THE RELATIONSHIP BETWEEN HIV INFECTION AND ACUTE DEEP VEIN THROMBOSES.

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in

in Haematology

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DECLARATION

I, Susan Louw, declare that this research report is my own unaided work. It is being submitted for the degree of Masters of Molecular Medicine and Haematology at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

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..... day of ....., 2011

DEDICATION

To my husband, Louis and my children, Werner and Betessa, for their patience and understanding.

To my mother, Du Borette Louw, for always believing in my ability.

To Professor Barry Jacobson for being an insightful and knowledgeable supervisor.

PUBLICATIONS ARISING FROM THIS STUDY

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ABSTRACT

Objective: HIV infection is a global pandemic with approximately 37 million adults infected worldwide.[1] Numerous abnormalities predisposing to a hypercoagulable state have been described in patients with HIV infection and include deficiencies of antithrombotic proteins and the presence of procoagulants. The abnormalities have been described to correlate with the degree of HIV associated immunosuppression as well as with the presence of concomitant infections and / or neoplastic disorders. The conclusion of several studies[2-3] has been that although evidence pointed towards a relationship between HIV infection and venous thrombotic disease, more studies were indicated to further elucidate this link. The majority of studies reporting on the documented prothrombotic abnormalities in HIV infection were conducted in first world cohorts.

The objective of the study was to determine the prevalence of underlying HIV infection in patients presenting with acute deep vein thrombosis (DVT) without the presence of traditional risk factors for DVT. This prevalence was compared to the HIV prevalence in a sex, age and race matched control group without symptomatic DVT. In addition, the possible pathophysiological mechanisms for DVT development in this cohort are detailed.

Methods: Consecutive adult patients presenting to the Charlotte Maxeke hospital casualty with lower limb acute deep vein thrombosis (DVT) were invited to participate in the study. Voluntary HIV testing of the participants were performed after counselling and consent with appropriate referral for further management if HIV result was positive. A record review was performed and information regarding the presence of commonly encountered traditional risk factors for the development of DVTs. The control group was an age, sex and race matched cohort to establish the prevalence of HIV infection in a matched population without symptomatic DVTs. A review of the literature to identify the possible underlying causative factors linking HIV and DVT was conducted.

Results: The HIV prevalence in the DVT group who consented to HIV testing and who had no traditional risk factor for DVT development (22 patients) was 81% (95% CI 0.67 - 0.96). The HIV prevalence in a matched control group without symptomatic DVTs was found to be 4% (95% CI 0.039 – 0.041). All the DVT patients who consented to HIV testing were active, community integrated members of the society. The average CD4 cell count of the HIV

positive patients with acute DVTs was 247 /mm^3. Two of the HIV positive patients with DVTs were on ART (anti-retroviral therapy) and 4 were also diagnosed with pulmonary tuberculosis. Traditional DVT risk factors identified in the HIV infected DVT cohort other than tuberculosis were immobilisation and carcinoma.

Conclusion: A prothrombotic state is present in HIV infected individuals giving rise to an increased prevalence of thrombotic complications with potentially fatal consequences. The risk of DVTs in the general population is 0.10 % a year[2] but the current and other studies indicate that the prevalence in HIV positive patients is significantly increased. From this thesis it is clear that there is no available evidence evaluating thromboprophylaxis specifically in HIV-infected individuals. The available thrombosis treatment guidelines lack recommendations in this growing sub-population. Important treatment decisions are therefore left to medical attendants without clear guidelines. HIV infection in the ARV era is a chronic disease with a clearly prothrombotic tendency. Future studies and guidelines should further define the thrombotic risk in the HIV infected population and direct treatment and prophylaxis.

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TABLE OF CONTENTS PAGE

LIST OF TABLES

ABBREVIATIONS

ACCP	American College of Chest Physicians
ACLAs	Anticardiolipin antibodies
AIDS	Acquired Immunodeficiency Syndrome
APC	Activated protein C
APLAs	Antiphopholipid antibodies
Arg	Arginine
ART	Anti-retroviral treatment
ARVs	Anti-retrovirals
AT	Antithrombin
C4-BP	Complement factor 4 Binding Protein
CAM	Cell adhesion molecule
CD4	Cluster of differentiation four
CMV	Cytomegalovirus
CRD	Chronic renal disease
DIC	Disseminated Intravascular Coagulation
DS	Dermatan sulphates
DVT	Deep vein thrombosis
DVTs	Deep vein thromboses
eNOS	Endothelial nitric oxide synthetase
EPCR	Endothelial Protein C Receptor
FISH	Fluorescence in situ hybridisation

FVIII	Coagulation factor VIII
FMD	Flow mediated dilatation
GAGs	Glycosaminoglycans
GSH	Glutathione
HC II	Heparin Co-factor II
HDLc	High-density lipoprotein cholesterol
HIV	Human Immunodeficiency Virus
HS	Heparin sulphates
hsCRP	Highly specific C-reactive protein
HUVEGs	Human umbilical vein endothelial cells
IL-6	Interleukin-6
IMT	Intima media thickness
ITP	Idiopathic Thrombocytopenic Purpura
LA	Lupus anticoagulant
LMWH	Low molecular weight heparin
MCP-1	Monocyte chemotactic protein-1
MHC	Major histocompatibility complex
MMP	Metalloproteinase
mtDNA	Mitochondrial deoxyribonucleic acid
NHL	Non-Hodgkin lymphoma
PAI-1	Plasminogen activator inhibitor-1
PC	Protein C
PS	Protein S

PSGL-1	P-selectin glycoprotein lligand-1
ROS	Reactive oxygen species
SLE	Systemic lupus erythematosis
sTM	Soluble form of thrombomodulin
TB	Mycobacterium tuberculosis
TF	Tissue Factor
TFPI	Tissue Factor Pathway Inhibitor
TG	Thrombin Generation
TNF	Tissue Necrosis Factor
tPA	Tissue-plasminogen activator
TTP	Thrombotic thrombocytopenic purpura
UFH	Unfractionated heparin
VEGF	Vascular endothelial growth factor
VL	Viral load
VTE	Venous thrombo-embolic
vWF	von Willebrand factor
WHO	World Health Organisation

1. Introduction

1.1 Background

HIV infection is a global pandemic with approximately 37 million adults infected worldwide and sub-Saharan Africa is severely affected. South Africa has never experienced an epidemic of the magnitude of HIV/AIDS with an estimated 600 new infections occurring per day.[1] A number of studies have been conducted to estimate the prevalence of HIV infection in the South African population. The most recent study, the South African National HIV Prevalence, HIV Incidence, Behaviour and Communication Survey was conducted in 2005 and provides estimates of the first nationally representative HIV prevalence in South Africa. The study, which involved more than 15 000 South Africans, showed an overall HIV prevalence of 10% which means that 4.8 million people older than 2 years were living with HIV/Aids in South African homes in 2005. These figures indicate that South Africa continues to have the largest documented number of people infected with HIV in the world. This epidemic continues to pose challenges to health care providers and policy makers.

Diverse clinicopathological processes have been linked to infection with HIV. These abnormalities have mainly been documented in First world Caucasian populations. HIV is associated with an increased risk of opportunistic infections, various malignancies as well as arterial and venous thrombotic disease. The literature contains various reports of thrombotic episodes in HIV infected individuals. Numerous abnormalities predisposing to a hypercoagulable state have been described in patients with HIV infection (Table 1):[2-3]

Table 1: Documented abnormalities in HIV predisposing to hypercoagulability:[2-3]

Deficiencies of antithrombotic proteins	• Protein S • Protein C • Antithrombin • Heparin-cofactor II
Presence of procoagulants	• Antiphospholipid antibodies • Increased coagulation factor VIII
Miscellaneous	• Elevated von Willebrand factor • Cellular microparticles • Endothelial cell activation • Concurrent malignancies and opportunistic infections

The first reported cases of HIV infection in South Africa occurred in 1982 with 2 distinct HIV epidemic patterns. Initially the infection was prevalent in White homosexual males. Their infections were caused by HIV-1 clade B. By 1989, the second epidemic was recognised primarily in the Black heterosexual population and the HIV-1 clade involved was mainly C.[4] The identification of different HIV groups relates to HIV viral genetic and molecular heterogenicity with distinct groups of HIV-1 viruses having been identified. One of these subgroups, group M (Major) is responsible for the majority of infections worldwide. Within group M, 9 subgroups or clades designated A through to K have been identified. Currently clade B constitutes the majority of infections in the first world with the African and Indian epidemic consisting largely of infections by clade C. Clade B and C are represented in the Asian epidemic.[5]

Subtype diversity in co-receptor usage, disease progression and transmissibility has been documented. Increasing evidence suggests that all clades of HIV

probably display similar sensitivity to antiviral drugs. However, viruses from some subtypes and/or geographical regions may have a greater propensity to develop drug induced resistance. In addition, differences in regard to replication capacity or fitness may exist among various HIV subtypes and these differences may become magnified under conditions of drug resistance. Immunological pressures may also play an important role in the evolution of viral subtypes that impact on ultimate drug resistance profiles.[6]

Racial differences in host response to HIV infection of the same clade also exist. Chronic renal disease (CRD) and end-stage renal disease (ESRD) are important co-morbid conditions that are prevalent in HIV-infected individuals. An American based study[7] assessed the incidence and progression of HIV-related CRD in an African-American cohort comparing the results with a white cohort. Disparities were documented in HIV-related ESRD between the racial groups. In HIV-infected individuals, the risk of ESRD was approximately 50-fold higher in African Americans than in Whites. Although the racial differences in the natural history of CRD that underlie this discrepancy have not been well characterised, the results of this study suggest a more aggressive natural disease history in African-Americans.

The underlying pathogenesis of the abnormalities predisposing to a hypercoagulable state in individuals with HIV infection is probably multifactorial and may include reduced synthesis of proteins, presence of inhibitory antibodies, abnormal distribution between free and bound moieties, losses of protein due to renal abnormalities and endothelial damage.[3]

The factors contributing to a hypercoagulable state in HIV are discussed in the section that follows. Traditional DVT risk factors are also reviewed as they will add to the thrombotic risk in HIV infected individuals.

1.2 Protein S

1.2.1 Physiologic action

Protein S, a vitamin K dependent protein produced in the liver, endothelial cells and megakaryocytes, acts in vivo as a natural non-enzymatic co-factor for activated protein C (APC) in the degradation of phospholipid bound coagulation factors Va (FVa) and VIIIa (FVIIIa).[8-9]

Proteolytic inactivation of FVa by the protein S / C complex occurs at the amino acid arginine at position 306 and 506 (Arg^{306} and Arg^{506}). Initially the majority of the FVa molecules are cleaved at Arg^{506} yielding partially active intermediates followed by complete inactivation through cleavage at Arg^{306}. Protein S enhances APC-catalysed cleavage at Arg^{306} approximately 20 times and further also counteracts factor Xa (FXa) protection of FV proteolysis at Arg^{506} by APC.[9]

Protein S also enhances the inactivation of coagulation factor VIIIa (FVIIIa) by APC, especially in the absence of coagulation factor IXa (FIXa). Proteolytic inactivation by APC of FVIIIa at Arg^{336} and more specifically Arg^{562} is increased 3 fold in the presence of protein S. If FIXa is however present, protein S is only a weak enhancer of FVIIIa inactivation, unless coagulation factor V (FV) is present. Protein S and FV therefore acts synergistically in APC proteolysis of FVIIIa although the exact underlying molecular pathways remain unclear.[9]

Protein S also forms a 1:1 stoichemetric complex with one of the regulatory proteins of complement, the C4-binding protein (C4-BP). Initially it was thought that the bound form of protein S is inactive and unavailable to act as a co-factor for APC.[10] Recent studies have however demonstrated that protein S bound to C4-BP (PS-C4-BP) is still able to augment the proteolysis of FVa at Arg^{306} more than 10 fold. The rate of cleavage of FVa by APC at Arg^{506} is inhibited by PS-C4-BP 4 fold. The net effect of the PS-C4-BP complex is an overall decreased augmentation of APC with resultant reduced inactivation of FVa. The PS-C4-BP also enhances APC inactivation of FVIIIa. In contrast to free protein S, it does not

synergise with FVa. These observations suggest that rather than inhibiting the activity of protein S, C4-BP may alter the function of protein S.[9]

In addition to its anticoagulant activity through its APC co-factor role, protein S also has APC independent anticoagulant action by inhibiting thrombin formation via the tissue factor pathway inhibitor (TFPI) pathway. TFPI down regulates the activity of tissue factor (TF) by forming a bimolecular complex with FXa and subsequently an inactive quaternary complex consisting of coagulation factors VIIa, Xa, TF and TFPI. Protein S accelerates the formation of the inactive FXa / TFPI complex 10 fold. Protein S is also involved in the inflammatory process and cellular apoptosis.[9]

1.2.2 Protein S deficiency

Protein S levels vary considerably with age, sex, lipid levels, hormonal and smoking status. Protein S levels are for example lower in pregnancy and in women on the oral contraceptive pill, especially preparations containing desogestral and cyproterone acetate. Other acquired causes of decreased protein S levels include disseminated intravascular coagulation (DIC), liver synthetic dysfunction, certain autoimmune diseases, infections such as HIV and warfarin therapy.[9]

In addition to the acquired causes of protein S deficiency, congenital deficiencies do occur but are rare (a prevalence of less than 0.5% of the population) with various usually autosomal dominant genetic mutations being responsible. In addition, genetic polymorphisms in the protein S and C4-BP genes contribute to variations in total and free protein S levels respectively and further contribute to inter-individual variability in protein S levels.[9]

Congenital protein S deficiency has been firmly established as a risk factor for venous thrombo-embolism (VTE) with a 5 – 10 fold increased risk in family

studies. Acquired protein S deficiency has also been demonstrated to be a risk in the development of VTE often acting in conjunction with other prothrombotic factors.[9]

1.2.3 Protein S deficiency in HIV infection

The prevalence of protein S deficiency in HIV infected patients has been reported in various studies to be between 20 and 80%.[8] The underlying pathogenesis and consequences of protein S deficiency in HIV infection have been investigated by various studies as detailed in the section to follow.

HIV associated protein S deficiency involves not only a reduction in the free fraction but also total protein S levels.[2] Reduction in the free protein S fraction may relate to increased levels of C4b-BP, the level of which increases during infection and / or inflammation.[11]

The reduction in total and free protein S levels in HIV might be related to T-cell apoptosis resulting in the formation of microparticles which bind and eventually remove protein S from the circulation.[10]

An autoimmune basis for the acquired protein S deficiency has been postulated in various studies. Lafeuillade et al.[8] evaluated the role of anti-protein S antibodies in protein S deficiency in 55 HIV positive patients. The authors demonstrated the presence of anti-protein S antibodies to be linked to the reduced protein S levels ($p=0.01$). The conclusion of this and other studies[12-13] are that protein S antibodies occur frequently in patients who are infected with HIV.

Erbe et al.[14] demonstrated a statistically significant ($p=0.011$) association between decreased protein S levels in an HIV positive cohort consisting of 49 consecutive

patients. These patients had either opportunistic infections (such as Cryptococcal meningitis and tuberculosis) or concomitant malignancies (such as lymphoma). Protein S levels responded to treatment of the secondary infections and / or malignancies. A positive correlation between decreased protein S levels and increased CRP and decreasing viral load was also established.

Another postulated mechanism of protein S deficiency in HIV is decreased synthesis as described by Hooper et al.[15] Tumour necrosis factor-alpha (TNFα) levels are increased in HIV infection and have also been reported to induce a procoagulant state on the surface of endothelial cells. In this study the authors demonstrated down regulation of protein S synthesis in a human endothelial cell line secondary to exposure to recombinant TNF-alpha (rTNF-alpha).

1.3 Protein C

1.3.1 Physiologic action

Protein C is an endogenous vitamin K dependent serine protease which exhibits antithrombotic properties. Protein C is converted to activated protein C (APC) when thrombin complexes with thrombomodulin, an endothelial surface glycoprotein. The activation of protein C is facilitated by endothelial protein C receptor (EPCR) present mainly on blood vessel endothelium. The concentration of circulating APC, when endothelial function is normal, is proportional to the amount of thrombin and protein C present. In severe sepsis, the host response results in generalised systemic dysfunction of the endothelium with suboptimal activation of protein C. This was the impetus for the development of recombinant human activated protein C (drotrecogin), as a treatment option in patients with severe sepsis.[16]

Protein C exerts its anticoagulant effect through inhibition of activated coagulation factors V and VIII, a function augmented by protein S, with resultant decreased thrombin generation. The antithrombotic function of protein C also

further involves enhanced fibrinolysis via its inhibition of plasminogen activator inhibitor 1 (PAI-1).[16]

In addition, APC exhibits multiple other biological activities and is an important modulator of severe infection. Inhibition of thrombin generation is indirectly anti-inflammatory. Direct anti-inflammatory and anti-apoptotic actions of protein C involve interaction with its receptor, endothelial protein C receptor (EPCR). EPCR is present on endothelium, certain white blood cells (neutrophils, monocytes and eosinophils) and respiratory epithelium. The direct anti-inflammatory actions involve decreasing the interaction between leucocytes and the endothelium and reducing chemotaxis of leukocytes in response to chemokines.[16]

1.3.2 Protein C Deficiency

Protein C deficiency is a well accepted risk factor for venous thrombo-embolic disease and can either be congenital or acquired.

Congenital protein C deficiency is a rare and heterogeneous condition with a prevalence of 0.2 to 0.4% in healthy blood donors and approximately 1.5 % in first time patients with thrombo-embolic disease. Thrombo-embolic disease in patients with congenital protein C deficiency tends to occur at a younger age and often in combination with other acquired risk factors such as pregnancy. Congenital protein C deficiency can be divided into type I (concordant reduction in protein C activity and antigen levels) and type II (reduced protein C activity but normal antigenic levels). A total of 160 different mutations have been identified with both clinically recessive as well as dominant forms. There is no ethnic distribution of congenital protein C deficiency. The increased tendency towards thrombotic disease is present even in the heterozygous form of the disease with a 8 – 10 fold increased risk. The rare finding of homozygous protein

C deficiency is frequently associated with purpura fulminans, a severe form of DIC presenting in the neonatal period.[17]

Acquired protein C deficiency is usually secondary to liver synthetic dysfunction, vitamin K deficiency, warfarin therapy or increased consumption related to thrombosis or DIC. HIV infection is now also a well established secondary cause of protein C deficiency.[14]

1.3.3 Protein C deficiency in HIV infection

Protein C deficiency has been documented to be present in up to 14% of HIV infected individuals[14] but has not been as clearly delineated as that of protein S deficiency.[18] The underlying cause, as in HIV related protein S deficiency, is probably multi-factorial. The protein C deficiency in HIV comprises both decreased levels of total (antigenic) as well as active (functional) protein C.[3]

A trend towards normalisation of protein C activity has been demonstrated in HIV positive patients when opportunistic infections have been treated successfully and anti-retroviral therapy has been initiated.[14] The association between superimposed infections in HIV positive patients and reduced APC activity may be related to the development of anti-phospholipid antibodies which interfere with the interaction between protein C and its target receptors.[2] In addition, protein C consumption is probably increased during acute infection relating to its anti-inflammatory function.[11] It has therefore been hypothesised that the decline in protein C levels in HIV may be secondary to a low-grade consumptive coagulopathy rather than decreased synthesis or altered metabolism.[19]

1.4 Antithrombin

1.4.1 Physiological action

Antithrombin (AT), a hepatocyte synthesised serine protease, is the most important physiological inhibitor of activated coagulation factors II, IX, X, XI and XII. Inhibition of the activated coagulation factors occurs by the formation of an equimolar complex between AT and the activated coagulation factor, a process accelerated by heparin.[3]

1.4.2 Antithrombin deficiency

Homozygous congenital antithrombin deficiency is not compatible with life. Heterozygous deficiencies are heterogeneous with two distinct subtypes described: Type I and II. No ethnic data on the distribution of antithrombin deficiency is available.[17]

Type I congenital deficiency is associated with both a reduction in functional and immunological antithrombin levels with more than 80 point mutations and 12 major gene deletions underlying these deficiencies. The prevalence of type I antithrombin deficiency is estimated to be 0.2% of the general healthy population and 1% of patients presenting with first time VTE. Type I deficiency is clearly related to increased risk of VTE occurring at a median age of 26 years of age.[17]

Type II congenital deficiency of antithrombin is associated with reduced functional but normal immunogenic levels of antithrombin. Various mutations have been described affecting different functional domains of antithrombin including the heparin binding site domain. The prevalence of type II antithrombin deficiency in healthy blood donors has been reported to be 1.45 per thousand. The risk of VTE in type II deficiency is lower than that in type I deficiency.[17]

Acquired causes of antithrombin deficiency include consumptive coagulopathy (DIC) with increased utilisation, nephrotic syndrome with increased renal loss, protein-losing-enteropathies with increased gastro-intestinal loss and liver disease with decreased synthesis. Increased cleavage by proteolytic enzymes may also contribute to acquired antithrombin deficiency as can heparin therapy.[2-3, 19]

1.4.3 Antithrombin deficiency in HIV infection

Acquired antithrombin deficiency in HIV infection is probably caused by multiple mechanisms including malnutrition, decreased liver synthesis and increased renal loss secondary to nephrotic syndrome with the antithrombin levels being inversely proportional to the degree of proteinuria. Inactivation by proteolytic enzymes has also been postulated to be a contributing factor. The exact contribution of these various factors in lowering antithrombin levels in HIV infection is unknown.[2-3] HIV associated malignancies may also play an aetiological role in HIV antithrombin deficiency.[18]

1.5 Heparin cofactor II

1.5.1 Physiological role

Heparin cofactor II (HC II) is synthesised in the liver and is a natural glycoprotein thrombin inhibitor with a structure very similar to antithrombin. HC II inactivates both free and clot based thrombin but has no inhibitory effect on any of the coagulation proteins other than thrombin.[20] The anticoagulant activity of HC II is enhanced by glycosamine glycans (GAGs) such as dermatan and heparin sulphates (DS & HS) which are naturally present in vessel walls and synthesised by cells such as fibroblasts.[2, 21] Results of various studies support the hypothesis that HCII interacts with DS & HS in the vessel wall after injury of the endothelium and that this interaction contributes to the regulation of thrombus formation in vivo.[21]

1.5.2 Heparin cofactor II deficiency

Congenital deficiency of HC II has been reported to be associated with recurrent arterial thromboses although the aetiological link between HC II deficiency and VTE remains controversial.[19] Although experimental work has demonstrated that HC II can contribute as much as 20 – 30% to thrombin inhibition during thrombus formation, neither mice nor humans deficient in HC II exhibit hypercoagulable states during normal conditions.[20-21] Recent data suggest that the primary physiological function of HCII is to inhibit thrombin's non-haemostatic roles e.g. development of atherosclerosis with HC II protecting against arterial atherosclerosis and preventing restenosis post thrombolysis, balloon-angioplasty or stenting procedures. Elevated levels of HCII has therefore been shown to protect against atherosclerosis and restenosis.[20] In contrast patients with low plasma concentrations of HCII do not appear to be at increased risk for venous thrombosis, but they may be predisposed to development of atherosclerosis and neo-intima formation following angioplasty and stent placement.

1.5.3 Heparin cofactor II deficiency in HIV infection

A higher prevalence of acquired HC II deficiency has been demonstrated in HIV infected patients with CD4 counts below 200 x 10^6 per liter compared with HIV patients with higher CD4 counts and HIV uninfected people. This suggests a possible association between HC II deficiency and immunodeficiency.[19, 22] The postulated mechanisms of HC II deficiency in HIV infection include decreased synthesis, proteolysis, neutralising antibodies and redistribution from the intravascular to the extravascular space secondary to endothelial cell dysfunction.[2, 19] No study has, however, clearly demonstrated a link between HC II deficiency in HIV and the documented prothrombotic state in HIV.[18]

1.6 Antiphospholipid antibodies

1.6.1 Role in disease

Antiphospholipid antibodies (aPLAs) including lupus anticoagulants (LA) and anticardiolipin antibodies (aCLAs) are a family of acquired auto-antibodies directed mainly against negatively charged phospholipids bound to phospholipid-

binding proteins. They can occur in a background of another disease process such as autoimmune diseases e.g. systemic lupus erythematosis (SLE), infections e.g. HIV, drug exposure, neoplasms or haematologic diseases (then referred to as secondary aPLAs). aPLAs can also occur alone with no background disease process, then referred to as primary aPLAs. The principle clinical manifestations of aPLAs include venous and arterial thromboses, recurrent miscarriages and thrombocytopenia with LA being a stronger predictor of thromboses than aCLAs. The underlying pathophysiological mechanism of thrombosis is thought to involve interference with endothelial cell function, including decreased prostaglandin production and interference with endothelial cell mediated protein C activation via thrombomodulin.[3] Laboratory detection of these autoantibodies is based on both functional clot and ELISA based tests.[23-24] Functional assays involve demonstration of prolongation of phospholipid based coagulation assays such as dilute Russell viper venom time (DRVVT) due to interference by aPLAs.[3, 23-24]

The combination of antibodies detected in the laboratory and clinical manifestations may then fulfill the criteria for the diagnosis of antiphospholipid syndrome.[25]

1.6.2 Disease processes in HIV infection

The results of antiphospholipid antibody (aPLA) testing in HIV patients have yielded conflicting results as far as the prevalence and clinical significance of these antibodies are concerned. Some studies have demonstrated a high prevalence of aPLAs in individuals with HIV infection with aCLAs detected in up to 90% and LA in 70% of patients.[3] Lijfering et al.[11] however did not observe the same prevalence of LA and aCLAs in their study in which they measured markers of coagulation during the course of HIV infection in 109 consecutive HIV positive patients. They attributed this lower prevalence in their study to previous studies not following the same strict guidelines to diagnose lupus anticoagulant and anticardiolipin positivity. Other studies failed to detect these autoantibodies in any of the HIV positive patients in their cohorts.[23- 24] Various

studies have found a relationship between the prevalence of aPLAs and progression of HIV infection with the highest prevalence being detected during the final stages of the infection i.e. AIDS.[24] Klein et al.[2] in their review of the relationship between chronic HIV infection and venous thrombotic disease concluded that the presence of aPLAs in HIV-infected patients was not related to the stage of the infection as determined by the CD4 cell count and / or the HIV viral load.

The origin of aPLAs in HIV infection has been postulated to be either secondary to specific or non-specific stimuli. The specific antibody production being related to the release of phospholipid antigens from the cell membranes of necrosing host cells with aPLAs playing a scavenging role mopping up debris of self damage. Opportunistic infections such as CMV can also release phospholipid antigens initiating a similar antibody production process stimulated by a specific antigen.[18] Non-specific antibody production relates to the immune dysregulation in HIV infection secondary to regulatory CD4 positive T-helper cells being targeted and depleted by HIV resulting in a non-specific polyclonal gammopathy.[3, 23-24]

Studies attempting to establish the clinical significance of aPLAs in HIV infection have also drawn different conclusions. Although aPLAs have been commonly detected in patients with HIV infection, the clinical manifestations of the antiphospholipid syndrome are uncommon in patients with HIV infection but do occur. Only 5 cases of full blown antiphospholipid syndrome in HIV infection had been described in the English literature by 2004. Thromboses, arterial and venous, do however occur more frequently in HIV infected patients with aPLAs compared with other viral infections with associated aPLAs. This fact suggests more major disturbances of the immune system and the presence of other co-morbidities i.e. malignancies and opportunistic infections such as Pneumocystis jiroveci and Cytomegalo virus (CMV) during the course of HIV infection.[3, 23-24, 26] Other studies, such as that by Palomo et al.[27] for example, have failed to demonstrate an association between the presence of aPLAs and thrombosis in

HIV infection. The theoretically increased risk of thrombosis in the face of aPLAs can in part at least be explained by inhibition of APC by aCLAs.[2]

In SLE an association between the level of aPLAs and the degree of thrombocytopenia has been demonstrated but no such a relationship has been found in HIV infections.[3] The absence of such a relationship is probably due to the multifactorial pathogenesis of thrombocytopenia in HIV infection.

1.7 Coagulation Factor VIII

1.7.1 Physiological role

Coagulation factor VIII (FVIII) is synthesised in the liver and circulates in plasma in a non-covalent complex with von Willebrand factor (vWF) which protects it against degradation in the circulation, thus prolonging its half life. FVIII is activated by thrombin or factor X (FX) through cleavage at arginyl residues which results in a heterodimeric molecule consisting of 3 domains non-covalently linked through calcium ions. FVIII, in combination with coagulation factor IX (FIX), activates FX which in turn, together with factor V (FV), has prothrombinase activity. The main inhibitor of activated FVIII is protein C, a naturally occurring proteolytic enzyme. Deficiency of FVIII results in the sex-linked bleeding disorder, haemophilia A. Elevated FVIII is generally defined as a level greater than 150% of the level in normal reference plasma.[5, 28]

1.7.2 Elevated factor VIII levels

Increased levels of various clotting factors have been considered in the quest to identify risk factors which increase the incidence of thrombosis. Elevation of coagulation FVIII has been consistently found to be a risk factor in the development of both primary and recurrent VTE. The increase in risk is, however, not linear with the increase in thrombosis incidence only being present when FVIII levels exceed a level of approximately 230 – 250%. The prevalence of elevated basal FVIII level in the general HIV negative population has been reported to be 10%.[11]

Although elevation of FVIII can be an acute phase reaction, basal FVIII levels are genetically determined and seem to be linked to the underlying blood group with non-O individuals having higher basal FVIII levels. FVIII levels may be useful in determining the duration of anticoagulation therapy post thrombosis but this still has to be researched further.[28-29] Cosmi et al.[28] demonstrated a risk of recurrence that was more than 5-fold higher when basal FVIII levels were above the 90th percentile and 3-fold higher with basal FVIII levels above the 75th percentile of normal. In addition, Goldenberg et al.[30] demonstrated that the combination of elevated FVIII and D-dimer levels was predictive of poor outcomes including lack of complete recanulisation, recurrence of thrombosis and development of the post-phlebitic syndrome in children.

1.7.3 Elevated factor VIII levels in HIV infection

Elevated FVIII levels have been demonstrated to be present in up to 41% of HIV positive individuals, with the median FVIII level being higher in patients with AIDS defining illnesses and CD4 counts below 200 x 10^6 per liter compared with the earlier stages of HIV infection. A stepwise increase in FVIII levels has been demonstrated in a study by Levine et al.[24] This study, focusing on female patients without an underlying cause for elevation of acute phase reactants, demonstrated a median FVIII level of 116% in HIV negative women, 149% in those with asymptomatic HIV infection, 196% in those with immunologic AIDS and 211% in patients with clinical AIDS. The difference in FVIII levels between the different groups in this study was statistically significant with $p < 0.0001$.[24] In addition, elevated FVIII levels were associated with increased fibrinogen and decreased protein S concentrations, both factors in themselves being procoagulant. The rise in FVIII levels with advancement of the HIV infection may be due to the HIV infection itself, since the same cytokines that activate the coagulation system have been described to be operational in the setting of advancing HIV.[11]

1.8 Miscellaneous factors

1.8.1 Von Willebrand factor

Von Willebrand factor (VWF), a multimeric glycoprotein, plays a crucial role in platelet adhesion and aggregation, known to be the main initial steps in haemostasis. Each von Willebrand factor (vWF) molecule has binding sites for platelet glycoproteins, coagulation FVIII, for which it serves as a carrier molecule, and collagen. VWF is almost exclusively produced by endothelial cells and, as such, serves as a useful marker of endothelial cell damage and dysfunction. Considering the pivotal role of vWF in thrombogenesis, therapies that specifically inhibit vWF have been considered as potential anticoagulant agents.[31-32]

Release of excessive amounts of vWF has been linked to the development of thrombotic thrombocytopenic purpura (TTP), a microangiopathy occurring with a significantly increased incidence in HIV infected patients. Although the numbers reported in the literature are small, only 44% of HIV related TTP patients have been demonstrated to have a significant deficiency of ADAMTS 13, the metaloprotease responsible for cleaving large monomers of vWF. In addition, only 42% of these patients have a demonstrable ADAMTS 13 inhibitor. These findings are in contrast to those in idiopathic TTP in which most patients have a severe deficiency of ADAMTS 13. Of further interest is the fact that none of the patients with CD4 counts less than 100 cells per liter had a severe deficiency of ADAMTS 13.[33]

Administration of DDAVP to healthy volunteers has been shown to lead to release of vWF with transient decreases in ADAMTS 13 activity. As far as the pathogenesis of TTP in HIV is therefore concerned, it is hypothesised that ongoing release of vWF from endothelial cells leads to an ongoing clearance and relative deficiency of ADAMTS 13. Reports have also demonstrated D-dimer levels to be often elevated in patients with HIV related TTP[34] and in vitro studies have shown that the presence of thrombin leads to lower ADAMTS 13 activity.[33]

It is therefore possible that endothelial activation and / or damage as is discussed in 1.8.2 can lead to the release of excessive amounts of vWF, localised thrombin generation and consumption of ADAMTS 13 expressed phenotypically as TTP.[33] It also seems plausible that elevated vWF with the associated elevation in FVIII contributes to the HIV hypercoagulable state.

1.8.2 Endothelial cell activation

A link between infection and thrombosis via endothelial cell activation has been postulated. Pro-inflammatory cytokines probably play an important role in this process.[11, 35] Numerous studies have concluded that the thrombotic state in HIV infection is caused by endothelial injury and / or dysfunction. [3, 36-37]

As previously noted vWF levels are increased in HIV infection with the increase correlating with the degree of immunosuppression as measured by CD4 cell counts. As vWF is an endothelial cell product which is released as these cells are injured, the increased vWF concentration in the circulation serves as a surrogate marker of underlying endothelial cell damage.

Evidence of direct infection of the endothelial cells by HIV has been sought utilising immunohistochemical stains and fluorescence in situ hybridisation (FISH).[3] Although the presence of one of the HIV capsular antigens, p24, has been demonstrated in endothelial cells[38] it remains uncertain whether HIV directly infects and damages endothelial cells. In vitro infection of human umbilical vein endothelial cells (HUVECs) with HIV has been demonstrated.[39] These infected endothelial cells up-regulated the production of interleukin-6, interleukin-1β, and granulocyte colony-stimulating factor. These cytokines in turn then stimulate HIV replication. To date studies have, however, been unable to conclusively prove that HIV actively infects human endothelial cells in vivo.

HIV proteins can damage the endothelium by various mechanisms. The HIV viral genome encodes for approximately 15 mature HIV proteins which may interact with host targets. HIV is a double-stranded RNA retrovirus belonging to the lentivirus ("slow virus") group. The viral genome, packaged in a nucleocapsid core, is surrounded by a matrix and a glycoprotein-rich envelope. The HIV genome contains 9 main genes: gag, pol, env, tat, rev, vpu, vpr, vif and nef. Proteolytic cleavage of the gag-pol precursor protein yields the major structural components of the viral core (i.e. matrix p17, capsid p24, nucleocapsid p9 and p6, reverse transcriptase, protease, and integrase). Proteolytic cleavage of env produces the important envelope glycoproteins gp120 and gp41. The remaining genes encode for the regulatory proteins tat and rev and the accessory proteins vpu, vpr, vif, and nef. Table 2 provides a summary of the mature HIV proteins, their roles in the viral replication-cycle and known effects on endothelial cell homeostasis.[40]

Table 2: Effects of HIV-1 proteins on viral function and endothelial cell biology:[40]

Protein	Viral Function	Endothelial Cell Effects
Vpr	Block cell division	↑ apoptosis
Tat	Transcriptional transactivator of RNA	↑ apoptosis ↓ relaxation ↑ CAMs ↑ MCP-1 ↑ adhesion, regulate cytoskeleton ↑ permeability ↑ MMP ↑chemotaxis ↑ proliferation ↑ angiogenesis ↑ ROS
Vpu	Virus release; ↓CD4	↑ CAMs
gp120	Receptor binding	↑ apoptosis ↑ CAMs ↑ adhesion regulate cytoskeleton ↑ permeability ↑ vasoconstrictors ↑ ROS
Nef	↓ CD4 and Class I MHC; ↑ viral infectivity	↑ apoptosis

↑: increase; ↓: decrease; CAM: cell adhesion molecule; MCP: monocyte chemotactic protein-1; MMP: matrix metalloproteinase; ROS: reactive oxygen species; MHC: major histocompatibility complex.

Torriani et al.[41] assessed endothelial cell function in ARV naïve HIV infected patients by measuring flow-mediated dilation (FMD) of the brachial artery in a cohort of 82 patients. The results of this study demonstrated a significant improvement in FMD after initiation of ARVs in spite of blood lipid profiles worsening on ARVs. This study focussed on the arterial side of the vascular tree and concluded that patients with HIV-infection who met criteria for starting ART had impaired endothelial function. The cause for this dysfunction was postulated to relate either directly or indirectly to HIV infection with infected patients demonstrating increased expression of both vascular cell adhesion molecule-1

and E-selectin. There is further support of endothelial cell activation and dysregulation, as evidenced by increased plasminogen activator inhibitor-1 (PAI-1) antigen and tissue-type plasminogen activator (tPA) in ARV-naïve patients. These patients also demonstrate positive correlation with anti-p24 antibody levels and disease severity.[40]

The recent Strategies for Management of Antiretroviral Therapy (SMART) study revealed an increase in total mortality and possibly an increased risk of developing cardiovascular disease when ARV treatment was episodically discontinued.[42, 43] Prolonged antiretroviral therapy has been associated with major metabolic and cardiovascular disorders and the authors of this study evaluated the efficacy of episodic antiretroviral treatment based upon $CD4^+$ T-cell levels in 2 720 HIV positive patients. Unfortunately, interruption of antiretroviral therapy actually increased the incidence of major cardiovascular events in the study cohort.

A further marker of endothelial injury is the soluble form of thrombomodulin (sTM). As thrombomodulin is a transmembrane protein located on the surface of vascular endothelium, its elevated plasma levels occur only in the presence of damaged endothelial cells. Studies have demonstrated elevation in sTM in HIV infected but ARV naive patients which normalised on ARV therapy.[44]

Regardless of whether or not the HIV virus directly infects human endothelial cells the vascular endothelium is continually exposed to numerous viral stimuli in HIV infected patients. These stimuli include HIV infected cells ($CD4^+$ T cells, monocytes, and macrophages), free circulating HIV, viral proteins released upon host cell lysis, actively secreted viral proteins (e.g. tat and gp120) and viral-induced pro-inflammatory cytokines. All of these factors increase endothelial cell permeability and may facilitate monocyte invasion into the vessel wall. HIV-induced cytokines may also activate the endothelium, leading to enhanced production of reactive oxygen species (ROS), expression of cell adhesion

molecules (CAMs) and the release of procoagulants such as vWF and chemo-attractants.[40]

Although altered endothelial cell homeostasis plays a more important role in the pathogenesis of arterial thrombosis and this dissertation is essentially concerned with venous thrombosis the two disorders have many features in common. Evidence does exist that patients with venous thrombosis may be at greater risk for arterial events. The pathogenesis of both disorders share many factors including endothelial injury. While the evidence that arterial disease is a risk factor for venous thrombosis is inconclusive, arterial disease does appear to occur with a modestly increased frequency in patients with a history of VTE.[45]

18.3 Microparticles

Microparticles are small cellular remnants circulating in plasma. They mostly originate from platelets, monocytes and endothelial cells. In HIV infection there is an increase in microparticles which originate from CD4 lymphocytes. This is as a direct consequence of HIV infection with resultant CD4 lymphocyte apoptosis. Elevated concentrations of microparticles in HIV-infected individuals have been demonstrated in various studies and this elevation is associated with activation of the coagulation cascade.[2] The procoagulant properties of microparticles are believed to relate to their phospholipid rich surfaces and expression of other molecules, notably tissue factor and P-selectin.

Tissue factor plays a major role in the initiation of blood clot formation. The source of the tissue factor remains somewhat controversial, since the majority of experimental animal models of venous thrombosis involve overt damage to blood vessels. Damage to blood vessels results in exposure of subendothelial tissue factor, but vessel wall damage does not precede the vast majority of human venous thromboses.[46]

There are experimental models in which blood borne tissue factor, associated with blood cells or microparticles derived from blood cells, probably leucocytes, has been shown to be involved in the genesis of thrombus. From these experiments it seems plausible that microparticles bearing tissue factor can serve as the initiator of thrombosis.[46]

Under arterial and venous flow conditions, thrombus formation also appears to involve the adhesion molecule P-selectin. Experiments employing P-selectin blocking antibodies have strengthened this hypothesis. P-selectin facilitates cell-cell interactions via PSGL-1, a major ligand for P-selectin.[46]

Tissue factor and P-selectin therefore both appear to be important for thrombus formation. They are present on microparticles derived from monocytes.[46] Microparticles are elevated in HIV infection adding to the procoagulant state present in HIV infection.[2]

1.8.4 Secondary infections

Infection increases the risk of thrombosis. Certain infections are particularly associated with an increased risk including cytomegalovirus (CMV), Pneumocystis jiroveci and tuberculosis (TB).[3] These infections occur frequently in HIV infected patients.

In industrialised countries TB incidence and mortality has been consistently declining but globally the situation is continually worsening due to HIV co-infection and the increase of multi-drug resistant TB strains. TB is still responsible for more than 1.5 million deaths every year with underdeveloped countries bearing the brunt. HIV/AIDS is the single biggest risk factor for TB infection with up to two thirds of HIV infected people also being infected with TB.[47] The most resistant form of TB, XDR (extreme drug resistant) -TB is defined as resistance to isoniazid, rifamycin, fluoroquinolones and one of three

injectable second-line anti-tuberculosis agents: capreomycin, kanamycin or amikacin. XDR-TB cases are causing increasing alarm in South Africa where endemic HIV makes for a deadly combination. A report published in 2006 from a rural clinic in KwaZulu-Natal in South Africa, showed that MDR-TB was present in 221 (41%) of 542 patients with positive cultures for tuberculosis. 53 (9.7%) of these patients had XDR-TB. Among the XDR-TB patients with a known HIV status all were HIV seropositive. In that setting, XDR-TB was rapidly fatal with a median survival of 16 days after specimen collection. However, because the diagnosis of XDR-TB by drug susceptibility testing takes several weeks, these patients died before appropriate therapy could be administered.[48] Although venous thrombo-embolism is a relatively rare complication of infection with tuberculosis, it may be a potentially life-threatening event. An increased prevalence of DVTs has been documented both prior to (as a presenting feature of TB infection) as well as after initiation of anti-tuberculosis therapy. [49]

Infections with Pneumocystis have been linked to the presence of antiphospholipid antibodies including lupus anticoagulant (LA), which is detected in up to 96% of HIV positive patients with concomitant Pneumocystis infection and this opportunistic infection has been linked to clinical thromboses.[3]

CMV, a well established cause of thrombosis has been linked to peripheral thrombophlebitis, strokes, cerebral venous thrombosis, digital infarcts and pulmonary emboli. [3, 18] There are three mechanisms thought to be operational in the prothrombotic state associated with CMV infection. Firstly, CMV may lead to a thrombotic microangiopathy through its effect on the endothelium which is altered from an anti- to a prothrombotic surface. This change involves increased expression of tissue factor by these cells lining all blood vessels. Direct infection of endothelial cells with the CMV has been demonstrated in AIDS patients with thrombosis. A second possible mechanism involves induction of antiphospholipid antibodies formation. Finally it has been postulated that impaired fibrinolysis due to raised haemostatic parameters e.g. vWF is also operational.[3, 18] A direct anatomic correlation of the physical presence of secondary infections such as

CMV with underlying vascular lesions has not been clearly demonstrated. A review of the literature does however suggest a strong association between the two entities. The early treatment and prevention of these opportunistic infections in HIV infected patients may have an impact on thrombotic episodes in these individuals. [3, 19]

1.8.5 Malignancies

The association between neoplasms and thrombosis has been firmly established. [50-52] Venous thrombo-embolic disease (VTE) is a well known complication in patients with cancer. The association between occult or overt malignancy and thrombosis has been widely recognised since Trousseau in 1865.[53] The overall risk of symptomatic VTE is increased nearly 6 to 7 fold in cancer patients with a 20% prevalence of malignancy in elderly patients with newly diagnosed otherwise unprovoked VTE. VTE remains an important cause of death in cancer patients prior to and during the course of cancer therapy.[54]

Malignancies seen with clearly increased frequency in HIV infection include Kaposi Sarcoma, Non-Hodgkin Lymphoma (NHL), anal and cervical carcinoma.[19]

The pathophysiological mechanisms underlying the development of VTE in cancer patients are multifactorial including tissue factor (TF) expression by malignant cells, formation of TF-bearing microparticles and production of prothrombotic cytokines such as IL-1 and VEGF. Other mechanisms include reduction of naturally occurring coagulation inhibitors which results in activated protein C resistance, decreased fibrinolysis secondary to production of plasminogen activator inhibitor-1 (PAI-1) and increased adhesive interactions between tumour cells. Levels of the adhesion molecule P-selectin has been shown to be increased in many tumours and probably plays an important role in these cellular interactions. The increase in pro-inflammatory cytokines is responsible for increased levels of pro-inflammatory acute phase reactants such as fibrinogen,

vWF and FVIII. Endothelial damage and / or activation related to surgery, chemotherapy, intravenous catheters or direct vascular invasion by tumour cells are major contributing factors. Bed rest and extrinsic compression of blood vessels by either tumour masses or lymph nodes may result in vascular stasis with thrombus formation.[54-55]

The pathophysiology of cancer related thrombosis also involves other molecules such as cancer procoagulant which is an endopeptidase that directly activates coagulation factor X and causes platelet activation. This prothrombotic molecule is released by many tumour cells promoting thrombosis.[55]

Sood S.L.[54] recently reviewed the risk factors underlying the development of VTE in cancer patients and reconfirmed that the individual risk of thrombosis varies from patient to patient. The risk is dependent on a combination of the prothrombotic state induced by the specific cancer, underlying host factors and treatment modalities including placement of catheters, surgery and various anti-angiogenic or chemotherapeutic agents. The thrombosis risk is increased in the first few months after diagnosis of a malignancy and parallels the extent of the malignancy with distant metastases being associated with a significantly increased risk of VTE. Cancer patients with history of VTE have a higher mortality rate compared to those without thrombosis, supporting the hypotheses that activation of coagulation pathways may be an important mechanism for tumour metastasis.

VTE risk varies depending on tumour histology. Primary brain, pancreatic, ovarian, bone, gastric and lung cancers are the main causes of an increased risk. Haematological malignancies also have a high rate of VTE. Lymphoma, acute leukaemia and multiple myeloma patients on treatment with angiogenesis inhibitors have a significantly increased VTE risk.

Endogenous patient factors are additive to the VTE risk in cancer patients. Effective use of VTE thromboprophylaxis in patients with cancer is important to reduce morbidity and mortality, contain healthcare costs and improve quality of life. Although evidence based guidelines for thromboprophylaxis of hospitalised surgical and medical patients with cancer have been developed, recommendations for ambulatory cancer patients are less well established.[56]

Patients receiving chemotherapy have a 2 to 6 fold higher incidence of VTE. This increased risk is likely to be due to a combination of endothelial injury, induction of activated protein C resistance and cell destruction leading to increased exposure of procoagulant material. Surgery and insertion of venous catheters will further add to the VTE risk in cancer patients.[55]

Chemotherapeutic agents are also associated with other vascular abnormalities, such as TTP in non-HIV patients.[52] HIV infection itself has been associated with this vascular complication, the incidence of which can only be expected to be increased when HIV infected individuals are exposed to chemotherapeutic agents.[57] The association between malignancies and thrombosis is further discussed in 5.6.1 of this dissertation.

1.8.6 Bed rest

Rest, specifically bed rest, is an essential part of daily living with most people sleeping for 6 to 9 hrs per day and also resting for shorter periods at other times. Obviously, when ill, people are less ambulant.[58]

Research based definitions of the duration of clinically impotant bed rest differs between published studies, but 4 or more days is generally regarded as being significant. The prothrombotic risk of bed rest contributes to the documented increased prevalence of DVTs in hospitalised patients with up to 20% of medical and 40% of surgical in-patients developing thromboses.[59]

The normally functioning human however, is physically active for substantial portions of each day and increased rest is associated with loss of the beneficial effects of physical activity and might lead to serious complications. The complications of bed rest include skeletal muscle atrophy, joint contractures, pressure ulcers, osteoporosis, metabolic consequences such as insulin resistance, lung atelectasis with associated infection and thrombo-embolic disease.[58]

Virchow's triad comprises the three categories of factors that contribute to thrombo-embolic disease: blood flow, vascular injury and coagulopathy. It is well known that blood flow through extremities varies directly with muscle activity and it therefore follows that sustained inactivity from bed rest promotes venous stasis. Furthermore, compression of veins from contact of limbs with the bed also contributes to stasis and could potentially damage the vascular endothelium. Hence, bed rest is an important risk factor for thrombo-embolic disease.[58]

1.8.7 Pregnancy

The association between pregnancy and VTE has been well documented in the literature.[46] The pathophysiological mechanisms underlying the pregnancy hypercoagulable state involves elevated procoagulant factors, notably FVIII and fibrinogen, with a reduction in natural anticoagulants such as protein S and acquired activated protein C resistance (APCR).

1.8.8 Post surgery period

The risk of thrombosis after surgery varies depending on the type of surgery, underlying patient characteristics and duration of immobilisation.[59] Surgery is a potent inducing factor of acquired hypercoagulability and prophylactic anticoagulation to prevent thrombosis development in the post surgical population has been extensively studied and included in guidelines.[56]

1.8.9 Long distance travel

Any type of travel has the potential to increase the risk of DVT development with the duration being the key factor. Studies have documented that travel by air, car, train or bus for 4 hours or longer all double the risk for several weeks after travel.[59]

Long distance (> 8hrs) travel by air has been more extensively studied than that occurring by road. Although the mechanisms underlying traveller's thrombosis have not been fully elucidated, the World Health Organisation (WHO) has investigated the association between travel and VTE. The conclusion of this investigation confirmed that long distance air travel increases the risk of VTE 2-fold. They further concluded that the aetiological factors involved in traveller's thrombosis are immobility, reduced humidity, hypoxaemia due to the reduced ambient oxygen pressure and dehydration. VTE can also develop after long distance travel by car, bus or train.[60]

1.9 Venous Thrombo-embolism (VTE): Prophylaxis and treatment

Prophylactic anticoagulation with either low molecular weight heparin (LMWH) or unfractionated heparin (UFH) has been proven to be safe and effective in surgical patients and is widely used for the prevention of venous thrombo-embolism (VTE). According to the American College of Chest Physicians (ACCP) guidelines[56, 61], prophylactic anticoagulants should also be prescribed to medical patients who are at high risk of VTE i.e. deep vein thrombosis (DVT) and pulmonary embolism (PE). Scientific evidence of the effectiveness of anticoagulants for the prevention of VTE and reduction of mortality in this latter medical population group is accumulating. Studies have however documented universal low rates of appropriate VTE prophylaxis in at risk medical in-patients despite the presence of guideline recommendations and mounting evidence of its effectiveness.[62-63]

The reasons for the low thromboprophylaxis rates in at-risk medical patients compared with their surgical counterparts remain largely unclear. However, a number of studies have demonstrated the ability to improve thromboprophylaxis rates in this population via education[64-65] and the use of special tools such as electronic alerts to treating physicians.[66]

Recommendations of various VTE prophylaxis and treatment guidelines with respect to VTE prophylaxis in medical in-patients can be summarised as follows:

- All patients should have their VTE risk assessed and addressed upon hospital admission, change in level of care and discharge.
- All patients should have proper education regarding VTE risk, signs and symptoms of VTE and mechanical prophylaxis methods available.
- All patients should be encouraged to ambulate as early as possible and as frequently as possible.
- All non-ambulatory patients should have, at a minimum, mechanical prophylaxis – unless contraindicated.
- All patients with moderate to high risk of VTE should have pharmacologic prophylaxis – unless contraindicated.

VTE risk factors can be divided into predisposing factors (i.e. patient's characteristics) and exposing factors (i.e. some medical conditions, nature of surgical intervention etc). Common VTE risk factors listed in guidelines on the subject include:

- Prior history of VTE
- Active cancer or myeloproliferative disorder
- Extended immobility or estimated length of stay of 4 or more days
- Age greater than 65 years
- Thrombophilia – congenital or acquired
- Congestive heart and acute respiratory failure
- Acute infection

- Inflammatory bowel disease
- Nephrotic syndrome
- Rheumatoid / collagen vascular disorder
- Obesity (body mass index > 30)

1.10 Antiretroviral drugs

The year 2006 marked the 25th anniversary of the initial reports of a catastrophic illness later termed AIDS and also the 10th anniversary of highly active antiretroviral therapy (ART) which revolutionised the treatment of HIV. With this success came the challenges of managing patients on long-term treatment for HIV infection, the development of drug toxicity as well as drug resistance and the ageing HIV-infected population. Despite treatment advances and prevention and education programmes, the number of HIV infected people continues to increase especially in the vulnerable and resource poor populations of the world.[67]

Since the emergence of ARVs, HIV infected patients have demonstrated dramatic decreases in viral burden and opportunistic infections and an overall increase in life expectancy. There are 24 approved antiretroviral drugs targeting various viral proteins or critical points in the virus-host life cycle. These drugs are divided into 5 classes according to their main mechanism of action: the nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase inhibitors and entry inhibitors (EIs). NRTIs usually constitute the mainstay of antiretroviral treatment strategies. The current recommendations are initial combination therapy regimens consisting of 2 NRTIs e.g. zidovudine and stavudine or the less toxic second generation NRTIs tenofovir and abacavir with either 1 or 2 PIs e.g. ritonavir or 1 NNRTI e.g. nevirpine or efavirenz.[40]

Although ARVs have undoubtedly reduced the morbidity and mortality of HIV infection, multiple side effects have been linked to therapy with ARVs. These include peripheral lipo-atrophy, steatohepatitis, lactic acidosis, anaemia, myopathy, pancreatitis and peripheral neuropathy. Clinical and in vitro evidence

also strongly suggests that long-term ARV treatment can results in endothelial toxicity and vascular dysfunction. Although Torriani et al.[41] demonstrated improved endothelial function among treatment-naïve individuals with HIV started on 3 different ARV regimens, there is still a concern about the toxic effects of ARV therapy on endothelial cells (Table 3).

Table 3: Effects of ARVs on endothelial cell homeostasis:[40]

Drug class	Drug Name	Abbreviation	Effect on endothelium
NRTIs	Zidovudine	AZT, ZDV	↓ vasorelaxation
	Stavudine	d4T	↑ ROS
	Didanosine	ddI	↑ vasoconstrictor release
	Zalcitabine	ddC	↑ proliferation
	Abacavir	ABC	↑ permeability
	Emtricitabine	FTC	↓ mitochondrial function
	Lamivudine	3TC	↓ GSH
	Tenofovir	TDF	Not documented
NNRTIs	Nevirapine	NVP	Not documented
	Efavirenz	EFV	Not documented
	Delavirdine	DLV	Not documented
PIs	Ritonavir	RTV	↑ carotid IMT
	Atazanavir	ATV	↓ FMD
	Amprenavir	APV	↔ IMT
	Indinavir	IDV	↔ FMD
	Nelfinavir	NFV	↓ mitochondrial function
	Darunavir	DRV	↑ ROS
	Saquinavir	SQV	↑ mtDNA damage
	Fosamprenavir	FPV	↑ permeability
	Tipranavir	TPV	↓ vasorelaxation
	Lopinavir	LPV	↓ eNOS
Integrase Inhibitor	Raltegravir	RAL	Not documented
EIs	Enfuvirtide	ENF	Not documented
	Maraviroc	MVC	Not documented

↑: increase; ↓: decrease; ↔: no change; GSH: glutathione; HDLc: high-density lipoprotein cholesterol; ROS: reactive oxygen species; IMT: intima media thickness; FMD: flow-mediated dilation; eNOS: endothelial nitric oxide synthase; mtDNA: mitochondrial DNA.

Glucose intolerance and dyslipidemia have been documented to occur in patients treated with protease inhibitors (PIs). A 16% increased risk of myocardial infarction for each year of exposure to PIs has also been documented.[68] Previous reports have linked the use of PI containing ARV regimens to a hypercoagulable state. In a study by Koppel et al.[69] plasminogen activator inhibitor 1 (PAI-1) and fibrinogen levels were found to be significantly higher in patients receiving PI-containing ARV regimens. In addition, the increased plasma lipid levels and the lipodystrophy syndrome could play a procoagulant role. In a review article in 2004, Shen et al.[70] came to the conclusion that, based on epidemiological studies, a hypercoagulable state and associated venous thromboses were emerging as clinical issues in HIV infected patients with the use of PIs being particularly implicated.

The effects of the different classes of ARVs on endothelial function have also been studied and the results are summarised in Table 3. Clinical evidence for NRTI-induced vascular / endothelial toxicity is indirect and difficult to define because NRTIs are not prescribed as monotherapy. In addition, most experimental in vitro studies examining mitochondrial toxicity of NRTIs are conducted in liver cell lines due to their endogenous abundant mitochondria, the liver's involvement in NRTI metabolism and the documented hepatic dysfunction encountered in patients on NRTI treatment. Although in vitro studies linking NRTIs to endothelial cell-specific toxicity are therefore not as numerous, evidence does support a role for NRTIs in endothelial dysfunction. Aortas from mice treated with AZT, have been demonstrated to have reduced maximum endothelium-dependent relaxation. Future investigation into the multiple direct and indirect effects of NRTIs on mitochondrial gene expression and reactive oxygen species generation are indicated to accurately determine how this important class of drugs exerts its toxic effects on the endothelium.[40]

In contrast to the NRTIs, there are even fewer in vitro studies examining endothelial toxicity in response to NNRTI treatment. In fact, clinical studies on the cardiovascular effects of NNRTIs have demonstrated overall atherosclerotic

protective effect when switching to a NNRTI-based ARV regimen. This positive result relates to the improved lipid profiles and reduced blood pressure on NNRTI treatment.[40]

Controversy exists regarding PI-based therapy and cardiovascular risk. Discrepant results have been obtained when studying the correlation between PI exposure and endothelial dysfunction as evidenced by increased intima media thickness (IMT) of the carotid bifurcation and decreased flow mediated dilatation (FMD) of the brachial artery. Various explanations exist for these discrepant findings but despite these contradicting reports, long-term treatment with PIs has generally been associated with a number of cardiovascular side effects including a greater incidence of diabetes mellitus, lipodystrophy, hyperlipidemia and atherosclerosis with an increased risk of myocardial infarction. The molecular mechanisms of PI toxicity in endothelial cells probably involve mitochondrial disturbances as evidenced by reduced cellular respiration and ATP production, decreased mitochondrial membrane potential, increased mitochondrial production of ROS, altered nitric oxide (NO) metabolism and mitochondrial DNA damage. PIs also increase endothelial cell permeability and leukocyte adhesion in cell culture models. PI effects on non-endothelial cells may also contribute to cardiovascular risk. For example, PIs dysregulate fat cell homeostasis which may explain the high incidence of lipodystrophy and hyperlipidemia in HIV patients receiving PI based ARV regimens. Besides adipocytes, macrophages are also extra-endothelial targets of PI toxicity. PIs, most notably ritonavir, cause accumulation of cholesterol in macrophages by activating the unfolded protein response and by up-regulating the scavenger receptor CD36. PIs also increase expression of the pro-inflammatory cytokines TNF-α and IL-6. Thus, PI-mediated changes in gene expression, activation of cell signalling pathways and oxidative stress in endothelial and non-endothelial cells can negatively impact cardiovascular health.[40]

1.11 Conclusion

Given all the above evidence of the presence of a hypercoagulable state in HIV infection, Klein SK et al. [2] reviewed the evidence of a link between HIV infections and venous thrombotic disease. The authors of this systematic review found that there is limited evidence coupling these two conditions. Ten relevant epidemiological studies of venous thrombotic disease in HIV infections were identified. These studies were however retrospective cohort studies prone to selection bias with confounding variables not always mentioned and only three of the ten studies included a control group. Their conclusion was that although some evidence pointed towards a relationship between HIV infection and venous thrombotic disease, more studies were indicated to further elucidate this link.

Lijfering WM et al.[11] investigated 109 consecutive HIV-infected patients with a median follow-up period of 5 years and reconfirmed the presence of the prothrombotic factors discussed above with worsening of these parameters as HIV infection progressed to AIDS. Cytokines may play an important role in the worsening prothrombotic state as HIV progresses since the same cytokines which activate the coagulation system have been described to increase in concentration as HIV infection progresses. In addition, a clearly increased incidence of both venous and arterial thromboses was demonstrated as compared to the incidence in an HIV negative cohort. The annual incidence of venous (1.61%) and arterial thrombosis (0.87%) were 5- to 16-fold and 2- to 8-fold higher respectively compared with a healthy HIV uninfected cohort (i.e. 0.1% – 0.3% and 0.1% - 0.4%). The median age at onset of venous and arterial thromboses was also earlier in the HIV positive cohort. This study concluded that the results do suggest that HIV infected patients are at higher risk of thromboses but that further research was needed.

The vast majority of the studies reporting on the documented prothrombotic abnormalities in HIV infection as summarised in Table 4 were conducted in first world cohorts. Differences in HIV infection and its consequences between the first and third world do exist and is discussed further in section 5.1.

Table 4: Comparison of the prevalence of prothrombotic risk factors between HIV infected and uninfected individuals:[11, 71]

Category of prothrombotic abnormality	Specific abnormality	Reported frequency of congenital / acquired deficiency in the general population	Reported frequency in HIV positive population
Deficiency of anticoagulants	Protein S deficiency	Congenital: <0.5%	20 – 80%
	Protein C deficiency	Congenital: 0.2 – 0.4% (1.5% 1st VTEs)	14%
	Antithrombin deficiency	Congenital: 0.2%	??
	Heparin cofactor II deficiency	??	??
Presence of specific prothrombotics	Antiphospholipid antibodies	1-5%	70-90%
	Increased FVIII	Congenital: 10%	41%

2. Study objective

The objective of this cross-sectional study was to determine the prevalence of underlying HIV infection in patients presenting with acute deep vein thrombosis (DVT) without the presence of traditional risk factors for DVT. Presence of DVT risk factors including underlying cancer, prolonged bed rest (>4 days), pregnancy, infections (particularly tuberculosis), recent (within last month) surgery / trauma, advanced age (>65 years) and long distance travel (> 8 hrs) were sought. DVT risk factor determination was based on clinical patient assessment including history and examination. No laboratory testing for thrombophilia was performed.

The HIV prevalence in the cohort of patients with acute DVTs was compared to the HIV prevalence in a sex, age and race matched control group without symptomatic DVT.

This was a pilot study with an anticipated prevalence of 80% from observations made in the past.

3. Study populations, materials and methods

3.1 Case selection

Consecutive male and female adult patients presenting to the Charlotte Maxeke hospital casualty with lower limb acute deep vein thrombosis (DVT) confirmed on venous Doppler ultrasound were invited to participate in this cross sectional study. Eligibility criteria were as follow:

- Community integrated members of the society i.e. out of hospital patients presenting with acute DVTs
- Adult patients over the age of 18yrs
- Male and female patients

Voluntary HIV testing of the participants were performed after counselling and consent with appropriate referral for further management if HIV result was positive.

The presence of commonly encountered traditional risk factors for the development of DVTs i.e. underlying cancer, prolonged bed rest (>4 days), pregnancy, infections (particularly tuberculosis), recent (within last month) surgery / trauma, advanced age (>65 years) and long distance travel (> 8 hrs) was ascertained on patient history and examination.

3.2 Control selection

The control group was an age, sex and race matched cohort consisting of laboratory personnel not subordinate to the investigator. The prevalence of HIV infection in this matched population without symptomatic DVTs was determined. Exclusion of DVTs in the control population was based on the absence of classic DVT signs such as unilateral swelling, pain and redness of a limb.

3.3 Statistical analysis

The University of the Witwatersrand Epidemiology Data Centre assisted with analysis of the data.

3.3.1 Sample Size:

A sample size of 25 subjects with acute DVTs was deemed adequate by Professor P Becker of the Department of statistics of the Medical Research Council (MRC) to estimate the expected HIV prevalence of 80% to an accuracy within 15% of the 95% confidence level.

A sample size of 24 control subjects without DVTs was deemed adequate to estimate the expected HIV prevalence in the control group in this pilot study and to guide future research in this field.

3.3.2 Data analysis:

The prevalence of HIV is expressed as a percentage along with a 95% confidence interval (CI).

3.3.3 Calculation of prevalence:

Prevalence is a measure of how commonly a disease or condition occurs in a population at a particular point in time. The prevalence was calculated by dividing the number of subjects with the disease / condition at a particular time point by the total number of individuals examined. Prevalence was expressed as a percentage, calculated by multiplying the ratio by 100.

3. 4 Ethics

Ethics approval was obtained from the Human Research Committee of the faculty of Health Sciences, University of the Witwatersrand (protocol number M051029) and approval was unconditional. Both patients and controls were invited to participate in the study and verbal informed consent was obtained from both groups. The study was conducted over a 24 month period between 2005 and 2007.

4. Results

The demographics and clinical details of the patients presenting with acute DVTs are detailed in Table 5 and 6. Table 7 reflects the details of the control cohort i.e. a matched population without clinical evidence of DVTs.

Table 5: Demographics and clinical details of DVT patients (arranged per age):

No:	Sex	Age (yrs)	Race	HIV Status	CD4 ($x10^6$/L)	Position of DVT	Traditional DVT risk factors
1	F	22	Black	+	ND	AK DVT	
2	F	23	Black	+	190	AK DVT	
3	F	25	Black	+	23	AK DVT	PTB on Rx / immobile
4	M	26	Black	+	199	AK DVT	PTB not on Rx
5	M	27	Black	+	74	AK DVT	
6	F	27	Black	-	ND	AK DVT	PTB on Rx
7	F	28	Black	+	197	AK DVT	PTB on Rx / immobile
8	F	28	Black	+	800	AK DVT	
9	M	29	Black	ND	ND	AK DVT	PTB not on Rx
10	F	30	Black	+	98	AK DVT	
11	M	31	Black	+	339	AK DVT	
12	F	33	Black	+	52	AK DVT	
13	M	33	Black	+	105	AK DVT	PTB not on Rx
14	F	35	Black	+	582	AK DVT	
15	M	37	Black	+	272	Popliteal	
16	F	37	Indian	ND	ND	AK DVT	SLE arthritis / immobile
17	F	38	Black	+	225	AK DVT	
18	F	39	Black	+	178	AK DVT	
19	M	39	Black	+	151	AK DVT	
20	F	40	Black	+	264	AK DVT	
21	F	40	Black	+	21	AK DVT	
22	F	40	Black	+	378	AK DVT	
23	M	42	Black	ND	ND	AK DVT	
24	F	44	Black	+	172	AK DVT	
25	M	45	Black	+	451	AK DVT	
26	F	45	Black	+	155	AK DVT	
27	M	45	Black	+	810	AK DVT	
28	M	47	Black	+	43	AK DVT	
29	F	47	Black	+	170	AK DVT	Carcinoma of cervix
30	M	50	Black	-	ND	AK DVT	Fractured ankle /
31	F	51	Black	+	273	AK DVT	
32	F	52	White	ND	ND	AK DVT	
33	F	52	White	-	ND	Bilateral	
34	M	53	Black	-	ND	Bilateral	
35	M	56	Black	+	136	Popliteal	
36	F	57	Black	ND	ND	AK DVT	Ca cervix / immobile
37	M	59	White	-	ND	AK DVT	
38	M	64	White	ND	ND	Popliteal	Previous DVT
39	M	65	Coloured	ND	ND	AK DVT	Age > 65 yrs
40	F	66	White	ND	ND	AK DVT	Age > 65 yrs
41	M	68	White	ND	ND	AK DVT	Age > 65 yrs
42	F	68	Black	ND	ND	AK DVT	Age > 65 yrs
43	F	69	White	ND	ND	Popliteal	Ca vulva />65/immobile
44	F	72	Black	ND	ND	AK DVT	Age > 65 yrs
45	F	74	White	ND	ND	Popliteal	Injury/>
46	F	76	White	ND	ND	AK DVT	Age > 65 yrs
47	F	78	Black	ND	ND	AK DVT	Age > 65 yrs
48	M	79	Black	ND	ND	AK DVT	Age > 65 yrs /

F: Female; M: Male; +: positive; -: negative; ND: Not done; AK: Above knee;
PTB: pulmonary tuberculosis; SLE: systemic lupus erythematosus; 0: No risk factor identified

Table 6: Demographics and clinical details of DVT patients without traditional risk factors for DVT and who consented to HIV testing:

No:	Sex	Age (yrs)	Race	HIV Status	CD4 ($x10^6$/L)	Position of DVT
1	F	22	Black	+	ND	AK DVT
2	F	23	Black	+	190	AK DVT
3	M	27	Black	+	74	AK DVT
4	F	28	Black	+	800	AK DVT
5	F	30	Black	+	98	AK DVT
6	M	31	Black	+	339	AK DVT
7	F	33	Black	+	52	AK DVT
8	F	35	Black	+	582	AK DVT
9	M	37	Black	+	272	Popliteal
10	F	38	Black	+	225	AK DVT
11	F	39	Black	+	178	AK DVT
12	M	39	Black	+	151	AK DVT
13	F	40	Black	+	264	AK DVT
14	F	40	Black	+	21	AK DVT
15	F	40	Black	+	378	AK DVT
16	F	44	Black	+	172	AK DVT
17	M	45	Black	+	451	AK DVT
18	F	45	Black	+	155	AK DVT
19	M	45	Black	+	810	AK DVT
20	M	47	Black	+	43	AK DVT
21	F	51	Black	+	273	AK DVT
22	F	52	White	-	ND	Bilateral
23	M	53	Black	-	ND	Bilateral
24	M	56	Black	+	136	Popliteal
25	M	59	White	-	ND	AK DVT

F: Female; M: Male; +: positive; -: negative; ND: Not Done AK: Above knee

A total of 48 consecutive community integrated out-patients presenting to the Charlotte Maxeke hospital medical casualty with acute lower limb DVTs were enrolled in the study (Table 5).

The HIV prevalence in the deep vein thrombosis (DVT) group who consented to HIV testing i.e. 25 patients and who had no traditional risk factor for DVT development was 81% (95% CI 0.67 - 0.96) (Table 6). All the DVT patients who consented to HIV testing were active, community integrated members of the society.

The average CD4 cell count of the HIV positive patients with acute DVTs was 247 /mm^3 with a range of 21 to 810.

Two of the HIV positive patients presenting with DVTs were on ART (anti-retroviral therapy) at the time of developing a DVT.

Six of the patients presenting with acute DVTs were also diagnosed as suffering from pulmonary tuberculosis with 3 patients receiving anti-tuberculosis therapy at the time of presentation. HIV testing was done on 5 of the 6 patients and 4 of them tested HIV positive. The average CD4 count of these 4 patients was 131/mm^3.

Traditional DVT risk factors identified in the acute DVT cohort other than tuberculosis were immobilisation (1 patient), trauma (2 patients), carcinoma (2 patients), previous DVT (1 patient) and age over 65 years (10 patients).

In a matched control group the HIV prevalence was found to be 4% (95% CI 0.039 – 0.041). The control group consisted of healthy, active adults without symptoms or signs suggestive of DVT (Table 7):

Table 7: Demographics of control cohort (arranged according to age):

Number	Sex	Age (yrs)	Race	HIV result
1	M	20	Black	-
2	F	21	Black	-
3	M	21	Black	-
4	F	22	Black	-
5	M	23	Black	-
6	F	23	Black	-
7	F	24	Black	+
8	M	26	Coloured	-
9	F	28	Black	-
10	M	32	White	-
11	M	32	Black	-
12	F	33	Black	-
13	M	34	Black	-
14	M	36	Black	-
15	F	36	Coloured	-
16	F	36	Black	-
17	F	37	Black	-
18	M	38	White	-
19	M	42	Black	-
20	F	46	Black	-
21	F	48	Black	-
22	F	52	White	-
23	F	58	White	-
24	F	60	Black	-

F: Female; M: Male; +: positive; -: negative.

Table 8: Comparison of demographics of DVT patients without traditional risk factors and control cohort:

Demographic parameter	DVT patients (consented to HIV testing and no traditional DVT risk factors)	Control cohort (No symptoms of DVTs)
Number of subjects	25	24
Average Age (Age range)	39 (22 – 59)	36 (20 – 60)
Sex	Females: 15 Males: 10	Females: 14 Males: 10
Race	Black: 23 (92%) White: 2 (8%) Coloured: 0	Black: 19 (79%) White: 3 (12.6%) Coloured: 2 (8.3%)

The prevalence of HIV infection in the population with DVTs who consented to HIV testing of 81% (95% CI 0.67 - 0.96) was therefore significantly higher than that in the control group with a HIV prevalence of 4% (95% CI 0.039 – 0.041).

According to the South African National HIV Prevalence, HIV Incidence, Behaviour and Communication Survey commissioned by the Nelson Mandela Foundation in 2005, the estimated prevalence of HIV infection in the general South African population above the age of 2 years was 10%. Again the HIV prevalence in the DVT patient cohort was significantly higher than that in the general population i.e. 81% vs. 10%. Although the DVT patient group was not completely representative of the general South African population as far as age was concerned, other demographics were similar and the prevalence of HIV infection in these 2 groups can also be compared.

5. Discussion

5.1 Worldwide differences in the HIV epidemic

The HIV epidemics in the first and the third world differ in the prevalence of HIV subtypes. There also are documented differences in host susceptibility, co-morbidities and disease manifestations between race and socio-economic groups. Published studies on the prevalence, underlying cause and prognosis of DVTs in HIV infection have largely been performed in the first world in mainly Caucasian populations.[7, 72]

In this study, twenty two (22) of the twenty five (25) DVT patients with no traditional DVT risk factors and who consented to HIV testing were found to be HIV positive. All these patients were of African descent.

As mentioned above, published studies on the prevalence, underlying cause and prognosis of DVTs in HIV infection have largely been performed in the first world in Caucasian populations and further research is needed in sub-Saharan Africa. The current study therefore is a pilot study to direct future studies in this regard.

5.2 The HIV prothrombotic state and role of ARVs

5.2.1 Documented prothrombotic abnormalities in HIV infection

Section 1 of this dissertation details the various abnormalities predisposing to a hypercoagulable state in patients with HIV infection. In summary, these include deficiencies in anticoagulant factors such as protein S and C, antithrombin and Heparin-cofactor II as well as an increase in or presence of procoagulant factors such as antiphospholipid antibodies (Lupus Anticoagulant and anticardiolipin antibodies) and FVIII / vWF (Table 4). Endothelial cell activation and presence of microparticles may also contribute. Concurrent malignancies and secondary infections worsen the procoagulant profile as would the presence of traditional DVT risk factors such as immobilisation, pregnancy advanced age and long distance travel.[3]

5.2.2 Prothrombotic profile with HIV disease progression

The average CD4 cell count of the HIV positive patients with acute DVTs who consented to HIV testing in this study was 247 x 10^6 cells per liter. The average CD4 count in this cohort was therefore slightly higher than that reported in the literature in HIV patients presenting with thromboses. Klein et al.[2] in their review in 2005 for example demonstrated the risk of DVT to be increased in HIV positive patients with AIDS or with CD4 counts below 200 x10^6 per liter.

Progression of HIV infection occurs along a wide spectrum. At the extremes of this spectrum some patients rapidly progress to clinical disease, while others behave as the poorly understood long-term non-progressors. In between these two poles, lies the majority of the infected population.[73-74] Anti-retroviral therapy (ART), where freely available, has significantly transformed infection with HIV from a terminal, life-threatening illness to a chronic manageable disease. The natural history of treated HIV infection and the long-term efficacy of antiretroviral agents remain however unknown. The entire spectrum of short and long-term ART-related side effects also needs to be elucidated.

Various studies[3, 11, 24, 75-77] have documented worsening coagulation profiles with progression of HIV infection. Median factor VIII concentrations were found to be higher in patients with AIDS (CD4 cell counts < 200 x 10^6 per liter) than in patients with a non–AIDS-defining illness. Median free protein S concentrations were lower in the first group. These changes correlated with increasing fibrinogen concentrations.[11] The worsening laboratory parameters translated into higher risks of thrombosis in HIV positive patients with AIDS versus infected patients without AIDS defining disease processes.[19, 78-79]

The aetiological link between HIV disease progression and the worsening prothrombotic state is largely unknown but a link between infection and thrombosis via endothelial activation has been suggested. Changes in the cytokine profile coupled with increases in acute phase reactants and opportunistic

infections probably also play a role.[35, 80] The same cytokines responsible for endothelial activation are also incrementally up-regulated during the course of HIV infection.[15, 81] The pro-inflammatory cytokines implicated in this process include tumor necrosis factor-α, interleukin-1 and interleukin-6. Not only do they have an effect on the inflammatory process but they also activate coagulation and down-regulate the production of fibrinolytic proteins.[82-83]

As HIV infection progresses, there is a concomitant progression of laboratory abnormalities consisting of increased concentrations of procoagulant proteins and decreased concentrations of anticoagulant proteins. This increasing procoagulant profile results in an increased incidence of venous and arterial thrombosis.[11, 84-88]

The procoagulant proteins which have been implicated are FVIII and fibrinogen and they are both acute phase reactants.[84-85, 87] As far as deficiencies of anticoagulants are concerned, low concentrations of protein C have been reported in various infections including HIV infection. Decreased protein C levels during infection possibly related consumption during its action as an anti-inflammatory mediator. Although inherited protein C deficiency has been demonstrated to be a strong risk factor for venous thrombosis, the risk associated with acquired protein C deficiency has not been as well documented. Nonetheless, protein C deficiency also worsens with HIV disease progression.[89]

Normally 60% of protein S is bound to C4BP and it is only the remaining approximately 40% of this anticoagulant protein which is physiologically optimally active as an anti-coagulant.[90] During active infection, the concentration of C4BP increases to up to 400% of its normal baseline concentration. Various studies have documented concentrations of protein S to be decreased in HIV-infected patients with the levels worsening as HIV infection advances.[13-14, 24, 91]

In conclusion, there are multiple acquired and usually persistent thrombophilic abnormalities present in HIV-infected patients. The frequency and severity of

these thrombophilic abnormalities increase with the progression to AIDS and probably contribute to the high prevalence of venous and arterial thrombosis in HIV-infected patients, especially in advanced disease.[11, 24]

5.2.3 Antiretroviral drugs (ARVs) as prothrombotic factors

Only 6 of the 26 HIV infected patients presenting with DVTs in the current cohort were receiving ARVs at the time of presenting with a DVT. This figure represents 23% of the DVT cohort that tested positive for HIV and 42% of the patients eligible for ARVs i.e. patients with CD4 counts below 200 $x10^6$ per liter. These figures probably reflect the inaccessibility of ARVs in South Africa. Inadequate public awareness as well as a lack of participation in HIV voluntary counselling and testing programmes probably also contribute.

Although the benefits of ARV therapy are beyond dispute, further exploration into the precise mechanisms of ARV drug toxicity is warranted. Since both the hypercoagulable state and endothelial dysfunction in HIV infected individuals are most likely multifactorial processes, the exact contributions by the viral infection itself and the drugs used to treat it, are needed.

5.3 The TB prothrombotic state

In the current cohort of 48 patients presenting with acute DVTs, 3 patients were on treatment for pulmonary TB at the time of presenting with a DVT. An additional 3 of the DVT cohort were subsequently diagnosed with pulmonary TB. Four of the 6 patients with tuberculosis tested positive for infection with HIV.

In a retrospective analysis in a South African hospital in the mid 1980s, White et al.[92] found DVTs in 3.4% of TB patients within the first two weeks after initiation of anti-tuberculosis therapy. The prevalence rate of DVTs occurring in patients receiving rifampicin as part of their ant-TB treatment regimen was increased by approximately 5%.

TB can cause thrombosis by various mechanisms such as local vascular invasion, venous compression by enlarged lymph nodes or by producing a transitory hypercoagulable state. Robson et al.[93] suggested that elevated plasma fibrinogen and factor VIII, impaired fibrinolysis coupled with decreased levels of antithrombin and reactive thrombocytosis appeared to favour the development of DVT in pulmonary TB. There is also data supporting a relationship between the prothrombotic state in TB infections and a presence of antiphospholipid antibodies and protein S deficiency present during this infection. The protein S deficiency is secondary to decreased liver production and increased levels of C4-BP reducing the free fraction of protein S.

Vascular endothelium may be primed to become prothrombotic by mycobacterial products. The host monocyte–macrophage system is induced to synthesise large amounts of cytokines including tumour necrosis factor alpha (TNFα) and interleukin-6. Cytokine production further increases during bacterial death related to anti-tuberculosis therapy. These cytokines block the protein C anticoagulant pathway and induce tissue factor (TF) production on endothelium and monocytes. Interleukin 6 also stimulates new platelet formation with these platelets displaying increased sensitivity to thrombin activation and increased pro-coagulant activity.[49]

The resultant procoagulant state during TB infection improves during the first month of therapy and therefore prophylactic anticoagulation therapy should always be considered at initiation of anti-tuberculosis therapy and for the first 3-4 weeks thereafter. Dose adjustment of anticoagulant drugs may also be necessary to achieve therapeutic and prophylactic anticoagulation levels because of the induction of the drug metabolising enzyme, cytochrome P450, by rifampicin, a cornerstone anti-tuberculosis drug. In addition, this drug may also contribute to the hypercoagulable state by decreasing production and increasing clearance of anticoagulant proteins i.e. antithrombin, protein S and C.[94] Patients receiving anti-tuberculosis therapy require frequent INR monitoring and increased warfarin doses are often needed to maintain a therapeutic warfarin effect. Abruptly

stopping rifampicin could be potentially hazardous resulting in over anticoagulation. Full induction of drug-metabolising enzymes is reached about 1 week after starting rifampicin and the induction dissipates roughly 2 weeks after discontinuing this anti-tuberculosis drug.[49]

In conclusion, an association between the inflammation induced by TB and a hypercoagulable state has been described. Therefore, the occurrence of DVT or pulmonary embolism should be considered in patients with TB and suggestive symptoms especially during the first weeks of treatment. Early diagnostic suspicion of VTE is important to ensure appropriate diagnosis and prompt treatment in order to prevent fatal outcomes. A high index of suspicion is needed in patients that are thought to be responding poorly to anti-tuberculosis treatment and have other predisposing factors, such as concomitant HIV infection. Patients who need prolonged in-hospital admission should be carefully monitored and the use of prophylactic anticoagulation should be considered.

5.4 HIV voluntary counselling and testing (VCT)

In the DVT cohort reported on here, 16% of patients under the age of 65 years refused HIV testing after counselling. A similar proportion of eligible people without DVTs approached to participate in the control arm of the study refused to participate when informed that VCT for HIV would be required.

Various studies[95-97] have investigated participation in VCT programmes. These studies concluded that the fear of receiving HIV-positive results make individuals postpone testing, resulting in inappropriate delays in seeking treatment. This has resulted in patients presenting in advanced disease stages.

Uganda is one of the countries in Africa with relative success in the HIV/ AIDS prevention arena. The HIV/AIDS epidemic in Uganda developed silently and took advantage of the war ravaged healthcare services leaving over 50% of the

population without primary healthcare in the early 1980s. The HIV prevalence increased to an estimated 15% in the mid 1990's. With VCT and a campaign termed ABC (Abstinence, Be faithful and Condomise) the trend turned sharply downward to 5% in the 2003. Stigma however remains an important role player even in a country such as Uganda where roughly half the people surveyed in 2005 indicated that if a family member contracted HIV they would prefer to keep it secret.[98] Stigmatisation suffered by people living with HIV/AIDS can arise from the patient themselves, from the family and can also have its origin in the larger community with men viewed as the traditional breadwinners having their positions in society most threatened. Recent studies show transport fares, denial of HIV-positive results and marital dissolution to be additional barriers to VCT.[99-100]

Living with HIV/AIDS carries not only stigma but also the emotional strain of being unable to provide and care for dependents and fear of pain and suffering. Nyanzi-Wakholi et al.[98] reported that many women indicated the desire to end intimate sexual relationships on testing HIV-positive whilst men in similar situations indicated the need to affirm their relationships so as to have a caretaker.

While the role of VCT in curbing the HIV/AIDS epidemic remains debatable, research has demonstrated that VCT not only informs individuals of their HIV sero-status but personalises the risk of infection and encourages treatment seeking.[99-101]

Nyanzi-Wakholi et al.[98] reported that men were especially reluctant to undergo VCT and preferred to postpone VCT until they were evidently sick. They acknowledged preferring to live in doubt of their status rather than living with the certainty of being HIV infected. Most women reported deciding to undergo VCT after they or their partners and / or children developed HIV/AIDS-related illnesses. Individuals in their study expressed fear that they will commit suicides if they were to discover that they were HIV positive. Access to free care from

HIV/AIDS support organisations for patients with a positive result did however serve as an incentive to undergo testing. Various studies[102-104] emphasise the crucial role of pre-and post-test counselling and informed support groups in enabling people testing HIV-positive to understand and accept their status, adhere to treatment regimens and live positively.

5.5 Anticoagulation in the HIV infected patient

VTE prophylaxis with both low molecular weight heparin (LMWH) and unfractionated heparin (UFH) have been proven to be safe and effective in medical in-patients at risk of VTE.[61-62, 105-106] LMWH has several important advantages over UFH when used for thromboprophylaxis including less adverse side-effects like major bleeding and heparin-induced thrombocytopenia.[107-109] It also offers more convenience with once-daily dosing in pre-filled syringes. It is however more costly.

These advantages are even more important in the HIV positive patients who often present with thrombocytopenia relating to HIV induced autoimmune peripheral platelet destruction. In addition, once daily administration of LMWH decreases the exposure of nursing staff to contaminated needles. Although LMWH costs more per dose, the total hospital cost has been shown to be lower and may be related to fewer major bleeding episodes occurring in patients receiving LMWH as opposed to UFH.[110]

5.6 Traditional risk factors for DVT development

This dissertation aims to explore the prevalence of HIV infection in newly diagnosed DVT patients without any of the traditional risk factors for the development of DVT. However traditional risk factors for DVT development were present in both DVT patients who tested positive and negative for HIV infection and in those refusing HIV testing. The pathophysiologic mechanisms involved in the most common traditional risk factors for DVT development are therefore discussed.

5.6.1 Underlying malignancy

One of the HIV positive patients in this cohort presenting with a DVT was on treatment for cervical cancer, which is considered to be an AIDS defining illness. One patient in the cohort with acute DVT had underlying carcinoma of the cervix and another of the vulva but the HIV status of both these patients was not determined. The extent to which the patients in this cohort presenting with DVTs were investigated to exclude underlying malignancy is also not known.

Neoplastic diseases clearly seen with an increased frequency in patients with HIV infection are Kaposi sarcoma and non-Hodgkin's lymphoma, the latter also being and AIDS defining illness. Cervical carcinoma is also an AIDS defining disease process. In addition, there also appears to be an increased incidence of Hodgkin's disease, multiple myeloma, leukaemia, melanoma and oral, lung and anal carcinoma in HIV infected ptaients.[67]

It is therefore clear that the mechanisms underlying cancer thrombosis share many similarities with the HIV thrombotic state and therefore co-existence of these disease processes poses a significant thrombosis risk. Appropriate and safe use of anticoagulation therapy, whether therapeutic or prophylactic, in the HIV positive cancer patient can be complicated by the presence of thrombocytopenia which is frequently present in HIV patients. Thrombocytopenia can also be worsened by certain malignant processes and chemotherapy. The conclusion of a recent study involving 1 514 in-patients post bone marrow transplantation demonstrating that DVTs occurred with regular frequency in patients with platelet counts below 50×10^9 per liter.[54]

5.6.2 Immobilisation / Bed rest

In the current cohort of patients with acute DVTs described here, 8 of the 48 patients (16%) admitted to significant bed rest immediately prior to their presentation. All these patients also suffered from other DVT risk factors including tuberculosis, arthritis, carcinoma and age greater than 65 years.

The current study aimed to explore the causal relationship between HIV infection and DVTs and therefore the presence of confounding factors such as prolonged bed rest was explored. A history of bed rest was present in 8 of the total cohort of patients presenting with acute DVTs and 3 of these patients also had underlying HIV infection. The reason for bed rest included musculoskeletal injuries, carcinoma and tuberculosis. The remainder of the patients with DVTs and documented HIV infection i.e. 22 patients, were community integrated active members of society at the time of developing a DVT and did not have significant histories of bed rest.

5.6.3 Pregnancy

All the female patients of reproductive age in this cohort of DVT patients underwent a pregnancy test and none were found to be pregnant.

The concurrence of HIV infection and pregnancy can be expected to operate synergistically in creating a prothrombotic environment.

5.6.4 Post surgery period

None of the patients in the HIV positive DVT cohort had undergone surgery within the 6 months preceding development of a DVT.

5.6.5 Advanced age

The average age of the HIV positive patients presenting with a DVT was 44 years with the age range being 22 – 56 years. Therefore, although increasing age is a well known risk factor for the development of VTE, especially beyond the age of 65 years, it was not a contributory DVT risk factor in the HIV positive cohort.

5.6.6 Long distance travel

None of the patients in this DVT cohort were exposed to long distance travel of 4 hours or more, within the month preceding the onset of DVT.

Again it can be expected that underlying HIV infection and long distance travel will operate synergistically to create a hypercoagulable state.

5.7 Venous thrombo-embolism (VTE) prophylaxis and treatment guidelines

Although all the patients in the DVT cohort discussed in this dissertation were outpatients, many HIV infected individuals are admitted to hospital during the course of their diagnosis and management.

Infection with HIV is not frequently recognised as an additive risk factor for VTE in both surgical and medical patients. A search of the Medline database found the South African version of these guidelines published in 2008[111] to be the only one to list HIV infection as a risk factor for the development of VTE. This guideline classifies infection with HIV as imparting a high VTE risk. The 9 contains a sample of the guidelines reviewed.

Table 9: Thromboprophylaxis guidelines: HIV infection as a risk for VTE:

Source	Year published	HIV included as a VTE risk factor
American College of Chest Physicians (ACCP)[61,56]	2008	No
American Society of Clinical Oncology (ASCO) [112]	2007	No
American Academy of Orthopaedic Surgeons (AAOS) [113]	2008	No
National Institute of Clinical Excellence (NICE) (UK) [114]	2009	No
Australian Government National Health and Medical Research Council (NHMRC) [115]	2003	No
Southern Africa Society of Thrombosis and Haemostasis (SASTH) [111]	2009	Yes

The absence of HIV in the list of VTE risk factors to be considered in medical in-patients probably reflects the prevalence of HIV in these predominantly first world countries.

Underlying HIV infection has also been excluded from studies attempting to elucidate effectiveness of thromboprophylaxis in medically ill patients. In a retrospective study titled "Thromboprophylaxis in medically ill patients at risk for venous thrombo-embolism" Burleigh et.al.[116] included over 12 million patients from the Solucient's ACTracker Inpatient Database between 2001 and 2004. All these patients had indications for thromboprophylaxis and rates of

thromboprophylaxis and mortality were compared between groups. The results demonstrated a significantly lower risk-adjusted mortality rate ($p < 0.001$) in the more than 2 million medical patients with indications for thromboprophylaxis who received the appropriate prophylaxis compared to those who did not. Patients with HIV infection were however excluded from the study. Unfortunately no reason was provided for this exclusion but it may have related to the frequent underlying haematological abnormalities detected in association with HIV infection as one of the other exclusions are given as "blood disorders".

5.8 Bilateral DVTs

The prevalence of bilateral DVT in patients with single limb symptoms has been previously investigated and the results reported are variable but incidences reaching 32 % have been documented.[117-119]

Casella et al.[120] studied the prevalence of DVTs in 157 in-patients with clinical suspicion of DVT. Following duplex Doppler scanning, the presence of DVT was confirmed in 57 (36.3%) of the patients with 11 patients (19.3% of all DVTs) having bilateral thromboses. No isolated DVT contra-lateral to the symptomatic limb was observed. Risk factors for DVT development studied were age, sex, prolonged immobilisation, chronic venous occlusive disease, stroke, lower limb trauma, post operative state (up to 30 days), hormonal therapy, pregnancy and active HIV infection. In addition, the presence of cancer and thrombophilia were also recorded but no active search for these 2 risk factors was embarked upon by the study investigators. Active HIV infection and ileo-femoral thrombosis (i.e. proximal thromboses) were identified as risk factors for the development of bilateral DVTs. The association between active HIV disease and acute DVT of both limbs showed a relative risk of 3.9 (range of 1.5-10.17) for bilateral disease in comparison with non-HIV patients with DVT symptoms. The authors also recommended that patients with positive duplex findings of DVT extending to the common femoral veins in the symptomatic limb should undergo examination of the contra-lateral side.

Our study did however not confirm the findings of Casella et al.[120]. All the patients in the current cohort detailed in this dissertation underwent bilateral lower limb duplex Doppler examination with 2 of the patients having bilateral DVTs. Both of these patients consented to HIV testing and both tested negative for infection with HIV. Proximal DVTs i.e. ileo-femoral thromboses were present in both these patients.

Doubt still remains as to the cost-benefit ratio of performing bilateral Doppler studies in suspected DVTs with studies concluding both in favour of and against bilateral scanning. Studies by Naidich[121], Lohr[122] and Prandoni[123] for example concluded that the high prevalence of bilateral thromboses warrants bilateral scanning whereas other authors[124-128] concluded that the adoption of routine bilateral scanning would increase costs without providing benefit or result in changed treatment.

Casella et al.[120] estimated the sensitivity and specificity of clinical examination in identifying bilateral thromboses to be 27.2% and 93.3% respectively with the positive and negative predictive values of clinical examination in identifying bilateral disease being 50.0 % and 84.3%, respectively. The authors concluded that clinical examination is not a reliable diagnostic method to diagnose bilateral DVT.

As to the question whether or not documentation of the bilateral extent of a DVT offers benefit, it is important to realise that before the advent of Doppler scanning, phlebography (venography) was the investigation of choice. Bilateral investigation was thus rarely performed, because phlebography represented additional risks and costs. Although phlebography is still considered the gold-standard diagnostic tool for lower limb DVT, especially for scientific studies, it has been almost abandoned in day to day clinical practice and replaced by duplex Doppler scanning. The latter diagnostic method offers lower cost, improved availability and virtual absence of complications. Actual described values of

accuracy are higher than 95% and allow effective evaluation of even infragenicular veins.[129-130] The diagnosis of DVT in an asymptomatic contra-lateral limb allows for early measures to avoid the development of symptomatic post thrombotic syndrome (PTS) with its social and economical burden. The costs of bilateral duplex scanning in patients with confirmed unilateral DVT may be largely offset by future savings. An unidentified contra-lateral thrombosis may lead to misinterpretation of future clinical manifestations, eventually causing confusion between chronic venous obstructions and acute thrombosis. If placement of an inferior vena caval filter is indicated, documentation of the bilateral extent of a DVT will prevent the puncturing and manipulation of guide wires and sheaths through a thrombosed vessel.

In conclusion, an association between active HIV infection and the presence of bilateral DVTs has been demonstrated and various studies have argued the benefits of documenting the extent of thromboses. Further studies and recommendations in this regard are warranted in view of our findings which did not confirm these previous observations.

6. Conclusion

The HIV prevalence in the deep vein thrombosis (DVT) group who consented to HIV testing and who had no traditional risk factor for DVT development was 81% (95% CI 0.67 - 0.96). This HIV prevalence was significantly higher than that in a matched population group without clinical DVTs with a HIV prevalence of 4%.

Traditional risk factors for DVT development were identified both in the HIV positive and negative DVT groups. These factors included tuberculosis, immobilisation, trauma, cancer, previous DVT and age over 65 years. Presence of these factors in HIV infected patients probably adds to the hypercoagulable state.

From this thesis it is clear that there is no available evidence evaluating thromboprophylaxis specifically in HIV-infected individuals. The available thrombosis treatment guidelines lack recommendations in this growing sub-population. Important treatment decisions are therefore left to medical attendants without clear guidelines. HIV infection in the ARV era is a chronic disease with a clearly prothrombotic tendency. Future studies and guidelines should further define the thrombotic risk in the HIV infected population and direct treatment and prophylaxis.

7. APPENDIX: Copy of ethics clearance certificate

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