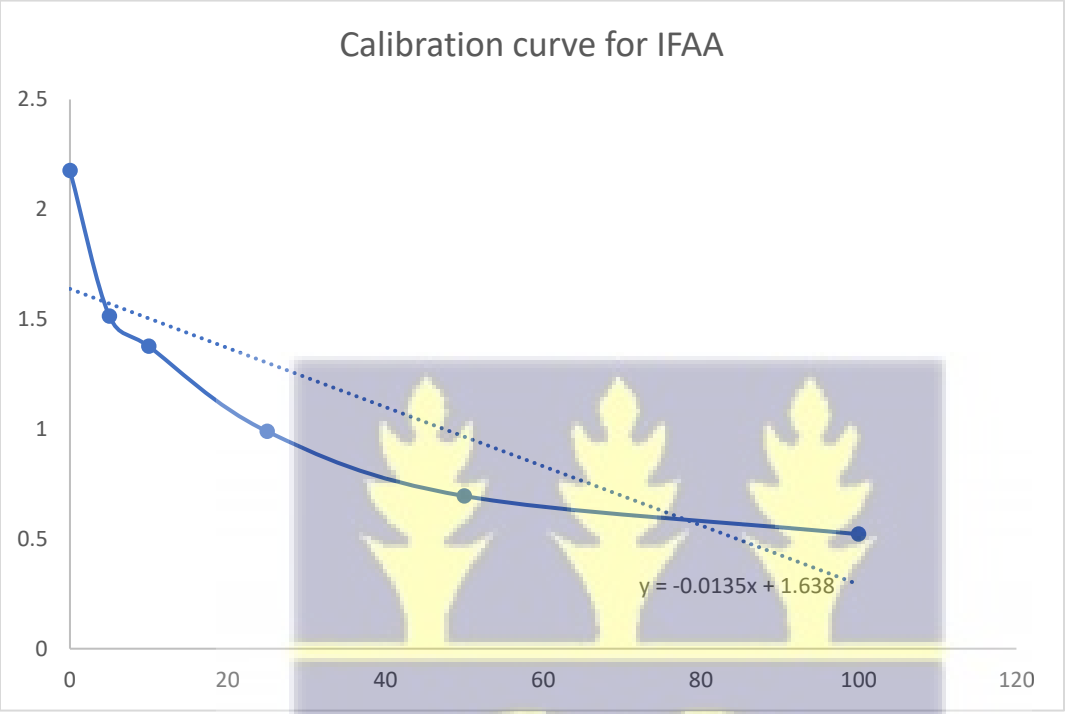
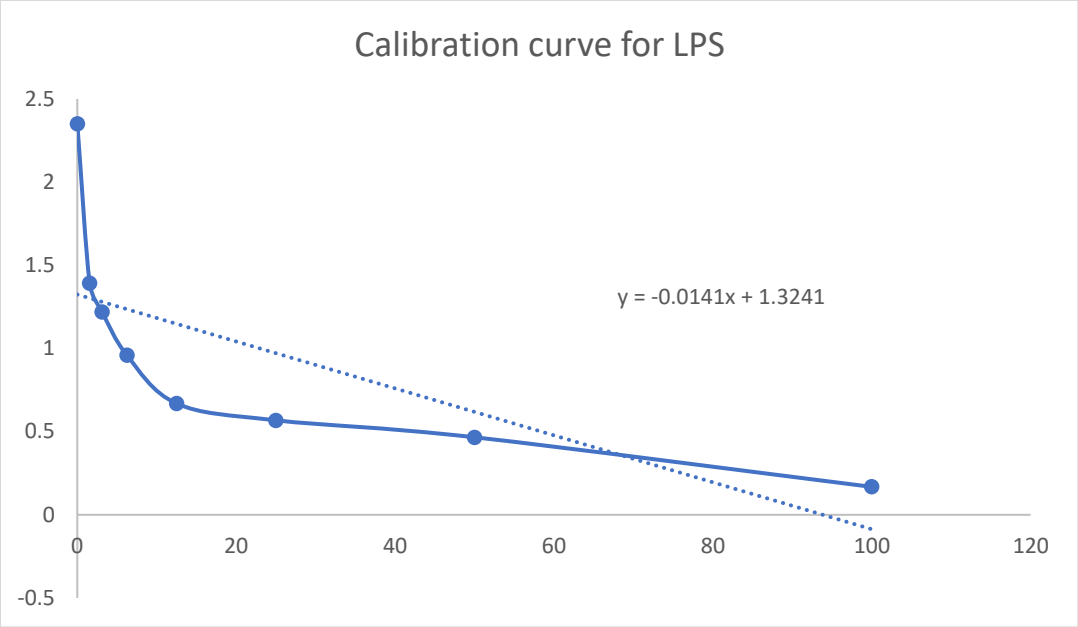
# APPENDIX

**Appendix Figure 1**: Calibration curve of intrinsic factor autoantibodies and Lipopolysaccharide






**Appendix Table 1:** Absorbance of Serum Lipopolysaccharide at 450nm

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 0.008 | 0.272 | 0.791 | 0.445 | 0.787 | 1.256 | 0.746 | 0.688 |
| 0.095 | 0.113 | 0.551 | 0.249 | 0.583 | 1.294 | 0.875 | 0.976 |
| 0.060 | 0.995 | 0.692 | 0.309 | 0.769 | 1.315 | 1.304 | 1.315 |
| 0.044 | 0.251 | 0.354 | 0.461 | 0.883 | 1.259 | 1.252 | 1.281 |
| 0.598 | 0.903 | 0.082 | 0.849 | 1.020 | 1.243 | 0.857 | 1.280 |
| 0.033 | 0.261 | 0.743 | 0.365 | 0.641 | 1.188 | 1.293 | 1.001 |
| 0.082 | 0.538 | 0.736 | 1.078 | 0.885 | 1.303 | 0.656 | 1.317 |
| 0.224 | 0.453 | 0.186 | 0.613 | 0.732 | 0.876 | 1.324 | 1.321 |
| 0.067 | 0.515 | 0.684 | 0.553 | 0.485 | 0.851 | 1.250 | 1.087 |
| 0.682 | 0.041 | 0.674 | 0.442 | 0.559 | 1.286 | 1.315 | 1.247 |

HIV Positive HIV Negative

**Appendix Table 2:** Concentration of Serum Lipopolysaccharide (ng/mL)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 9.332 | 7.462 | 3.781 | 6.235 | 3.809 | 0.482 | 4.097 | 4.514 |
| 8.717 | 8.589 | 5.483 | 7.625 | 5.256 | 0.213 | 3.188 | 2.471 |
| 8.965 | 2.334 | 4.483 | 7.199 | 3.937 | 0.065 | 0.140 | 0.063 |
| 9.079 | 7.611 | 6.880 | 6.121 | 3.128 | 0.460 | 0.511 | 0.305 |
| 5.150 | 2.987 | 8.809 | 3.370 | 2.157 | 0.578 | 3.316 | 0.311 |
| 9.157 | 7.540 | 4.121 | 6.802 | 4.845 | 0.964 | 0.218 | 2.289 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 8.809 | 5.575 | 4.171 | 1.745 | 3.114 | 0.151 | 4.738 | 0.050 |
| 7.802 | 6.178 | 8.072 | 5.043 | 4.199 | 3.177 | 0.010 | 0.024 |
| 8.916 | 5.738 | 4.540 | 5.469 | 5.951 | 3.358 | 0.520 | 1.678 |
| 4.554 | 9.100 | 4.611 | 6.256 | 5.426 | 0.268 | 0.065 | 0.549 |

HIV Positive HIV Negative

**Appendix Table 3:** Absorbance of Serum Intrinsic Factor Autoantibodies at 450nm

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 0.492 | 0.564 | 0.768 | 0.532 | 1.024 | 0.840 | 1.012 | 1.448 |
| 0.564 | 0.396 | 0.188 | 0.480 | 1.212 | 0.972 | 1.156 | 1.408 |
| 0.540 | 0.196 | 0.588 | 1.092 | 0.912 | 1.488 | 1.392 | 1.192 |
| 0.520 | 0.492 | 0.856 | 1.280 | 0.780 | 0.988 | 1.360 | 1.128 |
| 0.440 | 0.520 | 0.404 | 1.320 | 1.108 | 1.336 | 1.260 | 0.664 |
| 0.468 | 0.408 | 0.628 | 1.452 | 0.632 | 1.168 | 1.296 | 0.956 |
| 0.440 | 0.520 | 0.724 | 0.332 | 0.568 | 1.156 | 1.020 | 1.404 |
| 0.508 | 0.544 | 0.556 | 0.356 | 0.692 | 0.932 | 1.428 | 0.924 |
| 0.516 | 0.752 | 0.744 | 0.904 | 1.048 | 1.456 | 1.036 | 1.36 |
| 0.536 | 0.832 | 0.732 | 0.356 | 0.808 | 1.296 | 1.300 | 1.244 |

HIV Positive HIV Negative

**Appendix Table 4:** Concentration of Serum Intrinsic Factor Autoantibodies (ng/mL)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 84.889 | 79.556 | 64.444 | 81.926 | 45.481 | 59.111 | 46.370 | 14.074 |
| 79.556 | 92.000 | 107.407 | 85.778 | 31.556 | 49.333 | 35.704 | 17.037 |
| 81.333 | 106.815 | 77.778 | 40.444 | 53.778 | 11.111 | 18.222 | 33.037 |
| 82.815 | 84.889 | 57.926 | 26.519 | 63.556 | 48.148 | 20.593 | 37.778 |
| 88.741 | 82.815 | 91.407 | 23.556 | 39.259 | 22.370 | 28.000 | 72.148 |
| 86.667 | 91.111 | 74.815 | 13.778 | 74.519 | 34.815 | 25.333 | 50.519 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 88.741 | 82.815 | 67.704 | 96.741 | 79.259 | 35.704 | 45.778 | 17.333 |
| 83.704 | 81.037 | 80.148 | 94.963 | 70.074 | 52.296 | 15.556 | 52.889 |
| 83.111 | 65.630 | 66.222 | 54.370 | 43.704 | 13.481 | 44.593 | 20.593 |
| 81.630 | 59.704 | 67.111 | 94.963 | 61.481 | 25.333 | 25.037 | 29.185 |

HIV Positive HIV Negative

Blue shade= Pernicious Anaemia

Yellow shade = No Pernicious Anaemia