COAGULATION SYSTEM ABNORMALITIES IN HUMAN IMMUNODEFICIENCY VIRUS (HIV) POSITIVE AFRICAN (BLACK) PATIENTS WITH ACUTE UPPER SEGMENT DEEP VEIN THROMBOSIS (DVT) OF THE LOWER LIMBS

By

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PREFACE

This study is the original work of the author and has not been submitted in any form to another university. Where use is made of the work of others, it is acknowledged in the text.

The research described in this dissertation was carried out in the Department of Haematology, College of Health Sciences, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Durban, and under the supervision of Dr V Poovalingam.

Ethical approval to conduct this study was granted by the Ethics and Professional Standards Subcommittee of the University of KwaZulu-Natal.

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ABSTRACT

Background

Several case reports and studies have alluded to an increased prevalence of venous thrombosis in human immunodeficiency virus positive (HIV-positive) patients. Although a relationship between HIV infection and thrombotic disease has been suggested, the mechanisms predisposing to thrombosis have not been fully elucidated.

Aim

A prospective study, to determine possible coagulation factor abnormalities that could explain the predisposition to thrombosis in HIV-infected African (Black) patients, was undertaken.

Method

African (Black) patients, with acute upper segment deep vein thrombosis (DVT) confirmed by duplex ultrasound, were enrolled. Patients who had recognisable risk factors such as recent surgery, pregnancy or malignancy, were excluded. After informed consent, blood samples were taken for baseline tests as well as a thrombophilia screen. The control group comprised known HIV-positive African (Black) patients without DVT. Patients with DVT who were found to be HIV-negative were also analysed.

Analysis was done in 2 parts: HIV-positive patients with and without thrombosis and HIV-positive and negative patients with thrombosis were compared.

Results

Part A: HIV-positive patients with and without thrombosis

Of the 77 patients with DVT, 50 patients tested HIV-positive. These 50 patients (HIV-positive DVT-arm), as well as 56 controls (HIV-positive, no DVT), were enrolled into the study. The groups were well matched with regard to age, sex and cluster designation 4 (CD4) count. On univariate analysis, significant findings in the DVT-arm were a history of active tuberculosis on treatment, low protein C levels and a positive qualitative D-dimer, whereas on

multivariate analysis, only tuberculosis and an elevated D-dimer proved to be significant.

Part B: HIV-positive and negative patients with thrombosis

There were 20 HIV-negative patients with DVT who met our inclusion criteria Limited assessment was done on this group owing to unavailability of some data.

The mean age of the HIV positive DVT group was significantly lower than the HIV-negative group with DVT (31.78 vs. 41.45 years; p=0.005).

There was no significant difference in the prevalence of tuberculosis between the HIV-positive and HIV-negative patients with thrombosis (p = 0.269).

Mean protein C levels were reduced in the HIV-positive group and normal in the HIV-negative group. They were significantly lower in the HIV-positive patients compared to the negative group (p=0.02).

Conclusion

The findings of the study suggest a relationship between HIV, its complications and DVT. Although this study confirms HIV infection as a risk factor for thrombosis, clear pathogenetic mechanisms remain to be elucidated. In our population, tuberculosis appears to be an important risk factor predisposing patients to the development of DVT, both in the HIV-positive and negative population. Further studies will need to be done to confirm this hypothesis.

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1. **INTRODUCTION**

Thrombosis of veins and arteries, together with complicating embolic phenomena, are perhaps the most important causes of sickness and death in the developed world at the present time. Venous thromboembolic (VTE) disease is the third most common cardiovascular disease after atherosclerotic heart disease and stroke.[1]

Deep vein thrombosis principally affects the lower limbs and is highly age dependant. It is exceptional in childhood and may reach 1% per year in the very elderly.[2]

Venous thromboembolism results from the interaction of multiple risk factors. These may be gene-environment interactions or multiple environmental or acquired risk factors.[2] Important pathogenetic mechanisms in venous thrombosis are stasis and hypercoagulability.

Thrombophilia is the term used to describe disorders of haemostasis, either congenital or acquired, which predispose to the development of venous thrombosis. The heritable abnormalities include those due to deficiency of the natural anticoagulants antithrombin, protein C and protein S, as well as those resulting in increased procoagulant activity such as factor V Leiden and G20210A prothrombin polymorphism. Acquired conditions that predispose to thrombosis include pregnancy, immobility, surgery and inflammatory states.[2]

There have been several clinical studies and case reports describing the development of deep vein thrombosis in HIV-positive patients in the absence of classic thrombophilic risk factors such as advanced age, immobility, family history and malignancy. Several intersecting mechanisms associated with HIV infection are described that may lead to vasculopathy and hypercoagulability. HIV infection may be directly implicated in that it causes endothelial damage with the release of cytokines and tissue factor. HIV infection is also associated with decreased levels of protein C, S and antithrombin.[3] Many of these studies are retrospective small studies and there is still uncertainty regarding the incidence and risk factors for thrombosis in HIV-positive patients, including the importance of disease stage, associated opportunistic infections and antiviral therapy.

KwaZulu-Natal is the epicentre of a devastating HIV epidemic affecting sub-Saharan Africa. There are no studies describing the prevalence and pathophysiology of thrombosis in HIV-positive African (Black) patients in this region. Therefore, a study was initiated to examine the prevalence of DVT in this population group, as well as to test for possible coagulation factor abnormalities that predispose to thrombosis.

2. REVIEW OF THE LITERATURE

Venous thromboembolism (VTE) is a cause of considerable morbidity and mortality.[4] The major manifestations of venous thrombosis are deep vein thrombosis (DVT) of the lower limbs and pulmonary embolism. Thrombosis may also occur in other veins and involve the upper limbs, liver, cerebral sinus, retina and the mesenteric veins. Major complications of venous thrombosis of the lower limbs are acute death from pulmonary embolism and a disabling post-thrombotic syndrome.[5] The fatality rate of venous thrombosis, mainly due to pulmonary embolism, ranges from 1% in young patients to 10% in older patients.[6]

The annual incidence of venous thrombosis is 1 - 3 per 1000 individuals per year.[5] The frequencies of VTE described in the literature strongly depend on the patient populations being studied and on the sensitivity and specificity of the methods used for diagnosis.[7] Rates of venous thromboembolism increase dramatically as the population ages, with 1/10 000 events in the young (< 40 years old) to 1 per 1000 in the elderly (> 75 years old).[8]

Virchow, in the 19th century, postulated three major causes of thrombosis: changes in the vessel wall, changes in blood flow and changes in blood composition.[9] This broad classification is still valid today. In venous thrombosis, however, the most important factors are stasis and prothrombotic abnormalities.[6] Risk factors for the development of venous thrombosis can

be inherited or acquired. Table 1 lists the important causes of venous thrombosis.[5] Acquired and genetic causes frequently interact.

Most commonly, thrombosis is the result of more than one “hit”.[11] The likelihood of developing thrombosis would depend on the type and the number of predisposing factors involved. Acquired risk factors still play a major role in the burden of venous thrombosis even though their impact has lessened because of the implementation of prophylactic anti-thrombotic strategies.[6] The term *thrombophilia* is used to describe the familial or acquired disorders of the haemostatic mechanism which are likely to predispose to thrombosis.[12] Patients with inherited thrombophilia generally present with thrombosis at an early age (< 45 years), recurrent thrombosis, or with thrombosis at unusual sites.

Table 1: Risk factors for venous thrombosis [5]

Acquired	Inherited	Mixed/Unknown
Bed rest	Antithrombin deficiency	High levels of factor VIII
Pregnancy	Protein C deficiency	High levels of factor IX
Major surgery	Protein S deficiency	High levels of factor XI
Orthopaedic surgery	Factor V Leiden (FVL)	High levels of fibrinogen
Malignancy	Prothrombin 20210A	High levels TAFI
Oral contraceptives	Dysfibrinogenaemia	Low levels of TFPI
Hormone replacement therapy		APC-resistance in the absence of FVL
Antiphospholipid syndrome		Hyperhomocysteinaemia
Myeloproliferative disorders		
Polycythaemia vera		
Central venous catheters		
Age		
Obesity		

TAFI, thrombin activatable fibrinolysis inhibitor; TFPI, tissue factor pathway inhibitor; APC, activated protein C; FVL, factor V Leiden; PCI, protein C inhibitor; PAI-3, plasminogen activator inhibitor type 3

Inherited Thrombophilia

A familial tendency to venous thrombosis was first described in the early 1900s when pedigrees with a large number of individuals with venous thrombotic events suggested heritable hypercoagulability. Egeberg [13] described the first family with an identified hereditary tendency to thrombosis caused by antithrombin deficiency in 1965. Subsequently in the 1980s, protein C and protein S deficiency were recognised as causes underlying familial thrombophilia.[6] Over the last 10 years, several new defects have been identified which are commoner, but less thrombogenic when compared to deficiencies of protein C, protein S and antithrombin.

The frequency of the major inherited forms of hypercoagulability varies substantially within healthy populations and among patients with venous thrombosis. Factor V Leiden and the G20210A mutation in the prothrombin gene are common among healthy Whites, but extremely rare among Asians and Blacks.[10] Since Factor V Leiden and the G20210A mutation are relatively common, they can occur together with other thrombophilias. Deficiencies of antithrombin, protein C and protein S are found in less than 1% of the population.[10] Table 2 illustrates the relative frequencies of these disorders.

Table 2: Prevalence of thrombophilic defects

	Patients with venous thrombosis (%)	General population (%)
Antithrombin deficiency	1 - 2	0.1 - 0.3
Protein C deficiency	2 - 3	0.2 - 0.5
Protein S deficiency	2 - 3	0.2 - 0.5
Factor V Leiden	10 - 20	3 - 7
Hyperhomocysteinaemia	10 - 20	2 - 6
Prothrombin 20210A	5 - 6	1 - 3
High factor VIII concentrations	10 -15	6 - 8

Numerous mutations have been described in patients with a deficiency of protein C, protein S or antithrombin. Type I defects (low activity and low antigen level) predominate in patients with a deficiency of protein C or S, whereas both type I and type II (low activity and normal antigen level) defects are common in patients with antithrombin deficiency.[10] Deficiencies of protein C, protein S and antithrombin increase the risk of thrombosis by about 10-fold in heterozygotes. Of these deficiencies, the highest risk is associated with antithrombin deficiency.[6]

First identified in 1994, factor V Leiden is the most common genetic prothrombotic defect. It is one of the causes of resistance to activated protein C (APC). The resistance is due to a defect in the APC cleavage site on factor V, caused by a single arginine to glutamine mutation at position 506. The

relative risk of thrombosis for carriers is thought to be increased 7-fold for heterozygotes and 80-fold for homozygotes.[4]

The prothrombin gene mutation was first discovered in 1996. The defect is a G to A nucleoside transition at position 20210 of the prothrombin gene. Heterozygote carriers of the gene were found to have approximately 30% higher prothrombin levels than healthy controls, and presumably this is the mechanism by which it exerts its thrombotic effects.[4]

Mildly elevated levels of homocysteine are associated with an increased risk of thrombosis. The mechanism by which hyperhomocysteinaemia affects the risk of thrombosis is unknown. Hyperhomocysteinaemia can be caused by genetic disorders affecting the trans-sulphuration or remethylation pathways of homocysteine metabolism, or by acquired causes such as folic acid deficiency, vitamin B_{12} deficiency, vitamin B_6 deficiency, renal failure, hypothyroidism, increasing age and smoking.[10]

When investigating for hereditary thrombophilia, it is important to be aware of the acquired causes of reduced levels of the factors. Liver disease, oral anticoagulants, disseminated intravascular coagulation, vitamin K deficiency and nephrotic syndrome are some of the causes of reduced levels of protein C, protein S and antithrombin. Also, the timing of the testing is important. In the acute phase of deep vein thrombosis, the levels of protein C, S and antithrombin could be reduced due to utilisation and, therefore, testing immediately after diagnosis, would not be helpful. It is advisable to test after

the acute phase has resolved. It is most practical to test about two weeks after oral anticoagulation has been stopped.[15]

Acquired Thrombophilia

Acquired conditions that predispose to thrombosis are listed in Table 1.

Venous stasis is an important pathogenetic factor in venous thromboembolism. It contributes to post operative thrombosis and is a factor in thrombosis in paralysed limbs as well as limbs splinted in plaster casts. In postoperative patients, the procoagulant responses to tissue trauma (such as increased fibrinogen and factor VIII concentrations in plasma) and reactive thrombocytosis may also play a part. The highest prevalence of VTE occurs in major orthopaedic surgery, with the lower limb being the commonest site for venous thrombosis.[16]

Indwelling venous devices are a significant cause of deep venous thrombosis. Up to 60% of patients with central venous catheters will develop a complication, thrombosis being one of the most common.[16]

Cancer is a major risk factor for venous thromboembolism. It is estimated that almost 15% of cancer patients will have a thromboembolic event. Cancer patients have a high risk of venous thromboembolism after surgery and some forms of chemotherapy may also increase that risk. The risk of thrombosis varies with the type of cancer, with ovarian, brain and pancreatic cancers having the highest rates. [16]

Iatrogenic venous thromboembolism may be due to pharmaceuticals and venous thromboembolism associated with the use of the combined oral contraceptive pill is of particular importance because it affects young, healthy women. Oestrogen-containing preparations induce a state of reduced sensitivity to activated protein C, and this appears to be more marked with the third generation contraceptives than second generation preparations. Hormone replacement therapy use is also associated with VTE. In this case, the absolute risk is higher due to the greater background prevalence of venous thrombosis in older women. [16]

Antiphospholipid syndrome is an important thrombophilic condition that is associated with considerable morbidity and mortality. The essential features are arterial or venous thrombosis, or recurrent pregnancy loss occurring in a subject who has a positive antiphospholipid antibody, and/or a positive lupus anticoagulant. Antiphospholipid syndrome may occur in association with another chronic systemic auto-immune disease, usually systemic lupus erythematosis, when the term secondary antiphospholipid syndrome is used.[16] Antiphospholipid antibodies have also been present in association with a large number of infectious diseases, such as syphilis, HIV infection, malaria and hepatitis C, but are not usually associated with clinical complications. [17]

Elevated levels of prothrombin, factor VIII, factor IX, factor XI as well as thrombin activatable fibrinolysis inhibitor(TAFI) are all associated with an increased risk of thrombosis. Levels of these factors exceeding the 90th

percentile of the distribution in the general population, are associated with a 2 to 3 fold increase in the risk of thrombosis. Although little is known about the origins of such elevated levels, it is likely that they are a combination of genetic and acquired causes. Familial clustering of elevated levels of factor VIII has been shown.[5]

Thrombosis in Black subjects

Deep vein thrombosis has been historically perceived as a disorder that affects mainly Whites, a belief partly reinforced by the low incidence of hereditary prothrombotic mutations in Blacks. Some of the congenital thrombophilic disorders such as factor V Leiden and the prothrombin gene mutation are extremely rare in Blacks.[14]

Understanding of the epidemiology and risk factors for venous thromboembolism in Blacks is limited.[34] There are, however, recent studies that show that venous thrombosis is equally common amongst a variety of racial groups, including Black patients, although the predisposing factors appear to differ.[34] .

A prospective study [59] was performed during the 2 year period 2000-2001 at Kings College, London to ascertain possible ethnic differences in the prevalence of DVT. Of the 850 patients who were assessed for suspected DVT, 219 patients were found to have DVT. Of the White subjects, 27.7% had confirmed DVT relative to 22.8% of the Black subjects. The average age was 58 years for White persons and 49.3 years for Black persons. Of the 178 subjects tested, factor V Leiden was found in 21.8 % of White subjects whereas no Black subjects had the mutation. They demonstrated no

significant difference in the incidence of VTE in Blacks and Whites.[59] Patel et al. [35,36] found that significant factors in Black patients included elevated D-dimers as well as elevated factor VIII levels. Their study comprised 125 Black patients, 61 with a history of DVT and 64 control subjects. Patients with a history of DVT had completed treatment for DVT at least 3 months previously and had no intercurrent illness. All subjects were found to have normal thrombophilia screens, which included testing for protein C, protein S, antithrombin, factor V Leiden and lupus anticoagulant. Median D-dimer was significantly higher in cases than controls.[35] They also assessed 100 black patients with DVT and 100 black controls for elevated factor VIII levels and found that 34% of patients with DVT had an elevated factor VIII level compared to 10% of controls. In those Black patients with factor VIII below the 90th centile, the odds ratio for the risk of VTE was 4.64. Conventional thombophilia testing was found to be relatively uninformative in Black subjects with VTE, revealing a genetic risk factor in only 9.1% of Blacks with VTE as opposed to 30% of Whites. They proposed that raised factor VIII is a major risk factor for VTE in Blacks with prevalence and odds ratio exceeding that in Whites.[36] Pieper et al. [37] also found significantly higher levels of D-dimer in Black persons when assessing a group of community dwelling elderly persons.

The Gate (Genetic attributes and thrombosis epidemiology) study is an ongoing case control study in Atlanta designed to examine racial differences in venous thomboembolism (VTE) aetiology and pathogenesis. Between 1998 and 2001, 370 patients with confirmed VTE and 250 control subjects were

enrolled. Family history of VTE was reported with equal frequency by cases of both races, despite known genetic factors for VTE being rare in African-Americans. This suggests an as yet unknown genetic component amongst blacks and underscores the need for research that specifically addresses risk factors and aetiologic mechanisms for VTE in Blacks.[57]

In a Californian study, 17991 patients with idiopathic deep vein thrombosis and 5573 patients with secondary thromboembolism were evaluated for ethnic origin. The incidence of idiopathic deep vein thrombosis was almost 30% higher in African-Americans than in White persons. Similarly, after surgical or medical diagnoses, African-Americans had a higher relative risk for thromboembolism than White persons.[56]

HIV Infection

Human immunodeficiency virus infection is a chronic and progressive disease that starts as a primary infection and progresses to life-threatening opportunistic infections, malignancies and wasting. An effective immune system may be maintained for many years in patients with HIV infection, but in the majority of cases, the disease progresses and leads to CD4 cell depletion and vulnerability to opportunistic infections.[18]

Acquired immunodeficiency syndrome (AIDS) represents the range of opportunistic infections, malignancies and wasting manifestations that occur with profound immune depletion (World Health Organisation [WHO] stage 4).[18]

By the end of 2005, it was estimated that 38.6 million people world-wide were living with HIV infection and about 4.1 million people were newly infected with HIV. An estimated 2.8 million lost their lives to AIDS by the end of 2005. The prevalence of HIV infection in South Africa is the highest in the world and shows no evidence of a decline. The Joint United Nations Programme on HIV/AIDS (UNAIDS), reporting on the global HIV/AIDS epidemic, estimated that in 2005, 5.5 million (range 4.9 – 6.1 million) people or 18.8% of the population were living with HIV/AIDS in South Africa. Almost one in three pregnant women attending public antenatal clinics tested HIV-positive in 2004. The average life expectancy in South Africa is expected to fall from 60 years to 40 years between 1998 and 2008.[19]

Thrombosis in HIV-positive patients

At the time of the commencement of this study, there were few reports on venous thrombosis in HIV-positive patients. These were either case reports or small studies attempting to elucidate the problem.

The reported incidence of venous thromboembolism in patients with HIV infection ranges from 0.25% to 0.96% in clinical studies, with a 17% incidence at autopsy.[20,21]

One of the earliest reports of VTE in HIV-infected individuals was by Lafeuillade et al., who reported 2 cases of thrombosis in HIV-positive patients.[22] The first patient was a 29-year-old homosexual man who had

thrombosis of the iliac vein and the inferior vena cava. The second patient was a 58-year-old man who presented with ischaemia of the hands and was found to have thrombi in the interdigital arteries. On further investigation, both patients were found to have free protein S deficiency. To evaluate the significance of protein S deficiency, they proceeded to study the prevalence of protein S deficiency in 71 HIV-infected patients and found that 22 (31%) had low free protein S levels. Using a functional assay, the mean protein S was 43%. There was no correlation between low levels and the stage of disease. Neither was there any history of thrombosis in any of the patients studied. They postulated that protein S deficiency could be due to abnormal endothelial cell function secondary to HIV infection and that further studies would be required to evaluate the pathophysiology as well as the clinical significance of the deficiency of protein S.

Bissuel et al. conducted a prospective analysis of 63 HIV-infected patients at various stages of the disease and compared them to a matched control group of 24 HIV-negative healthy subjects.[23] The mean age of the patients was 34.1 years. Forty-one (41) of the 63 patients had low free protein S levels. A significant decrease in free protein S levels was found in HIV-positive patients, compared to the control group ($p = 0.0001$). Free protein S levels were significantly lower in patients with acquired immuno-deficiency syndrome (AIDS) when compared to the group who did not have AIDS ($p = 0.0001$).

In addition, total protein S levels were lower in HIV-positive patients compared to controls. On evaluation of total protein S levels in the HIV-positive group, no statistical difference between the patients with AIDS and those without AIDS

was found. There was a significant correlation between total and free protein S values ($p = 0.0006$) but free protein S levels remained lower than total protein S. Only 3 HIV-positive patients had protein C deficiency and one of the 39 patients tested had antithrombin deficiency. Despite the significant deficiency in free protein S, only three patients presented with a thrombotic event. All three patients had full blown AIDS and were deficient in protein S. One of the patients had cytomegalovirus infection, which could have predisposed to thrombosis, and the other two had other active infections. The details of the infections were not specified.

Stahl et al.[24] examined the possible predisposing factors to thrombosis in 25 randomly selected HIV-positive patients with a mean age of 38.6 years.
Their mean CD4 count was 226/mm^3. Three of the 25 patients gave a history of thrombosis, occurring after HIV seroconversion. Coagulation studies showed that all three of the patients with thrombosis and 16 of the 22 patients without thrombosis had decreased free protein S levels. Mean free and total protein S levels were lower compared to healthy controls. Decreases in protein S levels did not correlate with CD4 levels. Inflammation may result in an increase in C4b-binding protein, which can shift the protein S from the free to the bound form. In patients with decreased free protein S levels, C4b-binding protein was not increased, thus excluding inflammation as a cause for the decreased levels of free protein S. Of note all 25 patients had normal protein C, antithrombin and plasminogen levels. In addition, none of the patients were positive for a lupus inhibitor as measured by the tissue thromboplastin test. IgM anti-cardiolipin antibodies were positive in 1 of 18

patients and IgG antibodies were positive in 18 of 24 patients. The authors concluded that the pathogenesis of decreased protein S remained unclear and postulated that disturbances in endothelial function may play a role in the aetiology of protein S deficiency. They also suggested that protein S deficiency might be a predisposing factor in the development of thrombosis.

Hassel et al.[25] evaluated the occurrence of antiphospholipid antibodies and free protein S deficiency in HIV-infected men and sought to determine the possible role of these abnormalities in the development of thrombosis. They studied 74 HIV-infected men at all stages of infection. Antiphospholipid antibodies were detected in 63 (86%) of the patients tested. Sixty-five per cent (65%) had elevated IgG anticardiolipin (aCL) antibodies and 32% had elevated IgA aCL antibodies. Only 14% of patients tested had elevated IgM aCL antibodies (10 of 67 patients) and a positive dilute Russell viper venom test (6 of 43 patients). Low total protein S was detected in eight patients (11%), five of whom had low levels of free protein S. Overall there was a significant linear correlation between total and free protein S levels ($p = 0.004$), but isolated deficiency of free protein S was detected in 23 additional patients. C4b-binding protein was measured and was not found to be a contributing factor for the decreased free protein S. There was no correlation between free protein S (PS_F) levels and anticardiolipin antibody titres. There was also no correlation between these parameters and CD4 count, medication use or infections. The overall incidence of thrombosis in this group was 18%. Development of thrombosis was not significantly correlated with antiphospholipid antibodies or free protein S deficiency. They concluded that

further studies were required to elucidate the thrombotic risk in HIV-positive patients.

The presence of lupus anticoagulants and anticardiolipin antibodies has been reported in association with AIDS. The prevalence of lupus anticoagulant in HIV-positive patients has been reported to be as high as 53 - 70% in some series and has been noted to be absent in others. Lupus anticoagulant has been associated with thrombosis in certain disease states, such as systemic lupus erythematosis, but the association with thrombosis in HIV infection appears to be rare. Anticardiolipin antibodies have been reported in 46% to as many as 90% of patients with HIV infection, but have been rarely associated with thrombotic events.[26]

Sorice et al. evaluated protein S levels in patients with HIV infection, none of whom had thrombosis.[27] They tested 35 patients with HIV infection and found 23 (65.7%) had reduced free protein S activity and 20 (65.7%) had reduced free protein S antigen. They found that free protein S levels, tested either by activity or antigenic method, were reduced in HIV-positive patients when compared to controls. They also found that protein S levels were significantly lower in patients with CD4 counts less than 100 cells/mm^3. In an attempt to ascertain the cause of the reduced protein S levels, they tested for anti-protein S antibodies and found that 28% of patients had anti-protein S antibodies. There was a higher prevalence of antibody positivity in symptomatic patients as well as in patients with protein S levels below 50%. Their findings

suggested that one of the causes of reduced protein S levels was the presence of specific antibodies.

In view of the uncertainty regarding the causes of thrombosis in HIV-infected patients, Feffer et al. analysed 52 HIV-positive individuals and screened them for possible predisposing factors for the development of thrombosis.[28] None of the patients had thrombosis. Patients were grouped into 3 categories based on their CD4 counts. They found decreased levels of protein C and free protein S, along with elevated levels of total protein S and von Willebrand factor. The increased total protein S and von Willebrand factor were thought to be due to endothelial injury resulting from HIV infection. These results correlated with the degree of immunosuppression as measured by CD4 counts. The relation of free protein S levels and the patients' CD4 levels concurs with the results of Bissuel et al.[23] and differs from those reported by Stahl et al.[24] and Lafeuillade et al.[22] Also, contrary to the findings by Stahl et al. and Lafeuillade et al., they found an inverse relationship between free protein S and C4b-Binding-Protein (C4b-BP). They also detected a significant elevation of D-dimers in patients with inflammatory/neoplastic disease and proposed that a low grade consumptive coagulopathy was responsible for the decline in protein C levels. They postulated that the predisposition to thrombosis was as a result of a combination of consumptive coagulopathy, endothelial damage and a decline in free protein S.

In a review by Laing et al., the authors alluded to the difficulty in establishing the absolute risk and incidence of thrombosis in HIV-positive patients, since

asymptomatic thrombi may not be diagnosed and fatal pulmonary emboli missed unless a post-mortem was performed.[14] The other area of difficulty was to elucidate the possible causative factors in these patients. Many of these patients were chronically ill and debilitated and probably had multiple predisposing factors for the development of thrombosis.

A longitudinal review of over 100 medical clinics was done by Sullivan et al. in which they assessed 42 935 patients with HIV infection.[29] A total of 335 instances of thromboses were documented in 273 patients. Forty three (43) patients had more than one thrombotic event. The incidence of thrombosis was 2.6/1000 person years in this group. The incidence was higher in patients with clinical AIDS as compared with immunological AIDS. Factors significantly associated with thrombosis were age ≥ 45 years, diagnosis of cytomegalovirus infection or other AIDS-defining opportunistic infections, hospitalisation and the prescription of megesterol acetate or indinavir. They were unable to evaluate the relative importance of the predisposing factors for thrombosis as they did not have information on variables such as protein S and anticardiolipin antibodies.

Saif et al. evaluated 131 HIV-positive patients retrospectively.[30] Ten (10) patients experienced 15 episodes of unexplained deep vein thrombosis. All were male with a mean age of 39.3 years. Six patients had lower extremity deep vein thrombosis, 3 developed pulmonary embolism and the others had subclavian or axillary vein thrombosis. A history of catheter placement was found in one patient who developed subclavian vein thrombosis. Two of the

patients had a malignancy: one had non-Hodgkin's lymphoma and the other had Kaposi's sarcoma. Three patients were bedridden at the time of the thrombotic disease. Only 5 out of the 10 patients were evaluated for a hypercoagulable state. Abnormal findings were found in 3 of these patients. One had anti-thrombin deficiency attributable to HIV-associated nephropathy, another had protein C deficiency and the third had positive anti-phospholipid antibodies. Thirteen (13) of the 131 patients were on protease inhibitors and only 2 patients in this group developed thromboembolism. A significant correlation between thrombotic events and CD4 counts was found. Nine (9) of 10 patients with thrombosis had a CD4 count of < 200 cell/mm^3. The authors concluded that AIDS appeared to predispose to thrombosis but that further studies were required to elucidate the mechanism involved in HIV-related thrombosis. Factors postulated as having a role in the predilection for thrombosis included abnormalities in the haemostatic pathway, AIDS-related opportunistic infections and malignancies.

Saber et al. conducted a retrospective review of HIV-infected patients with DVT from January 1995 to January 2000.[31] Of 4 752 HIV-positive patients admitted, 45 (0.95%) were found to have DVT. Thirty-six (36) patients were males and 9 were female, with a mean age of 43 years. Of the 45 patients, 38 had infectious complications, with the most common infection being cytomegalovirus. Other infections encountered were pneumocystis carinii, toxoplasmosis, candidiasis, hepatitis B or C and mycobacterium avium. Thirteen (13) patients developed a malignancy, the most common being Kaposi's sarcoma (7 patients). In their review, 95.6% of the patients were

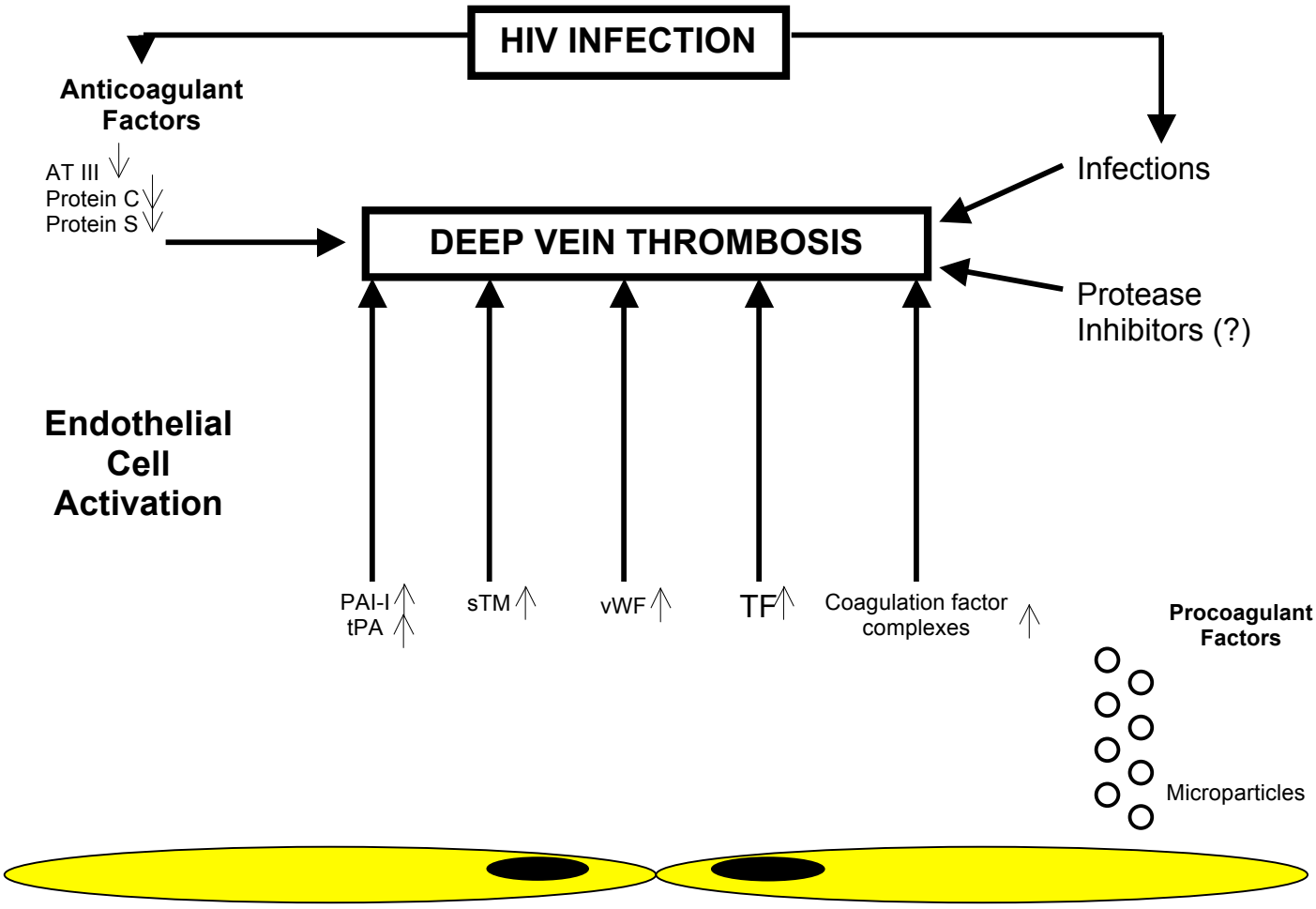
ambulatory at the time of diagnosis. Four patients gave a history of recent surgery (within one month of diagnosis of DVT). Data on hypercoagulability was obtained in 11 patients only. Protein S deficiency was found in 3 patients, anti-thrombin deficiency in 2, and 2 patients had anti-cardiolipin antibodies. Thrombosis occurred in the popliteal vein in 20 patients (44.4%), femoral vein in 23 patients (51.1%) and ileo-femoral in 2 patients (4.5%). Twelve of the 45 patients had more than one episode of DVT. According to Rosendaal, one in 1000 adults in the general population suffers from DVT.[32] In Saber's study, he found that almost one in 100 patients had DVT in the HIV-infected group.[31] He attributed the higher risk of DVT in this group to the wide range of comorbidity associated with HIV. He is also of the opinion that hypercoagulability, opportunistic infections and malignancies make patients with HIV more prone to developing thrombosis and that the interactive synergistic effect of these risk factors may explain the exaggerated thrombotic phenomenon in these patients.

Copur et al.[20] in a retrospective study (between July 1998 and June 1999) of patients with venous thromboembolism and/or HIV infection, reported a frequency of VTE in patients with HIV infection, regardless of age, of 2.8% compared to 1.8% in the non-HIV infected patients. The difference was not statistically significant ($p = 0.16$). However, when the study population was divided into those less than 50 years and those greater than 50 years, a significant interaction between HIV infection and VTE was found. In those patients < 50 years, the frequency of VTE was 3.31% in the HIV-positive patients compared to 0.53% in the HIV-negative patients ($p < 0.0001$). The

rate of VTE in the older group of HIV- negative patients (3.04%) was greater than in the younger HIV-negative patients (0.53%) ($p < 0.0001$). Risk factors for VTE (pregnancy, malignancy, obesity, surgery, and immobilisation) were present in 30% of HIV-positive patients < 50 years of age, in contrast to 60% of HIV-negative patients < 50 years of age. They concluded that VTE is a potential cause of increased morbidity in HIV-positive individuals less than 50 years and that HIV should be considered as a possible risk factor in young patients with idiopathic VTE. However, the reason for the association remained unclear.

Many of the above studies have alluded to the increased prevalence of venous thromboembolism in HIV-positive patients. The incidence was increased two- to ten-fold in comparison with a healthy population of the same age. A variety of predisposing mechanisms have been postulated. However, these studies were mainly retrospective cohort studies that were prone to selection bias, confounding factors were not always mentioned, diagnostic work-up was not always clear and data was sometimes incomplete.[33] Figure 1 summarises the postulated predisposing factors for the development of thrombosis in HIV-positive patients.

Figure 1: Predisposing Factors for thrombosis in HIV-infected patients



Klein S.K., Slim E.J.: The Journal of Medicine; 2005 vol. 63, no.4, 129-136

3. PATIENTS AND METHODS

AIM

A prospective study, to determine possible coagulation factor abnormalities that could explain the predisposition to thrombosis in HIV-infected African (Black) patients.

Evaluation and comparison of levels of coagulation factors in:

- HIV positive patients with and without DVT
- HIV positive and negative patients with DVT

SUBJECTS

African (Black) patients over 18 years of age with acute upper segment deep vein thrombosis were considered for entry into the study. Patients with DVT were recruited from the diagnostic vascular laboratory, wards and clinics at King Edward VIII Hospital.

The control group was made up of patients who were known to be HIV-positive but without any symptoms or signs suggestive of thrombotic disease. These patients were recruited from the HIV clinic and the wards at King Edward VIII hospital.

Exclusion Criteria

- past history of thrombophilia
- obvious cause of thrombosis, e.g., malignancy
- diabetes mellitus
- post-operative states
- chronic deep vein thrombosis
- any patient already commenced on anticoagulants
- patients who refused HIV testing

.

PLAN OF INVESTIGATION

STUDY DESIGN

Patients were analysed in two groups:

Group A

A case controlled study group comprising HIV-positive patients with and without thrombosis.

Group B

An analytical cross sectional group comprising HIV-positive and HIV-negative patients with thrombosis.

.DESIGN AND EXPERIMENTAL PROCEDURES

Patients with suspected DVT were subjected to duplex ultrasound at the vascular laboratory at King Edward VIII Hospital.

Upper segment deep vein thrombosis of the lower limbs is defined as venous thrombosis of the iliac, femoral and or popliteal veins, i.e., thrombosis above the popliteal trifurcation.

Any patient positive for acute upper segment DVT, who did not have any obvious risk factors as outlined in the exclusion criteria, was enrolled in the study.

Known HIV-positive patients without symptoms of DVT were enrolled into the control arm. Duplex Ultrasound was not performed in this group.

Informed consent for enrolment in the study was obtained from all patients. Copies of the informed consent forms are in the appendix (appendices b and c).

INTERVIEW

The following information was elicited from each eligible patient:

- name
- age
- gender
- relevant past medical history
- relevant family history
- relevant past or family history of coagulation disorders
- drug history
- current medical illness/symptoms
- duration of presenting symptoms
- HIV status (if previously tested)
- site of thrombosis

A copy of the data collection form is in the appendix (appendix a).

BLOOD TESTS

After obtaining informed consent, patients were counselled prior to HIV testing. (Patients who tested HIV-positive and who wished to be informed of the result, received post-test counselling).

Venous blood was drawn for the following tests:

(1) Full blood count (FBC)

- 3ml of blood collected in a tube containing ethylenediamine tetra-acetic acid (EDTA)
- FBC was assayed via standard Beckman coulter routine (STKS)

(2) Urea and electrolytes (U&E) and liver function tests (LFT)

- 3ml of blood collected in a plain tube
- U&E and LFT were processed via Beckman Syncoun CX7

(3) HIV assay

- 3ml of blood collected in a plain tube

(4) Antinuclear factor (ANF) and anti-cardiolipin antibodies (ACA)

- 3ml of blood collected in a plain tube
- ANF and ACA were processed by the immunology laboratory using an in-house standardised assay.

(5) **Protein C, total protein S, anti-thrombin (AT) , activated protein C resistance, prothrombin index/partial thromboplastin time (PI/PTT), qualitative D-dimer and lupus anticoagulant.**

- 2 X 5ml of blood collected in tubes containing 3.2% sodium citrate
- PI/PTT/Fibrinogen: analysed using ACL 300 (Ilex S.A.)
- Fibrinogen was measured with the derived method
- Qualitative D-dimer screen was performed using a latex agglutination test for qualitative determination of fibrin D-dimer. We utilised the Minutex D-dimer kit.[55]
- Citrated blood was obtained for protein C, protein S, APC resistance, AT and lupus anticoagulant. Samples were spun, aliquoted and stored at -70^0 C
    - Anti-thrombin: done with a kit: chromogenic method
    - Protein C: kit method: SA Scientific: chromogenic method
    - Functional Total Protein S: kit method: ILEX clottable technique
    - Lupus anticoagulant: Reagent: Russel viper venom
    - APC Resistance kit method: SA Scientific

All of the above were processed on the ACL 300 machine.

(6) CD4 count

- 3ml of blood collected in an EDTA tube
- CD4: specimens were subjected to monoclonal antibodies after being aliquoted in 100ul portiSample incubated for 30 minutes, then passed through the Coulter workstation. Red cells were lysed and white cells stabilised. Samples were passed through the Coulter Epics II Profile flow cytometer.

All specimens were subjected to routine quality control in the laboratory.

ETHICAL ISSUES

The study protocol was submitted and approved by the research ethics committee prior to commencement of the study. Informed consent was obtained from all participants in the study.

STATISTICAL ANALYSIS

Ms Eleanor Gouws (statistician) was consulted regarding sample size and design.

Assistance with data analysis was obtained from Ms Tonya Esterhuizen (biostatistician) on completion of the study. The statistical analysis was performed using the SPSS statistical package. Categorical variables were compared by the Fisher's exact test with continuity correction when appropriate.

Analysis of Risk Factors

The risk factors were categorised as follows:

Risk Factor	Category 1	Category 2
Tuberculosis	No Tuberculosis / ?Tuberculosis	Tuberculosis on treatment
Platelets	normal/low:<450x 10^9/l	high: ≥ 450 X 10^9/l
Fibrinogen	normal / low: < 4.5g/l	elevated: ≥ 4.5g/l
D-dimer	negative D-dimer < 500	positive D-dimer ≥ 500
ANF	negative	positive
ACA	negative	positive
Lupus anticoagulant	negative : < 45 seconds	positive: ≥ 45 seconds
Protein C levels:	elevated or normal: ≥ 70 %	low: < 70%
Total protein S:	elevated / normal: ≥ 60%	low: < 60%
Anti-thrombin:	elevated / normal: ≥ 80%	low: < 80%
Globulins	low/normal: ≤ 32g/l	elevated: > 32g/l
CD 4 counts	CD4 ≥ 200cells/mm^3	CD4 < 200 cell s/mm^3

ANF, antinuclear factor; ACA, anti-cardiolipin antibody; CD4, cluster designation 4

Analysis was done as follows:

Group A

To identify possible risk factors, the baseline characteristics of HIV-positive patients who had deep vein thrombosis were compared with the baseline characteristics of HIV positive patients without deep vein thrombosis. The risk factors were tested for association of HIV and DVT using log rank tests.

Group B

Eligible patients with DVT who were found to be HIV-negative were compared to the HIV-positive group with thrombosis. Odds ratio (OR) with 95% confidence intervals (CI) were calculated separately for each parameter by means of backward elimination based on likelihood ratio tests. All tests were two-sided and $p \leq 0.05$ was considered statistically significant.

Tuberculosis was included in all models. Variables with a p value of ≤ 0.05 on univariate analysis were entered into multivariate logistic models. When an association was identified, a composite model was built with baseline covariates to evaluate the effect of adjusting for other important covariates.

The baseline covariates analysed were:

(1) Sex

(2) Tuberculosis

(3) CD4 count < 200 and ≥ 200 cells/mm^3 (for the HIV-positive cohort)

4. RESULTS

A total of a hundred and thirty three (133) African (Black) patients were enrolled from November 1997 to May 2002 at the King Edward VIII hospital in Durban. Recruitment of patients was via the vascular laboratory, outpatient clinics or by referral of inpatients by doctors who were managing these patients. None of the patients had a history of previous deep vein thrombosis, a family history of thrombosis, malignancy or recent surgery. None of the female patients were on oral contraceptive agents. All subjects were antiretroviral therapy naïve.

Of the 133 patients, seventy seven (77) were found to have acute upper segment deep vein thrombosis of the lower limb. Fifty five (55) of the 77 patients with DVT were HIV-positive. Fifty six (56) HIV-positive patients without thrombosis formed the control arm. Five patients from the HIV-positive DVT arm were excluded from the study because of inconclusive data. Eventually 128 patients were analysed.

As outlined earlier, analysis was done in 2 parts:

Group A: HIV-positive patients with and without DVT

Group B: HIV-positive and negative patients with DVT

GROUP A: HIV-positive with DVT vs. HIV-positive without DVT

ENROLLMENT AND BASELINE CHARACTERISTICS (Table 3)

Demographic characteristics

Fifty HIV-positive patients with deep vein thrombosis (who formed the HIV-positive DVT-arm) were enrolled on to the study.

The control arm consisted of 56 patients who were HIV-positive without a history of DVT.

In the HIV-positive DVT-arm, 19 patients had right-sided DVT, 25 had left-sided DVT and 6 patients had bilateral DVT.

There were more females in the control-arm than in the DVT-arm (40 vs. 30), but this was not statistically significant ($p = 0.215$).

The mean age of the patients was 32 years in both arms. Forty-two (42) of the 50 patients in the HIV Pos DVT arm were less than 40 years of age and 22 of these were ≤ 30 years. Figure 1 depicts the age breakdown of patients in the HIV-positive DVT-arm.

CD4 Counts

CD4 counts were available in 30 of the 50 patients in the HIV-positive DVT-arm and in 50 of the 56 patients in the control-arm. The mean CD4 count was lower in the HIV-positive DVT-arm (202.70 vs. 233.74 cell/ mm^3). The difference was not statistically significant ($p = 0.461$). Of the patients tested, 17 (56.7%) in the HIV-positive DVT-arm and 26 (52%) in the control-arm had CD4 counts < 200 cells/mm^3.

Comorbidities

Twenty-two (22) of the patients in the HIV-positive DVT-arm and 11 patients in the control-arm were on treatment for tuberculosis (TB) at presentation. This information was obtained from the patients. Details of duration, type of TB treatment and site of TB were not obtained. No details on the exact drug regimen were obtained. The difference between the HIV-arm and the control-arm was statistically significant ($p = 0.015$).

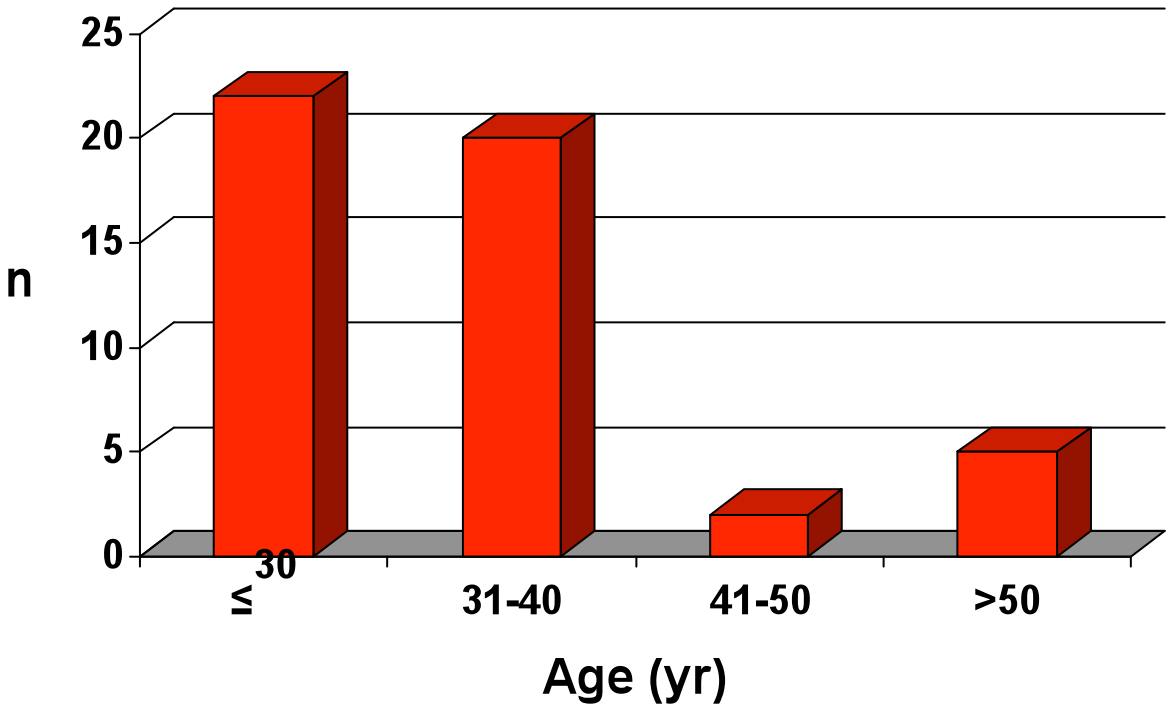
As per the exclusion criteria, none of the patients enrolled had a history of malignancy or recent surgery.

Table 3: Baseline characteristics of GROUP A patients

Baseline Characteristics	HIV-POSITIVE DVT-arm n (%)	HIV-POSITIVE No DVT n (%)
Total number	50 (100)	56 (100)
Sex		
Female	30 (60)	40 (71)
Male	20 (40)	16 (29)
CD4 Count		
No. of patients analysed	30	50
< 200 cells/mm^3	17 (34)	26 (46)
≥ 200 cells/mm^3	13 (26)	24 (43)
Mean cells/mm^3 (SD)	202.70 (124.2)	233.74 (208.2)
Tuberculosis	22 (44)	11 (19.6)
Site of DVT		
Right side	19 (38)	0 (0)
Left side	25 (50)	0 (0)
Bilateral	6 (12)	0 (0)
Age - years (SD)	32.43 (11.97)	32.86 (9.62)

n = number; SD = standard deviation

Figure 2: Age breakdown of HIV-positive patients with DVT



HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS

Table 4: Comparison of mean values for haematological and biochemical parameters between HIV-positive DVT-arm and control-arm

Baseline Characteristics	HIV-positive DVT-Arm (SD)	HIV-positive No DVT (SD)	p-Value
Haemoglobin [g/dl]	8.76 (2.76)	9.94 (2.51)	0.024*
WCC [$x10^9$/l]	7.11 (3.20)	5.82 (2.35)	0.020*
Platelets [$x10^9$/l]	293.73 (130.59)	246.64 (121.34)	0.058
Urea [mmol/l]	4.03 (1.41)	5.9 (7.09)	0.102
Creatinine [umol/l]	64.55 (16.46)	88.95 (78.27)	0.056
Albumin [g/l]	19.79 (7.60)	28.39 (9.13)	<0.001*
Globulin [g/l]	59.78 (11.06)	55.8 (16.17)	0.200

* Statistically significant at 0.05 level
DVT = deep vein thrombosis; SD = standard deviation; WCC = white cell count

The mean haemoglobin was significantly lower in the HIV-positive DVT-arm ($p = 0.024$).

The mean platelet count was higher in the HIV-positive DVT-arm, but was within the normal range and the difference was of borderline statistical significance ($p = 0.058$). In the HIV-positive DVT-arm, only one patient had an elevated platelet count ($>450 \times 10^9$ /L).

The mean white cell count was higher in the HIV-positive DVT-arm (7.11 vs. 5.82), but within the normal range. The difference was statistically significant ($p = 0.020$).

The mean urea and creatinine values in both arms were within the normal range.

The mean globulin level was increased in the HIV-positive DVT-arm compared to controls, but this was not statistically significant ($p = 0.20$). In the HIV-positive DVT-arm, 17 of 37 patients (46%) analysed had elevated globulin levels, compared to 16 of 50 patients (32%) in the control arm.

Mean albumin levels were lower in the HIV-positive DVT-arm and this was statistically significant ($p < 0.001$).

Only 3 out of the 82 HIV-positive patients analysed had a positive anti-nuclear factor (ANF). Two (2) of these patients were in the HIV-positive DVT-arm.

The anticardiolipin antibody (ACA) was positive in 18 of the 46 patients (39%) tested in the HIV-positive DVT-arm and 26 of the 47 patients (42%) tested in the control arm. The difference was not statistically significant ($p = 0.834$).

COAGULATION STUDIES (Tables 5, 6)

The mean international normalised ratio (INR) and partial thromboplastin time (PTT) were within the normal range in both the HIV-positive DVT-arm and control-arm and there was no statistical difference between the two arms. The mean fibrinogen was slightly lower in the HIV-positive DVT-arm (4.08 vs. 4.24).

The mean total protein S level was lower in the control arm compared to the HIV-positive DVT-arm (69.29% and 81.15% respectively) and the difference was statistically significant ($p = 0.047$). However, both levels were within the normal range (60 - 140 %).

The mean protein C level (66.9%) was reduced in the HIV-positive DVT-arm and normal in the control-arm (80.38%). The difference between the two groups was statistically significant ($p = 0.016$). Twenty six (26) of 43 patients (60.1%) analysed in the HIV-positive DVT-arm had low protein C levels as compared to 19 of 50 patients (38%) in the control-arm.

The mean antithrombin levels were normal in both the control and HIV-positive DVT-arms and there was no statistically significant difference between the two arms ($p = 0.661$).

Of the 41 patients analysed in the HIV-positive DVT-arm, 4 had an elevated lupus anticoagulant. Seven (7) of the 50 patients tested in the control-arm had an elevated level.

The mean activated protein C resistance values were normal in both groups. Of the 26 patients analysed in the HIV-positive DVT-arm, all had normal activated protein C resistance levels.

Table 5: Mean values: Coagulation studies HIV-positive patients

INR, international normalised ratio; PTT, partial prothrombin time;

Baseline Characteristic	HIV-positive DVT-Arm (SD)	HIV-positive No DVT(SD)	p-Values
INR	1.14 (0.25)	1.10 (0.21)	0.372
PTT [sec]	35.77 (9.47)	34.47 (6.44)	0.443
Fibrinogen [g/l]	4.08 (1.39)	4.24 (2.11)	0.659
Protein S [%]	81.15 (35.45)	69.29 (20.79)	0.047*
Protein C [%]	66.93 (26.87)	80.38 (25.73)	0.016*
Antithrombin [%]	102.56 (25.59)	100.47 (19.39)	0.661
APCR	2.91 (0.69)	3.05 (0.60)	0.373

APCR, activated protein C resistance

* Statistically significant at 0.05 level

Table 6: D-dimer levels

D-dimer Level	HIV-positive DVT	HIV-positive No DVT
≤500	8	38
1000	13	5
2000	4	5
4000	4	2
8000	2	0

In the HIV-positive DVT-arm, 23 of 31 patients (74%) analysed had positive D-dimer screens (> 500ng/ml). Twelve of the 40 control patients (24%) had positive D-dimer screens. The difference between the HIV-positive DVT-arm and the control-arm was statistically significant ($p < 0.001$). Table 6 depicts the breakdown of the D-dimer levels in both arms.

BIVARIATE AND MULTIVARIATE ANALYSIS OF DATA (Tables 7 and 8)

Table 7(a): Bivariate analysis of risk factors in HIV-positive patients

Baseline Characteristics	**HIV-positive DVT-Arm** n (%)	**HIV-positive No DVT** n (%)	**p-Value**
Gender			
Female	30 (60)	40 (71)	0.215
Male	20 (40)	16 (29)	
History of Active TB			
Tuberculosis			
Possible tuberculosis/	22 (44)	11 (19.6)	0.011*
No tuberculosis	28 (56)	45 (80.4)	
Platelets(x10^9/l)			
High: ≥ 450	2 (4.1)	3 (5.4)	1.00
Normal/Low: < 450	47 (95.9)	53 (94.6)	
D-dimer(qualitative)			
Positive	23 (74.2)	12 (24)	<0.001*
Negative	8 (25.8)	38 (76)	
Antinuclear Factor			
Positive	2 (5.6)	1 (2.2)	0.579
Negative	34 (94.4)	45 (97.8)	
Anticardiolipin Antibody			
Positive	18 (39.1)	20 (42.6)	0.834
Negative	28 (60.9)	27 (57.4)	
CD4			
< 200 cells/mm^3	17 (56.7)	26 (52)	0.817
≥ 200 cells/mm^3	13 (43.3)	24 (48)	
Globulins			
High	17 (45.9)	16 (32)	0.185
Low	20 (54.1)	34 (68)	

Table 7(b): Bivariate analysis of risk factors

Baseline Characteristics	HIV-positive DVT-Arm n (%)	HIV-positive No DVT n(%)	p-Value
Protein C			
Elevated/Normal	17 (39.5)	31 (62)	0.038*
Low	26 (60.5)	19 (38)	
Protein S			
Elevated/Normal	29 (70.7)	35 (67.3)	0.823
Low	12 (29.3)	17 (32.7)	
Antithrombin			
Elevated/Normal (≥80%)	36 (87.8)	46 (93.9)	0.461
Low	5 (12.2)	3 (6.1)	
Fibrinogen			
Elevated	17 (36.2)	15 (29.4)	0.523
Normal/Low	30 (63.8)	36 (70.6)	

* Statistically significant at 0.05 level

DVT = deep vein thrombosis

Table 8: Comparison of HIV-positive patients with DVT and without DVT on multivariate analysis

Risk Factor	OR (95% CI)*	p-Value
Tuberculosis	3.24(1.281-8.231)	0.030*
D-dimer	3.858(1.208-12.319)	<0.001*

* Statistically significant at 0.05 level
OR = odds ratio; CI = confidence interval

Both arms were well matched with regard to age and sex.

The factors that were significant on bivariate analysis were active tuberculosis on treatment, low protein C levels and a positive D-dimer screen (Table7).

On multivariate analysis, only tuberculosis and D-dimer positivity were found to be significant (table 8).

Group B: HIV-positive and negative patients with DVT

BASELINE CHARACTERISTICS: GROUP B (Table 9)

Patients with upper segment DVT who did not have any identifiable risk factors as mentioned in our exclusion criteria were recruited into the study. When tested for HIV status, the majority of these patients were HIV-positive, i.e., 50 patients. Only 22 patients were HIV-negative. The HIV-negative patients with thrombosis were compared to the HIV-positive cohort with thrombosis.

The mean age of the HIV-negative DVT group was significantly higher than the HIV-positive group with DVT (p=0.005). There were relatively more males in the HIV-negative arm compared to the HIV-positive arm, but the difference was not statistically significant.

There was no significant difference between the 2 arms with regard to site of DVT. However, a higher percentage of patients in the HIV-negative arm had bilateral thrombosis (31% vs. 12%). Four of the 13 patients with bilateral DVT (2 in the HIV-positive group and 2 in the HIV-negative group) had a history of tuberculosis on treatment and a further 3 patients were being investigated for possible tuberculosis.

Seven of the 22 patients in the HIV-negative group gave a history of being on treatment for tuberculosis. There was no significant difference in the prevalence of tuberculosis between the HIV-positive and HIV-negative patients with thrombosis (p = 0.269).

Table 9: Baseline characteristics of HIV-positive and HIV-negative patients with DVT

Baseline characteristics	**HIV-positive with DVT** n (%)	**HIV-negative with DVT** n (%)	**p-value**
Number of Patients Evaluated	50	22	
Mean Age	31.78	41.45	0.005
Sex Female Male	 30 (60) 20 (40)	 8 (36.4) 14 (63.6)	 0.064
History of Tuberculosis on treatment	22 (44%)	7 (31.8%)	0.269
Site of DVT Right Left Bilateral	 19 (38) 25 (50) 6 (12)	 5 (23) 10 (45) 7 (31)	 0.722 0.205

Group B: HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS (Table 10)

The mean platelet count was higher in the HIV-negative patients with thrombosis compared to the HIV-positive group with thrombosis and this was of borderline significance (p =0.06).

Mean fibrinogen levels were significantly higher in the HIV-negative group (p = 0.05).

Mean protein C levels were reduced in the HIV-positive group and were significantly lower in the HIV-positive patients compared to the negative cohort (p=0.02).

Mean protein S levels were normal in both groups, but significantly lower in the HIV-positive group (p =0.04).

Mean antithrombin levels were normal in both groups and there was no significant difference between them (p=0.72).

Mean albumin levels were significantly reduced in the HIV-positive patients with thrombosis compared to the HIV-negative group (p=0.02).

Mean globulin levels were significantly higher in the HIV-positive patients (p =<0.001)

Table 10: Mean values haematological and biochemical parameters in HIV-positive and negative patients with DVT

Baseline characteristics	HIV-positive with DVT	HIV-negative with DVT	p-value
Platelets	293.7	366.8	0.06
Fibrinogen levels	4.08	4.9	0.05
Protein C	66.93	85.79	0.02
Protein S	81.15	99.61	0.04
Antithrombin	102.56	99.77	0.72
Albumin	19.79	25.57	0.02
Globulins	55.78	44.56	< 0.001

BIVARIATE ANALYSIS OF RISK FACTORS: GROUP B (Table 11)

On bivariate analysis, significantly more patients in the HIV-positive group with thrombosis had reduced protein C levels compared to the HIV-negative cohort with thrombosis (p =0.011).

Also, significantly more patients in the HIV-positive DVT group had reduced protein S levels compared to the HIV-negative group. Of note, none of the patients in the HIV-negative group had reduced protein S levels.

Both the HIV-positive group and the HIV-negative group had reduced antithrombin levels in 15% of the patients tested (2 of 13 in the HIV-negative group and 6 of 41 in the HIV-positive group).

Only one of the 16 patients tested in the HIV-negative group had elevated globulin levels compared to 17 of the 37 patients in the HIV-positive cohort. The difference was statistically significant (p=0.005).

Table 11: Bivariate analysis of risk factors in HIV-positive and negative patients with DVT

Baseline characteristics	HIV-positive with DVT	HIV-negative with DVT	p-Value
Protein C levels			
Number Tested	43	14	
Low Levels	26	3	0.011
Elevated/Normal Levels	17	11	
Protein S levels			
Number Tested	41	18	
Low Levels	29	0	0.010
Elevated/Normal Levels	12	18	
Antithrombin			
Number Tested	41	13	
Low Levels	6	2	0.947
Elevated/Normal Levels	35	11	
Globulins			
Number Tested	37	16	
Low Levels	20	15	0.005
Elevated/Normal Levels	17	1	

5. DISCUSSION

Venous thrombotic disease can be a serious and potentially fatal complication of HIV infection. Clear insight is needed into the risk of venous thrombotic disorders in HIV-infected patients in order to plan the optimal interventions to prevent DV. This entails an understanding of the relationship between HIV and DVT, the mechanisms responsible for DVT in HIV-infected individuals and the risk factors for DVT in HIV-infected individuals.

There are uncertainties regarding the incidence of and the risk factors for thrombosis in HIV-infected patients.[3] A number of studies have suggested that the risk of deep vein thrombosis was higher in HIV-infected individuals. Klein et al. [33] performed a systematic review of the literature and identified ten relevant epidemiological studies that investigated the risk of venous thrombosis in HIV-infected patients. The incidence was increased 2- to 10-fold in comparison to a healthy population of the same age. However, these studies were mainly retrospective cohort studies that were prone to selection bias. Confounding factors were not always mentioned and in many studies, control populations were missing. They concluded that an increased risk of venous thrombotic events could be explained by the presence of a hypercoagulable state characterised by an increase in procoagulant factors such as endothelial tissue factor expression and thrombogenic properties of microparticles, and a decrease in anticoagulant factors (including antithrombin and heparin cofactor 2) and abnormalities in the protein C pathway.

Furthermore, the risk of venous thromboembolism was associated with an increased risk of infection and was weakly associated with highly active anti-retroviral therapy (HAART). The evidence pointed towards a relationship between HIV infection and venous thrombotic disease, but they concluded that this association needed to be established in well-designed epidemiological studies.[33]

This study of African (Black) HIV-infected patients attempted to ascertain possible coagulation system abnormalities as risk factors for deep vein thrombosis in Black HIV-positive patients. All the patients were antiretroviral therapy naïve. None of the patients had recognised risk factors for thrombosis such as malignancy or previous surgery.

Altogether 126 patients were enrolled onto the study. There were 72 patients in the DVT-arm and 56 patients in the control-arm (HIV-positive patients without thrombosis). In the DVT-arm there were 50 patients who were HIV-positive whilst 22 patients were HIV-negative. Six (6) of the patients in the HIV-positive DVT-arm and seven patients in the HIV-negative thrombosis group had bilateral thromboses.

Comparative analysis was done in two parts. In group A HIV-positive patients with and without thrombosis were analysed, whilst in group B HIV-positive and negative patients with thrombosis were analysed. In group A there were more females compared to males both in the HIV-positive DVT-arm (30 of 50) as well as in the control-arm (40 of 56), but this was not statistically significant. Of

the subjects with proven thrombosis (Group B), there were more males in the HIV-negative thrombosis arm compared to the HIV-positive thrombosis arm and this was of borderline significance (p =0.064).

All the patients and control subjects in the cohort were African (Black) patients who developed thrombosis in the absence of the classic well-recognised risk factors such as surgery and malignancy. Understanding of the epidemiology and risk factors for venous thromboembolism in the Black population is limited.[34] Venous thrombosis has historically been perceived as a disease restricted to White populations, a belief reinforced by the low incidence of known hereditary prothrombotic mutations in Blacks together with the lack of diagnostic services in underdeveloped countries. Furthermore, data on thrombosis in Africa is limited [60] and our findings are an important contribution to research on thrombosis in Blacks in Africa.

Recent reports suggest that venous thromboembolism is common across a variety of racial groups including Africans.[34] In a prospective study performed to ascertain possible ethnic differences in the prevalence of DVT, no significant difference was found in Black subjects compared to Whites.[59] White et al. [56] reported an almost 30% higher incidence of idiopathic venous thrombosis in African-Americans compared to Whites.

The mechanisms for predisposition to thrombosis in Blacks, however, may differ. Some of the congenital thrombophilic disorders such as factor V Leiden and the prothrombin gene mutation are extremely rare in Blacks.[14]

Patel et al. [35,36] found that significant factors in Black patients included elevated D-dimers as well as elevated factor VIII levels. Pieper et al. [37] also found significantly higher levels of D-dimer in Black persons when assessing a group of community dwelling elderly persons. Significantly elevated qualitative D-dimer levels were found in our HIV-positive patients with thrombosis (74% of HIV-positive patients with thrombosis had an elevated D-dimer compared to 24% of HIV-positive patients without thrombosis). The significance of this as a predisposing factor is unclear as D-dimer levels can also be elevated in acute thrombosis.[6] D-dimer results were not available for the HIV-negative cohort with thrombosis and analysis was not possible. Further studies would need to be done in our population to establish baseline levels and to repeat the test in patients with DVT after the acute event has subsided.[35]

The mean age of the HIV-positive patients in this study was 32 years (32.43 in the DVT-arm and 32.86 in the control-arm). Only 5 patients in the HIV-positive DVT-arm were over 50 years of age. Twenty-two (22) patients in the HIV-positive DVT-arm were ≤ 30 years of age. When comparing HIV-positive and negative patients with thrombosis, the HIV-negative group was significantly older than the HIV-positive group.(41.45 vs. 32 years). The mean age of our HIV-positive patients with thrombosis was even lower than that of Saber [31] (43 years) and Saif [30] (39 years). The incidence of thrombosis strongly depends on age. It is a very rare disorder in the young and a common affliction in the elderly. Venous thrombosis is a multicausal disease; more than one risk factor needs to be present before thrombosis occurs. Thrombosis occurs when a sufficient number of risk factors are present simultaneously.

The younger an individual, the more risk factors are needed to precipitate thrombosis.[38] In children, 3 or 4 risk factors are required before thrombosis occurs, whereas in individuals aged 55 years and older, thrombosis will almost invariably occur when two or more risk factors are present. The number of risk factors needed to cause thrombosis decreases with age, which itself appears as a risk factor for thrombosis.[38] DVT is uncommon in young patients unless predisposing factors such as malignancy and congenital thrombophilia are present.[38] HIV-positive patients with thrombosis in our study did not have any of the recognised risk factors for thrombosis, i.e., malignancy, hereditary thrombophilia and recent surgery. Considering that no other predisposing factors were identified, HIV infection and its complications are a strong consideration as risk factors for thrombosis. This postulate is reinforced by the retrospective study by Copur et al. in which the frequency of VTE was significantly higher in HIV-positive patients under 50 years compared to HIV-negative patients under 50 years (3.315 vs. 0.53%; $p < 0.0001$).[20] Also, the rate of VTE was significantly greater in the older HIV-negative patients compared to the younger HIV-negative patients ($p < 0.0001$). Among HIV-positive patients under 50 with VTE, risk factors for VTE were present in 3 of 10 patients (30%) as compared to 21 of 35 patients (60%) in the HIV-negative group under 50 years.

There is uncertainty regarding the importance of the stage of HIV infection in the predisposition to thrombosis. Some studies have suggested that patients with clinical or immunological AIDS were more predisposed to deep vein thrombosis.[33] There are, however, other studies that found no statistically

significant increased risk for patients with a CD4 count < 200 cells/mm^3 compared to those with a CD4 count > 200 cells/mm^3.[3,29]

The mean CD4 count in our study was lower in the HIV-positive DVT-arm when compared to the control-arm but the difference was not statistically significant (p = 0.461). Seventeen (17) of the 30 patients (57%) tested in the DVT-arm had CD4 counts <200 cells/mm^3. The findings in this study are limited as only 30 patients in the DVT-arm had their CD4 counts done and clinical HIV staging was not done. The lack of data regarding the clinical stage of HIV infection and the small number of patients tested makes analysis difficult and may have precluded us from showing the impact of AIDS on the occurrence of DVT.

Although tuberculosis was not one of the primary determinants of this study, a history of active tuberculosis on treatment was an independent risk factor for DVT in the HIV-positive group as determined by multivariate analysis. The risk of DVT was increased 2.9-fold for patients with tuberculosis. Significantly, 31.8% of patients in the HIV-negative cohort with thrombosis had a history of tuberculosis on treatment. Six (6) of the patients in the HIV-positive DVT-arm and seven patients in the HIV-negative thrombosis group had bilateral thromboses. Analysis of these patients revealed tuberculosis as a possible risk factor. Four of the 13 patients (2 in the HIV-positive group and 2 in the HIV-negative group) had a history of tuberculosis on treatment and a further 3 patients were being investigated for possible tuberculosis. The significance of

this is unclear, as these patients were not fully investigated for abdominal pathology.

The global burden of tuberculosis was estimated at 9 million cases in 2004. It is estimated that 80% of tuberculosis occurs in sub-Saharan Africa and Asia. Sub-Saharan Africa is one of the areas with the highest burden of both TB and HIV infection. South Africa has the largest number of people living with HIV infection in the world and ranks ninth on a list of twenty-two high burden TB countries (i.e., incidence rate of 558 per 100 000).[40] Worldwide, tuberculosis is one of the common opportunistic diseases in those infected with HIV.[40] People at risk for HIV infection are also at risk for tuberculous infection. Numerous reports and epidemiological studies have confirmed the association between HIV and tuberculosis.[39] HIV infection impairs cellular immunity, the latter being responsible for containing latent tuberculosis and new exposure to *Mycobacterium*.[41] The link between tuberculosis and DVT in this study suggests that tuberculosis may be an important predisposing factor in the pathogenesis of DVT both in the HIV-positive and negative patients in an area where tuberculosis and HIV are endemic.

The association between tuberculosis and deep vein thrombosis has previously been reported. However, this finding has never been validated in the HIV-infected population. Deep vein thrombosis is clinically observed and can be confirmed with laboratory methods in 3 to 4 % of patients with pulmonary tuberculosis (PTB). The real incidence may, however, be closer to 10% because it is thought to be clinically unclear in most patients.[42]

There are several postulates on why patients with tuberculosis are predisposed to deep vein thrombosis. Severe pulmonary tuberculosis is

characterised by an acute phase response and a hypercoagulable state.[42] Experimental studies have shown that peripheral blood mononuclear cells in PTB can be readily induced to produce interleukin-1 (IL-1), IL-6 and tumour necrosis factor-alpha (TNF-α). It is likely that the vascular endothelium could be primed as a result of the interaction between mycobacterial products and the host monocyte-macrophage system, which then synthesises large amounts of TNF-α and IL-6. These cytokines induce hepatic acute phase responses that alter levels of coagulation factor proteins such as fibrinogen and factor VIII. It has been shown that the risk of DVT is four times higher in patients with fibrinogen levels over 5g/L.[42] Turken et al. looked for possible haemostatic disturbances that predisposed to venous thrombosis in patients with PTB.[42] They found that patients with PTB had decreased antithrombin, decreased protein C, increased fibrinogen and increased platelet aggregation, and that these levels improved with treatment of the PTB. Also, plasminogen activator inhibitor-1 levels were high and remained high even after treatment of PTB was commenced. Similarly, Robson et al. have found that elevated plasma fibrinogen with impaired fibrinolysis together with a decrease in antithrombin and a thrombocytosis would favour the development of DVT in patients with PTB.[43] Deep vein thrombosis can occur due to venous obstruction caused by retroperitoneal lymphomas and malignant masses. In HIV-infected patients, tuberculosis is frequently extrapulmonary and disseminated. Sites commonly affected in extrapulmonary tuberculosis are lymph nodes, intestine, peritoneum, genitourinary tract and bones.[39] Tubercular lymph nodes may cause inferior vena cava obstruction and thrombosis.[38] A report by White suggests that rifampicin therapy is yet

another procoagulant factor in patients with PTB.[44] Rifampicin is associated with proliferation of smooth endoplasmic reticulum of the hepatocyte and with the induction of cytochrome p450. This could alter the balance of anticoagulant and procoagulant proteins produced by the liver.[44] Therefore, PTB leads to alteration in haemostasis which, together with immobility associated with hospitalisation and anti-tuberculous therapy, predisposes to DVT. The diagnosis of tuberculosis in this study was ascertained by the patient's history of current therapy for tuberculosis and was not confirmed by microbiological or tissue diagnosis. Considering that the diagnosis of TB was not the primary aim of the study, there are shortfalls that need to be addressed in further studies. Reliance was placed on the patient's clinical history for the documentation of tuberculosis. In further studies details of diagnosis of tuberculosis will need to be ascertained. Also, the site and possible dissemination of TB will need to be assessed. Careful assessment of treatment in patients already on anti-tuberculosis therapy will be required in view of the possible thrombogenic role of rifampicin.[44] The recognition of tuberculosis as a risk factor could make a case for prophylaxis against deep vein thrombosis in this population. Further studies are indicated both in HIV-positive and negative patients with tuberculosis to investigate accurately the real risk for the development of DVT as well as the possible pathogenesis of DVT in these patients .

The mean globulin levels were increased both in the HIV-positive DVT-arm and the control-arm and there was no statistically significant difference between the two arms. However, when comparing the HIV-positive and

negative patients with DVT, globulin levels were significantly increased in the HIV-positive cohort.

The mean haemoglobin as well as albumin levels were significantly reduced in the HIV-positive DVT-arm ($p = 0.024$ and < 0.001 respectively). This might signify more advanced HIV infection in this cohort. More investigations are needed to identify the possible mechanisms.

Mean fibrinogen levels were within normal limits in both HIV-positive groups and no significant difference was noted on univariate analysis. However, when comparing HIV-positive and negative patients with thrombosis, mean fibrinogen levels were elevated and significantly higher in the HIV-negative group of patients ($p = 0.05$).

ANF, ACA and lupus anticoagulant were not found to be significant risk factors predisposing to DVT in this study. Eighteen (18) patients in the HIV-positive DVT group and 20 patients in the control group had positive ACA screens. There have been several reports [49,50,51] commenting on the significance of a positive ACA screen in HIV-positive patients. Elevated anticardiolipin antibodies are found in 20-70% of HIV-positive patients. However, despite the high prevalence of ACA and lupus anticoagulant, the clinical manifestations of classic antiphospholipid antibody syndrome are distinctly unusual.[51] It is postulated that APL antibodies encountered in HIV infection do not share the features of immune APL antibodies and that they are an epiphenomenon in infectious disease. In infectious disease,

alloimmune rather than autoimmune antibodies are found, and these have not been associated with thromboembolic events.[49]

Mean protein C levels were significantly reduced in the HIV-positive DVT-arm compared to the HIV-positive controls (p = 0.016) as well as when compared to the HIV-negative patients with thrombosis (p =0.02). Interpretation of the low protein C level is complicated by the fact that levels can be reduced in acute thrombosis [4] as well as in patients with PTB.[42] In our study, 52% of patients in the DVT-arm who had TB and 66% of patients in the control-arm who had TB, had decreased protein C levels. We were unable to repeat these tests after the acute thrombotic phase as these patients were lost to follow up.

Mean total protein S levels were higher in the HIV-positive DVT-arm compared to the control-arm (81.15 vs. 69.29). However, they were both within the normal range. When comparing HIV-positive and negative patients with thrombosis, total protein S levels were significantly lower in the HIV-positive cohort. Although previous studies [33,45] suggested that protein S was a risk factor for thrombosis in HIV-positive patients, no significant association between protein S and thrombosis was observed in this study. Results could, however, have been affected by missing data. In this study, protein S levels were assayed in only 41 of the HIV-positive patients with DVT. Another possible limitation was that we did not measure free protein S levels as only total protein S assays were available at the time in our laboratory. Free protein S levels are more reliable than total protein S. However, studies have shown a correlation between free protein S and total protein S levels.[23]

The mean activated protein C resistance (APCR) values were normal in the HIV-positive thrombosis group as well as the control group. All 26 patients analysed in the DVT-arm had normal APCR levels. Factor V Leiden is the primary cause of activated protein C resistance.[15] This finding correlates with other studies which have shown that the prevalence of factor V Leiden is highest amongst people of European origin and is very rarely found amongst Blacks.[46,47,48]

Mean antithrombin levels were normal in the HIV-positive DVT-arm, the control-arm as well as the HIV-negative group with thrombosis.

Many studies [22,23,24,25] , including ours have found decreased levels of protein C, S or antithrombin, both in HIV-positive patients with DVT and those without DVT. Bissuel et al. evaluated 63 HIV-positive patients and of these, 41 had low free protein S levels.[23] Yet only three of these patients had a thrombotic event. All three patients had full-blown AIDS and had active infections. Stahl found 19 patients with reduced free protein S levels and only three of them had thrombosis.[24] Some of the studies showed low protein S levels in patients with HIV, yet none of the patients had thrombosis.[22,28] Feffer et al. found decreased protein C and free protein S in patients with HIV, yet none of them had thrombosis.[28] These findings suggest that reduced levels of protein C, S and antithrombin do occur in HIV-positive patients, but that this alone is not enough to result in thrombosis. Venous thrombosis is a multicausal disease; more than one risk factor needs to be present before thrombosis occurs. Thrombosis occurs when a sufficient number of risk factors are

present simultaneously. It suggests that a combination of factors, which include infections, malignancy and immobilisation, together with reduced anticoagulant factors, result in the development of thrombosis. It is possible that different combinations of factors are responsible in each patient.

There were limitations in this study which will need to be addressed in future studies. The original intention of this study was to have enrolled an additional arm of HIV-negative subjects with proven DVT to interrogate possible specific associations with HIV infection. However, only 22 HIV-negative patients with DVT that met our inclusion criteria presented to the hospital during this period. We were only able to extract limited data for this group, as some of the data was unavailable.

As alluded to earlier, owing to logistical problems, many patients in the study did not have CD4 counts and protein C and S levels done.

We performed total protein S levels in the study. Both free and total protein S levels may be more informative in future studies.

Ideally, coagulation testing should have been done after the acute thrombotic phase. We were unable to repeat these tests at a later time because of poor follow-up.

We also did not ascertain from patients whether they had been immobilised and, if so, for how long.

Tuberculosis has emerged as an important risk factor for DVT in this study. As alluded to earlier, in future studies there will have to be careful screening for tuberculosis in patients with DVT.

We did not assess our patients for other infections associated with immune deficiency. Many of these infections are considered an additional risk factor for thrombosis. Squizzato, Gerdes et al. [53] reviewed published human studies on the influence of cytomegalovirus (CMV) infection on the coagulation system. They found that it was likely that CMV is involved in the pathogenesis of thrombosis but that further studies were required to evaluate the role of CMV as a risk factor for thrombosis. In vitro experiments have also suggested that other viruses such as Herpes simplex virus type 1 and 2, respiratory syncytial virus and adenovirus, might have procoagulant effects as well.[52] Also, although none of our patients at the time of assessment gave a history of malignancy, we did not definitively exclude malignancy as a predisposing factor for thrombosis.

Although none of patients were on antiretroviral therapy, this is an additional factor that will have to be taken into consideration in future studies. George et al. found that the incidence of venous thrombotic events increased dramatically from 0.19% before the introduction of protease inhibitors to 1.07% afterwards.[53] However, recently Fultz et al. reported finding no significant increase.[54] Thus, it is not yet clear whether antiretroviral therapy is associated with an increased risk of DVT. The postulated mechanisms could be related to increased levels of plasminogen activator inhibitor-1, fibrinogen

and lipid. Further studies would be required to establish whether HAART adds to the risk of DVT.[33]

This study highlights the importance of HIV and tuberculosis as possible risk factors for the development of DVT. In a population such as ours in Sub-Saharan Africa , where the prevalence of both tuberculosis and HIV is high, confirmation of our findings will have important implications in terms of prophylaxis against DVT in vulnerable populations. Further studies are therefore required to address the limitations of our study and add to the understanding of this important topic.

6. CONCLUSIONS

HIV-positive and negative African (Black) patients with deep vein thrombosis as well as HIV-positive patients without DVT were analysed to identify possible coagulation factor abnormalities predisposing to the development of deep vein thrombosis in HIV-positive patients.

A compelling argument for HIV being a significant predisposing factor in the pathogenesis of DVT is that 50 of the 77 randomly referred patients with DVT were HIV-positive and that the HIV-positive group were young patients with no other recognised risk factor for the development of DVT. The mean age of the HIV-positive patients with DVT was 32 years. The HIV-negative group with DVT were significantly older than the HIV-positive group (42 vs. 32 years). The mean CD4 count in the HIV-positive patients with thrombosis was 202 cells/mm^3. A low CD4 count was not found to be a significant predisposing factor for thrombosis.

Mean protein C levels were significantly reduced in the HIV-positive DVT-arm compared to the HIV-positive group without thrombosis. Protein C levels were significantly reduced on bivariate, but not on multivariate analysis. When comparing the HIV-positive and HIV-negative patients with thrombosis, mean protein C levels were significantly reduced in the HIV-positive patients. Mean protein S levels were normal in both DVT-arms as well as the control-arm. However, the mean protein S levels was significantly lower in the HIV-positive patients with thrombosis compared to the HIV-negative thrombosis group.

Mean antithrombin levels were normal in the HIV-positive and negative DVT groups as well as in the control-arm and there was no statistically significant difference between the groups.

A positive D-dimer was significant both on bivariate and multivariate analysis in HIV-positive patients with thrombosis compared to the HIV-positive control group.

Although not a primary aim of the study, tuberculosis was a significant factor predisposing to the development of DVT in the HIV-positive patients both on univariate and multivariate analysis. Twenty-two (22) of the 50 HIV positive patients (44%) with DVT gave a history of being treated for tuberculosis. Also important is that 7 of the 22 patients (31.8%) analysed in the HIV-negative group with thrombosis had a history of tuberculosis on treatment. With both HIV and tuberculosis being endemic in our population, tuberculosis might be an important risk factor predisposing to DVT both in the HIV-positive and negative patients. Further studies are needed to confirm this finding as, in this study, the diagnosis of tuberculosis was made only from the history obtained from the patient.

Patients with classic thrombophilic risk factors such as malignancy and recent surgery were excluded from the study. It is well known that HIV-positive patients have an increased incidence of malignancy. This would further increase the risk of DVT in this group of patients.

The findings of his study add credence to postulates by other authors that the pathogenesis of thrombosis in HIV-infected patients is multifactorial. Venous thrombosis is a multicausal disease; more than one risk factor needs to be present before thrombosis occurs. Thrombosis occurs when a sufficient number of risk factors are present simultaneously. It suggests that a combination of factors, which include infections, malignancy and immobilisation, together with reduced anticoagulant factors, result in the development of thrombosis. It is possible that different combinations of factors are responsible in each patient. There are several intersecting mechanisms associated with HIV and its complications that may predispose these patients to thrombosis. HIV may contribute to thrombosis by direct as well as indirect means. HIV infection could cause hypercoagulability by affecting endothelial function, causing perturbation of fibrinolysis as well as by causing reduction of levels of protein C, S and antithrombin. Opportunistic infections such as tuberculosis as well as cytomegalovirus could also predispose to thrombosis. Patients with HIV infection are also predisposed to the development of cancer which further predisposes patients to thrombosis. Additionally, there are suggestions that antiretroviral therapy, especially protease inhibitors, could predispose to thrombosis.

The lack of consensus regarding the pathogenesis of thrombosis in HIV-positive patients could partly be explained by the variability and multiplicity of predisposing factors depending on the stage of infection, associated infections, malignancy as well as drug history. This study highlights the importance of HIV and TB as possible risk factors for the development of

DVT. Further studies are indicated to confirm these postulates so that appropriate prophylactic measures can be instituted.

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APPENDIX

(a) DATA SHEET

Date:	Hospital No.:	
Name:	Age:	Sex:
Address:		
Telephone No: (Home)		
Clinical Problems:		
Past History:		
Family History:		
Drug History:		

(b) CONSENT FORM: ENGLISH

You have deep vein thrombosis. You will have blood tests done on you. These blood tests are performed on all patients with this condition. The reason we require your consent is that we shall be using the results for a study on this condition. You will be treated in the same way as all patients with deep vein thrombosis. However, in your case we shall do an additional test for human immunodeficiency virus as we want to establish if there is any relation between this disease and immunodeficiency virus infection.

You have a right to refuse performance of this test. If you agree to the performance of the tests you have the right to choose whether or not you wish to be informed of the results.

All results will be treated in confidence.

You also have the right to withdraw from the study at any time. If you refuse to participate in the study, your further management will not be prejudiced.

(c) CONSENT FORM: ISIZULU

Unesifo esidala ukuba libe yihlule phakathi emithanjeni yakho. Kukhona amagazi okuzodinga sizodinga siwathathe ukuze sense ama test athile. Lama “test” ayenziwa kubo bonke abantu abanaiesisfo, Indlela ozolashwa nhayo ngeke yehlike kuleyo abanye anbtu abelashwa ngayo. Kuzodingeka futhi sithathe igazi ukuze siyohola igciwane lengculazi, lokhu sikwenzela ukubu kesibheke ukuthi lesisifo asihambelani yini naleligciwane.

Unegunya lokwala ukwenziwa kwalama “test”. Uma kwenzeka uvuma sense lama “test” uwena oyosho noma uyathanda yini ukuyazi imiphumela yawo. Uma ufuna siyokutshela, uma ungafuni ngeke siklutshele, Lolulwazi esizolwazi ngawe loyokwaziwa yithi kuphela, amukho amunye omunye omunty oyoke azi. Kuyilungelo lakho ukuba usho noma yinini ukuthi ufuna ukuyeka ukuba sisebenzise imiphumela yakho kuloluphenyo.

Noma ungafuni ukuba kuloluhlelo lokufunda ngalesisifo, lokho akusho ukuthi usozobe usuyaekwa ukwelashwa.

(d) .NORMAL RANGES

- Haemoglobin (Hb) 11.5 -13.5 g/dl (females)
  13.5- 15.5 g/dl (males)
- White cell count $4 – 11 \times 10^9$ /L
- Platelets $150- 450 \times 10^9$ /L
- International normalised ratio (INR) < 1.2
- Partial Thromboplastin Time (PTT) 29-45 sec
- Fibrinogen 1.5-4.5 g/L
- D-dimer
  - negative < 500ng/ml
- Antinuclear factor negative
- Anticardiolipin antibody negative

- Lupus anticoagulant < 45 sec
- Protein C 70 – 140 %
- Protein S 60 – 140 %
- Antithrombin 80 – 120 %
- APC resistance 2 - 5
- CD4 count 550 –1955 cells/mm^3
- Globulin 20 – 32 g/L
- Albumin 32 – 50 g/L
- Urea 2.5 – 6.6 mmol/L
- Creatinine 53 –115 umol /L