Pathological Clotting and Deep Vein Thrombosis in Patients with HIV

Brandon S. Jackson
[1] Department of Surgery, University of Pretoria, Pretoria, South Africa

Etheresia Pretorius*
[2] Department of Physiological Sciences, Stellenbosch University, Stellenbosch, Matieland, South Africa

* **Address for correspondence**

Etheresia Pretorius, PhD
Department of Physiological Sciences
Stellenbosch University, Stellenbosch, Private Bag X1, Matieland, 7602
South Africa
Email: resiap@sun.ac.za

Abstract

The number of people infected with human immunodeficiency virus (HIV) is rapidly increasing and the majority of those infected are living in sub-Saharan Africa. Some hallmarks of HIV are inflammation and upregulation of inflammatory markers. A pathological coagulation system may accompany these inflammatory changes and potentially result in venous thromboembolism such as a deep vein thrombosis (DVT). In this review, the authors describe the inflammatory profile in HIV, the treatment regimens currently in place in South Africa, and in particular how HIV affects the hematological system, with specific focus on platelets, red blood cells (RBCs; erythrocytes), and fibrin(ogen). They also discuss the presence of DVT in HIV, focus on screening tests, and suggest a more proactive approach to track the inflammatory profile of HIV patients, by specifically using parameters that might point to pathological coagulation; these should involve platelet, RBC, and fibrin(ogen) analysis. They conclude by suggesting that including coagulation function tests to study the effect of treatment interventions would improve outcomes in these individuals, as it could help in the diagnosis of thromboembolic disease. Furthermore, this approach could streamline treatment strategies due to improved monitoring. A better understanding of hypercoagulability of HIV-infected patients is therefore urgently needed. In conclusion, the authors suggest a panel of pathology tests that should be considered as standard procedures when HIV is present.

Keywords: human immunodeficiency virus - deep vein thrombosis - antiretroviral medication - inflammation - coagulation

The number of people recorded with human immunodeficiency virus (HIV) infection is over 70 million and half of this population have already died. The mortality from HIV-related illnesses in 2016 alone was at 1 million people.[1] Sub-Saharan Africa has just under 21 million people infected with HIV, which therefore comprises nearly 60% of the worldwide cases.[2] New HIV infections are ~2.6 million per year globally.[3] HIV is a retrovirus that targets the immune system and primarily destroys and functionally impairs T lymphocyte cells and monocytes/macrophages.[4] [5] Furthermore, the viral replication and the antigenic stimulation by the virus stimulates host inflammatory responses,[4] [5] resulting in an upregulation of circulating proinflammatory cytokine concentrations. These inflammatory cytokines include well-studied biomarkers like interleukin-6 (IL-6), IL-1β and tumor necrosis

factor-α (TNFα).[6] [7] [8] [9] [10] One of the hallmarks of inflammation (which include a pathological circulating inflammatory marker profile) is a hypercoagulable state, and an increased propensity for abnormal clotting or thromboembolic events, like deep vein thrombosis (DVT). As HIV infection is associated with inflammation, as well as DVT and pathological clotting propensity, the focus of this review is to discuss the inflammatory HIV patient profile, how medication affects the occurrence of the hypercoagulable state, and how to address these events. The next few paragraphs will give a general overview of HIV, including various classifications, followed by a review of the literature on DVT and the abnormal clotting propensity during HIV infection.

HIV and Its Classification

An untreated HIV infection results in an exhausted and weakened immune system.[4] The deteriorating immune system is reflected by the low CD4+ (T lymphocyte) count and an increased risk of opportunistic infections.[11] The World Health Organization (WHO) immunological classification of HIV-infected patients is depicted in [Table 1], according to the absolute number of CD4+ lymphocytes (per mm^3). Acquired immunodeficiency syndrome (AIDS) is the term used for the severely immunocompromised patients.[2]

Table 1 WHO immunological classification according to the absolute number of CD4+ lymphocytes

HIV immunodeficiency classification	CD4+ cells (per mm^3)
Nonsignificant	Greater than 500
Mild	Between 350 and 499
Advanced	Between 200 and 349
Severe	Lower than 200

Note: Adjusted from World Health Organization (WHO).[11] WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children. HIV/AIDS Program: Strengthening health services to fight HIV/AIDS. World Health Organization Library Cataloguing-in-Publication Data 2007, 16.

In 2007, the WHO published a clinical staging system of HIV/AIDS.[11] The clinical criteria are divided into four stages. Asymptomatic patients are classified as stage 1. Those presenting with mild clinical features are classified as stage 2. Stage 3 represents advanced symptoms and Stage 4 represents severe symptoms. Stages 3 and 4 are associated with advanced HIV infection (see [Table 2]).[11] From stage 2 onward, inflammation and a pathological inflammatory marker profile are predominant characteristics in all the concurrent conditions listed in [Table 2].

Table 2 WHO clinical staging of HIV/AIDS

Clinical stage 1
• Asymptomatic with/without persistent generalized lymphadenopathy
Clinical stage 2
• Recurrent respiratory tract infections • <10% weight loss • Herpes zoster • Recurrent oral ulceration • Angular cheilitis • Papular pruritic eruptions • Fungal nail infections • Seborrheic dermatitis
Clinical stage 3
• > 10% weight loss • More than a month's duration of unknown cause of chronic diarrhea • Persistent fever of unknown cause >1 mo (temperature >37.6°C) • Oral hairy leukoplakia • Persistent oral candidiasis • Pulmonary tuberculosis • Acute necrotizing ulcerative stomatitis, gingivitis, or periodontitis • Severe bacterial infections • Unexplained anemia, thrombocytopenia, or neutropenia
Clinical stage 4
• Pneumocystis pneumonia • HIV-related wasting syndrome • Severe recurrent bacterial pneumonia • Infection with chronic herpes simplex >1 mo (orolabial, genital or viscera, or anorectal) • Oesophageal or airway candida infection • Kaposi's sarcoma • Extrapulmonary tuberculosis • Cytomegalovirus infection • HIV encephalopathy • Toxoplasmosis of the central nervous system • Extrapulmonary Cryptococcus infection • Progressive multifocal leukoencephalopathy • Disseminated nontuberculous mycobacterial infection • Chronic cryptosporidiosis (with diarrhea) • Disseminated mycosis (coccidiomycosis or histoplasmosis) • Chronic isosporiasis • Recurrent nontyphoidal Salmonella bacteremia • Lymphoma or other solid HIV-associated tumors • Atypical disseminated leishmaniasis • Invasive cervical carcinoma • HIV-associated nephropathy or cardiomyopathy that is symptomatic

Note: Adjusted from World Health Organization (WHO).[11] WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children. HIV/AIDS Program: Strengthening health services to fight HIV/AIDS. World Health Organization Library Cataloguing-in-Publication Data 2007, 16.

HIV Prevalence and Antiretroviral Usage in South Africa

The prevalence of HIV in South Africa, as well as worldwide, has escalated the already overwhelming influx of patients into hospitals. Highly effective antiretroviral (ARV) therapy combinations were introduced around 1995.[12] In South Africa, there are multiple classes of ARVs available (see [Table 3]). First-line ARV regimen, as recommended by the Southern African HIV Clinicians Society and conforming with international guidelines, includes the combination of two nucleoside/nucleotide reverse transcriptase inhibitors (NRTI) and a nonnucleoside reverse transcriptase inhibitor (NNRTI). The following combinations are recommended:

- Lamivudine (3TC) with tenofovir (TDF), zidovudine (AZT), or abacavir (ABC).
- Emtricitabine (FTC) with tenofovir in a fixed-dose combination or with the addition of efavirenz as a combined pill.[13]

Table 3 Classes of ARV therapy available in Southern Africa

Class	Mechanism of action	Specific action	ARV therapy
Nucleoside/nucleotide reverse transcriptase inhibitors	Reverse transcriptase inhibitor	Inhibits the HIV RNA transcription to DNA by mimicking DNA building blocks	Zidovudine, lamivudine, didanosine, abacavir, stavudine, tenofovir, emtricitabine
Nonnucleoside reverse transcriptase inhibitors	Reverse transcriptase inhibitor	Blocks the binding site of reverse transcriptase	Efavirenz, nevirapine, etravirine
Protease inhibitors	Protease inhibitor	Inhibits the maturation stages of replication resulting in viral particles that are not infective	Atazanavir, indinavir, lopinavir/ritonavir, saquinavir, darunavir
Integrase strand transfer inhibitors	Viral integration inhibitor	Inhibits proviral DNA being transferred into the host DNA	Raltegravir

Abbreviations: ARV, antiretroviral; DNA, deoxyribonucleic acid; RNA, ribonucleic acid.
Source: Adapted from Meintjes et al.[13]

In Pretoria (South Africa), the first-line ARV regimen combination most frequently used is efavirenz (NNRTI), emtricitabine (NRTI), and tenofovir (NRTI). ARVs suppress HIV replication, but have also multiple side effects, specifically the NRTIs and the protease inhibitors (PIs). The most common side effects include lactic acidosis, pancreatitis, hypersensitivity reactions, and cardiovascular abnormalities.[3] [14] [15] [16] [17] ARVs have been associated with coagulation abnormalities in HIV-positive patients (see [Table 4]). NRTI with NNRTI regimen, and the NRTI with PIs regimen have demonstrated decreased concentrations of endothelial markers (von Willebrand factor [VWF], soluble vascular adhesion molecule 1 [sVCAM-1], and soluble intercellular adhesion molecule 1 [sICAM-1]). The NRTI with PI regimen has also been associated with an increase in fibrinogen concentrations.[18] [19] [20] [21] The next few paragraphs will discuss in more detail the physiological reasons for these coagulation abnormalities.

Table 4 Coagulation parameters and ARV regimens

Parameters	NRTI regimen	NRTI + NNRTI regimen	NRTI + PI regimen
Coagulation markers: PT, aPTT, VWF, factor VIII, fibrinogen, D-dimer, and endogenous thrombin potential	No significant differences	No significant differences between combinations	Increased fibrinogen levels
Endothelial markers: VWF, sVCAM-1, and sICAM-1	No information	Decreased	Decreased
Platelet markers: CD40 ligand and P-selectin	No information	No significant differences	No significant differences

Abbreviations: aPTT, activated partial thromboplastin time; ARV, antiretroviral; NNRTIs, nonnucleoside reverse transcriptase inhibitors; NRTIs, nucleoside/nucleotide reverse transcriptase inhibitors; PIs, protease inhibitors; PT, prothrombin time; sICAM-1, soluble intercellular adhesion molecule 1; sVCAM-1, soluble vascular adhesion molecule 1; VWF, von Willebrand factor.
Source: Compiled from Baker et al,[18] Wolf et al,[19] and Jong et al.[20,21]

Circulating Inflammatory Markers and Their Effects on the Hematological System

As mentioned in the introduction, inflammation is one of the hallmarks of a hypercoagulable state. A hypercoagulable state can be considered as a condition where the components of coagulation are increased/activated and/or the anticoagulation pathways impaired/inactivated resulting in a hematological system that is prone to thromboembolism. Hypercoagulable conditions include protein C deficiency, antiphospholipid syndrome, increased VWF, and malignancy. Coagulation abnormalities are clinically measured by plasma-based, activated partial thromboplastin time [aPTT], and prothrombin time [PT], as sometimes converted to an international normalized ratio [INR]) as well as whole blood (viscoelastic tests) based coagulation tests. A combination of these tests is usually performed as there are limitations of each test (see [Table 5]).[22] [23] [24]

Table 5 Commonly used coagulation tests and their limitations

Coagulation tests	Limitations (selected references provided)
Bleeding time	Patients using antiplatelet therapy, operator dependent, frail skin
PT	Laboratory variations in PT results vary with the type of reagent used in combination with the instrument
aPTT	Laboratory variations in aPTT results vary with the type of reagent used in combination with the instrument
INR	INR differences due to incorrect prothrombin time ratios; incorrect MNPT and ISI assignment within the INR calculation; incorrect thromboplastin reagent/test system for the ISI/MNPT assignment
Thromboelastography	Unable to identify platelet adhesion and VWF abnormalities

Abbreviations: aPTT, activated partial thromboplastin time; INR, international normalized ratio; ISI, international sensitivity index; MNPT, mean normal prothrombin time; PT, prothrombin time; VWF, von Willebrand factor.
Source: Compiled from Kamal et al,[22] Poller,[23] Franz.[24]

Platelets are important role players in a hypercoagulable state during inflammation and their activation occurs in three stages (initiation, extension, and perturbation).[25] [26] [27] Besides hemostasis, platelets also appear to have a role in the immune system.[28] Low-grade inflammatory conditions are associated with a high mean platelet volume (MPV).[27] Platelets can also be classified as natural inflammatory cells. They have the ability to aggregate/gather around microorganisms, and to assist with the removal from the blood.[28] In the presence of infection, platelets become activated and their production is increased by certain inflammatory mediators, such as IL-6.[27] Platelets aggregate with leukocytes in areas of ischemia/reperfusion injury.[29] The activated platelets interact with and signal to

other inflammatory cells, such as leukocytes, and release their granules in high concentrations. Between 0.26 and 7.6% of HIV-infected patients will have venous thromboembolism (VTE) and activated platelets have been suggested as the etiology.[28]

Several chemokines are stored in very significant amounts in α granules in the platelet cytoplasm and they possess virucidal and suppressive activity against HIV.[30] Activated platelets release cytokines such as IL-1-β, IL-7, IL-8, platelet activating factor (PAF), and transforming growth factor (TGF)-β.[28] The chemokines released, activate leukocytes, specifically neutrophils and monocytes. P-selectin, a cell adhesion molecule whose surface expression is mediated by thrombin, appears on activated platelets, thereby increasing the platelet's adhesion to endothelial cells and to neutrophils. Activated platelets release a proinflammatory-mediator CD40 ligand, which induces the production of IL-6, IL-8, and the synthesis of tissue factor (TF).[27] Platelets therefore play a role in surveillance and amplifying the immune response during viral infections. However, platelets and cytokines, such as IL-18, can also cause dysregulation of the immune response to HIV.[28]

Platelets attach to the actual HIV virus, via two receptors, fibronectin and chemokine receptor type 4 (CXCR4), a fundamental coreceptor required for the virus to enter into cells. Activated platelets can then endocytose the HIV virus. P-selectin is then expressed on the platelet membrane surface, which can activate surrounding macrophages. This leads to enhanced viral clearance by the activated macrophages.[31] On the other hand, platelets can also enhance the spread of the virus. Platelets attached to HIV can infect CD4+ cells directly or endocytose and deliver the viral particles elsewhere in the body.[28]

Thrombocytopenia (defined here as a platelet count of less than 100×10^9/L) is a common manifestation of HIV infection. Severe thrombocytopenia is classified as less than 50×10^9/L, and there is a high risk for spontaneous bleeding below 10×10^9/L.[30] Thrombocytopenia in the presence of HIV can be caused by megakaryocyte infection by the HIV virus in the bone marrow, bone marrow infiltration from opportunistic infections or lymphoma, hypersplenism, myelosuppressive effects of medications, or by immune-mediated peripheral platelet-specific antiglycoprotein antibodies.[28] Thrombocytopenia can occur when the glycoproteins on the platelet membrane surface are targeted by the antibodies that are supposed to be directed toward the viral glycoproteins.[30] The platelet–leucocyte interaction can result in aggregates different from the usual platelet aggregates that may affect hemostasis. This platelet–leucocyte interaction has not been visualized in detail previously.[32] [33] Another mechanism resulting in thrombocytopenia is the increased inflammatory markers. It is well known that circulating inflammatory markers have a pronounced pathological effect on platelet structure and function.[34] [35] [36] [37] [38] [39] [40] The usual morphology of activated platelets is characterized by membrane blebbing, platelet spreading, and extensive pseudopodia formation.[36] These ultrastructural changes have been documented in disease (e.g., asthma, cancer, thrombotic disease, bleeding disorders) and aging.[41] In the presence of increased circulating inflammatory markers, platelets present with irregular, disrupted membranes with shedding of procoagulant vesicles. These morphological features are suggestive of platelet apoptosis, cell death.[34] [35] [36] [37] [38] [39] [40] HIV infection has also been associated with similar morphological features.[42] Platelet destruction is predominant early in the HIV disease course, whereas decreased platelet production is the main cause later in the disease course.[43]

Antiretroviral therapy usually improves HIV-related thrombocytopenia, but the effect may not be the same for all HIV-infected patients.[28] Before ARVs, the prevalence of HIV-

associated thrombocytopenia was 5 to 30%, but with ARVs the prevalence is now 3.2%. HIV-infected patients may, however, have an increased risk of bleeding with ARVs, which indicates that ARVs, or the different combinations of ARVs, alter the nature of the observed thrombocytopenia.[30] Zidovudine, NRTI, results in resolution of 24 to 34% of HIV-associated thrombocytopenia and a partial response in 33 to 44%. Didanosine commonly results in a rise in the platelet concentration. Protease inhibitors (PIs) are generally associated with an increased rate of thrombosis; however, increased bleeding risks have also been documented.[30]

A study of four HIV-infected patients using scanning electron microscopy (SEM) was reported in 2008 by the University of Pretoria which examined the ultrastructural changes of platelet aggregates. There were areas of intact membranes adjacent to membrane blebbing with areas where the membrane was torn compared with the control platelet aggregates, which showed smooth membranes. Both HIV-positive and HIV-negative platelet aggregates had pseudopodia and openings of the open canalicular system channels. HIV-infected patients therefore have platelet aggregates that appear apoptotic, which could be due to direct viral damage or to patient antibody cross-reaction.[42] Not much research has been documented on the platelet ultrastructure in HIV patients while on ARV therapy. One study looked at the morphology of platelets with the use of an immunomodulator, Canova (manufactured by Canova do Brazil), in HIV patients. Canova is a herbal drug that augments the activation of the patient's immune system. Patients treated with Canova had intact platelet morphology, including decreased platelet membrane blebbing and intact smooth membranes. The findings suggest that the use of Canova prevents the destructive effects of the virus.[44] The effects of ARVs may show similar effects on platelet ultrastructure as Canova.

Platelets are also influenced by physical and chemical properties of red blood cells (RBCs). If damaged, RBCs can release adenosine diphosphate (ADP) and therefore exert prothrombotic properties by activating platelets. Damaged RBCs may stimulate platelet granule release, which will recruit additional platelets into the developing thrombus. The interaction between platelets and RBCs is initiated by platelet activation. As the platelets become activated, the coagulation pathways are stimulated resulting in the fibrin network trapping more platelets and RBCs. The interaction of activated platelets with RBCs therefore has a positive feedback on the coagulation system.[45]

Fibrinogen expresses binding sites for the membrane receptors of cells involved in inflammation, and enhances adhesion of the RBCs, by binding to the RBC membrane.[45] RBC aggregation thereby increases in the localized presence of fibrinogen. Fibrinogen, arguably one of the most important circulating plasma proteins, is a large centrosymmetric glycoprotein. Thrombin cleaves the fibrinogen molecules and thereby releasing fibrinopeptides A and B. Fibrinopeptide A is an early indicator of fibrin formation. Cleavage of the fibrinopeptides allows fibrin monomers to attach forming protofibrils. Bundles of protofibrils form the insoluble fibrin fibers.[27] [46] [47] The fibrin clot consists of thick fibers which form the majority of the clot, and thin minor fibers which are arranged among the major fibers.[48] The thickness of the fibrin fibers appears to be influenced by thrombin concentration.[32] Antithrombin inactivates thrombin and proteolysis of the fibrin fibers into soluble degradation products occurs in the presence of plasmin.[26]

The nature of the clot can be influenced by changes in the concentration of fibrinogen; a higher concentration of fibrinogen will lead rapid clot formation with decreased spaces between the fibrin strands. Clot characteristics can also be influenced by fibrinogen amino

acid sequence changes and divalent cations (e.g., iron ions), changes that interact with fibrinogen and its conversion into fibrin. Ionic strength can influence the clot pore size, as fibrinogen at a neutral pH has a negative charge. In diabetes, for example, the nature of fibrinogen can change due to glycation.[49] Hormonal changes, such as an increase in estrogen, also cause a granular fibrin network.[45]

Fibrin clot proteolysis in hypercoagulable conditions may be inhibited or delayed. The delayed/inhibited breakdown of the fibrin clot may be due to inflammatory cellular elements that are trapped in the fibrin network that stimulates a firm clot formation resistant to antithrombotic agents.[50] This resistant fibrin clot is associated with an altered fibrin network on SEM investigation.[48] In stroke patients, it was noticed that an abnormal matted fibrin network was present even without the addition of thrombin, indicating the presence of other plasma factors. In disease-free individuals, this abnormal matted fibrin network can only be recreated when thrombin is added to citrated blood.[51] A thick matted layered fibrin network is seen which is due to the increased volume of the thin minor fibrin fibers. The result is the appearance of the thick major fibers being covered by a net of thickened minor fibers. These fibrin changes are documented in patients with inflammatory or other conditions such as arthritis, asthma, and smoking.[48]

Recently, fibrin(ogen) protein has been noted to change to an amyloid-like structure, in the presence of circulating proinflammatory agents, such as iron, gram-negative bacteria lipopolysaccharides (LPS), and gram-positive bacteria lipoteichoic acids (LTA), which are soluble peptidoglycan and muropeptides.[52] This structural change involves pathology noted in the actual protein structure. Unagitated human fibrin contains $30 \pm 3\%$ α-helices, $37 \pm 4\%$ β-sheets, and $32 \pm 3\%$ loops, turns, and random coils.[53] In general, amyloid protein structure is characterized by the increased formation of β-sheets, compared with α-helices; and this is observable using X-ray reflections.[54] [55] [56] [57] [58] Recently, fluorescent amyloid markers were also used to show amyloid in pathologic fibrin clots.[52] [59] [60] As part of considerable studies on atypical blood clotting,[49] [50] [61] [62] [63] [64] [65] [66] we recently found[66] [67] that the atypical fibrin clotting was in fact amyloid in nature. Although clearly fibrin(ogen) structure is of great importance in the pathology of HIV, we could not find much literature regarding the structure and function of it in HIV.

HIV, the Accompanying Proinflammatory State and the Development of Deep Vein Thrombosis

Deep vein thrombosis is associated with increased concentrations of inflammatory markers, whether the patient is infected with HIV or not. Raised plasma levels of C-reactive protein (CRP), D-dimer, TNFα, IL-6, IL-8, and monocyte chemotactic protein have been found in patients with DVT, especially at the onset of DVT.[68] [69] [70] [71] Inflammation and coagulation are closely linked. Inflammation can activate coagulation by cytokine- (IL-6 and TNFα) induced TF expression, downregulating protein C and inhibiting fibrinolysis. Conversely, coagulation factors and products (thrombin and fibrin) can also activate inflammation.[71] [72] The proinflammatory state in HIV-infected patients, with upregulated cytokines and other inflammatory molecules present in the blood,[73] [74] [75] [76] [77] [78] [79] [80] [81] [82] [83] [84] is therefore strongly associated with DVT formation. However, the medical treatment of DVT remains the same for HIV-infected and HIV-negative patients.[85] The elevated inflammatory markers in the plasma are therefore a good measuring tool to assess inflammation in HIV-infected patients.

Screening for a hypercoagulable state in HIV patients that may develop a DVT is not usually performed, unless the patient presents with a symptomatic VTE disease. However, prophylactic DVT screening and/or early detection of a DVT in HIV-infected patients would improve patient care and decrease morbidity and mortality. A better understanding of hypercoagulability will therefore result in improved patient care in HIV-infected patients.

Up to 30% of general surgery patients may develop a symptomatic or nonsymptomatic DVT.[86] A systematic review of HIV-positive patients in 2005 found a DVT incidence ranging from 0.19 to 18%, whereas in the HIV-negative population the DVT risk is reported to be approximately 0.0005% (5/10,000).[87] Up to 40% of patients with DVT can develop PE.[88]

Patients with HIV are more prone to a hypercoagulable state compared with HIV-negative patients. Factors (Virchow's triad) predisposing to a procoagulable state have been documented in HIV-infected patients such as increased antiphospholipid antibodies, lupus anticoagulant, VWF, and D-dimers. Deficiencies include proteins C and S, heparin cofactor II, and antithrombin.[89]

Elevated concentrations of activated platelets (raised levels of P-selectin, microparticles, and aminophospholipids) are present in HIV-positive patients. With the addition of elevated levels of VWF, these activated platelets easily aggregate and adhere to each other and the surrounding endothelium.[87] There is a positive correlation; the more severe the viral immune suppression, the more severe the coagulation abnormalities. The risk of thrombosis can therefore also occur in uncommon sites and in those patients who do not have known risk factors for DVT.[14]

Conclusion

Although there are adequate tests available for screening for DVTs when this is already suspected by the clinician, a more proactive approach is needed to track the inflammatory profile, and specifically the parameters that might point to pathological coagulation; and these should include platelet, RBC, and fibrin(ogen) analysis. Hypercoagulable changes are present in the body long before an actual thromboembolic event occurs. Using techniques to study fibrin(ogen), as a marker of coagulation, might also be of great value. The importance of the investigations into the molecular and fiber-level origins of hypercoagulability has only recently been realized. Platelet ultrastructure in HIV-positive patients has shown differences compared with HIV-negative patients, but previous study samples have been small. Besides the morphology and ultrastructure of platelets and fibrin, the interaction of activated platelets with fibrin, as well as the interactions with red blood cells and white blood cells, could have important implications of hypercoagulability. Ultrastructural investigation should be used as a complementary investigation with the tests more commonly used for diagnosing coagulation abnormalities. This will result in a more complete method of understanding and diagnosing hypercoagulability and hypofibrinolysis. A predictive screening assessment of HIV-infected patients could then possibly be determined for those at risk of a thromboembolic event, including DVT. Additional tests would greatly improve understanding of the effect of ARVs on fibrin(ogen) structure as well as platelet and RBC functioning during coagulation. We suggest a panel of tests (shown in [Table 6]) that could be included. Although some of the equipment/tests are traditionally seen as more research orientated (e.g., SEM), there are desk-top machines available. Thromboelastography (TEG) is also currently used as point-of-care instruments. Multiplex panels like a V-PLEX Vascular

Injury Panel could also be used as a standard biomarker test. Such panels typically measure four biomarkers like serum amyloid A, CRP, VCAM-1, and ICAM-1 that are important in acute inflammation and tissue damage as well as numerous other biological processes.

Table 6 Proposed tests that could be included as standard tests during HIV/AIDS hospital visits[a]

Hematological markers	Traditional coagulation tests	Global clotting tests[b]	Ultrastructural tests
Full blood count (especially platelet count and mean platelet volume)	aPTT	Viscoelastic tests (TEG/ROTEM/Sonoclot instruments) and/or thrombin generation assay and/or clot waveform analysis	Scanning electron microscopy (desktop type)
Ultrasensitive CRP	PT/INR[c]		
ESR			
Fibrinogen concentration			
D-dimer			
Biomarker multiplex panels; e.g., vascular injury panels			

Abbreviations: aPTT, activated partial thromboplastin time; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; INR, international normalized ratio; PT, prothrombin time; ROTEM, rotational thromboelastometry; TEG, thromboelastography.

[a]These are suggested tests that we believe in time will help develop a better understanding of the pathophysiology of HIV, especially as related to derangements in hemostasis. We recognize that the evidence base for usage in this setting is currently limited.

[b]These tests are generally utilized in research studies, but are increasingly being developed for use in clinical investigations.

[c]The INR is intended for monitoring of vitamin K antagonists (VKAs) and has not been validated for other assessments of hemostasis. It may be preferential to utilize a PT ratio, as has sometimes been proposed for use in end-stage liver disease for transplant evaluation.[90] The PT ratio is simply the patient's PT divided by the mean normal prothrombin time, as otherwise used in the INR ratio. Unlike the INR, the PT ratio does not include an adjustment with an "international sensitivity index," as applied to VKA monitoring.

Coagulation screening methods together with vascular injury panels could be used for early diagnoses of thromboembolic disease in HIV patients and could greatly impact to streamline treatment strategies due to improved monitoring of those strategies. It will also give insight into the general inflammatory status of the patients, and will not only track disease progression but also give insights into medication compliance and the effect of treatment regimes. A better understanding of hypercoagulability of HIV-infected patients is therefore urgently needed.

Conflicts of Interest

None.

Acknowledgments

We thank the National Research Foundation (NRF) of South Africa (E Pretorius: Rated researcher grant) as well as the Medical Research Council (MRC of South Africa [E Pretorius: grant holder]) for supporting this research. The funding organizations did not have any involvement in the research. We thank Professor Taole Mokoena (Head of the department of Surgery, Faculty of Health Sciences, University of Pretoria, South Africa) for his support.

Competing Interests

None.

References

1 World Health Organisation. Global health observatory data. Available at: http://www.who.int/gho/hiv/en/ . Accessed January, 2018

2 Simon V, Ho DD, Abdool Karim Q. HIV/AIDS epidemiology, pathogenesis, prevention, and treatment. Lancet 2006; 368 (9534): 489-504

3 Dieffenbach CW, Fauci AS. Thirty years of HIV and AIDS: future challenges and opportunities. Ann Intern Med 2011; 154 (11) 766-771

4 Appay V, Sauce D. Immune activation and inflammation in HIV-1 infection: causes and consequences. J Pathol 2008; 214 (02) 231-241

5 Hunt PW. HIV and inflammation: mechanisms and consequences. Curr HIV/AIDS Rep 2012; 9 (02) 139-147

6 Weiss L, Haeffner-Cavaillon N, Laude M, Gilquin J, Kazatchkine MD. HIV infection is associated with the spontaneous production of interleukin-1 (IL-1) in vivo and with an abnormal release of IL-1 α in vitro. AIDS 1989; 3 (11) 695-699

7 Molina JM, Scadden DT, Byrn R, Dinarello CA, Groopman JE. Production of tumor necrosis factor alpha and interleukin 1 beta by monocytic cells infected with human immunodeficiency virus. J Clin Invest 1989; 84 (03) 733-737

8 Emilie D, Peuchmaur M, Maillot MC. , et al. Production of interleukins in human immunodeficiency virus-1-replicating lymph nodes. J Clin Invest 1990; 86 (01) 148-159

9 Birx DL, Redfield RR, Tencer K, Fowler A, Burke DS, Tosato G. Induction of interleukin-6 during human immunodeficiency virus infection. Blood 1990; 76 (11) 2303-2310

10 Lafeuillade A, Poizot-Martin I, Quilichini R. et al. Increased interleukin-6 production is associated with disease progression in HIV infection. AIDS 1991; 5 (09) 1139-1140

11 World Health Organization. WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children. HIV/AIDS Programme: Strengthening health services to fight HIV/AIDS. World Health Organization Library Cataloguing-in-Publication Data 2007. :16

12 Mocroft A, Vella S, Benfield TL. et al. Changing patterns of mortality across Europe in patients infected with HIV-1. EuroSIDA Study Group. Lancet 1998; 352 (9142): 1725-1730

13 Meintjes G, Maartens G, Boulle A. et al. Guidelines for antiretroviral therapy in adults by the Southern African HIV Clinicians Society. Southern African Journal of HIV Medicine 2012; 13 (03) 114-134

14 Saif MW, Greenberg B. HIV and thrombosis: a review. AIDS Patient Care STDS 2001; 15 (01) 15-24

15 Bates CM. HIV medicine: drug side effects and interactions. Postgrad Med J 1996; 72 (843) 30-36

16 Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents Living with HIV. Department of Health and Human Services. Available at: http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf . Updated October 17, 2017 . Accessed January 18, 2018

17 Subbaraman R, Chaguturu SK, Mayer KH, Flanigan TP, Kumarasamy N. Adverse effects of highly active antiretroviral therapy in developing countries. Clin Infect Dis 2007; 45 (08) 1093-1101

18 Baker JV, Neuhaus J, Duprez D. , et al; INSIGHT SMART Study Group. Changes in inflammatory and coagulation biomarkers: a randomized comparison of immediate versus deferred antiretroviral therapy in patients with HIV infection. J Acquir Immune Defic Syndr 2011; 56 (01) 36-43

19 Wolf K, Tsakiris DA, Weber R, Erb P, Battegay M. Swiss HIV Cohort Study. Antiretroviral therapy reduces markers of endothelial and coagulation activation in patients infected with human immunodeficiency virus type 1. J Infect Dis 2002; 185 (04) 456-462

20 Jong E, Meijers JCM, van Gorp ECM, Spek CA, Mulder JW. Markers of inflammation and coagulation indicate a prothrombotic state in HIV-infected patients with long-term use of antiretroviral therapy with or without abacavir. AIDS Res Ther 2010; 7 (09) 9

21 Jong E, Louw S, Meijers JCM. et al. The hemostatic balance in HIV-infected patients with and without antiretroviral therapy: partial restoration with antiretroviral therapy. AIDS Patient Care STDS 2009; 23 (12) 1001-1007

22 Kamal AH, Tefferi A, Pruthi RK. How to interpret and pursue an abnormal prothrombin time, activated partial thromboplastin time, and bleeding time in adults. Mayo Clin Proc 2007; 82 (07) 864-873

23 Poller L. International normalized ratios (INR): the first 20 years. J Thromb Haemost 2004; 2 (06) 849-860

24 Franz RC. ROTEM analysis: a significant advance in the field of rotational thrombelastography. S Afr J Surg 2009; 47 (01) 2-6

25 van Rooy MJ, Pretorius E. Metabolic syndrome, platelet activation and the development of transient ischemic attack or thromboembolic stroke. Thromb Res 2015; 135 (03) 434-442

26 Guyton AC, Hall JE. Human Physiology and Mechanisms of Disease. 6th ed. Philadelphia, PA: W.B. Saunders ; 1997; 299–300

27 Swanepoel AC, Nielsen VG, Pretorius E. Viscoelasticity and ultrastructure in coagulation and inflammation: two diverse techniques, one conclusion. Inflammation 2015; 38 (04) 1707-1726

28 Torre D, Pugliese A. Platelets and HIV-1 infection: old and new aspects. Curr HIV Res 2008; 6 (05) 411-418

29 Van Der Spuy WJ, Pretorius E. A place for ultrastructural analysis of platelets in cerebral ischemic research. Microsc Res Tech 2013; 76 (08) 795-802

30 Metcalf Pate KA, Mankowski JL. HIV and SIV associated thrombocytopenia: an expanding role for platelets in the pathogenesis of HIV. Drug Discov Today Dis Mech 2011; 8 (1-2): e25-e32

31 Gresele P, Falcinelli E, Sebastiano M, Baldelli F. Endothelial and platelet function alterations in HIV-infected patients. Thromb Res 2012; 129 (03) 301-308

32 Pretorius E, Oberholzer HM, Smit E, Steyn E, Briedenhann S, Franz CR. Ultrastructural changes in platelet aggregates of HIV patients: a scanning electron microscopy study. Ultrastruct Pathol 2008; 32 (03) 75-79

33 van Rooy MJ, Pretorius E. Platelet interaction with erythrocytes and propensity to aggregation in essential thrombocythaemia. Lancet 2016; 387 (10024): 1210

34 Pretorius E. The role of platelet and fibrin ultrastructure in identifying disease patterns. Pathophysiol Haemost Thromb 2008; 36 (05) 251-258

35 Bester J, Matshailwe C, Pretorius E. Simultaneous presence of hypercoagulation and increased clot lysis time due to IL-1β, IL-6 and IL-8. Cytokine 2018; 110: 237-242

36 Bester J, Pretorius E. Effects of IL-1β, IL-6 and IL-8 on erythrocytes, platelets and clot viscoelasticity. Sci Rep 2016; 6: 32188

37 Page MJ, Bester J, Pretorius E. Interleukin-12 and its procoagulant effect on erythrocytes, platelets and fibrin(ogen): the lesser known side of inflammation. Br J Haematol 2018; 180 (01) 110-117

38 Page MJ, Bester J, Pretorius E. The inflammatory effects of TNF-α and complement component 3 on coagulation. Sci Rep 2018; 8 (01) 1812

39 Pretorius E, du Plooy JN, Bester J. A comprehensive review on eryptosis. Cell Physiol Biochem 2016; 39 (05) 1977-2000

40 Pretorius E, Olumuyiwa-Akeredolu OO, Mbotwe S, Bester J. Erythrocytes and their role as health indicator: using structure in a patient-orientated precision medicine approach. Blood Rev 2016; 30 (04) 263-274

41 Pretorius E, Oberholzer HM, van der Spuy WJ, Meiring JH. Age-related changes in fibrin networks and platelets of individuals over 75: a scanning electron microscopy study showing "thrombotic preparedness". J Thromb Thrombolysis 2010; 29 (03) 271-275

42 Pretorius E, du Plooy J, Soma P, Gasparyan AY. An ultrastructural analysis of platelets, erythrocytes, white blood cells, and fibrin network in systemic lupus erythematosus. Rheumatol Int 2014; 34 (07) 1005-1009

43 Parinitha S, Kulkarni M. Haematological changes in HIV infection with correlation to CD4 cell count. Australas Med J 2012; 5 (03) 157-162

44 Pretorius E, Smit E, Oberholzer HM, Steyn E, Briedenhann S, Franz RC. Investigating the ultrastructure of platelets of HIV patients treated with the immuno-regulator, Canova: a qualitative scanning electron microscopy study. Histol Histopathol 2009; 24 (04) 399-405

45 Swanepoel AC, Pretorius E. Erythrocyte-platelet interaction in uncomplicated pregnancy. Microsc Microanal 2014; 20 (06) 1848-1860

46 Totora GJ, Grabowski SR. Principles of Anatomy and Physiology. 8th ed. New York, NY: Harper Collins College Publishers ; 1996: 564-570

47 Pretorius E, Lipinski B. Differences in morphology of fibrin clots induced with thrombin and ferric ions and its pathophysiological consequences. Heart Lung Circ 2013; 22 (06) 447-449

48 Pretorius E, Oberholzer HM, van der Spuy WJ, Swanepoel AC, Soma P. Scanning electron microscopy of fibrin networks in rheumatoid arthritis: a qualitative analysis. Rheumatol Int 2012; 32 (06) 1611-1615

49 Pretorius E, Bester J, Vermeulen N. et al. Poorly controlled type 2 diabetes is accompanied by significant morphological and ultrastructural changes in both erythrocytes and in thrombin-generated fibrin: implications for diagnostics. Cardiovasc Diabetol 2015; 14: 30

50 Kell DB, Pretorius E. The simultaneous occurrence of both hypercoagulability and hypofibrinolysis in blood and serum during systemic inflammation, and the roles of iron and fibrin(ogen). Integr Biol 2015; 7 (01) 24-52

51 Pretorius E, Swanepoel AC, Oberholzer HM, van der Spuy WJ, Duim W, Wessels PF. A descriptive investigation of the ultrastructure of fibrin networks in thrombo-embolic ischemic stroke. J Thromb Thrombolysis 2011; 31 (04) 507-513

52 Pretorius E, Page MJ, Hendricks L, Nkosi NB, Benson SR, Kell DB. Both lipopolysaccharide and lipoteichoic acids potently induce anomalous fibrin amyloid formation: assessment with novel Amytracker™ stains. J R Soc Interface 2018; 15 (139) 20170941

53 Litvinov RI, Faizullin DA, Zuev YF, Weisel JW. The α-helix to β-sheet transition in stretched and compressed hydrated fibrin clots. Biophys J 2012; 103 (05) 1020-1027

54 Maji SK, Wang L, Greenwald J, Riek R. Structure-activity relationship of amyloid fibrils. FEBS Lett 2009; 583 (16) 2610-2617

55 Eisenberg D, Jucker M. The amyloid state of proteins in human diseases. Cell 2012; 148 (06) 1188-1203

56 Morris KL, Serpell LC. X-ray fibre diffraction studies of amyloid fibrils. In: Sigurdsson EM, Calero M, Gasset M, eds. Amyloid Proteins: Methods and Protocols, 2nd ed. New York, NY: Humana Press

57 Tycko R, Wickner RB. Molecular structures of amyloid and prion fibrils: consensus versus controversy. Acc Chem Res 2013; 46 (07) 1487-1496

58 Langkilde AE, Morris KL, Serpell LC, Svergun DI, Vestergaard B. The architecture of amyloid-like peptide fibrils revealed by X-ray scattering, diffraction and electron microscopy. Acta Crystallogr D Biol Crystallogr 2015; 71 (Pt 4): 882-895

59 Pretorius E, Page MJ, Engelbrecht L, Ellis GC, Kell DB. Substantial fibrin amyloidogenesis in type 2 diabetes assessed using amyloid-selective fluorescent stains. Cardiovasc Diabetol 2017; 16 (01) 141

60 Pretorius E, Mbotwe S, Kell DB. Lipopolysaccharide-binding protein (LBP) reverses the amyloid state of fibrin seen in plasma of type 2 diabetics with cardiovascular co-morbidities. Sci Rep 2017; 7 (01) 9680

61 Bester J, Soma P, Kell DB, Pretorius E. Viscoelastic and ultrastructural characteristics of whole blood and plasma in Alzheimer-type dementia, and the possible role of bacterial lipopolysaccharides (LPS). Oncotarget 2015; 6 (34) 35284-35303

62 Kell DB, Pretorius E. Serum ferritin is an important inflammatory disease marker, as it is mainly a leakage product from damaged cells. Metallomics 2014; 6 (04) 748-773

63 Pretorius E, Vermeulen N, Bester J, Lipinski B, Kell DB. A novel method for assessing the role of iron and its functional chelation in fibrin fibril formation: the use of scanning electron microscopy. Toxicol Mech Methods 2013; 23 (05) 352-359

64 Pretorius E, Bester J, Vermeulen N, Lipinski B, Gericke GS, Kell DB. Profound morphological changes in the erythrocytes and fibrin networks of patients with hemochromatosis or with hyperferritinemia, and their normalization by iron chelators and other agents. PLoS One 2014; 9 (01) e85271

65 Pretorius E, Kell DB. Diagnostic morphology: biophysical indicators for iron-driven inflammatory diseases. Integr Biol 2014; 6 (05) 486-510

66 Pretorius E, Mbotwe S, Bester J, Robinson CJ, Kell DB. Acute induction of anomalous and amyloidogenic blood clotting by molecular amplification of highly substoichiometric levels of bacterial lipopolysaccharide. J R Soc Interface 2016; 13 (122) 20160539

67 Kell DB, Pretorius E. Proteins behaving badly. Substoichiometric molecular control and amplification of the initiation and nature of amyloid fibril formation: lessons from and for blood clotting. Prog Biophys Mol Biol 2017; 123: 16-41

68 Fox EA, Kahn SR. The relationship between inflammation and venous thrombosis. A systematic review of clinical studies. Thromb Haemost 2005; 94 (02) 362-365

69 Roumen-Klappe EM, den Heijer M, van Uum SHM, van der Ven-Jongekrijg J, van der Graaf F, Wollersheim H. Inflammatory response in the acute phase of deep vein thrombosis. J Vasc Surg 2002; 35 (04) 701-706

70 Folsom AR, Lutsey PL, Astor BC, Cushman M. C-reactive protein and venous thromboembolism. A prospective investigation in the ARIC cohort. Thromb Haemost 2009; 102 (04) 615-619

71 O'Brien M. The reciprocal relationship between inflammation and coagulation. Top Companion Anim Med 2012; 27 (02) 46-52

72 Petäjä J. Inflammation and coagulation. An overview. Thromb Res 2011; 127 (Suppl. 02) S34-S37

73 Baker JV. Chronic HIV disease and activation of the coagulation system. Thromb Res 2013; 132 (05) 495-499

74 Funderburg NT, Lederman MM. Coagulation and morbidity in treated HIV infection. Thromb Res 2014; 133 (Suppl. 01) S21-S24

75 Peters L, Neuhaus J, Duprez D. et al; INSIGHT SMART Study Group. Biomarkers of inflammation, coagulation and microbial translocation in HIV/HCV co-infected patients in the SMART study. J Clin Virol 2014; 60 (03) 295-300

76 Wohl DA, Arnoczy G, Fichtenbaum CJ. , et al. Comparison of cardiovascular disease risk markers in HIV-infected patients receiving abacavir and tenofovir: the nucleoside inflammation, coagulation and endothelial function (NICE) study. Antivir Ther 2014; 19 (02) 141-147

77 Graham SM, Mwilu R, Liles WC. Clinical utility of biomarkers of endothelial activation and coagulation for prognosis in HIV infection: a systematic review. Virulence 2013; 4 (06) 564-571

78 Nordell AD, McKenna M, Borges AH, Duprez D, Neuhaus J, Neaton JD. ; INSIGHT SMART, ESPRIT Study Groups; SILCAAT Scientific Committee. Severity of cardiovascular disease outcomes among patients with HIV is related to markers of inflammation and coagulation. J Am Heart Assoc 2014; 3 (03) e000844

79 Mooney S, Tracy R, Osler T, Grace C. Elevated biomarkers of inflammation and coagulation in patients with HIV are associated with higher Framingham and VACS Risk Index Scores. PLoS One 2015; 10 (12) e0144312

80 Funderburg NT. Markers of coagulation and inflammation often remain elevated in ART-treated HIV-infected patients. Curr Opin HIV AIDS 2014; 9 (01) 80-86

81 Young LC, Roediger MP, Grandits G. et al. Relationship between inflammatory and coagulation biomarkers and cardiac autonomic function in HIV-infected individuals. Biomarkers Med 2014; 8 (09) 1073-1083

82 Borges AH, Weitz JI, Collins G. et al; INSIGHT SILCAAT Scientific Committee. Markers of inflammation and activation of coagulation are associated with anaemia in antiretroviral-treated HIV disease. AIDS 2014; 28 (12) 1791-1796

83 Green SA, Smith M, Hasley RB. et al. Activated platelet-T-cell conjugates in peripheral blood of patients with HIV infection: coupling coagulation/inflammation and T cells. AIDS 2015; 29 (11) 1297-1308

84 Baker JV, Huppler Hullsiek K, Bradford RL, Prosser R, Tracy RP, Key NS. Circulating levels of tissue factor microparticle procoagulant activity are reduced with antiretroviral therapy and are associated with persistent inflammation and coagulation activation among HIV-positive patients. J Acquir Immune Defic Syndr 2013; 63 (03) 367-371

85 Jackson BS, Mokoena T. Comparison of the therapeutic dose of warfarin in HIV-infected and HIV-uninfected patients: a study of clinical practice. BMJ Open 2017; 7 (02) e013709

86 Chalmers RTA, Cross KS, Byrne D, Moores C. Essentials of Vascular Surgery, a Pocket Reference. 2nd ed. Scotland: Vascutek Terumo; 2003: 98-100

87 Eyal A, Veller M. HIV and venous thrombotic events. S Afr J Surg 2009; 47 (02) 54-56

88 Oh T, Bersten AD, Soni N. Oh's Intensive Care Manual. 5th ed. London, UK: Elsevier Health Science; 2003. ;29:351–352

89 Bibas M, Biava G, Antinori A. HIV-associated venous thromboembolism. Mediterr J Hematol Infect Dis 2011; 3 (01) e2011030

90 Tripodi A. Liver disease and hemostatic (dys)function. Semin Thromb Hemost 2015; 41 (05) 462-467