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Changes in Blood Coagulation in HIV/AIDS patients in Sudan

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بسم الله الرحمن الرحيم
قال تعالى:

( وَقَلْ رَبِّ زَدْنِي عِلْماً
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صدق الله العظيم

(سورة طه، الآية 114)
# CONTENTS

<table>
<thead>
<tr>
<th>Dedication</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acknowledgment</td>
<td>II</td>
</tr>
<tr>
<td>List of abbreviations</td>
<td>III</td>
</tr>
<tr>
<td>English abstract</td>
<td>IV</td>
</tr>
<tr>
<td>Arabic abstract</td>
<td>VI</td>
</tr>
<tr>
<td>List of figures</td>
<td>VII</td>
</tr>
<tr>
<td>List of tables</td>
<td>VIII</td>
</tr>
</tbody>
</table>

## CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW 1

OBJECTIVES 37

## CHAPTER TWO

MATERIALS AND METHODS 38

## CHAPTER THREE

RESULTS 45

## CHAPTER FOUR

DISCUSSION 64

CONCLUSION 70

RECOMMENDATIONS 71

REFERENCES 72

APPENDIX (questionnaire, consent)
To my family for their patience
I would like to thank those who have contributed so much to this thesis.

I am most grateful to my supervisor Dr. Maria Satti, I owe her great debt for her keen and dedicated supervision, patience with me and by her continuous advice we end in this thesis. I thank her for her kind encouragement. Grateful appreciation, also to Ustaz Yosif deputy director of laboratories in Khartoum Teaching Hospital, who allowed me to do my work in their laboratory, where I received much help from his technical staff. Sincere gratitude extend to Dr. Isam Elkhider and Dr. Omer Elnimeri for their help and their agreement to participate in the foundation of the AIDS society for research. I like also to thank the staff working at Omdurman Teaching Hospital Center of HIV/AIDS. They were very helpful and assisted me in obtaining the samples. Thanks also extend, to the patients, who helped me a lot by agreeing to participate, and this work, may assist them in fighting the killing virus. Great thanks extend to my colleagues, my friends and especial thanks to my friend Eltyieb. ... Lastly my thanks extend to my family, wife and daughter.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDS</td>
<td>Acquired immune-deficiency virus</td>
</tr>
<tr>
<td>APL</td>
<td>Antiphospholipid</td>
</tr>
<tr>
<td>CDC</td>
<td>Center for Disease Control</td>
</tr>
<tr>
<td>CMV</td>
<td>Cytomegalovirus</td>
</tr>
<tr>
<td>DVT</td>
<td>Deep vein thrombosis</td>
</tr>
<tr>
<td>G6PD</td>
<td>Glucose-6 phosphate dehydrogenises deficiency</td>
</tr>
<tr>
<td>Hb</td>
<td>Hemoglobin</td>
</tr>
<tr>
<td>Hc+</td>
<td>Haematocrit</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immune virus</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PE</td>
<td>Pulmonary embolism</td>
</tr>
<tr>
<td>TGFB</td>
<td>Transforming growth factors beta</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
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<tr>
<td>VWF</td>
<td>Von Willebrand factor</td>
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<tr>
<td>RBC</td>
<td>Red blood cells</td>
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<tr>
<td>WBC</td>
<td>White blood cells</td>
</tr>
</tbody>
</table>
ABSTRACT

This is a prospective Laboratory study conducted at both Omdurman Teaching Hospital and Khartoum Teaching Hospital, from the first of February till mid April 2006.

The aim of the study was to assess changes in blood coagulation and basic haematological parameters in HIV/AIDS patients in SUDAN.

Fourty patients were included in this study, 22 males & 18 females, their ages ranged between 18-65 years.

Basic haematological parameters namely HB, WBC, platelet counts were done. Also porthrombin time , activated partial thromboplastin time, antiphospholipid antibodies and CD4 counts, were carried out.

Anemia was found in 57.5% of patients, but the type of anemia were not determined. There was leucopenia in (45%) patients. Thrombocytopenia was detected in 20% of patients.

In haemostatic profile, no changes were detected regarding prothrombin time or activated partial thromboplastin time. IgM antiphospholipid antibodies were found in 22.5% of patients, and
IgG antiphospholipid in 75% of patients. These antibodies were not associated with any clinical manifestations.

CD4 count was done in 35& low in 34 patients (85%) (less than 200/ul) while normal in 1 patient (2, 5%).

The knowledge of these hematological abnormalities in HIV/AIDS patients allows more rational care of these patients.
للحصول على المعلومات المطلوبة، تم القيام بدراسة شاملة تشمل 40 حالة من المرضى الذين وقعوا في فترات مختلفة من عام 2006. هذه الدراسة تتعلق بخصائص الدم، وخاصة الشكل الكرياتي، ونسبة الخلايا النشطة، والزمن العلال، والدموم، والخلايا البيضاء، والللمفاوية، CDs4، والشحم، ومضادات (IgM وIgG) التي يمكن تفسيرها.

تشمل هذه الدراسة أيضًا تقييم الفحصات الدخانية والخليطية، وتحديد كميات الأوكسجين في الدم، وأساليب الفحص الأخرى ذات الصلة.

النتائج أظهرت أن هناك نسبة عالية من العواقب السلبية للمرض، والتي تشمل تقلبات في الإنتاجية، وانخفاض في الطاقة، ومشاكل في الجهاز العصبي. وقد تم ملاحظة أن بعض المرضى قد تأثرت بصحة جيدة، وهي حالة تتعلق بشكل خطير.

توصيات الدراسة تشمل الحاجة لتطوير أدوية جديدة لعلاج هذه الاضطرابات، وتحديث إرشادات التشخيص والعلاج، والبحث عن أسباب أخرى في ظل انخفاض حالات الإصابة والوفيات.
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Fig.</th>
<th>Description</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1.</strong></td>
<td>Distribution of studied population according to gender</td>
<td>49</td>
</tr>
<tr>
<td><strong>2.</strong></td>
<td>Distribution of studied population according to age</td>
<td>50</td>
</tr>
<tr>
<td><strong>3.</strong></td>
<td>Distribution of studied population according to residence</td>
<td>51</td>
</tr>
<tr>
<td><strong>4.</strong></td>
<td>Distribution of studied population according to marital status</td>
<td>52</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

| Table 1: Distribution of studied population according to employment | 53 |
| Table 2: Distribution of studied population according to duration of illness | 53 |
| Table 3: Distribution of studied population according to tribe | 54 |
| Table 4: Distribution of studied population according to educational level | 55 |
| Table 5: Distribution of studied population according to symptoms | 56 |
| Table 6: Distribution of studied population according to symptoms of bleeding | 56 |
| Table 7: Distribution of studied population according to ARV used | 57 |
| Table 8: Distribution of studied population according to infection | 57 |
| Table 9: PTT and platelet groups | 58 |
| Table 10: Distribution of Hb in study population | 58 |
| Table 11: Correlation between bacterial infection and IGM group | 59 |
| Table 12: Correlation between bacterial infection and IGG group | 59 |
| Table 13: Correlation between bacterial infection and CD4 group | 60 |
| Table 14: Correlation between viral infection and IGM group | 60 |
| Table 15: Correlation between CD4 and WBCS | 61 |
| Table 16: Correlation between platelets and CD4 | 61 |
INTRODUCTION AND LITERATURE REVIEW

AIDS (Acquired Immune Deficiency Syndrome) is the most serious of all infections known to man. Since its discovery in 1981, the disease has attracted attention from health care professionals around the globe. The disease is now viewed as a threat to social and economic fabric of the world community. Currently 30-40 million people are infected with HIV most of them are in Sub-Saharan Africa and South East Asia. Serious apprehensions about the problem and effective preventive measures have reduced its prevalence in the developed countries. Despite a global awareness and prevention campaign, one person still dies of HIV every 3 minutes around the world. Estimated numbers of HIV/AIDS carriers in SUDAN are six hundred thousands, but the reported cases are only 11,500 patients.

1.1. Virology:

There are two main types of human immunodeficiency viruses; HIV-1, HIV-2 & both cause AIDS. They belong to retroviruses of Lent viruses Subfamily they are similar in terms of viral properties.
HIV-1 contains single-stranded RNA genome that is 9 Kilo bases in length and contains 9 genes that can cause 15 different protein s.(1)

The major viral proteins are classified as structural protein (Gag, pol and Env), regulatory protein (Tat and Rev) and accessory proteins (Vpu, Vpr, Vif and Nef). The functional structural proteins bind to host cell, intracellular synthesis of provirus by reverse transcription, provirus integration into host cell genome, and viral assembly and release. They do this by encoding the enzymes in Pol (protease, transcriptase and integrase, while Gag gene encode 54KD precurss protein that is cleared by viral protease to form viral core protein: p24, p17, p9 and p7. The Env gene form the icosahedral virus of HIV, this membrane derived from host cell. The surface contain glycoprotein 120 that linked to 41 glycoprotein both derived from a 160 KD pre protein that encoded by ENV gene.

Three major classes of HIV have emerged: M (main), N (new) and O (outlier). Among M group viruses, which account for >90% of HIV infections world wide, there are 9 such types called clades, designated by Letters A-D, F-H, J and K as well as many recombinant
Forms. (clade B common in America and western Europe, while clade A dominant in Africa). (2)

1.2. Transmission and Risk Factors:

HIV is transmitted by sexual contacts with infected partner, by parental drug use with contaminated needle, by exposure to infected blood or blood. (3)

The primary method of spread of HIV worldwide is by sexual exposure whether it heterosexual or homosexual intercourse, this factor associated with increased viral burden in semen include advanced HIV disease, high HIV-RNA in blood, CD4 count of less than 200 ml and presence of seminal fluid leucocytosis. (4)

In women, factors that influence the level of HIV-1 in female genital tract include stage of HIV disease, menstruation, hormonal parameter, vaginal secretion, age, HIV-RNA leveling plasma. (5) The presence of other sexually transmitted disease facilitated infection of HIV. Sharing needles and syringes is an important mode of transmission among drug abusers specially cocaine abusers. Also transmission through infected blood products causes a great risk of
transmission of HIV and account for the initially high prevalence of HIV infection among patients with hemophilia.

This way of transmission has been sharply reduced by modern techniques of screening blood and blood products.

Mother to child transmission occur in approximately 25% of live birth to HIV-infected mother viral regimens of antiretroviral, can reduced the rate of parental transmission by 50% or more.

Breast feeding is also a risk factor for HIV transmission. One third of cases of mother to child transmission result from breast feeding, and risk increase with the duration of breast feeding. Thus great recommendation has been made by CDC to reduce the rate of transmission from mother to child through many measures, such as giving ARV during pregnancy or at delivery or giving ARV to babies in the first week of life, also elective cesarean section and avoidance of breast feeding.

1.3. Life cycle of HIV:
Can be divided into many steps HIV-1 gp 120 bind the CD4 surface membrane protein, resulting in further binding to the chemokine CCR5 receptor. CD4 lymphocyte, monocyte, macrophage, Langerhans cells, follicular dendritic cells, megakaryocyte and thymic cells express CD4 and chemokine receptors molecules are susceptible to infection by HIV-1. Then virus envelope fuses with the host cell membrane.

The HIV life cycle in the host cell can be divided into several steps, binding-fusion, entry, transcription, integration, and replication.

Binding: HIV-binds to cells via enzymes between the HIV envelope glycoproteins and the host cell receptors CD4 molecules and co-receptors. The receptors are the CD4 antigen found and some T lymphocyte and immunophage monocyte, glial cells of the brain and Langerhans cells. The co-receptors are CCR5 and CXCR5. These receptors and co-receptors determine which cell the HIV virus will infect.

Fusion: The HIV envelope protein gp 120 binds to the host cell receptors and co-receptor on the outside of the cell. This is result in
insertion of gp 41 into cell member of the host cell, with fusion of two members.

**Entry:** The virus Particle leaves it members behind (uncoating) and the core virus is released into the cytoplasm of the host cell. The host cell enzymes infect with the core of the virus, resulting in the release of viral enzymes.

Reverse transcription: For the virus to multiply, the viral (single-strand) RNA must first be converted into (double-strand) DNA. This is done by the viral enzyme reverse transcriptase, which changes the single-strand viral RNA into double-strand DNA.

Integration and Replication: The viral DNA is then able to enter the host nucleus and the viral enzyme integrase is used to insert the viral DNA into the host cell's DNA and this is called integration. Once a cell is infected, it is remain infected for life because the viral genetic material is integrated into the cells DNA. The host cells are then used as a machine to produce more viral DNA "replication".

Budding: The many viral DNA particles (provirus) that are produced using the host cell machinery gather at the membrane of the CD4 cell.
The proviral particles push through the cell membrane by budding, taking the lipid bilayer with them, ready to form new virus particles.

Maturation: The gp 160, embedded in the cell membrane, is cleared by the enzyme protease to produce functional gp 41, to form a mature virus, which is then ready to infect a new cell.

1.4. Pathogenesis of HIV infection:

HIV damages the immune system of body, leaving the infected person (vulnerable to a variety of infection "called opportunistic infection") this impairment includes the decreased lymphocyte count mainly CD4, also decreased lymphocyte proliferation to soluble antigen (6,7) decreased response in immunoglobulin synthesis, impaired and delayed hypersensivity, decreased interferon gama production and decreased T cell mediated cytotoxic of virally infected cells.

The depletion of CD4 T cell by HIV results from direct cytopathic effect of HIV and CD4. The host immunological response against HIV infected lymphocyte also may contribute to progressive loss of CD4 lymphocyte by antibody mediated and cytotoxic T cell
mediated mechanism. Non infected lymphocyte are also became "innocent by standard" targets for immunological destruction by binding free gp120 to their surface CD4 protein. Another continuing mechanism, include defective production of cytokine IL2 and exaggerated expression of inhibitors of T lymphocyte proliferation such as "TGFB".\(^8\)

A number of defects in hum oral immunity have been associated with HIV infection, polyclonal hyper agammaglobulinemia, regulation of B lymphocyte is associated with increase in autoimmune phenomena and associated with increase in risk of B-cell lymphoma,\(^9\) in addition to increase frequency of positive antiglobulin test, antibodies against, neutrophil, Lymphocyte and platelets also been reported.

Also accessory immune cells, such as monocyte, macrophage and follicular cells of lymphocyte express CD4 antigen all are infected by HIV. Monocyte and macrophage are resistant to HIV induced cytotoxicity and serve as chronic reservoir of HIV expression, while functional defect in chemotoxis is observed. Progressive loss of
follicular cells is observed by time, resulting in increasing plasma, viraemia.

Natural killer "NK cells" activity is decreased on HIV patients while their numbers are normal,\(^{(9)}\) defect in NK activity result from deficiency in signal for cell activation.

1.5. Diagnosis of HIV Infection:

Detection of HIV specific antibodies is screening tests:

1- **Screening test by ELSA:**
   - b. Simple test.

2- **Confirmatry test:**
   - Western Blot. 2- Indirect Immune Fluroscene "IFA".
   - 3- Radio Immune. Prediction early before antibodies. Assay "RIPA".
   - Ag detection: it is p24 detected
   - Nuclic Acid Detection: by PCR for proviral DNA RT- PCR to detect HIV-RNA.

1.6. Course and Outcome of HIV:
In untreated person with HIV, there is progression of disease by gradual depletion of immune function and eventual development of rather nonspecific symptoms, followed by specific infection and neoplastic disease.\(^{(10)}\)

Primary HIV infection this defined by the time period from initial infection with HIV to development of antibody response detectable by standard tests occurring in 50-90% of patients this begins 1-3 wk "range 5 days – 3 months" after primary infection and usually lasts 1-2 wk. Prominent symptoms include fever 80%, rash 51% and ulcer 37%, arthralgia 54%, pharyngitis 44%, loss of appetite 54%, weight loss 32%, malaise 68%, Myalgia 49% and generalized lymphadenopathy may occur. These symptoms are similar to infectious mononucleosis.\(^{(10)}\) All symptoms of this acute retroviral syndrome subsided within several weeks, chronic HIV infection is the asymptomatic phase of HIV infection or the second phase before AIDS. It is defined by the period after acute HIV infection during which CD4 count and viral load change dramatically balance between replication and immune response of host occur. So there is little or no manifestation of HIV infection, this time between initial infection and
development of AIDS may take a long period averaging 10 years even in absence of treatment. Lastly the advanced symptomatic HIV disease or AIDS, this occur with time with more extensive fatigue, fever, weight loss, night sweating and eventually development of opportunistic infections, & candidiasis of bronchi, trachea, lung, and oesophagus, and cryptococcosis, cryptosporidium, cytomegalovirus, herpes, histoplasmosis, isosporiasis, mycobacterium avium, mycobacterium tuberculosis, pneumocystis carinii pneumonia, toxoplasma of the brain, progressive multifocal leucoencephapathy and many neoplasm such as lymphoma of the brain, immunoblastic lymphoma, Kaposi sarcoma, lymphoma of brain and cervical cancer.

ALL these considered as AIDS defining condition and according to the CDC criteria; AIDS is defined by either diagnosis of one of AIDS defining condition or by measurement of CD4 count less than 200/mm cell (normal between 600-1200 cell).

By the phase of AIDS median survival life is short less than 5 years. Some factor affect progression of HIV disease these include host factors and viral factors and they altered the course of progression of the disease.
Whereas infection of HIV is a multi system disease, haematological abnormalities are amongst the commonest clinicopathological manifestations of AIDS. Pathogenesis of these changes is multi factorial including direct invasion by virus, aggregates, HIV- related infections, HIV – associated malignancies and drugs.

1.7. Normal hematopoiesis:

Pluripotent stem cells in bone marrow are the basic units of hematopoiesis and possess dual ability to replicate and also to differentiate into committed progenitors i.e. CFU-UEMM under influence of various cytokine like GM-CSF, erythropoietin, and thrombopoietin giving rise to more committed progenitors. These colony forming units eventually generate the mature forms like granulocytes monocytes, erythrocytes and platelets respectively. In addition to the role of stem cells and hematopoietic factors in hematopoiesis, bone marrow microenvironment is important, this includes T cell and other factors that stimulate normal hematopoietic cell lines, these such as stem cell factors, platelets legend, IL3 and pan growth factor. They active stem cells and initiate progenitors and act
on differentiation. They are all secreted by T lymphocyte, Macrophages also secrete TNF and ILI which act on fibroblasts and endothelial cells. Fibroblast and endothelial cells secrete GM-(S), which secrete G-CSF & MCSF.

1.7.1. Normal haemostasis and blood coagulation:

It is the physiological balance that maintains blood in fluid state and prevents spontaneous hemorrhage on the one hand and intracellular thrombosis on the other hand. It the is result of interplay of five major components: platelets, coagulation factors, coagulation inhibitors, fibrinolysis and blood vessels.\(^{(11)}\)

Platelets derived from megakaryocyte of bone marrow circulate at concentration of 150- 400 x 10^9. They are flattened disc 2-4 mm in diameter, non nucleated have a surface membrane of phospholipids involved in the coagulation reaction. On the membrane are receptors for collagen, thrombin, ADP, adrenaline and prostacyclin. Invaginated from of this surface membrane are channels through which the platelet secretes the contents of its granules these granules are two groups’ alpha and dense granules. The alpha contain platelet factor B thromboglobulin which are anti heparin &
also contain Fibrinogen and factor V & XIII and VWF. The dense granules contain 5HT, ADP which enhance platelets aggregation by exposure of Fibrinogen binding sites on platelets.

The main function of platelets is function of primary plug or primary homeostasis; this is initiated when platelets adhere, using specific platelets collagen receptors glycoprotein IIa to collagen fibers in vascular endothelium. This adhesion is mediated by VWF which link between platelets glycoprotein Lib/IX/X and collagen. The platelets are then activated and release the contents of their granules into plasma, in turn activating other platelets and white blood cells.

The platelets undergo a change in their shape which expresses a phospholipids surface for the coagulation factors that require it. Fibrinogen links adjacent platelets by forming link via glycoprotein IIb/IIa. In addition, thrombin activates platelets.

1.7.2. Coagulation Factors:

Coagulation cascade involve series of enzymatic reaction leading to conversion of soluble plasma fibrinogen to fibrin clot. The coagulation factors are primarily synthesized in the liver and are
either enzyme precursor factors XII, XI, IX and thrombin or cofactors "V and VIII" except fibrinogen which is degraded to form fibrin.

The enzymatic part from factor XII is a serine protease and hydrolyzes peptide bond this cascade is divided into "extrinsic and intrinsic" pathways leading to final common pathway to form fibrin.

1.7.3. Coagulation Control:

The physiological anticoagulant mechanisms fall into two main groups which inhibit serine proteases of coagulation "Serpins" such as anti thrombin III or heparin cofactor II which degrades thrombin, FXa, XIIa, Xla and IXa.

It is constantly active, but it is adhesion to these factors is increased by presence of heparin surface (glycosaminoglycon) or the administration of heparin. Quantitive or qualitative deficiency of antithrombin (in born or acquired) leads to thrombophilia. Other serine protease inhibitors are C1 esterase inhibitors, α2 antiplasmin, α2 macroglobublin and tissue factor pathway inhibitors.

The second group are those that neutralize activated coagulation factors (protein C system) which are serine dependant protease and composed of protein C,S and thrombomodulin. This
degrades the cofactors Va and VIIIa. Quantitative or qualitative deficiency of either "protein C or protein S" may lead to thrombophilia. Protein C also enhances fibrinolysis.

1.7.4. Fibrinolysis:

Fibrinolysis like coagulation is a normal haemostatic response to vascular injury. Plasminogen a B-globulin pro-enzyme in blood & tissue fluid is converted to serine protease plasmin by activators either from vessel wall (intrinsic activation) or from tissue (extrinsic activation). This important route follows the release of tissue plasminogen activators (tPA) from endothelial cells: TPA is serine proteases that bind to fibrin. This enhances its capacity to convert thrombin-blood plasminogen into plasmin. This fibrin dependence of tPA strongly localizes plasmin generation by tPA to fibrin clot.

Release of tPA occur after such stimuli as trauma, excessive or emotional stress. Activated protein C stimulates fibrinolysis by destroying plasma inhibitor of tPA. On the other hand thrombins inhibit fibrinolysis by activating thrombin-activated fibrinolysis inhibitors TAF1.
Plasmin is capable of digesting fibrinogen, fibrin, factor V and VIII and many other proteins. Cleavage of peptide bonds in fibrin and fibrinogen produce small fragments D and E which can be detected in plasma of patient with DIC.

Important natural inhibitors other than plasminogen activation inhibitors are $\alpha_2$ antiplasmin and $\alpha_2$ macroglobulin.

1.8. Anaemia in HIV:

Anemia in HIV infection is multifactorial; some of the causative mechanisms are:

- Anemia of chronic disease.
- HIV-related infections.
- Hemolysis.
- Drug toxicity.
- Malignant myelo infiltration.
- Gastrointestinal bleeding.
- Vitamin $\text{B}_{12}$ deficiency.
- Hypogonadism
• BM failure.

These are the most frequent causes of anemia in HIV-infected patients (12). Red cells are mostly normocytic and normochromic; there may be mild anisocytosis. Primary defect is the failure of the bone marrow to produce normal number of erythrocytes (hypoplasic anemia). Some of the postulated mechanisms are:

• Direct toxic effect of HIV on hematopoietic stem cells and bone marrow microenvironment.

• Abnormal expression of inhibitory cytokines.

• Relative deficiency of erythropoietin.

• Defective iron metabolism and reutilization.

There is a direct cytotoxic effect of HIV on hematopoietic stem cells as well as on stromal microenvironment of the bone marrow. This leads to decreased production of G-CSF and IL-3 resulting in defective erythropoiesis (13). A study from John Hopkins School of Medicine showed that treating severe anemia especially with erythropoietin reduces the risk of early death in HIV infected anemic patients (14).
Inhibitory effect of increased activity of IL-1, TNF- and IFN-gama on bone marrow erythropoiesis has also been postulated as a contributory factor towards marrow hypoplasia. There is good evidence that serum concentration of endogenous IFN-gama correlates inversely with the hemoglobin level in patients with HIV infection\(^{(15)}\). Secretion of these cytokines is most likely triggered by the underlying chronic inflammation. In a large epidemiological study conducted at CDC HIV/AIDS division, the reports indicate that 28% of men & 31% of women with asymptomatic HIV were anemic (Hb <14 and 12g/dl respectively) this rose to 87% &77 respectively for patients with clinical AIDS\(^{(16)}\).

Another important mechanism of anemia in AIDS is decreased erythropoietin\(^{(17)}\). Levels of erythropoietin were elevated in HIV-infected persons with anemia; the increase was less than that seen in the HIV sero-negative patients with anemia of the same severity\(^{(18)}\).

Iron deficiency is also well documented in patients with AIDS. 50% of children with HIV infection have low serum iron\(^{(19)}\). Iron deficiency in AIDS has also been linked to intestinal
malabsorption as well as poor intake of iron. Chronic gastrointestinal infections may also contribute to malabsorption of iron in patients with AIDS.

The opportunistic infections can lead to defective hematopoiesis in HIV-infected patients; the commonest being Parvovirus B19 and Mycobacterium avium complex (MAC). Parvovirus antibodies have been isolated in 64% of HIV-infected patients, MAC infection was diagnosed in 18% of patients with HIV disease (20).

Other less common infectious causes of anemia in HIV-infected patients include T.B, histoplasmosis, and cryptococcosis and pneumonocytosis carinii.

Hemolytic anemia in AIDS is not uncommon; it is develop as a consequence of:

- G6PD deficiency.
- Hemophagocytic syndrome.
- Auto-antibodies against RBCs.
- Thrombtic thrombocytopenic purpura.
- Coincidence Hepatitis B and C infection.
- Hypersplenism due to advanced liver disease.

Direct anti globulin (coomb's) test is positive in 37% of HIV infected patients.\(^{(21)}\) Also haemolysis can occur in HIV infected patients receiving drugs like Trimethprime sulfamethoxazole and dapsone particularly in G6PD deficiency.

Drugs induced anemia constitutes more than 20% of anaemia in patient with HIV. The most common drugs are AZT (Zidovudine).\(^{(22)}\) Trimethoprim Sulphamethoxazole. AZT usually causes macrocytic anemia. Less common causes of anemia like CMV colitis and malignancy like Kaposi by gastrointestinal blood loss and lymphoma causing anemia by infiltrations of BM. \(^{(23)}\)

Hypogonadism is relatively common in HIV infected men. Estimation is recommended if anemia is associated with signs of Hypogonadism.\(^{(24)}\)

1.8.1. Neutropenia in HIV infection:

This is common in HIV infected person. It occurs in 13% of patients in asymptomatic-HIV infected persons and rises to 44% in clinical AIDS.\(^{(25)}\)
In a study carried out in 1997, it was suggested that there was strong correlation between the level of absolute Neutrophil count (ANC) and need for hospitalization by bacterial infection.\textsuperscript{(26)} In a detailed study in 62 HIV infected patients, 24\% with ANC below 1000/ml develop infectious complication within 24 hours of the onset of neutropenia.\textsuperscript{(27)} It is postulated that there is a 2-3 fold risk of bacterial infection in HIV-infection patients with ANC < 1000 cell/ml, the risk increases by 7 to 9 times when the count falls below 500 cell/ml.\textsuperscript{(28)} Other factors associated with increased risk of infection is presence of central venous line, recent history of neutropenia and low CD4 count.

In addition to neutropenia defective granulocytic functions have also been documented in HIV infection contributing to defective phagocytosis. It is however widely believed that these finding are not of much clinical significance.\textsuperscript{(29)}

Some important causes of neutropenia in HIV setting are:-

- Drug toxicity.
- HIV-related infection.
- Nutritional deficiency.
- Autoimmune destruction.

- Malignant infiltration of bone marrow.

Most of drugs cause direct BM suppression. An autoimmune mechanism involving antibodies against white cells has been suggested but studies have shown that anti granulocytic antibodies are not related to increased incidence of neutropenia in HIV-infected persons.\(^{(30)}\)

Wide range of drugs in AIDS causes leucopenia. This is usually dose dependent though it has also been reported as an idiosyncratic phenomenon. AZT treated patients develop neutropenia in 16%. Chemotherapy used for non Hodgkin’s lymphoma is also a cause of neutropenia in those patients& also in patients of Kaposi sarcoma.\(^{(31)}\)

Gancyclovir used for HIV related CMV infection causes neutropenia.\(^{(32-33)}\) Pentamidine & trimethoprim- sulfamexozole used for PCP (pneumocystic carnii pneumonia). Also causing neutropenia in AIDS patients, like methotrexate, interferon Alfa amphotericinB, cyclophosphamide, etc.
Also important mechanism of neutropenia is decrease numbers of progenitor’s cell, and decrease serum level of colony stimulating factors (GC-CSF).

1.8.2. Thrombocytopenia in HIV infection:-

Association of HIV-infection with thrombocytopenia was recognized long ago. In 1984, a physicians' handbook listed thrombocytopenia along with fever, weight loss, fatigue and swollen lymph glands as a signal that a patient might have AIDS.(34)

**Incidence:-**

Thrombocytopenia is believed to be present in as many as 40% of HIV-infected person. In approximately 10% of patients, it may be the first sign of AIDS.(35) In a multicenter AIDS cohort study among 1500 HIV positive individuals 6.7% had platelets count of less than 150,000 ml on at least one semiannual visit.(36)

In a center for disease control study in 1996(37) one year incidence of thrombocytopenia (platelets count < 50,000 ml) in HIV infection was 8.7% in clinical AIDS, 3.1% In immunological AIDS
(CD4 cell count < 249ml) and 1.7% in non-clinical, non immunological HIV-infection (CD4 cell count > 700ml).

**Pathophysiology and clinical course:**

HIV-related thrombocytopenia though often asymptomatic, may present with petechiae, ecchymoses, epistaxis, menorrhagia, gingival bleeding and bleeding in the gastrointestinal tract and in the CNS. According to a study conducted in 1992, thrombocytopenia in AIDS can be divided into four clinical subsets, of 52 HIV patients it was noted that 17% had acute ITP like illness, 40% had chronic ITP like disease, while 35% and 8% of patients had splenic sequestration type and hypoplastic thrombocytopenia respectively. Hence thrombocytopenia in HIV infection may be result from defective haemtopoisis but in many instances it is due to hematological abnormality related to increased platelets destruction.

Some important mechanisms of HIV associated thrombocytopenias are:

1- **Immune thrombocytopenia "HIV-ITP"**:

ITP in HIV-related patients like ITP in a similar mechanism and is operative. Marked increase in platelet associated IgG, IgM, C3,
C4 level have been demonstrated in blood of HIV-infected persons. Cross reactive antibodies between HIV gp 160/120 and platelet gp IIb/IIIa have been found. Platelet antibodies are not helpful in establishing the diagnosis of immune medicated thrombocytopenia, as there are false positive and false negative results. Instead, response to treatment with intravenous immunoglobulin "IVIg" and to IV anti D is regarded as among reliable diagnostic tools in HIV-ITP.

2-Defective thrombopoiosis: this is thought to be direct infection of bone marrow and due to direct suppression of megakorocyte by the virus. Megakorocyte express both CD4 and CCRS receptors which enable them to engulf HIV particles.\(^{(38)}\)

3- Drug induced thrombocytopenia: the two drugs in the treatment of HIV-infection associated thrombocytopenia are Gancyclovir and pentamidine. Grancyclovir is associated with dose dependent cytopenia including thrombocytopenia.

4-HIV-related infection: opportunistic infection like cryptococcosios also produces thrombocytopenia in HIV-infected persons.5- Also neoplastic condition affect bone marrow, can lead to thrombocytopenia.6- Other rare causes of thrombocytopenia include
alcohol ingestion, splenomegaly, liver disease and Thrombotic thrombocytopenic purpura.

1.8.3. Coagulation abnormalities in HIV/AIDS:

Coagulation abnormalities in the setting of HIV infection are not common though there is an increased risk of thrombosis \(^{(39)}\). They are still of considerable importance. In the CDC study it was found that overall incidence of thrombotic episodes were 2.6 per100 HIV infected person.\(^{(40,41)}\) The incidence of thrombotic events\(^{(42)}\) was 3.31\% in those younger than 50 years old versus 53\% in those 50 years or more. Retrospective study from the University of Connecticut Health Center \(^{(43)}\) found 7.6\% (10 of 131) HIV-infected with an unexplained DVT or pulmonary embolism (PE). The majority of the thrombotic events occurred in those with CD4 counts less than 200/mm\(^3\), and those with the concurrent presence of opportunistic infections or acquired immunodeficiency syndrome (AIDS)-related neoplasm. Thus thrombosis is a significant and probably under-recognized complication of HIV infection.

A variety of hemostatic abnormalities have been identified in patients with HIV infection that provide mechanisms for
hypercoagulable state and increased likelihood of thrombosis. Best known are the endothelial-related changes that include the presence of antiphospholipid and anti-cardiolipin antibodies and microangiopathy.\(^{(44)}\) Anti phospholipids antibodies (APL) are proteins directed against different phosphor-containing lipids. The best known of these are anticardiolipin antibodies (APL) and lupus anticoagulants. Studies have shown APL to be present in 82 to 92% of patients with AIDS. APL are related to increased occurrence of both venous and arterial thrombosis.

Less well publicized are a variety of changes in the physiologic anticoagulant system as a result of HIV infection.\(^{(45-46)}\) Decreased anticoagulant activities due to deficiencies in protein S, protein C, antithrombin, and heparin cofactor II, as well as decreased fibrinolysis due to increased levels of plasminogen activator inhibitor have been well described. It has been proposed that these changes are largely the result of disseminated intravascular-coagulopathy and endothelial injury/activation.\(^{(47,48)}\) Supporting this hypothesis is a report from Erbe and colleagues\(^{(49)}\) that documented an increased incidence of deficiencies in both protein C\(^{(50)}\) and protein S\(^{(43)}\) in HIV-
infect-patients with an acute opportunistic infection, when compared to 8% and 54%, respectively, on follow up investigations after the acute events.

Protein S deficiency is the most consistent coagulation abnormality in HIV-infected patients. The prevalence is reported to be as high as 76%. Some investigators have shown a correlation between low protein S levels and low CD4 counts, while others have not. The prevalence of protein S deficiency (both total and functional) was greater in those CD4 counts less than 200/mm³ compared to those with CD4 counts above 200/mm³.

The relationship between protein S levels and CD4 counts may simply be a reflection of the duration of the HIV infection. While small case series have reported thrombotic vents in protein S-deficient HIV patients, Hassell and colleagues did not find a correlation between protein S deficiency and thrombosis. It must be concluded that the significance of low protein S levels in infected patients is indeterminate.

The frequency of protein S alterations/deficiencies has led to an extensive pursuit of potential mechanisms. Protein S is synthesized
by the endothelium, hepatocytes, and megakaryocytes. Endothelial injury in HIV infection\(^{57,58}\) has been proposed as a reasonable etiologic basis for the decreased protein S in HIV-infected patients. Because free protein S is reduced in inflammatory states largely due to increased levels of C4b-binding protein in HIV-infected patients, and this issue and found no difference in the C4b-binding protein among normal controls. Sugarman and co-workers also failed to establish a relationship between protein S values and C4b-binding protein in HIV-infected children.\(^{54}\)

In addition, increasing IgG anticardiolipin antibody level was correlated with decreasing level of free protein S antigen in adults.\(^{52}\) This relationship was not identified in children.\(^{53}\) Despite the data in children, a working hypothesis that increased protein S clearance occurs secondary to autoimmune antibodies may be operative in the high prevalence of protein S deficiency in HIV-infected patients.

Another potential mechanism of the hypercoagulable state relates to platelet activation. Soluble P-selectin, a marker of platelet activation, is also noted to be elevated in HIV-infected patients and may contribute to the thrombotic tendency.\(^{59,60}\) Schecter and
colleagues(61) reported that the HIV envelope protein, gp120, activates human arterial smooth muscle cells to express tissue factor, the initiator of the coagulation cascade. The activation of smooth muscle cells by gp120 may play an important role in the prothrombotic phenotype of the HIV with infected patients.

In two epidemiological studies of thrombosis in HIV-infected patients,(43,46) the use of protease inhibitors was associated with thrombotic events. A review of the literature by safe and colleagues(43) found 45 HIV/-infected patients with thrombotic events; 11 of the 45 were taking at least one protease inhibitor.

Among these 11 patients, portal vein thrombosis developed in two that led to portal hypertension and variceal bleeding. The offending medication was indinavir, and no bleeding was observed after the protease inhibitor was discontinued.(62) An interesting suggestion by these authors was that because aspartyl proteases such as renin, endothelin, and cathepsin D are involved in the regulation of coagulation,(63,64) and because the HIV protease is an aspartyl protease, inhibition of the proteases by indinavir may lead to a prothrombotic state.
Further support for this comes from Henry and colleagues, who reported two young patients in whom severe coronary artery occlusion developed after starting protease inhibitors. In addition, the 26-year-old patient (included in the Saif review) had angina 4 weeks after starting ritonavir and saquinavir.

These authors did suggest that lipodystrophy due to the protease inhibitors was the cause of the premature coronary artery disease; however, the short duration of the therapy would suggest that other mechanisms are involved.

George and colleagues reviewed the other seven patients in the Saif review. PE developed in seven of 650 HIV-infected patients. It is noteworthy that protease inhibitors were not available before that time.

The mean time to the development of venous thromboembolism was 72 days after starting a protease inhibitor.

No patient had a concurrent infection at the time of the thrombotic event. Three patients required high doses of warfarin to maintain a therapeutic International Normalized ratio, and the same
three patients had recurrent thrombosis or extension of thrombosis while on warfarin and the protease inhibitor.

One patient had a history of thrombosis (before institution of the protease inhibitor); one had recent surgery but was ambulatory for 9 weeks; one was obese and had increased anticardiolipin antibodies; one had a family history of DVT and also had increased anticardiolipin antibodies; one other patient had a family history of PE and was also taking hormonal replacement therapy.

These data can be compared to historical controls before 1996, where only two cases of unexplained DVT were identified in 1050 patients.

Saif and colleagues\(^{67}\) speculated that because the protease inhibitors are metabolized by the cytochrome P450 system, they may interfere with hepatic regulation of the thrombotic proteins, leading to a prothrombotic state in some patients.

However, Pulik and colleagues\(^{50}\) reported, in abstract form, the results of coagulation studies in non-hemophiliac HIV-infected patients treated with protease inhibitors.
There were no changes in activated partial thromboplastin time, prothrombin time, fibrinogen, von Willebrand factor antigen, ristocetin cofactor activity, factor VIII activity, or factor XI antigen.

Majluf-Cruz and colleagues performed a retrospective study of their HIV-infected patients.\textsuperscript{(68,69)} Similar to the study by George and colleagues,\textsuperscript{(68,69)} the prevalence of venous thromboembolism appeared to have increased significantly since the protease inhibitors were introduced into clinical practice.

In addition, they found a high incidence of protein S deficiency in this particular patient cohort. However, only functional protein S activity was examined; antigenic protein S was not determined.

While previous studies we reviewed have established a quantitative deficiency of protein S, additional data on C4b-binding protein and protein S antigen would have been informative.

Majluf-Cruz and colleagues also reported two patients who had acquired transient activated protein C resistance.\textsuperscript{(69)}

It would have been interesting to know the factor VIII activity at the time of the acquired activated protein C resistance because
elevated factor VIII can consume the activated protein C added for the assay and result in a falsely defined abnormality.

Elevated factor VIII activity, which can be a result of acute phase reaction, is associated with an increased risk of thrombosis.\textsuperscript{(69)}

And interesting aspect of the Majluf-Cruz report\textsuperscript{(69)} is that the use of aspirin in this cohort of thrombophilic patients resulted in no further episodes of thrombosis, including those patients who had recurrent thromboses while taking oral anticoagulants.

The Data Collection on Adverse Events of Anti-HIV Drugs (DAD) study group recently reported its prospective observational study on the effects of combination antiretroviral therapy on the risk of myocardial infarction.\textsuperscript{(70)}

The incidence of myocardial infarction increased with longer exposure to combination antiretroviral therapy.

These observations, along with the P-selection studies, lend incidence to the suggestion that platelet activation is an important pathophysiologic mechanism of the prothrombotic, state in HIV-infected patients.
To date, there are little formative data on the effect of protease inhibitors on platelet function. Such data might provide a basis for aspirin use as part of the primary therapy for HIV-infected patients with thrombophilia, particularly those placed on therapy, with protease inhibitors.

Clearly a hypercoagulable state and thrombosis are emerging as clinical issues in HIV-infected patients.

Although commonly multifactorial issues are projected as the clinical basis and the focus turns to management, the recent growing evidence of associated thrombophilia and correlated platelet activation as well as potential interactions with therapy with protease inhibitors. This lead to importance of examination of HIV infected patients.
1.9. Objectives:

1.9.1. General:
To find the incidence of hematological abnormalities in HIV/AIDS patients.

1.9.2. Specific:
1. To study & investigate coagulation profile and basic hematological parameters in HIV/AIDS patients.
2. To find out if laboratory investigation can predict a thrombotic events
3. To find if there an association between CD4 level & some hematological parameters.

1.10. Justification:
In the literature there is documented evidence of hemorrhagic & hypercoagulable state in the HIV infection. This has not been looked into in Sudanese patients & it would be important to see if
such abnormalities exist so as to be aware of them & diagnose & treat them early.
MATERIALS AND METHODS

2.1: Subjects:

2.1.1. Study design:

This is a prospective laboratory study. It is conducted at Omdurman Teaching Hospital (OTH C) which has the largest center for screening and management of patients with HIV/AIDS in Sudan.

2.1.2. Study duration:

Data were collected in the period from first of February till the mid of April 2006.

2.1.3. Study Population:

The study population was known HIV/AIDS patients who were on regular follow up at OTHC. 40 such patients were studied.

2.1.4. Inclusion Criteria:

Any HIV+ve patient, whether hospitalized or outpatient.

2.1.5. Exclusion Criteria:

1. Patients less than 18yr.
2. Those patients who refused testing.
3. Clinically suspected HIV patient with negative tests.

2.2. Tools and methods:
2.2.1. Consent: See appendix(1)

Verbal and written was taken from the hospital administration (By directors of HIV/AIDS center) and the patients.

2.2.2. Questionnaire:

See appendix (2).

2.2.3. Clinical Examination:

All patients with HIV/AIDS were examined systemically.

2.2.4. Samples:

Blood collection under a septic condition from anticubital fossa veins, (4 ml) using disposable plastic syringe and divided into two containers:

- 2mls into EDTA container for measurement of hematological values and $CD_4$ count.
- 2mls into Tri Sodium citrate container for coagulation profile

2.2.5. Tests done:

For the target patients the following tests were done:

- Hemoglobin (HB).
- Hematocrit (Hct).
- White cell counts
- Platelets count.
- APTT, PT.
- ApL(IgM&IgG)
- CD4 counts

### 2.2.6.1. Instruments used:

Hb, Hct, platelets were measured by an automated blood counter (Sysmex Kx-21, which also measured white cell counts and differentials.

### 2.2.6.2. Coagulometer:

Prothrombin time and activated partial thromboplastin were done by semi automated coagulometer.

### 2.2.6.3: ELISA:

Antiphospholipid antibodies were done by ELISA (enzyme linked immunosorbant assay) with serum or plasma as sample material.

### 2.2.6.4. CD4 counter machine:
CD4 count: Is done by an automated CD4 counter (trade name Cyflow counter). It is an automated blood cell counter used specifically for determination of CD4, CD8 and CD45.

The principle: cells are separated in suspension and stained with fluorescent markers and quantity of the dye uptake is proportional to the number of antigen molecules of a single cell, then light from laser excite cell fluorescence and scattered by the cell. This fluorescence signal are displayed in histogram. Normal range of CD4 lies between 400-1200 cell /L.

2.2.6.1. Sysmex Kx-21

This is an automated multi-parameter blood cell counter for in vitro diagnostic use in clinical laboratories.

The sysmex Kx-21 processes approximately 60 samples per hour and displays on the LCD screen the particle distribution curve of WBC, RBC and platelets, along with data of 18 parameters as analytical results.

The sysmex Kx-21 employs three detector blocks and two kinds of reagents for blood analysis. The WBC detection block using
DC detection method measures the WBC count. The RBC detection block also using DC detection method takes the RBC count and platelets counts. The Hb detection block measures the HB concentration using a non cyanide hemoglobin method.

2.2.6.2. Coagulometer:

The Amelung KC4 a micro coagulation analyzer is a semi automated machine designed for the determination of prothrombin time (PT), activated partial thobomoplastine time (APTT), fibrinogen concentration determined by Clauss methodology, and other clotting assays.

**Principle:** KC4 a micro coagulation analyzer measures the time from the addition of start reagents to the onset of fibrin formation in the cuvette. The specimen 0, I ml of plasma is placed in the cuvette and time measurement is stated either automatically or manually with the addition of reagent. As the conversion of fibrinogen to fibrin occurs, the fibrin strand pulls the ball away from its position. This position change of the ball triggers an impulse in the magnetic sensor which electronically stops the time measurement.
A control sample from a normal person was run with each batch of sample.

- Reagents in PT: thromboplastin+CaCl$_2$.
- Reagents in PTT: Kaolin+phospholipid solution+CaCl$_2$.
  - The reagents for PT and PTT were purchased from DiaMed AG 1785, Switzerland (Appendixes 3 & 4).

2.2.6.3 Anti phospholipids antibodies:

This was done by the DRG antiphospholipid screen (ELISA 3591) for quantitative measurement of IgG or IgM class auto antibodies directed against phospholipids. It is an indirect solid phase enzyme immunosorbant assay. Micro plate wells are coated with mixtures of highly purified negatively- charged phospholipids: cardiolipin, phosphotidyl serine, phosphtidyl inositol and phosphatidic acid. B$_2$ Glycoprotein 1 as cofactors for binding. Test sera or plasma are applied and left for 30 minutes at room temperature.

An antihuman IgG or IgM horseradish peroxidase conjugates solution was pipetted into the well. After 15 minutes, washes to remove the unbound antibodies. On addition of the substance TMB
(3, 3, 5, 5-Teramethyl-benzidine), a colour develops only in those wells in which the enzyme is present, indicating the presence of IgG or IgM. The reaction is stopped by addition of dilute hydrochloric acid and the absorbance is then measured at 450nm.

The concentration of IgG or IgM is directly proportional to the colour intensity of the test sample. See appendix (4).

2.3. **Statistical analysis:**

Data were analyzed by computer software statistical package for social science (SPSS) programme. Chi square was used to compare the association levels.
RESULTS

Fourty patients were studied, 22 were males & 18 were females (Fig.1).

Distribution of studied group according to age 4 were between 18-25 (10%), 15 (37.5%) were between 26-35, & 18 (45%) were more than 35 years. (Fig. 2).

Distribution of region of studied group; Khartoum 30(75%), central Sudan 3(7.5%), Eastern Sudan 2(5%) western Sudan 2(5%) and Southern Sudan 3 (7.5%), (Fig. 3).

Distribution of marital status of studied groups 39 patients were studied: married 27(67.5%), Single 4(10.0%), Divorced 5(12.5%) and widowed 3 (7.5%) (Fig. 4).

Distribution of occupation and employment 39 patients out of 40 were studied; 18(45)were employed and 21(52.5%) were unemployed (Table 1).
Distribution of duration, of illness of studied groups; weeks 1(2.5%), years 35 (87.5%) out of 40 patients. (Table 2).

Distribution of symptoms of studied groups; fever was present in 17 (42.5%), fatigue in 21(52.5%) , Loss of weight, in 29(75.5%) , diarrhea in 21 (57.5%) (Table 3).

Distribution of symptoms of bleeding of studied groups; Hematuria was found only in one, case 1(2.5%); epistaxis, 1(2.5%), melena 1(7.5%) and both Hematuria and malena in 1(2.5%) (Table 5).

Distribution of drugs taken by the studied groups out of 40 patients; ARVS were taken by 29 patients (72.5%) and not taken in 11(27.5%) patients (Table 6).

other drugs taken by the studied groups; Septrin by 40- (100%) Septrin and Anti TB 22(55%), Septrin + Anti TB + Flagyl 2 (5%).

Distribution of inter current infection among the studied group were; Bacterial infection 12(30%), Viral infection in 2 patients (5%) and fungal infection in 9 (22.5%) (Table 7).
Investigation results:
Laboratory results:

Hematological findings in the studied patients:

White cell count in studied patients: Out of 36 patients, 18 (45%) had low count, while 15 (37.5%) with normal count and 3 (7.5%) with high cell count.

Platelet count in studied patient: Out of 33 patient, 8 (20%) had a low platelets count (<150,000), 22 (55%) had a normal count and 3 (7.5%) had a high count. (Table 8).

Prothrombin time and partial thromboplastin time: 33 patients were tested & all were found to be within normal. (Table 8).

Anti phospholipids anti bodies in studied patients were classified: IgM was normal in 24 patients (60%) and high in 9 (22.5%). While IgG was normal in 3 patients (7.5%) and high in 30 (75.0%) (Table 8).

CD4 count is studied patients: out of 35 patient 34 (85%) were low (less 200mm^3l) while was normal in 1 (12.5%) (Table 8).
Haemoglobin value in studied patients: out of 36 patient, 23 (57.5%) had low Hb level, while 5-12.5% had normal level and 8 (20.0%) with high Hb level (Table 9).

Tables 10-14 show the correlations between infection and IGM, IGG and CD4 groups.
Fig. 1: Distribution of studied population according to Gender

Female 18 (45%)

Male 22 (55%)
Fig. 2: Distribution of studied population according to age

Bar chart showing the percentage of the studied population across different age groups. The age groups are 18-25, 26-35, and >35 years. The percentages are as follows:
- 18-25 years: 10% (4 people)
- 26-35 years: 37.5% (10 people)
- >35 years: 50% (10 people)
Fig. 3: Distribution of studied population according to residence

- 30 (75.00%)
- 3 (7.5%)
- 2 (5.0%)
- 2 (5.0%)
Fig. 4: Distribution of studied population according to marital status

- Married: 27 (69.20%)
- Single: 4 (10.30%)
- Divorced: 5 (12.80%)
- Widow: 3 (7.70%)
Table 1: Distribution of studied population according to employment

<table>
<thead>
<tr>
<th>Employee</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>18</td>
<td>45.0%</td>
</tr>
<tr>
<td>Not</td>
<td>21</td>
<td>52.5%</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>97.5%</td>
</tr>
</tbody>
</table>

Table 2: Distribution of studied population according to duration of illness

<table>
<thead>
<tr>
<th>Duration</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weeks</td>
<td>1</td>
<td>2.5%</td>
</tr>
<tr>
<td>Years</td>
<td>35</td>
<td>87.5%</td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td>90.0%</td>
</tr>
</tbody>
</table>
Table 3: Distribution of studied population according to tribe

<table>
<thead>
<tr>
<th>Tribe</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central</td>
<td>4</td>
<td>10.0%</td>
</tr>
<tr>
<td>North</td>
<td>11</td>
<td>27.5%</td>
</tr>
<tr>
<td>East</td>
<td>3</td>
<td>7.5%</td>
</tr>
<tr>
<td>West</td>
<td>17</td>
<td>42.5%</td>
</tr>
<tr>
<td>South</td>
<td>4</td>
<td>10.0%</td>
</tr>
<tr>
<td>Foreigner</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>100.0%</td>
</tr>
</tbody>
</table>
Table 4: Distribution of studied population according to educational level

<table>
<thead>
<tr>
<th>Education</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Khalwa</td>
<td>1</td>
<td>2.5%</td>
</tr>
<tr>
<td>Primary</td>
<td>8</td>
<td>20.0%</td>
</tr>
<tr>
<td>Secondary</td>
<td>10</td>
<td>25.0%</td>
</tr>
<tr>
<td>Higher secondary</td>
<td>12</td>
<td>30.0%</td>
</tr>
<tr>
<td>None</td>
<td>9</td>
<td>22.5%</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>100.0%</td>
</tr>
</tbody>
</table>
Table 4: Distribution of studied population according to symptoms

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>17</td>
<td>42.5%</td>
</tr>
<tr>
<td>Fatigue</td>
<td>21</td>
<td>52.5%</td>
</tr>
<tr>
<td>Loss of weight</td>
<td>29</td>
<td>72.5%</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>21</td>
<td>52.5%</td>
</tr>
</tbody>
</table>

Table 5: Distribution of studied population according to symptoms of bleeding

<table>
<thead>
<tr>
<th>Symptoms of bleeding</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematuria</td>
<td>1</td>
<td>2.5%</td>
</tr>
<tr>
<td>Epistaxis</td>
<td>1</td>
<td>2.5%</td>
</tr>
<tr>
<td>Malena</td>
<td>1</td>
<td>2.5%</td>
</tr>
<tr>
<td>Haematuria + malena</td>
<td>1</td>
<td>2.5%</td>
</tr>
</tbody>
</table>
Table 6: Distribution of studied population according to ARV used

<table>
<thead>
<tr>
<th>ARV uses</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>29</td>
<td>72.5%</td>
</tr>
<tr>
<td>No</td>
<td>11</td>
<td>27.5%</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Table 7: Distribution of studied population according to infection

<table>
<thead>
<tr>
<th>Infection</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>15</td>
<td>45.5%</td>
</tr>
<tr>
<td>No</td>
<td>18</td>
<td>54.5%</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>100%</td>
</tr>
</tbody>
</table>
### Table 8: Coagulation profile, APL[antibodes] and CD4

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal (%)</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>APL/IgM</td>
<td>24 (60.0%)</td>
<td>-</td>
<td>9 (27.3%)</td>
</tr>
<tr>
<td>APL/IgG</td>
<td>3 (7.5%)</td>
<td>-</td>
<td>30 (75.0%)</td>
</tr>
<tr>
<td>CD4</td>
<td>1 (2.5%)</td>
<td>34 (85.0%)</td>
<td>-</td>
</tr>
<tr>
<td>PTT</td>
<td>33 (82.5%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Platelets</td>
<td>22 (55.0%)</td>
<td>8 (20.0%)</td>
<td>3 (7.5%)</td>
</tr>
<tr>
<td>PT</td>
<td>33 (82.5%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>WBC</td>
<td>18 (45%)</td>
<td>15 (37.5%)</td>
<td>3 (7.5%)</td>
</tr>
</tbody>
</table>

### Table 9: Distribution of Hb in study population

<table>
<thead>
<tr>
<th>Hb level</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>11 (27.5%)</td>
<td>5 (12.5%)</td>
</tr>
<tr>
<td>Low</td>
<td>23 (57.5%)</td>
<td>23 (57.5%)</td>
</tr>
<tr>
<td>High</td>
<td>2 (5.0%)</td>
<td>8 (20.0%)</td>
</tr>
</tbody>
</table>
### Table 10: Correlation between infection and APL IGM group

<table>
<thead>
<tr>
<th>Infection</th>
<th>IGM Groups</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>High</td>
</tr>
<tr>
<td>Yes</td>
<td>9 (66.7%)</td>
<td>6 (37.5%)</td>
</tr>
<tr>
<td>No</td>
<td>15 (83.3%)</td>
<td>3 (16.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>24 (72.7%)</td>
<td>9 (27.3%)</td>
</tr>
</tbody>
</table>

P = 4.09

### Table 11: Correlation between infection and APL (IgG) group

<table>
<thead>
<tr>
<th>Infection</th>
<th>IGG Groups</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>High</td>
</tr>
<tr>
<td>Yes</td>
<td>2 (13.3%)</td>
<td>13 (86.7%)</td>
</tr>
<tr>
<td>No</td>
<td>1 (5.6%)</td>
<td>17 (94.4%)</td>
</tr>
<tr>
<td>Total</td>
<td>3 (9.1%)</td>
<td>30 (90.9%)</td>
</tr>
</tbody>
</table>

P = 1.36
Table 12: Correlation between infection and CD4 group

<table>
<thead>
<tr>
<th>Infection</th>
<th>CD4 Groups</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Normal</td>
</tr>
<tr>
<td>Yes</td>
<td>15 (100.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>No</td>
<td>19 (95.0%)</td>
<td>1 (5.0%)</td>
</tr>
<tr>
<td>Total</td>
<td>34 (97.1%)</td>
<td>1 (2.9%)</td>
</tr>
</tbody>
</table>

P = 0.43

Table 13: Correlation between TWBCs and CD4 counts

<table>
<thead>
<tr>
<th>CD4</th>
<th>TWBCs</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Normal</td>
</tr>
<tr>
<td>Low</td>
<td>17 {54.8%}</td>
<td>11 *(3.5%)</td>
</tr>
<tr>
<td>Normal</td>
<td>1 (8.3%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>17 {53.1%}</td>
<td>12 (37.5%)</td>
</tr>
</tbody>
</table>

P = 0.09
Table 14: Correlation between platelets and CD4 counts

<table>
<thead>
<tr>
<th>CD4</th>
<th>platelets</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Normal</td>
</tr>
<tr>
<td>Low</td>
<td>8 (28.6%)</td>
<td>18 <em>(64.3%)</em></td>
</tr>
<tr>
<td>Normal</td>
<td>1 (5.3%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>8 (27.6%)</td>
<td>19 (65.5%)</td>
</tr>
</tbody>
</table>

P. = 0.07
DISCUSSION

Human immune deficiency virus infection is an illness with protean manifestations including hematological abnormalities with progression to AIDS state, & more abnormalities in different sites of the body is being noticed. Changes in blood coagulation in patients with HIV infection and AIDS occur frequently. These changes vary considerably between bleeding & hemorrhage to the rarely observed complication of hypercoagubility state.(71)

A variety of mechanisms have been proposed to explain the hypercoagubility in HIV & AIDS patients. These include the presence of antiphospholipids antibodies (APL) anticardiolipin ACL antibodies, decreased natural inhibitors, increased platelets activation and others.(71)

This study was designed to asse the coagulation state and other hematological manifestations in Sudanese HIV/AIDS patients. The studied populations consist of 22 males and 18 females. And most common age group lies between 18-35 years, which is the hypersexual age.
In the studied group the prevalence of anemia was 57.5%. The type of anemia was not determined. The incidence of anemia varies according to stages of HIV disease. Another contributing factors, such as chemotherapy, race, concurrent illness, and poor nutritional status.

Thrombocytopenia is the most common known hematological abnormality causing high morbidity and it affects patients from every risk group independantly of age, Sex, or stage of infections (72). This thrombocytopenia had been detected in 24.2% of our patients, while normal & high platelets were observe in 76.8 without any association with bleeding. HIV related thrombocytopenia is often asymptomatic but may present with petechiae, ecchymosis, epistaxis,menorrhagia, gingival bleeding and bleeding in the gastrointestinal tract and in the CNS.In approximately 10% of the patients ,it may be the first sign of AIDS.

In addition, there are some abnormalities of coagulation, mentioned in literature, such as prolongation of activated partial thromboplastin time, production of Lupus anticoagulant and anticardiolipin antibodies. Such a study was done by Temple school
of medicine USA. These changes were not all detected in our study. All our patients had normal PT &APPT. Similar results were found by Fezoui H, Garnier G, and France. They however found only thrombocytopenia and circulating anticoagulant like APL, ACL which were asymptomatic. In our study we found that there was high level of APL(IgG) in 30 patients out of 33 patients in whom this test was done (75%), and APL(IgM) was high in 9 patients (22.5%) These high levels of APL were not associated with any clinical symptoms. This is compatible with the French study.

35 patients had CD4 done, low CD4 was found in 34 patients (85%) while 1 patient (2.5%) had normal CD4. By this test, which is newly introduced in Sudan, these patients were categorized as AIDS, although most of them are clinically well. Many infections have been associated with antiphospholipid antibodies although pathogenic role for these antibodies has not usually been obvious except in a few cases. These infections could be viral like HIV, Parvovirus B19, Varicella, mumps, hepatitis C, and adenovirus. Other bacterial infections like leprosy, tuberculosis, salmonella, staphylococci, streptococci, spirochetes and other parasitic diseases like malaria,
kalazar and toxoplasmosis. These antibodies are rarely found in fungal infections.

In this study correlation between level of the APL antibodies (IgM - IgG) and infections (Bacterial, viral, fungal).

In 15 patients with infection, 9 patients showed normal levels of APL (IgM) (66.7%) and 6 patients showed high level (37.7%). This represent that all the infected patients are 45.5% out of 33 patients who did the test.

Another correlation was made between infection and presence of APL (IgG). Only 2 patients had normal level of IgG (13.3%) while the 13 had high level (86.7%) this represents 15 patients who were infected out of 33 patients who had the test done. HIV patients are predisposed to different infection by various microorganism, these will increased likelihood to thrombosis, by increasing the incidence of APL. This with other coagulation abnormalities increased risk of thrombosis and pulmonary embolism. In our study non of the patients had clinical evidence of thrombosis, we have not however done investigations e.g. Doppler, Ultrasonography & others to document venous thrombosis. patients
Further more two epidemiological studies showed higher risks for patients with AIDS or with a CD$_4$ count < 200 mm$^3$. Thus increased risk of DVT in HIV infected patients is probably caused by active ongoing triggering of the immune system by both HIV infection and superimposed infection. In this study an association between level of CD4 and infection had been done, 15(44.1%) patients had infection. All had a low CD4 count out of 35 patients did the test.

An important association between APL antibodies and level of CD$_4$ count showed a high level of APL IgM in 7 patients with 24.1%. In the group with APL IgG level, it is high in 26 patients (89.7%) and so it may show great association between the level of APL antibodies { IgM and IgG} with low CD4 counts.

In addition to this result Stahl found that increased level of APL (IgG) antibody was correlated with decreased level of free protein S antigen in adults. This decrease in proteins S was noted in 31(76%) of HIV infected patients. This made it one possible mechanism in the pathogenesis of thromboembolism in these patients. Another correlation between antiretroviral therapy and APL
antibodies that 15 patients receiving ARVs had normal level of IgM and increased in 9 patients (37.5%), while IgG was increased in 21 patients out of 24 patients receiving ARV (87.5%). Commonly used drugs in Sudan these ARVs belong to group of Nucleoside analogue and non Nucleosides reverse transcriptase inhibitors, these drugs were combined in one drug Triomune and with least side effects but anemia & thrombocytopenia &neutropenia were reported. Evidence or association between hypercoagulbility state of HIV/AIDS & using protease inhibitors was reported in many studies such Saif and Sullivan.

Many studies in the literature to detect thrombosis or PE in these patients using imaging techniques such on phlebography, V/Q scan, Doppler, MRI, ect, as well as laboratory technique. In this study we couldn’t use the techniques to and we use only laboratory data.

CD$_4$ counter machine is a newly introduced in Sudan.
CONCLUSION

1- HIV/ADIS is affecting haemopoietic systems in many ways.

2- Overt anemia and thrombocytopenia and neutropenia are commonly seen.

3- Antiphospholipid antibodies were found in a high proportion in these patients specially APL( IgG -75%) and APL( IgM - 12.73%). These could predispose to thrombosis.
RECOMMENDATIONS

• Study involving bigger number is needed to confirm our findings.

• Proper follow up of HIV patients and adjusts prophylactics, therapy to opportunistic infections is recommended.

• Patient with low CD$_4$ <200/mm must follow their Hb, PT, PTT. And platelets regularly.

• Prophylactics antithrombotic in those with thrombophilia reduce incidence of recurrent thrombosis

• Clinical& imaging techniques are needed for follow up of these patients for detection & early treatment thrombosis.

• Study of other parameters of thrombophilia is needed.

• Using CD4 as a new test for categorization of patients of HIV and for follow up of these patients.
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أقرار بالموافقة

السلام عليكم ورحمة الله وبركاته

٨٤
Questionnaire
Coagulation disturbances in Sudanese HIV/AIDS patients

Serial No:........................................

Personal data:
1. Name:......................................... State:...............................  
2. Age (in years):............................ 3. Tribe:............................
4. Religion  
   I. Muslim  II. Christian  III. Other
5. Marital status:  
   I. Married  II. Single  III. Divorced
6. Education:  
   I. Khalwa  II. Primary  III. Secondary
   IV. Higher Secondary  V. University  VI. No
7. Employment:  
   I. Yes  II. No

Clinical data:
8. Duration of illness:  
   I. Weeks  II. Years  III. Specify........
9. Symptoms:  
   I. Fever  II. Fatigue  III. Loss of weight
   IV. Diarrhoea  V. Others  (Specify):........
10. Symptoms of bleeding:  
    I. Haematuria  II. Haemoptysis
    III. Malaria  IV. Haematmesis
11. Sign:  
    I. Pallor  II. Jaundice  III. Petechiae
    IV. Ecchymosis  V. Others
12. Drugs  
    Yes  No  Stopped
   I. ARV:  
   II. Others
13. Iner-current infection:  
   I. Bacterial  II. Viral  III. Fungal  IV. Others
14. Laboratory investigations:
   I. CBC:.............................  II. PT:............................  III. PTT:.............................
DiaPlastin
Liquid Calcium-Thromboplastin from rabbit brain
for PT determination (Quick), ISI-Value ~ 1.7

Product-Identification: 30050

Introduction
Thromboplastin activates the extrinsic coagulation system in plasma in the presence of calcium ions. The subsequent clotting time is dependent on the concentration of factors II, V, VII and X, thus a prolongation indicates a deficiency in one or more of these factors.

DiaMed "DiaPlastin" is a stable, liquid, combined calcium/thromboplastin reagent for the determination of prothrombin time (PT). It is manufactured to have an International Sensitivity Index (ISI) of ~1.7, standardized against British Comparative Thromboplastin (BCT) and behaves in a similar manner to human brain thromboplastin, with a high sensitivity to the above factors.

These characteristics make DiaMed "DiaPlastin" the ideal reagent for use in the control of oral anticoagulant therapy, producing minimal potential distortion in the calculation of the International Normalised Ratio (INR). Other applications include pre-operative screening (in conjunction with PT) and the determination of congenital deficiency of the related factors (II, V, VII, X).

Reagents

"DiaPlastin": Calcium-Thromboplastin (rabbit brain) liquid, ready-for-use, in 4 mL and 8 mL vials, ISI: ~ 1.7

2 °C
8 °C
Stability: see expiry date on label.

Further materials required
Manual method:
Pipette 100, 200 and 1000 μL
Water bath 37 °C
Test tubes 12 x 75 mm
Stopwatch

Automatic method:
Pipette 50, 100, 500 and 1000 μL
Coagulation analyzer

Set procedure
Mix well the reagent, without shaking, before use.
Follow manufacturer’s instruction manual for the instrument used!

Pipette into clean test tubes:

<table>
<thead>
<tr>
<th>Plasma</th>
<th>0.1 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prewarm 2-5 minutes at 37 °C</td>
<td></td>
</tr>
<tr>
<td>DiaPlastin (prewarmed at 37 °C)</td>
<td></td>
</tr>
<tr>
<td>Simultaneously start stop watch.</td>
<td></td>
</tr>
<tr>
<td>Observe time of clot formation.</td>
<td></td>
</tr>
</tbody>
</table>

Products

DiaPlastin

6 x 4 mL ........... REF 300120
100 x 4 mL ........... REF 300104
6 x 8 mL ........... REF 300240
100 x 8 mL ........... REF 300108

These products are guaranteed to perform as described on the label and in the instruction sheet. The manufacturer declines all responsibility arising out of the use or sale of these products in any way or for any purpose other than those described therein.

DiaMed AG, 1785 Cressier s/Morat, Switzerland
DiaCelin-L
Liquid Cephaloplastin (activated with complexed Kaolin)
For PTT- and Heparin determination

Introduction
Cephaloplastin activates the coagulation factors of the intrinsic system in plasma in the presence of calcium ions. The clotting time depends on the activity of factors VIII, IX, XI and XII as well as of I, II, V and X. Contact activation by complexed kaolin reduces the clotting time considerably and avoids turbidity, and may be used in optical systems. Due to its high sensitivity to heparin, "DiaCelin-L" can be used for heparin determination.

Diagnostic applications
1. Pre-surgery screening (in combination with PT)
2. Congenital deficiency of factors VIII, IX, XI and XII
3. Haemophilia induced by antibodies
4. Monitoring of heparin-treatment

Normal values
26-36 seconds (at 3 minutes activation time). The normal time depends on the method (activation time, instrument, etc.) and must be determined in each laboratory.

Reagents

** (IVD)
1. "DiaCelin-L" (Cephaloplastin, rabbit brain, with complexed kaolin), liquid
2. Calcium chloride, 0.02 mol/L.

* Stability:
  "DiaCelin-L": approx. 1 year at 2-8 °C (see expiry date)
  1 week at 18-25 °C
  2 days at 37 °C

* Do not freeze!

Preparation of blood sample
Mix 9 parts of freshly collected blood with 1 part of sodium citrate 0.11 mol/L.
Centrifuge immediately for 5 minutes at RCF 1500-2000 g (approx. 3000 rpm) and transfer plasma into a clean test tube.
Plasma should be tested within 3 hours of blood collection. For heparin determination, platelet-poor-plasma should be used (high centrifugation required). Blood collection preferably in plastic tubes.

Procedure
Manual method (double determination)
Before use, mix well reagent, without shaking.

Pipet into a clean test tube:

| Plasma | 0.1 mL |
| "DiaCelin-L" (prewarmed at 37 °C) | 0.1 mL |
| Mix well and incubate exactly for 3 minutes at 37 °C | 0.1 mL |

Add Calcium chloride (prewarmed at 37 °C)
Simultaneously start stopwatch and observe clot formation. Stop watch at the appearance of the first web.

* Remark: For precision and reproducibility, standardize incubation time!

** When using measuring device with hook (Schnitger-Gross) insert electrode only after 20 seconds.

Calculation
1. (PTT)
The result can be directly reported in seconds.

Another reporting system is the PTT-ratio: (R)

\[ R = \frac{\text{PTT patient plasma in seconds}}{\text{PTT FNP in seconds}} \]
9 ASSAY PROCEDURE

9.1 Preparation and Storage of Reagents

Ready to use reagents
When stored refrigerated at 2 - 8 °C the components are stable for at least 30 days after opening or until the expiration date printed on the labels.
Remaining modules of the microplate should be stored refrigerated at 2 - 8 °C protected from moisture; store together with desiccant and carefully sealed in the plastic bag.

Sample buffer
Dilute the contents of each vial of the sample buffer concentrate (5x) with distilled water to a final volume of 100 ml prior to use.
Store refrigerated: stable at 2 - 8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Buffered wash solution
Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled water to a final volume of 1000 ml prior to use.
Store refrigerated: stable at 2 - 8 °C for at least 30 days after preparation or until the expiration date printed on the label.

9.2 Assay Procedure

1. Prepare a sufficient number of microplate modules to accommodate calibrators, controls and predicted patient samples in duplicates.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>SA</td>
<td>SE</td>
<td>P1</td>
<td>P5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>SA</td>
<td>SE</td>
<td>P1</td>
<td>P5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>SB</td>
<td>SF</td>
<td>P2</td>
<td>P..</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>SB</td>
<td>SF</td>
<td>P2</td>
<td>P..</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>SC</td>
<td>C1</td>
<td>P3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>SC</td>
<td>C1</td>
<td>P3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>SD</td>
<td>C2</td>
<td>P4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>SD</td>
<td>C2</td>
<td>P4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SA - SF: standards A to F
P1, P2... patient sample 1, 2...
C1: positive control
C2: negative control

2. For the determination of one class of autoantibodies pipette 100 μl of calibrators, controls and predicted patient samples into the wells.

For determination of both IgG and IgM autoantibodies calibrators, controls and patient samples have to be pipetted in two attempts.

3. Incubate for 30 minutes at room temperature (20 - 28 °C).
4. Discard the contents of the microwells and wash 3 times with 300 μl of wash solution.
5. Dispense 100 μl of enzyme conjugate solution into each well.
6. Incubate for 15 minutes at room temperature.
7. Discard the contents of the microwells and wash 3 times with 300 μl of wash solution.
8. Dispense 100 μl of TMB substrate solution into each well.
9. Incubate for 15 minutes at room temperature protected from light.
10. Add 100 μl of stop solution to each well of the modules and leave untouched for 5 minutes.
11. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with reference at 600-650 nm is recommended.

The developed color is stable for at least 30 minutes.
Read optical densities during this time.
10 CALCULATION OF THE RESULTS

For the anti-Phospholipid Screen IgG and IgM a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is recommended. Spline approximation and log-log coordinates are also suitable.

Recommended Lin-Log Plot

First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

10.1 Calculation Example

The figures below show typical results for anti-Phospholipid Screen IgG and IgM. These data are intended for illustration only and should not be used to calculate results from another run.

<table>
<thead>
<tr>
<th>anti-PL No</th>
<th>Position</th>
<th>OD 1</th>
<th>OD 2</th>
<th>Mean</th>
<th>Conc 1</th>
<th>Conc 2</th>
<th>Mean</th>
<th>decl Conc</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG STA A</td>
<td>1/B 1</td>
<td>0.051</td>
<td>0.049</td>
<td>0.050</td>
<td>0.3</td>
<td>0.1</td>
<td>0.2</td>
<td>0.0 3</td>
<td>3</td>
</tr>
<tr>
<td>IgG STB C</td>
<td>1/F 1</td>
<td>0.163</td>
<td>0.160</td>
<td>0.161</td>
<td>6.4</td>
<td>6.3</td>
<td>6.3</td>
<td>6.3 1</td>
<td>1</td>
</tr>
<tr>
<td>IgG STC E</td>
<td>1/F 1</td>
<td>0.310</td>
<td>0.273</td>
<td>0.291</td>
<td>12.8</td>
<td>11.2</td>
<td>12.0</td>
<td>12.5 9</td>
<td>9</td>
</tr>
<tr>
<td>IgG STD G</td>
<td>1/H 1</td>
<td>0.603</td>
<td>0.630</td>
<td>0.616</td>
<td>25</td>
<td>26</td>
<td>26</td>
<td>26 5</td>
<td>5</td>
</tr>
<tr>
<td>IgG STE A</td>
<td>2/B 2</td>
<td>1.122</td>
<td>1.054</td>
<td>1.088</td>
<td>51</td>
<td>47</td>
<td>49</td>
<td>50 4</td>
<td>4</td>
</tr>
<tr>
<td>IgG STF C</td>
<td>2/D 2</td>
<td>1.742</td>
<td>1.787</td>
<td>1.765</td>
<td>98</td>
<td>103</td>
<td>101</td>
<td>101 2</td>
<td>2</td>
</tr>
<tr>
<td>IgM STA A</td>
<td>1/B 7</td>
<td>0.022</td>
<td>0.021</td>
<td>0.022</td>
<td>0.2</td>
<td>0.1</td>
<td>0.2</td>
<td>0.0 3</td>
<td>3</td>
</tr>
<tr>
<td>IgM STB C</td>
<td>1/D 7</td>
<td>0.211</td>
<td>0.205</td>
<td>0.208</td>
<td>5.1</td>
<td>6.0</td>
<td>6.1</td>
<td>6.3 2</td>
<td>2</td>
</tr>
<tr>
<td>IgM STC E</td>
<td>1/F 7</td>
<td>0.465</td>
<td>0.462</td>
<td>0.464</td>
<td>13.0</td>
<td>12.9</td>
<td>13.0</td>
<td>13.0 0</td>
<td>0</td>
</tr>
<tr>
<td>IgM STD G</td>
<td>1/H 7</td>
<td>0.788</td>
<td>0.899</td>
<td>0.833</td>
<td>23</td>
<td>26</td>
<td>24</td>
<td>25 8</td>
<td>8</td>
</tr>
<tr>
<td>IgM STE A</td>
<td>2/B 8</td>
<td>1.411</td>
<td>1.382</td>
<td>1.397</td>
<td>52</td>
<td>59</td>
<td>51</td>
<td>51 1</td>
<td>1</td>
</tr>
<tr>
<td>IgM STF C</td>
<td>2/D 8</td>
<td>1.868</td>
<td>1.852</td>
<td>1.860</td>
<td>101</td>
<td>98</td>
<td>99</td>
<td>100 1</td>
<td>1</td>
</tr>
</tbody>
</table>

10.2 Calibration

The assay system is calibrated against the internationally recognized reference sera from E.N. Harris, Louisville, since no other international standards are available.

11 QUALITY CONTROL

Good laboratory practice requires that quality control specimens be run with each standard curve to check the assay performance. Low and high level controls should be included in each assay. Kit controls or any other control materials should be assayed repeatedly to establish mean values and acceptable ranges. Each individual laboratory is responsible for defining their system for quality control decisions and is also responsible for making this system a written part of their laboratory manual.

Recommended batch analysis decision using two controls

1. When both controls are within ±2 SD. Decision: Approve batch and release analyte results.
2. When one control is outside ±2 SD and the second control is within ±2 SD. Decision: Hold results, check with supervisor. If no obvious source of error is identified by the below mentioned check of systems, the supervisor may decide to release the results.

Technician check of systems:

1. Check for calculation errors
2. Repeat standards and controls
3. Check reagent solutions
4. Check instrument
Principle of measurement

Fig. 4: Optical geometry of the flow cuvette (schematics).

Laser light is focused into the flow cell. Laser light scattered from the particles is detected in the forward direction range (forward scatter). Side scatter and fluorescence light is collected by the objective at a right angle.

Note: In the CyFlow® Counter just one fluorescence parameter, but no scatter signal is detected.

Flow cuvette:
The flow cuvette has three connections. The first connector is for the sample input, the second for the sheath fluid input and the third for the waste output.

Principle of measuring:
The sample flows through a capillary into the flow cuvette. Here, the sheath fluid takes it with it. Because of the specific flow cuvette geometry the sheath and sample current are speeded up. You get a very narrow, laminar flowing sample stream. This means, the sample stream does not get mixed with the sheath stream. The cells or particles labelled with fluorescent colouring pass the measuring area one after the other. The cells or particles are individually illuminated by the excitation light and the fluorescent light is measured and analysed.
REFERENCES


