

https://dspace.org/

Coagulation profiles of HIV positive and negative paediatric patients undergoing dental extractions at Charlotte Maxeke Johannesburg Hospital.

Zeijlstra, Anne Elisabeth

2013-04-24
http://hdl.handle.net/10539/12684

Downloaded from a DSpace repository. https://dspace.org/

COAGULATION PROFILES OF HIV POSITIVE AND NEGATIVE PAEDIATRIC PATIENTS UNDERGOING DENTAL EXTRACTIONS AT CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL.

Anne Elisabeth Zeijlstra

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Medicine in the branch of Anaesthesiology

Johannesburg, 2012

DECLARATION

I, Anne Elisabeth Zeijlstra, declare that this research report is my own work, except to the extent indicated in the reference citation and acknowledgements. It is being submitted in partial fulfilment of the degree of Master of Medicine in the branch of Anaesthesiology at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Signed: _____

_____ day of _____, 2012

ABSTRACT

Paediatric Human Immunodeficiency Virus (HIV) and Acquired Immunodeficiency Syndrome (AIDS) remain a significant health care challenge in South Africa. Oral health and coagulation are only two of the many problems experienced by HIV positive paediatric patients.

This research report began with an observation that known HIV positive paediatric patients bled more than known HIV negative paediatric patients or those with unknown HIV status while undergoing dental extractions at Charlotte Maxeke Johannesburg Academic Hospital. The observation prompted a prospective, contextual, descriptive study looking at the coagulation profile (platelet count and thromboelastogram (TEG) profile (reaction time (r-time), clot formation time (K-time), alpha angle (α-angle) and maximum amplitude (MA)), CD4 counts and percentages and observed clinical bleeding in HIV negative, HIV positive not on antiretroviral treatment (ARVs) and HIV positive on ARVs paediatric patients presenting for dental extraction.

Over a two year period 47 HIV negative, 12 HIV positive not on ARVs and 17 HIV positive on ARVs paediatric patients were enrolled in the study using a consecutive, convenience sampling method. Each paediatric patient was given a standard inhalational general anaesthetic using sevoflurane and during intravenous cannulation the researcher drew blood from each child for analysis. A senior dentist from the Department of Paediatric Dentistry assessed bleeding in all cases.

The data obtained for each of the three study groups was compared using a one-way analysis of variance followed by pair wise comparison using the Bonferroni adjustment to address multiplicity. To deal with the big standard deviations and skewed data a one-way analysis of variance for ranks tested for differences between the groups. No statistically significant differences were found when comparing the groups for platelet count ($p = 0.2087$), TEG r-time ($p = 0.4738$), TEG K-time ($p = 0.6967$), TEG α-angle ($p = 0.7948$) or TEG MA ($p = 0.2982$). There was a statistically significant difference between the HIV negative and HIV positive not on ARVs groups ($p = 0.000$ and 0.004) and HIV positive on ARVs and HIV positive not on ARVs groups ($p = 0.000$ and 0.001) when comparing CD4 count and percentage.

Patient groups were compared with respect to bleeding complications using the Fisher's exact test. There was no statistically significant difference in observed bleeding between the three groups of paediatric patients. The entire HIV positive group was then compared for bleeding, and using the Welch t-test, adjusting for unequal variances it was found that there was statistically, significantly more bleeding in the HIV positive children with lower CD4 counts regardless of treatment with ARVs ($p = 0.0129$). These results were also confirmed using the Wilcoxon rank-sum test ($p = 0.0335$).

Although this study showed statistically significant bleeding in HIV positive paediatric patients with lower CD4 counts, the tests of coagulation used in the study were unable to define the underlying pathogenesis. Further research into coagulation in HIV positive paediatric patients is needed.

ACKNOWLEDGEMENTS

I would like to thank the following people for their contribution to this research report.

Juan Scribante for her supervision, help and enthusiasm.

Jacinta Shung for her supervision and encouragement.

Helen Perrie for her support, help and encouragement.

Professor Sid Setzer and the **staff** in the Maxillo-Facial and Dental ward and theatre, for accommodating the data collection process.

Professor Piet Becker, from the Medical Research Council of South Africa, for his involvement in the statistical analyses.

The **paediatric patients** who participated in the study and their **parents** and **caregivers**.

LIST OF ABBREVIATIONS

HIV	Human Immunodeficiency Syndrome
ARVs	antiretroviral treatment
TEG	thromboelastogram
TEG®	registered trademark of the TEG assay performed by the Haemoscope Corporation's machine
AIDS	Acquired Immune Deficiency Syndrome
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
HREC	Human Research Ethics Committee
NHLS	National Health Laboratory Services
aPTT	partial thromboplastin time
PT	prothrombin time
K-time	TEG clot formation time
MA	TEG maximum amplitude
r-time	TEG reaction time
α-angle	TEG alpha angle
AZT	zidovudine
WHO	World Health Organisation
ASA	American Society of Anaesthetists
ANOVA	analysis of variance
SD	standard deviation
95% CI	95% confidence interval

TABLE OF CONTENTS

DECLARATION.....ii

ABSTRACT.....iii

ACKNOWLEDGEMENTS.....v

LIST OF ABBREVIATIONS.....vi

TABLE OF CONTENTS.....vii

LIST OF FIGURES.....xi

LIST OF TABLES.....xii

LIST OF APPENDICES.....xiii

CHAPTER 1: OVERVIEW OF THE STUDY.....1

1.1 Introduction.....1

1.2 Problem statement.....3

1.3 Aims of the study.....3

1.4 Objectives of the study.....4

1.5 Research assumptions.....5

1.6 Location of the study.....8

1.7 Ethical considerations.....8

1.8 Research methodology.....8

1.8.1 Research design.....8

1.8.2 Study population and sample.....9

1.9 Significance of the study.....10

1.10 Validity and reliability of the study.....11

1.11 Potential limitations.....12

1.12 Outline of the research report.....12

1.13 Conclusion……13

CHAPTER 2: LITERATURE REVIEW.....14

2.1 Coagulation and tests of coagulation.....14

2.1.1 Coagulation and fibrinolysis……..14

2.1.2 Traditional tests of coagulation and platelet function…….17

2.1.3 Thromboelastography……19

2.1.4 HIV and TEG……26

2.1.5 Paediatric patients, coagulation and testing of coagulation

……26

2.2 Human Immunodeficiency Virus……28

2.2.1 HIV background……28

2.2.2 HIV epidemiology……..29

2.2.3 Effects of HIV on the haematological system, in particular, the platelets……31

2.2.4 Antiretroviral agents and coagulation……35

2.2.5 Dentistry and HIV……..38

2.2.6 Paediatric patients and HIV……40

2.3 Conclusion……44

CHAPTER 3: RESEARCH METHODOLOGY.....45

3.1 Problem statement.....45

3.2 Aims of the study……45

3.3 Objectives of the study……46

3.4 Location of the study……47

3.5 Ethical considerations……..47

3.6 Study design……47

3.7 Study population……48

3.8 Study sample……48

3.8.1 Sample statement……48

3.8.2 Sampling method……49

3.8.3 Inclusion criteria……49

3.8.4 Exclusion criteria……49

3.9 Procedure for data collection……50

3.10 Statistical analysis……54

3.11 Validity and reliability……54

3.12 Conclusion……55

CHAPTER 4: RESEARCH RESULTS……56

4.1 Sample realisation……56

4.2 Demographic data……56

4.3 Coagulation profiles of paediatric patients studied……59

4.3.1 Platelet count……59

4.3.2 Thromboelastogram r-time……60

4.3.3 Thromboelastogram K-time……61

4.3.4 Thromboelastogram α-angle……62

4.3.5 Thromboelastogram MA……63

4.4 The CD4 count and percentage of the study group……64

4.4.1 CD4 count……64

4.4.2 CD4 percentage……66

4.5 Assessment of bleeding……67

4.6 Conclusion……70

CHAPTER 5: DISCUSSION OF RESEARCH RESULTS……71

5.1 Sample realisation.....71

5.2 Demographic data.....71

5.3 Part 1.....72

5.4 Part 2.....73

5.5 Part 3.....74

5.6 Conclusion.....76

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS.....77

6.1 Summary.....77

6.2 Conclusions.....79

6.3 Limitations.....80

6.4 Recommendations.....82

6.5 Conclusion.....83

REFERENCE LIST.....95

LIST OF FIGURES

Figure 2.1 The coagulation cascade (19)……..……..15

Figure 2.2 The process of fibrinolysis (19)……..……...17

Figure 2.3 The thromboelastogram tracing (40)……22

Figure 3.1 Flow diagram of the data collection procedure……..53

Figure 4.1 Ages of paediatric patients studied divided into HIV negative, HIV positive and HIV positive on ARVs categories……..…….57

Figure 4.2 Pie chart of the number of patients assessed to have increased bleeding…….68

LIST OF TABLES

Table 1.1 CDC classification of immunological categories of HIV infection in children (4)……..6

Table 1.2 CDC classification of clinical categories of HIV infection in children (4)……7

Table 2.1: Comparative values: TEG® and ROTEM® (25)……24

Table 2.2: TEG reference values for paediatric patients (49, 51)……..……28

Table 4.1 Summary of extracted teeth……..59

Table 4.2 Summary of description of platelet count results……..……60

Table 4.3 Summary of description of TEG r-time results……61

Table 4.4 Summary of description of TEG K-time results……62

Table 4.5 Summary of description of TEG α-angle results……..63

Table 4.6 Summary of description of TEG MA results……..64

Table 4.7 Summary of the description of results for CD4 count……..……65

Table 4.8 Summary of the description of results for CD4 percentage……66

Table 4.9 Summary of data comparing HIV positive patients and CD4 count……70

LIST OF APPENDICES

Appendix 1: HREC approval……..84

Appendix 2: Information packet – parent/caregiver (HIV positive).....85

Appendix 3: Information packet – parent/caregiver (HIV unknown/HIV negative).....86

Appendix 4: Consent form……..87

Appendix 5: Consent form (HIV testing).....88

Appendix 6: Information packet – child (HIV unknown/HIV negative)……..89

Appendix 7: Information packet – child (HIV positive).....90

Appendix 8: Assent form.....91

Appendix 9: Data collection sheet.....92

CHAPTER 1: OVERVIEW OF THE STUDY

This chapter will introduce the study. This will be done by way of a background description followed by the setting out of the problem statement, aims and objectives of the study. Definitions relevant to the study will then be defined. A description of the location, ethical considerations, methodology, significance, validity and reliability of the study and potential limitations of the study will follow this. An outline of the research report will then be given.

1.1 Introduction

It is estimated that more than 50 million people in the world have become infected with Human Immunodeficiency Virus (HIV) and that 36 million of these infected people are living in sub-Saharan Africa (1). The Actuarial Society of South Africa estimates that 5,6 million South Africans are HIV infected, constituting approximately 15% of our population (2, 3). Almost 30% of new infections have been amongst the young adult female population who spread HIV during pregnancy, parturition and breastfeeding to their children. Consequently the epidemic in children is seen to parallel that of reproductive women (1). In 2005 2,3 million children worldwide were estimated to be HIV positive, however, there is no data to estimate the numbers in South Africa (4). It is estimated that 20-25% of patients infected with HIV will need to undergo surgery at some time during their illness; this includes children (1, 4).

Dental caries in children is an important primary health care issue. It is estimated that 40% of five-year-olds in the United Kingdom have had caries (5). In South

Africa, the 1999/2002 South African National Children's Oral Health Survey published a dental caries prevalence of 51% in four-to-five-year-olds (6). The incidence in HIV positive children may be higher as a result of immunosuppression. These children are also at risk for complications of caries, namely periodontal disease and systemic infection (7).

HIV can affect any of the human organ systems, including the haematological system. These effects on the haematological system include anaemia, leucopoenia, lymphoma, thrombocytopenia, bone marrow suppression, hypercoagulability, and Acquired Immune Deficiency Syndrome (AIDS)-related lymphoma (1, 4). These effects are as a result of infection by the virus itself, antiretroviral drugs (ARVs), impaired nutrition and bone marrow infiltration by neoplasia or opportunistic infection (1). HIV is known to affect the coagulation system.

There are numerous tests currently in use for establishing the integrity of the coagulation system. One of these tests is the thromboelastogram (TEG). The TEG reports an amplitude measurement of the examination of whole blood clot strength over time (8). There is currently no literature exploring the effects of HIV on the TEG in either the adult or the paediatric population groups.

1.2 Problem statement

It was observed clinically that children known to be HIV positive bled more than those with unknown or known negative status when undergoing dental extractions at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH). At the time of this research project there was no research determining whether these HIV positive children had abnormal coagulation profiles as assessed by TEG or platelet count and whether abnormalities in these parameters were the reason for the observed increase in bleeding.

1.3 Aims of the study

This study was conducted in three parts:

Part 1

The aim of this part of the study was to determine if there was a difference in the coagulation profile of HIV positive paediatric patients (those on ARVs and those not on ARVs) and HIV negative paediatric patients undergoing dental extractions at CMJAH.

Part 2

The aim of this part of the study was to compare the CD4 counts and percentages of HIV positive paediatric patients on ARVs and those not on ARVs with that of the HIV negative paediatrics patients undergoing dental extractions at CMJAH.

Part 3

The aim of this part of the study was to compare observed bleeding of the HIV positive paediatric patients (on ARVs and not on ARVs) with that of the HIV negative paediatric patients undergoing dental extractions at CMJAH.

1.4 Objectives of the study

The aims of the study were justified by the following objectives.

Part 1

- To describe the coagulation profile of HIV positive (on ARVs and not on ARVs) and HIV negative paediatric patients undergoing dental extractions.
- To compare the coagulation profiles of these patients.

Part 2

- To determine the CD4 counts and percentages of HIV positive (on ARVs and not on ARVs) and negative paediatric patients undergoing dental extractions.
- To compare the CD4 counts and percentages of the three groups.

Part 3

- To assess the observed bleeding in the HIV positive (on ARVs and not on ARVs) and the HIV negative groups of paediatric patients undergoing dental extractions.
- To compare the observed bleeding between the groups.

1.5 Research assumptions

The following definitions were used in this study.

Paediatric patient: for the scope of this study a paediatric patient was a patient aged between one and thirteen years presenting for dental extraction.

Bleeders: study patients observed to have excessive bleeding during dental extraction.

Non-bleeders: study patients observed to have a normal amount of bleeding during dental extraction.

Thrombocytopenia: a platelet count of less than 150 x 10^9/l (9, 10).

Clinically relevant bleeding for the scope of this study:

- loss of more than 10% of blood volume
- haemodynamic instability as a result of blood loss
- the need for admission as a result of blood loss
- the need for blood transfusion following blood loss.

Paediatric HIV infection: HIV infection in a child confirmed by the presence of the HIV RNA, DNA PCR, p24 antigen or HIV-specific antibodies detected in a serum sample of children over 18 months of age or at least six weeks after cessation of breastfeeding (4).

AIDS: the development of one or more AIDS defining conditions in the presence of HIV infection with or without a CD4 count of less than 200 x 10^6/l or less than 15% of the total lymphocyte count in the one to thirteen year age group.

The CDC classification of immunological and clinical categories of HIV infection in children (table 1.1 and table 1.2) were used in this research (4).

Table 1.1 CDC classification of immunological categories of HIV infection in children (4)

Immunological Categories	CD4 count (µl/%)		
	< 12 months	**1-5 years**	**6-12 years**
No evidence of suppression	>1500/>25	>1000/>25	>500/>25
Evidence of moderate suppression	750-1499/15-24	500-999/15-24	200-499/15-24
Evidence of severe suppression	<750/<15	<500/<15	<200/<15

Table 1.2 CDC classification of clinical categories of HIV infection in children (4)

Clinical categories	Signs and symptoms
n	No signs or symptoms or only one of category A event
A	Mildly symptomatic At least 2 of: lymphadenopathy, hepatomegaly, splenomegaly, parotitis, rash, ear, nose and throat infections
B	Moderately symptomatic Single episode of severe bacterial infections
	Lymphoid interstitial pneumonia or pulmonary lymphoid hyperplasia complex
	Anaemia, neutropenia, thrombocytopenia Cardiomyopathy, nephropathy, hepatitis, diarrhoea Candidiasis, severe Varicella/zoster or Herpes simplex virus infections
C	Severely symptomatic Two serious bacterial infections
	Encephalopathy: acquired microcephaly, cognitive delay, abnormal neurology
	Wasting syndrome: severe failure to thrive or downward crossing two weight centiles
	Opportunistic infections: Pneumocystis jirovecii, Cytomegalovirus, Toxoplasmosis, disseminated fungal infections
	Disseminated mycobacterial disease Malignancy: Kaposi's sarcoma, lymphoma

1.6 Location of the study

The study took place at CMJAH, Gauteng Province, South Africa. CMJAH is an academic hospital associated with the University of the Witwatersrand. It is a tertiary hospital acting as a referral hospital for a number of smaller regional hospitals.

1.7 Ethical considerations

Ethical approval for the study was sought from the Human Research Ethics Committee (HREC) (Appendix 1) of the University of the Witwatersrand, Johannesburg, Gauteng Province, South Africa.

Approval for the study was verbally obtained from the authorities of CMJAH.

After discussion and explanation of the study and giving of an information packet, written informed consent was obtained from the parent or legal guardian of each paediatric patient prior to admission into the study. Assent was sought from the paediatric patients who were six years or older. Appendices 2 to 8.

Patients and their guardians who had an HIV test for the purposes of the study received pre- and post-test counselling.

1.8 Research methodology

1.8.1 Research design

A prospective, contextual, descriptive research design was followed in this study.

1.8.2 Study population and sample

Study population

The study population was all paediatric patients presenting for dental extraction at CMJAH.

Study sample

Sample statement

In consultation with a biostatistician a sample size of 16 paediatric patients in each group was used. This sample had 90% power to detect a difference in means of 4.0 (one standard deviation) assuming that the common deviation is 3.3 (reference) using a two group t-test with a 0.05 two-sided significance level (11, 12, 13).

Sampling method

A consecutive convenience sampling method was used in this study.

The convenience sampling method was chosen because of the time constraints and the scope of the research. The most readily accessible paediatric patients presenting for dental extraction were included. It is acknowledged that a convenience sample cannot fully represent the study population (14).

The study further used a consecutive sampling method where every paediatric patient who presented for dental extraction was invited to take part in the study.

Consecutive sampling is the most reliable form of convenience sampling as research bias is limited (14).

Inclusion criteria

The following inclusion criteria were used for the study:

- known HIV positive paediatric patients on ARVs
- known HIV positive paediatric patients not on ARVs
- HIV negative paediatric patients.

Exclusion criteria

The following exclusion criteria were used for the study:

- refusal to give consent
- HIV positive or negative paediatric patients known to have a coagulation abnormality.

1.9 Significance of the study

The Declaration of Helsinki states that, “the purpose of biomedical research involving human subjects must be to improve diagnostic, therapeutic and prophylactic procedures and the understanding of the aetiology and pathogenesis of disease” (15).

This study increased the understanding of coagulation in HIV positive children. The identification of patients who are at increased risk of bleeding during dental extraction and surgery is desirable, as this will allow for preparation and better care of such patients.

The study may promote further study into bleeding in HIV positive children.

1.10 Validity and reliability of the study

The validity and reliability of the study were increased by the following measures.

In order to ensure that standard practices were adhered to and reliable results were obtained, one anaesthesiology technician trained in TEG analysis performed all the TEG analyses for this study. A single TEG machine (TEG®) was used to limit variance that might occur between machines. The machine was calibrated to manufacturer standards on each day that TEG analysis was performed to ensure accurate results were obtained.

Blood specimens drawn for HIV testing, platelet count and CD4 count and percentage were sent to the National Health Laboratory Services (NHLS) laboratory at CMJAH. In this accredited laboratory Good Laboratory Research Practices are adhered to ensuring reliable results from these tests.

The researcher personally collected all data prior to analysis (Appendix 9). This ensured that data was collected in a standard manner from reliable sources.

Assessment of an increase in observed bleeding was subjective. To limit the subjectivity of this observation only one senior member of the Department of Paediatric Dentistry assessed bleeding in all the paediatric patients who had dental extractions.

1.11 Potential limitations

The study was done contextually at CMJAH. This context may not allow for generalisation of the results of the study, however, it was an important study to be done in that context as it addressed an observed problem.

The limited number of study subjects could affect the ability to generalise the study results. It may, however, provide a baseline to direct further research.

The assessment of clinical bleeding was subjective. In order to standardise this observation bleeding was assessed by one senior dentist from the Department of Paediatric Dentistry at CMJAH.

1.12 Outline of the research report

The research report will be presented as follows.

Chapter 1 – Overview of the study.

Chapter 2 – Literature review of relevant aspects.

Chapter 3 – Discussion of the research methodology.

Chapter 4 – Presentation of the research results.

Chapter 5 – Discussion of the research results.

Chapter 6 – Conclusions and recommendations.

1.13 Conclusion

This chapter has introduced the study. The problem statement was stated followed by the aims and objectives of the study. Definitions relevant to the study were defined. The methodology to be used was briefly described, as were the location, ethical considerations, validity and reliability, potential limitations and significance of the study. Finally an outline of what will be covered in each chapter of the report was given.

CHAPTER 2: LITERATURE REVIEW

This chapter will be a discussion of current literature relevant to the study. It will be discussed under the following headings: Coagulation and tests of coagulation, and Human Immunodeficiency Virus.

2.1 Coagulation and tests of coagulation

2.1.1 Coagulation and fibrinolysis

When there is a balance between bleeding and thrombosis, haemostasis is normal. Coagulation relies on the interaction between endothelium, platelets, and coagulation factors (16). With injury to the endothelium tissue factor is exposed and interacts with factor VII. Von Willebrand factor exposes glycoprotein Ib for platelet binding, and stimulates exposure of glycoprotein IIbIIIa binding sites, resulting in further attachment of von Willebrand factor and fibrinogen (17, 18).

Dense granules from the platelets release ADP (resulting in platelet aggregation and disintegration), serotonin and thromboxane A_2 (causing vasoconstriction and calcium release). Alpha granules from the platelets release fibrinogen, thrombospondin, fibronectin, factor V and factor VIII. Phospholipid phosphatidylserine is exposed on the platelet surface and serves as a base for initiation of clot formation (17).

The interaction of tissue factor, calcium and factor VII on the platelet surface rapidly initiates coagulation. This complex activates factors IX and X. Factor XIa enhances factor X production with factor VIIIa acting as a co-enzyme (18, 19).

Factor Xa then converts prothrombin to thrombin with the facilitation of co-enzyme Va. Thrombin then cleaves fibrinogen, which forms fibrin, this then polymerises to form a fibrin clot. Factor XIII stabilises the clot by forming covalent bonds between the fibrin molecules (18, 19). This process is illustrated in figure 2.1.

Thrombin formation is enhanced by its own activation of co-enzymes V and VIII, activation of factor XI, and by encouraging platelet aggregation and disintegration (19).

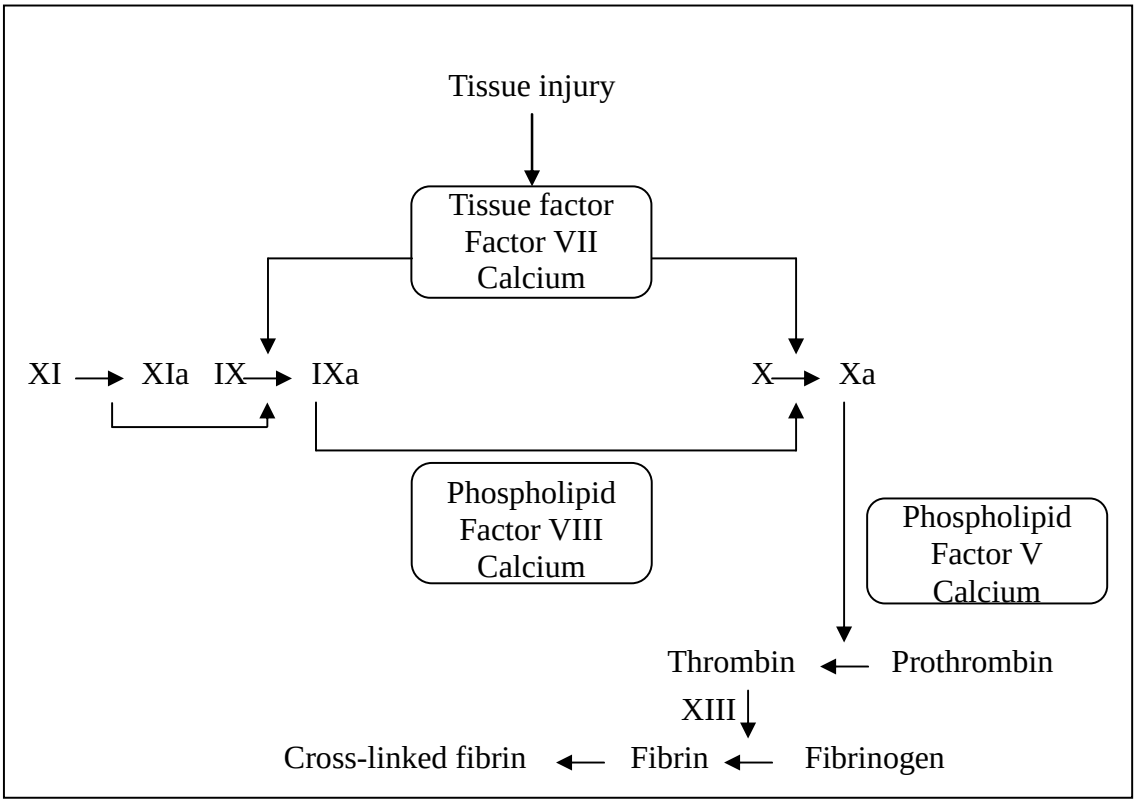


Figure 2.1: The coagulation cascade (19)

Inhibitors regulate the process of coagulation. Prostaglandin I_2 prevents platelet activation and causes vasodilatation. Antithrombin and heparan sulphate inactivate thrombin, factor IXa, factor Xa and factor XIa. Protein C, activated by the thrombin-thrombomodulin complex, inactivates factor Va and factor VIIIa in the presence of cofactor Protein S. Extrinsic Pathway Inhibitor binds factor Xa and tissue factor, inhibiting both. Finally, damaged endothelial cells release tissue plasminogen activator and urokinase, which stimulate the fibrinolytic pathway. Fibrinolysis is activated by tissue injury (18, 19, 20).

Tissue plasminogen activator and urokinase cleave plasmin from plasminogen and fibrin bonds. Plasmin degrades factor Va, factor VIIIa and glycoprotein Ib, and fibrin into D-dimers and fibrin degradation products. The negatively charged, exposed subendothelium activates factor XII, which then releases kallikrein from prekallikrein (19). Fibrinolysis is illustrated in figure 2.2.

Kallikrein activates plasmin from plasminogen and releases bradykinin from kininogen. This activates a pro-inflammatory process (19).

Plasminogen activator inhibitor 1, alpha 2 antiplasmin and alpha 2 macroglobulin are inhibitors of the fibrinolytic pathway (19).

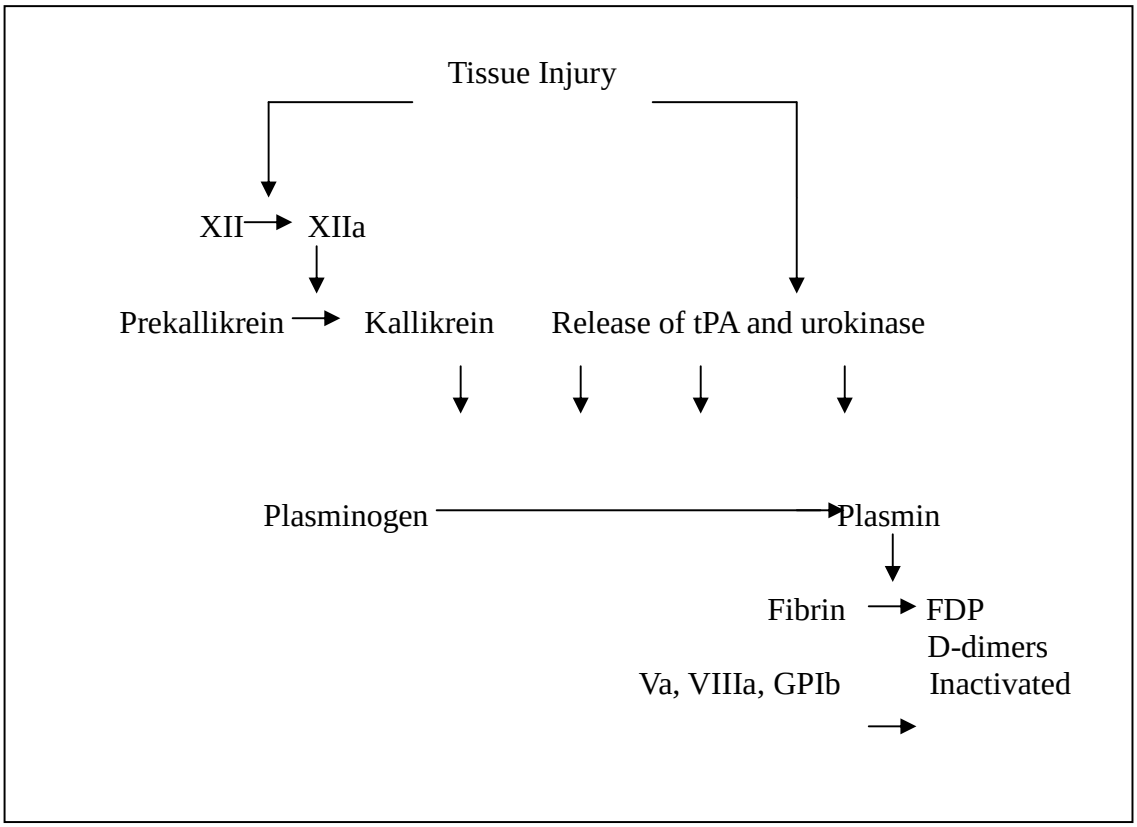


Figure 2.2: The process of fibrinolysis (19)

2.1.2 Traditional tests of coagulation and platelet function

The partial thromboplastin time (aPTT) and prothrombin time (PT) are tests used for coagulation screening. Both use exogenous reagents to measure the clotting time in plasma and do not reflect any interaction between cells, endothelium and fibrin. The tests are affected by high haematocrit, difficult venepuncture, and incorrect concentration with citrate. Watson and Greaves (21) maintain that these tests are insufficiently sensitive and specific to be used as principal screening tests for coagulation, and are not useful for platelet function screening. The aPTT is useful for the diagnosis of disseminated intravascular coagulation and for the quantitative assessment of factor VIII. The aPTT records only the beginning of the overall clotting process, leaving at least 95% unrecorded (22).

The bleeding time was described by Milian (in 23) in 1901 and by Duke (in 23) in 1910. It involves touching a piece of filter paper to the edge of a wound (of standard length, depth and direction on the forearm with a sphygmomanometer cuff inflated to 40 mmHg on that arm) at set time intervals and measuring the time taken for bleeding to stop. It is affected by emotion, temperature and exercise, and is poorly reproducible. The bleeding time is no longer thought to be a reasonable preoperative screening test of haemostatic function (21, 23).

Kratzer and Born (in 23) designed a platelet function analyser in 1985, which stimulates platelet adhesion and aggregation under high shear forces. This was followed by the Platelet Function Analyser-100 in 1995, which occludes an aperture by contact between platelets and agonists, thus stopping blood flow and giving a closure time. These tests were found not to be sensitive or specific enough to be used as screening tests for platelet disorders. They are, however, more sensitive than the bleeding time (21, 22, 23). The Platelet Function Analyser-100 is useful for the diagnosis of von Willebrand's Disease (22).

Platelet aggregometry was developed in 1962. Adhesion, aggregation, and platelet secretion of components of coagulation are evaluated. This is a useful test of platelet function defects and it assesses primary haemostasis. Qualitative defects in platelet function can further be analysed by measuring various parameters including intraplatelet adenine nucleotides (21, 23).

Flow cytometry of platelet surface glycoproteins evaluates circulating platelet activation and reactivity, diagnoses platelet disorders, and monitors efficacy antiplatelet agents (23).

Recently there has been a questioning of whether these tests are useful in the acute perioperative period. There is a time delay in receiving test results, they are performed in plasma and not in whole blood, and give limited information on platelet function (24). TEG monitoring is rapid, performed as a point of care test at body temperature and gives information about platelet function. It is, therefore, an assessor of the global coagulation mechanism (21, 24).

Watson and Greaves (21) conclude that the cornerstone of the assessment for bleeding risk is still a thorough clinical history and examination of the patient prior to surgical procedures.

2.1.3 Thromboelastography

Hartert (in 23) first described thromboelastography in 1948. It was used as a method to describe global haemostatic function from a single blood sample. The TEG is the trace produced from the measurement of viscoelastic changes associated with fibrin polymerisation (25). The clotting process, starting with fibrin formation and continuing to clot retraction and fibrinolysis is measured at the bedside (17, 24). Unlike traditional tests of clotting, the TEG is not based on static isolated endpoints. It measures the interaction of platelets and the clotting cascade.

TEG® is a registered trademark of the assay performed by the Haemoscope Corporation's instrument and ROTEM® is the registered trademark of Pentapharm GmbH (24). Both of these instruments produce a graphical representation of the measurement of visicoelastic changes during clot formation.

The TEG has been used for the following purposes.

- Assessment of haemostasis.
    - There have been reports of TEG abnormalities in thrombocytopenic pre-eclamptic women. Clot formation time (K-time) and maximum amplitude (MA) were reduced as the platelet count deceased (25, 26, 27).
    - TEG has been found to reflect the clinical state of haemostasis in neonates better than conventional tests of coagulation (25).
    - The TEG has been used for risk stratification of children with snakebite, by identifying clotting abnormalities (28).
- Monitoring of blood component therapy in surgery for adults and children. It has been used in the following types of surgery:
    - liver transplantation
    - cardiac surgery
    - trauma patients.
- The TEG has been seen to reduce the intraoperative use of fresh frozen plasma, blood and platelets. TEG analysis can also reduce the postoperative blood loss and need for relook surgery by determining whether bleeding is the result of a coagulation defect, or of inadequate surgical haemostasis (24, 25, 29, 30, 31, 32).

- Pharmaceutical monitoring.
    - Patients on treatment with low molecular weight heparins or heparinoids and direct thrombin inhibitors can be monitored by means of the TEG (24, 25).
    - Heaney et al. (33) showed that perioperative diclofenac does not adversely affect clot strength in children undergoing tonsillectomy.
- Hypercoagulability screening and assessment of thrombotic risk.
    - The TEG has been used to assess and manage postpartum coagulopathy that may exist for 24 hours post-delivery (25, 26, 27, 34).
    - The TEG has demonstrated hypercoagulability in paediatric patients with Sickle cell disease (35).
    - The TEG has demonstrated the existence of a hypercoagulable state in adult and paediatric neurosurgical patients (36).
- Monitoring of recombinant factor VIIa and activated prothrombin complex treatment in haemophilia (25). The TEG has also been used to assess coagulation in children with haemophilia (37).

The TEG is a bedside test that, within 30 minutes, can deliver a graphical representation of platelet function, coagulation proteases and inhibitors, and the fibrinolytic system (25). The TEG measures the interaction between platelets, clotting factors and fibrinogen as opposed to other coagulation tests which assess isolated portions of the coagulation cascade (34). The TEG is helpful in identifying a cause for bleeding and thus is a guide for coagulation therapy. The TEG better reflects haemostatic potential than traditional tests of coagulation (24, 38). In their study of 12 normal adult subjects Valeri and Ragno (39) concluded that the TEG

could not be used to predict the bleeding time as the bleeding time did not correlate with the TEG tracing.

The TEG is a graphical representation of thrombosis and lysis of a blood clot. When a TEG® is used 360 μℓ of blood is placed in an oscillating cup at 37°C. The cup oscillates through an angle of 4°45' every 10 seconds. A pin with a torsion wire is suspended in the cup; this is the detection device. As a clot forms a tracing, as seen in figure 2.3, is generated. Reaction time (r-time) is the time taken for initial clot formation. K-time is the coagulation time from 2-20 mm of clot size. The alpha angle (α-angle) is the slope of the TEG from the r-time to the K-time. MA is the maximum amplitude of the TEG and is the reflection of the absolute strength of the clot. A60 is the amplitude after 60 minutes and is a measure of clot lysis (17, 25, 40).

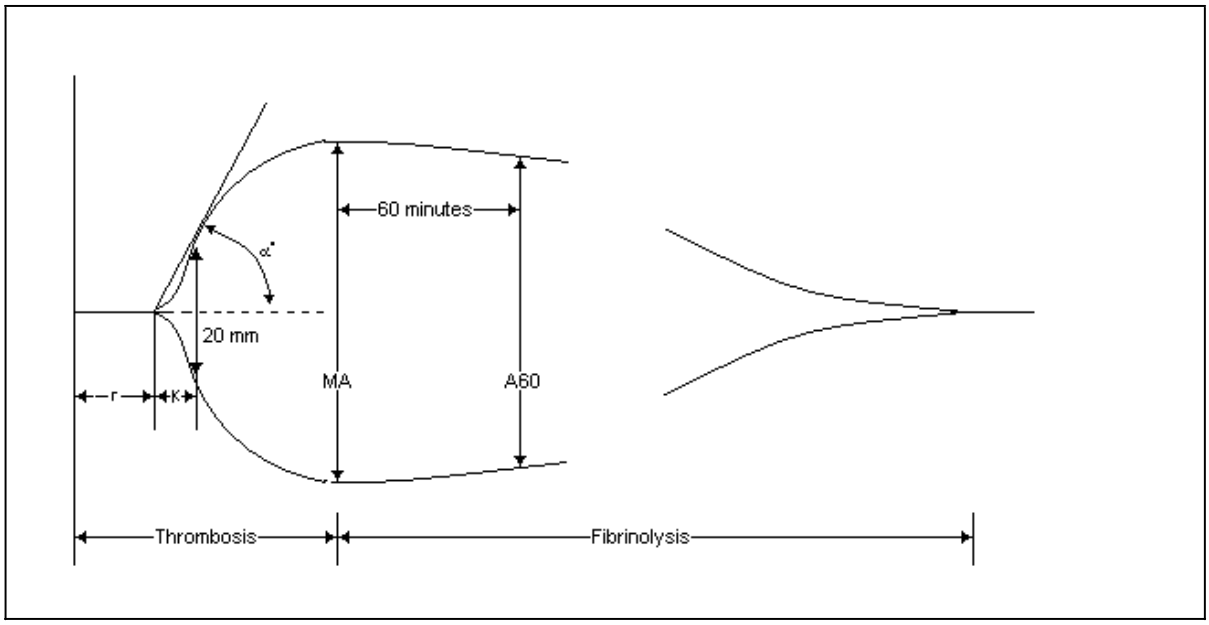


Figure 2.3: The thromboelastogram tracing (40)

The r-time is influenced by the intrinsic activity of clotting factors, fibrinogen, and platelets. In liver transplant surgery a shortened r-time may indicate the need for fresh frozen plasma infusion. MA is deceased by platelet abnormalities or deficiencies. The α-angle is a measure of the speed at which clot forms and is decreased by hypofibrinogenemia and thrombocytopenia; in liver transplantation this indicates a need for cryoprecipitate infusion (17, 24, 25, 40). A short r-time may be present with hypercoagulability syndromes, the K-time influenced by activity of clotting factors, fibrinogen and platelets, a decreased α-angle by thrombocytopenia, and MA altered by platelet abnormalities (40).

The ROTEM® differs from the TEG® as it uses an optical detection device system, not a torsion wire, initiates movement from the pin, not the cup, and is equipped with an electronic pipette (24).

The nomenclature and reference ranges for the TEG® and ROTEM® are different and are set out in table 2.1. The reliability of measurements from these two methods is within acceptable ranges (24). In their “head-to-head” comparison of the TEG® and ROTEM® systems Jackson et al. (41) found that the TEG® had the best compromise between user-friendliness, cost and usefulness in their setting. Normal values between centres are not standardised, however, if analysis within a centre is performed in a standardised manner, results are valid (25).

Table 2.1: Comparative values: TEG® and ROTEM® (25)

	TEG®	ROTEM®
Clotting time (min) (time to 2 mm amplitude)	r	CT
Clot formation time (min) (time from 2-20 mm amplitude)	K	CFT
Maximum clot strength	MA	MCF
Alpha angle	α (slope between r and K)	α (slope of tangent at 2 mm amplitude)
Maximum angle	-	CFR
Time to maximum strength	TMA	MCF-t
Amplitude at set time	A (A30, A60)	A (A5, A10…)
Clot elasticity	G	MCE
Maximum lysis	-	ML
Lysis at fixed time	CL30, CL60	LY30, LY45, LY60
Time to lysis	TTL (2 mm drop from MA)	CLT (10% of MCF)
Maximum lysis	-	CLR (maximum tangent post-MCF)
Measurement period	-	RT

The normal reference values for TEG in adults are as follows:

r-time	4-8 minutes (whole blood), 3-8 minutes (citrated/kaolin)
K-time	1-4 minutes (whole blood), 1-3 minutes (citrated/kaolin)
α-angle	47°-74° (whole blood), 55°-78° (citrated/kaolin)
MA	55-73 mm (whole blood), 51-69 mm (citrated/kaolin) (24)

Fresh whole blood has traditionally been used for the TEG. This fresh blood must be placed in the TEG within 4-6 minutes of venesection for analysis (31, 42). Celite can be added as an adjunct to accelerate the clotting process; it consists of inert silica particles that act as a surface to activate factor XII and platelets (34, 36). Normal ranges of parameters of the TEG have been established for fresh whole blood. Currently validation studies are being carried out on the use of citrated blood for the TEG. Citrate causes incomplete inhibition of coagulation cascade activation (25). It is suggested that citrated samples must rest for at least 30 minutes before analysis in the TEG begins in order for standardisation of results (24, 25). Citrated samples can be stored at room temperature or at 4ºC and must be recalcified before TEG analysis (42). Bowbrick et al. (42) showed no significant difference in TEG results of citrated whole blood kept at room temperature for 30 minutes or at 4ºC for 45 to 150 minutes when compared to fresh whole blood samples.

A temperature of 4˚C can cause platelet damage and induce coagulation. It is possible that the platelets do not play a significant role in the TEG profile unless there is a thrombocytopenia of $<66 \times 10^9$/l (16, 42). Orlikowski et al. (27) found that the TEG performed adequately as a screening test for coagulation when compared with traditional screening tests (platelet count, PT, aPTT and fibrinogen) and that platelet counts of more than 54×10^9/l were associated with adequate clot formation on the TEG.

Limitations of the TEG exist. The TEG cannot identify specific factors responsible for coagulopathy, results are not standardised between institutions and it has not

been validated (24, 31). TEG tests are performed under static conditions in a cuvette, thus there is no flow as in a vessel, and no interaction with endothelial cells (24). This necessitates careful interpretation of the TEG results. To ensure accuracy of the TEG there must be strict procedures for collection and handling of blood samples by trained personnel (24, 32).

Age and gender influence TEG tracings in adults (24). Miller et al. (43) showed that platelet counts and fibrinogen levels correlate with TEG tracings in adults and children over the age of 12 months. The aged tend toward a hypercoagulable state, as do women when compared to men (44).

2.1.4 HIV and TEG

There is currently no research on the effects of the haematological changes of HIV on the TEG.

2.1.5 Paediatric patients, coagulation and testing of coagulation

There are currently no publications on paediatric HIV infection and the TEG. The small amount of blood needed for TEG analysis may be beneficial for children (31). Normal values for TEG analysis using whole blood and analysis within six minutes of venesection in children have been established and are shown in table 2.2. Rajwal et al. (31) recommend that if citrated whole blood is to be used, normal ranges need to be established.

There is a functional difference in fibrinogen between children and adults, and a decrease in contact and vitamin K dependent clotting factors, and coagulation

inhibitors, especially in children less than 12 months of age when compared to adults. Children less than one year of age develop clot faster than adults (43). After 12 months fibrinogen matures and levels increase to adult levels, and interaction with platelets corresponds with that of adults, although maturation is not yet complete. The TEG (celite-activated) and heparinase-modified TEG tracings of healthy children and adult groups are comparable (31, 38, 43, 44, 45, 46, 47, 48). Age-specific reference ranges for kaolin-activated TEG tracings have recently been established (49). In children less than one year old there is functional maturity of the coagulation system seen by TEG monitoring, despite the lower factor levels. In children less than three months old the r-time is significantly shorter (45, 46, 50).

Oswald et al. (50) showed that there were no gender-related differences in ROTEM® parameters in all children younger than sixteen years in their study.

Current literature confirms that the inhalational anaesthetic agent Sevoflurane has no effect on platelet function or number (50).

Table 2.2: TEG reference values for paediatric patients (49, 51)

	Celite-activated TEG (children 4-9 years)	Citrated whole blood with calcium chloride (children 6.5 ± 4.8 years)	Adult
r-time (mins)	9.6 ± 2.5	8.7 ± 2.6	11.1 ± 3.0
K-time (mins)	2.8 ± 0.6	2.1 ± 0.7	2.7 ± 0.6
α-angle (degrees)	72.9 ± 4.2	61.6 ± 7.1	70.7 ± 3.9
MA (mm)	70.5 ± 3.3	59.6 ± 4.2	70.2 ± 3.7

2.2 Human Immunodeficiency Virus

2.2.1 HIV background

HIV is a lentivirus and a member of the human retrovirus subtype (1). Before 1986, when an international committee termed it HIV, there were various confusing names for the virus that was causing AIDS (52).

A theory of the origin of HIV is that a simian transmitted Simian Immunodeficiency Virus to humans in West Africa in 1931 (52, 53). HIV was identified in 1983, under much controversy, by Montagnier's research team in Paris and Gallo's research team in the United States of America (52, 54). The first cases of AIDS in the United States of America were described in June 1981 in adult, homosexual men and in 1982 in paediatric patients (4, 54, 55). A disease called 'Slim' disease was seen in Uganda following the opening of trade routes for truckers in 1982; it was characterised by diarrhoea and a wasting syndrome (52). AIDS-like symptoms were reported in both sexes in the Congo and Rwanda in 1984 (52).

HIV has a cytopathic action. Characteristically a person infected with this virus has a long latency period, with persistent viraemia, before being diagnosed with AIDS. If untreated 10% of patients will be diagnosed with AIDS within two to three years of infection, the remainder are diagnosed with AIDS within ten years of infection. It is this silent period that allowed HIV to spread unnoticed during the 1970's and has resulted in the pandemic seen today (53). By 1985 there were diagnostic kits available for the detection of HIV antibodies in infected persons (52).

In the United States of America AIDS changed from an unmentionable disease in the early 1980's to the leading cause of death in the young adult population by 1994 (54). The advent of antiretroviral drugs changed the mortality from HIV in the developed world. The first agent to be licensed was zidovudine (AZT), a reverse transcriptase inhibitor, in 1987 (54). Other drugs of the same class and then protease inhibitors followed this in 1995 (52, 54). Most recently cell-entry inhibitors and integrase inhibitors have been licensed (52).

HIV targets the immune system and selectively depletes the CD4 T helper cells. Infected persons are susceptible to mycobacterial, bacterial, viral and malignant diseases (1).

2.2.2 HIV epidemiology

It is estimated that more than 50 million people in the world have become infected with HIV (around 40 million are living with HIV) and that 36 million of these infected people are living in sub-Saharan Africa. This is 10% of the sub-Saharan

population. This is a high percentage of the population and will result in a deepening of poverty, and diminish food and health care resources (1, 4, 56). The burden on health care resources is large, with 50% of hospital beds in sub-Saharan Africa being occupied by patients with HIV-related diseases (56). The Actuarial Society of South Africa estimates that 5,6 million South Africans are HIV infected, that is at least 15% of our population (2, 3).

Almost 30% of new infections have been amongst the young adult female population who spread HIV during pregnancy, parturition and breastfeeding to their children. Consequently the epidemic in children is seen to parallel that of reproductive women. Nearly 10% of childhood mortality in sub-Saharan Africa (2004) is HIV-related (1, 56). In the developed world the incidence of vertical transmission has been reported to be as low as 1-2% with the use of antiretroviral agents and elective caesarean sections. In developing countries where such interventions are not always possible the following interventions may be acceptable in trying to reduce the rate of vertical transmission of HIV, vitamin and nutritional supplementation, vaginal cleansing, passive immunisation, and prophylaxis for chorioamnionitis (57).

Some children become infected by unsafe injections, sexual transmission, household contact, or by exposure to infected blood products (1, 57). In 2005 2,3 million children worldwide were estimated to be HIV positive and by 2006 this estimate had grown to more than three million (56). Nearly 90% of HIV infected children live in sub-Saharan Africa (58).

Access to antiretroviral agents has significantly decreased the morbidity and mortality associated with HIV/AIDS especially in the developed world. In the developing world, however, there are severe human and financial resource shortages thus increased morbidity and mortality as compared to developed countries. The World Health Organisation (WHO) aimed to provide three million people in the developing world with ARVs by 2005 (56). In a WHO report from 2010 it was estimated that 23% of the children living with HIV in the developing world were receiving ARVs (59)

It is estimated that 20-25% of patients infected with HIV will need to undergo surgery at some time during their illness; this includes children (1, 4). If the CD4 count is less 50 cells/μl at the time of surgery, it is suggested that the six month mortality is 13.3% and if less than 200 cells/μl, 0.8% (52).

2.2.3 Effects of HIV on the haematological system, in particular, the platelets

HIV affects almost every organ system in the human body. This includes the cardiovascular system, respiratory system, haematological system, renal system, nervous system, and growth and nutrition in children (4). The primary effects of HIV are on the haematological system (55).

There is a broad range of haematological manifestations of HIV infection. Characteristically there is a decrease in CD4 T lymphocytes in all untreated patients. This may be accompanied by anaemia (in 70% of patients), granulocytopenia (in 50-70% of patients), thrombocytopenia (in 10-40% of patients), and lymphopenia. These changes may be as a result of increased

peripheral destruction of cells or decreased production of cells because of direct bone marrow suppression by HIV, opportunistic infection, tumour infiltration or from treatment therapies. These therapies include antiretroviral, antimicrobial and antitumour agents (9, 55, 60, 61).

HIV itself suppresses the bone marrow. This is evidenced by an increase of cellular deficiencies with advancing disease. However, isolated thrombocytopenia is often the presenting manifestation of HIV infection and is thus not considered a criterion for advancing disease and progression to AIDS (60).

Infectious agents that infiltrate bone marrow and suppress cell production or cause reticuloendothelial dysfunction include: Mycobacterium avium intracellulare; Mycobacterium tuberculosis; Cytomegalovirus; Cryptococcus neoformans; and Histoplasma capsulatum (60).

The metastatic diseases, which infiltrate the bone marrow and thus contribute to the haematological manifestations associated with HIV infection, include Kaposi sarcoma and lymphoma (60).

Thrombocytopenia in AIDS has a complex, multifactorial aetiology, which includes the following: there is often immune mediated destruction of platelets (usually occurring earlier in the disease process) by circulating immunoglobulins, platelet bound IgG, complement or antibody complexes (there is cross-reaction between HIV gp120 and thrombocyte gp3a, and between HIV p24 antibodies and platelets) against platelet antigens. These antibodies are due to a defect in regulation of

antibody production characterised by HIV infection. B-cell regulation, activation and response are abnormal as a result of the HIV infection, or other viral infection (for example hepatitis B virus). Other possible aetiologies for the thrombocytopenia include decreased and defective production in the bone marrow (which also shortens platelet life-span), a side effect of drugs taken, syndromes mimicking haemolytic uraemic syndrome, thrombotic thrombocytopenic purpura, hypersplenism, disseminated intravascular coagulation, or an increase in the peripheral destruction and fragmentation of platelets (platelets are directly attacked by HIV through CD4 and CXCR4 receptors). There might be a decrease in thrombopoietin production in hepatocytes in those HIV infected patients with liver disease (9, 55, 60, 62).

Guidelines suggest that the treatment of thrombocytopenia only be instituted in those patients with very low platelet counts or those with severe bleeding. Treatment of thrombocytopenia with the standard approach of steroids, splenectomy, transfusion, and in the case of HIV antiretroviral agents, is controversial because of the immunosuppressive nature of these modalities. This often complicates treatment of HIV infected persons with thrombocytopenia. Some thrombocytopenic seropositive patients have responded well to intravenous gamma globulins, anti-Rh(D), platelet infusion, vincristine, danazol, or interferon alpha 2a. Treatment modalities for thrombocytopenia have been associated with accelerated disease progression, increased costs and transmission of infections (9, 60).

Thrombocytopenia is associated with rapid disease progression in the HIV infected person, which is a decrease in CD4 count and earlier diagnosis of AIDS. However, isolated thrombocytopenia may present at any stage of the disease (55). A 4% increase in mortality from bleeding has been observed in thrombocytopenic patients (9, 52). Platelets also play a part in inflammatory and immunological host defences by secreting antimicrobial peptides and expressing cytokines. Platelet internalisation of HIV has been suggested as a mechanism of viral clearance (61). In addition to immune-mediated thrombocytopenia, there are often antibodies to erythrocytes and granulocytes.

HIV is known to affect the coagulation system. Antiphospholipid antibodies and lupus-like anticoagulant of IgG type have been found in 20-70% of HIV positive people. This antibody is associated with active opportunistic infection in patients and these patients are seen to have a prolonged activated partial thromboplastin time. HIV-associated antiphospholipid antibodies do not have the same association with hypercoagulability that these antibodies do in HIV negative patients (60, 63).

Coagulation abnormalities in HIV positive patients may be due to the above antibodies and anticardiolipin antibodies, thrombocytopenia or platelet function abnormalities. The following bleeding abnormalities have been seen; prolonged bleeding time and abnormal platelet aggregation (55, 60). As well as the predisposition to an increased bleeding tendency, there have also been reports of hypercoagulability in HIV positive people. This is related to coexisting malignancies, autoimmune disease or as a result of drug therapy (1). Saif and

Greenberg (64) found the following abnormalities that predispose to hypercoagulability: protein C and S deficiencies; heparin cofactor II deficiency; antithrombin III deficiency; abnormalities of fibrinolysis; lupus anticoagulant; antiphospholipid antibody syndrome; malignancy; myeloproliferative disorders; autoimmune diseases; stasis; altered lipid metabolism and lipodystrophy; antiretroviral drugs; thrombotic thrombocytopenia purpura; and infections (62, 64). Saber et al. (65) added dehydration from diarrhoea, immobility, intravenous drug abuse, and Cytomegalovirus endothelial procoagulant activity as possible mechanism for HIV/AIDS associated hypercoagulability. Levine et al. (66) showed that advancing age as well as HIV disease, megestrol acetate or indinivir treatment, and progressive abnormalities of protein S and factor VIII leads to hypercoagulability.

Vitamin B_{12} or cyanocobalamin deficiencies are found in 20% of patients with AIDS (60). Vitamin B_{12} is necessary for erythropoiesis, DNA and myelin synthesis, and other metabolic processes. A deficiency manifests, amongst other symptoms, with a megaloblastic anaemia. If severe, vitamin B_{12} deficiency results in a pancytopenia (20, 40).

2.2.4 Antiretroviral agents and coagulation

ARV treatment has significantly modified the course of HIV disease. There is an improvement in quality of life and survival of people infected with HIV. The measurement of the CD4 count, and its increase (rapidly, at first and then more gradually) and normalisation (in the majority of cases), has generally been considered as a marker of successful therapy. This increase in CD4 count usually

corresponds with a decrease in the viral load. A patient's prognosis will approach that of an HIV negative individual when the CD4 count remains consistently high; more than 500 cells/mm^3 (67). ARVs are, however, not without side effects.

Long-term treatment with AZT is associated with bone marrow suppression and pancytopenia (1). AZT is a nucleoside reverse transcriptase inhibitor, which inhibits viral replication by acting as a false substrate for reverse transcriptase and thus terminating the DNA chain (68). AZT inhibition of DNA polymerases in bone marrow cells impairs haemopoietic cell production. Of patients on AZT, 34% will be anaemic, 16% will be neutropenic and 12% will have thrombocytopenia (usually with a 50% reduction in platelet count). Platelets are usually the spared haemopoietic line. Those patients with low CD4 counts prior to initiation of AZT and those with pre-existing cytopenias are more at risk from this side effect of AZT. If paracetamol is used concomitantly there is a further increase in the incidence of myelosuppression. AZT inhibition of DNA polymerases in bone marrow cells impairs haemopoietic cell production (60).

Protease inhibitors form an important component of antiretroviral regimes. Yazdanpanah et al. (69) found that there was an increased incidence of bleeding in HIV positive patients with congenital coagulation disorders who were on lopinavir-ritonavir-containing antiretroviral regimes. A mechanism for this bleeding has not been found, but may be associated with inhibition of the cytochrome P450 system by disruption of prostaglandin-mediated platelet activation, or an enhanced fibrinolytic response (69, 70). Ritonavir appears to be associated with the highest bleeding risk, followed by indinavir, and then saquinavir and nelfinavir. Bleeding

usually occurs soon after commencement of therapy, and the risk of bleeding decreases with time on the agent. Bleeding occurs into the joints or tissues and mucous membranes. Some will only have excessive bleeding with surgery or dental extractions. If severe the protease inhibitor is stopped and bleeding tendency resolves with time (70). There have been reports of increased bleeding in patients who do not have a hereditary bleeding disorder. Racoosin and Kessler (71) reported this, but found the incidence to be much lower than in the haemophiliac population. Nielsen (72) reported hypermenorrhoea in a case series. Lorenzi et al. (73) reported intracranial haemorrhage in two babies born to mothers on protease inhibitors. These babies were premature, which could also be an explanation for the haemorrhage. Koppel et al. (74) found increased levels of plasminogen activator inhibitor type 1 and fibrinogen in patients on protease inhibitors. These increased levels alter the regulation of fibrinolysis.

Protease inhibitor therapy has recently been implicated in deep vein thrombosis, pulmonary embolism, coronary artery disease and portal vein thrombosis. Protease inhibitors can thus result in a hyper- or hypocoagulable state. Hypercoagulability usually occurs at a mean of 72 days post initiation of therapy and is thought to be as a result of interference with hepatic regulation of coagulation proteins. Agents implicated included ritonavir, indinavir, nelfinavir, and saquinavir (63). Giuseppe (75) reported that 25% of patients on protease inhibitors have coagulation defects. Endothelial dysfunction, and increased fibrinogen, d-dimer, plasminogen activator inhibitor-1 and tissue-type plasminogen activator antigen, and deficiency of protein S (reported in up to 73% of HIV positive men) have been implicated in these coagulation defects. Coagulation side effects

of protease inhibitors occur less frequently than metabolic side effects. However, those patients with fat redistribution syndrome have increased procoagulant levels in their blood samples and increased fibrinolysis. It is suggested that the routine evaluation of coagulation is not advised until a benefit of screening is found. An awareness of possible disorders is needed (75, 76).

De Andrade et al. (77) showed an increase in bleeding at delivery of pregnant women on antiretroviral therapy. The antiretroviral agents increased tissue plasminogen activator antigen and plasminogen activator inhibitor type 1 levels, which lead to hyperfibrinolysis.

Suramin, dideoxyadenosine and dideoxyinosine are also myelosuppressive (60). Gancylovir, pentamidine and trimethoprim-sulfamethoxazole used to treat opportunistic infections associated with AIDS are bone marrow suppressant.

2.2.5 Dentistry and HIV

Early in the course of their disease 40-50% of HIV-infected persons will develop oral fungal, bacterial or viral infections (56). These infections are often the first signs of immunosuppression and disease progression (78). Early detection of oral disease and HIV testing and treatment can significantly decrease morbidity and mortality (79).

Oral diseases include the following: candidiasis, oral hairy leucoplakia, gingivitis, oral ulcers, Kaposi sarcoma, non-Hodgkin's lymphoma, histoplasmosis, penicilliosis and xerostomia. If treated early, the lack of these manifestations may

significantly improve the quality of life of HIV infected people (56, 80). In a South African cohort oral candidiasis, angular chelitis and oral hairy leucoplakia were the most common oral lesions in HIV infected people (81).

The dry mouth, as a result of decreased saliva, increases the incidence of dental caries (the saliva acts as a buffer to bacteria, contains secretory IgA, is a reservoir for calcium, phosphate and fluoride, and washes away plaque and food debris) and also decreases the quality of life as there is difficulty swallowing, chewing, tasting food, and speaking (7). There are also halitosis and aesthetic problems. Arendorf et al. (82) found that more than 25% of HIV-infected patients had oral soft tissue disease that necessitated treatment. Diffuse Infiltrative Lymphocytic Syndrome and drugs used to treat HIV and opportunistic infections may also cause xerostomia (56, 78, 81).

The mouth is a reservoir for pathogenic and non-pathogenic organisms. In the immunosuppressed patient these organisms multiply rapidly to cause opportunistic infections. There is a local spread of the pathogens and their toxins causing caries, necrotizing gingivitis, periodontitis, stomatitis, pharyngitis, and oesophagitis, or systemic spread causing blood and central nervous system infection (81).

It is suspected that pathogens that would not usually cause widespread disease in the immunocompetant are the source of severe disease in HIV-positive people; especially as the CD4 lymphopenia worsens (81). Silva-Boghossian et al. (78) investigated microorganisms in the saliva of HIV positive and negative children

and found that the majority of species tested for were more prevalent in the HIV negative group. The incidence of bacteria in those children on ARVs and those not ARVs were similar, although ARVs and drugs used to treat opportunistic infections may modify microorganisms found in the mouth. They found that HIV-positive children were able to mount a mucosal immune response to oral pathogens. An explanation of similar levels of salivary IgA in both groups was given.

HIV positive patients have been observed to bleed more during dental procedures as a result of HIV disease process or the consequences of drug therapy (83).

2.2.6 Paediatric patients and HIV

The HIV/AIDS pandemic is severely affecting children. Not only do they get sick, but also they are orphaned, suffer poverty and in many cases are deprived of a valuable, normal education (56). Most children are infected by vertical transmission from mother to child (15-45% of children born to HIV infected mothers will become HIV positive if no specific interventions to prevent transmission are used (58)), some by household contact, sexual abuse or intercourse, blood product infusion, scarification, or by contaminated needles (4, 84). Of the 1500 daily new HIV infections in children, 9 out of 10 will be in sub-Saharan Africa (84).

Almost all children born to HIV positive women are HIV-antibody positive at birth. This antibody usually becomes undetectable between nine and eighteen months of age. Infants less than 18 months are therefore diagnosed with HIV DNA

polymerase chain reaction, HIV peripheral blood culture and HIV p24 antigen, and not the standard anti-HIV IgG antibody tests used for older infants, children and adults (4).

There are unique features of HIV infection acquired perinatally. HIV-1 has very high replication rates that persist over time, and may lead to a rapidly progressive course towards AIDS with high viral loads. The half-life of the virus is longer in infants less than three months than older infants. It is thought that this is as a result of the immaturity of the immune system of infants (57, 85). AIDS accounts for 3% of deaths in children less than five years old (84).

As with adults, combination nucleoside protease inhibitor antiretroviral therapy is better than monotherapy. Only 8% of patients receiving antiretroviral therapy in South Africa are children (84). Three and four drug antiretroviral regimes have been found safe and effective in children, who experience greater immune recovery than adults because of an active thymus gland (57, 86). Adherence to drug regimens and long-term complications are of concern (87). Eligibility for ARVs has until recently been determined by clinical and immunological category, that is a CD4 percentage of less than 15 and/or a CDC clinical stage B or C (58). In 2010 the WHO published new recommendations for starting ARVs in children, as follows:

- infants and children younger than two years old should start ARVs immediately on diagnosis

- children from two years old to five years old should be started on ARVs when the CD4 percentage is less than 25 or CD4 count is less than 750 cells/mm^3
- children aged five or more should be started on ARVs when the CD4 count is less than 350 cells/mm^3 (88).

In 2008 fewer than 10% of children eligible for ARVs in sub-Saharan Africa were receiving these drugs, however, the WHO reported in December 2010 that this figure had risen to 21% (59, 89). Drug dosages need to be altered for paediatric metabolism and often drugs are not prepared in a form easily taken by children. There is less research into antiretroviral agents in children than in adults (75).

HIV affects many of the organ systems in children, however, little has been written about these manifestations. The haematological system is commonly affected. Consolini et al (90) found that 35% of children in their study group presented with haematological manifestations of HIV, 21% of these with thrombocytopenia. Haematological manifestations include: anaemia (the most common manifestation after CD4 count decrease); neutropenia; lymphopenia; thrombocytopenia; and eosinophilia. These manifestations are as a result of immune-mediated peripheral destruction of haematological cells, bone marrow infiltration, suppression and dysplasia, nutritional deficiencies, HIV replication, or medications (zidovudine, gancyclovir, trimethoprim-sulfamethoxazole) (4, 85, 90).

Thrombocytopenia is one of the earliest manifestations of HIV infection in children, with up to 50% developing this complication before one year of age (it is, however,

rarely the first clinical sign of disease in children; 5,8% versus 10% in adults (90)). Others develop thrombocytopenia later in the disease process (9). A study in Lagos, Nigeria showed that thrombocytopenic children have more advanced disease. Although these results were not significant as the sample size was not large enough, they have also been shown in a CDC study (91). Children with thrombocytopenia are placed in CDC clinical category B (4). Consolini et al (90) found that children with anaemia and thrombocytopenia associated with HIV had a significantly worse prognosis than those without. Children are unlikely to have any significant bleeding if the platelet count is more than 20-30x10^9/l. It is suggested that the thrombocytopenia only be treated if there is significant bleeding (10). The response of platelets to antiretroviral treatment in children has not been as good as in adults, and as with adults, AZT, didanosine, lamivudine and enfuvirtide have been shown to cause thrombocytopenia (4, 9, 92).

Ellaurie (93) reported thrombocytosis (a platelet count of >500 x10^9/l) in 6% of children from a cohort of 400 patients. There were no thrombotic complications with these patients. It is thought that the chronic nature of the viral infection with HIV, as well as nutritional, metabolic, immune and neoplastic diseases, trauma, drugs, splenectomy, iron deficiency anaemia, and acute blood loss, may contribute to the aetiology of the thrombocytosis (92, 93).

Dental caries in children is an important primary health care issue. It is estimated that 40% of five year olds in the United Kingdom have had caries, making it the most common chronic disease in that country (5, 7). In South Africa, the 1999/2002 South African National Children's Oral Health Survey published a

dental caries prevalence of 51% in four-to-five year olds (6). The incidence in HIV positive children (in whom the incidence of xerostomia is higher than in adult infected patients) and those with other chronic disease is higher as a result of immunosuppression. Although HIV positive children have a lower incidence of caries producing microorganisms (Streptococcus mutans, Streptococcus salivarius and Lactobacillus acidophilus) in their saliva, the incidence of caries is higher than in HIV negative children. These children are also at risk for complications of caries, namely periodontal disease and systemic infection (7, 78, 80). The most common oral manifestations in HIV positive children are oral candidiasis (angular chelitis, pseudomembranous, erythematous and hyperplastic), gingivitis, linear gingival erythema, parotid enlargement, herpes simplex and leucoplakia (78). In immunocompromised children the risks associated with untreated caries and other oral infections necessitates the provision of preventative dental care (7).

The issue of paediatric HIV disclosure has been studied in South Africa. Moodley et al. (94) concluded that disclosure of HIV status might improve the quality of life, longevity, care and treatment of infected children.

2.3 Conclusion

This chapter has focused on a discussion of currently available literature on coagulation and HIV relevant to the study.

CHAPTER 3: RESEARCH METHODOLOGY

This chapter will present the methodology for this research report.

3.1 Problem statement

It was observed clinically that children known to be HIV positive bled more than those with unknown or known negative status when undergoing dental extractions at CMJAH. At the time of this research project there was no research determining whether these HIV positive children had abnormal coagulation profiles, as seen by an abnormal TEG or thrombocytopenia, and whether this was the reason for the observed increase in bleeding.

3.2 Aims of the study

This study was conducted in three parts:

Part 1

The aim of this part of the study was to determine if there was a difference in the coagulation profile of HIV positive paediatric patients (those on ARVs and those not on ARVs) and HIV negative paediatric patients undergoing dental extractions at CMJAH.

Part 2

The aim of this part of the study was to compare the CD4 counts and percentages of HIV positive paediatric patients on ARVs and those not on ARVs with that of the HIV negative paediatrics patients undergoing dental extractions at CMJAH.

Part 3

The aim of this part of the study was to compare observed bleeding of the HIV positive paediatric patients (on ARVs and not on ARVs) with that of the HIV negative paediatric patients undergoing dental extractions at CMJAH.

3.3 Objectives of the study

The aims of the study were justified by the following objectives.

Part 1

- To describe the coagulation profile of HIV positive (on ARVs and not on ARVs) and HIV negative paediatric patients undergoing dental extractions.
- To compare the coagulation profiles of these patients.

Part 2

- To determine the CD4 counts and percentages of HIV positive (on ARVs and not on ARVs) and negative paediatric patients undergoing dental extractions.
- To compare the CD4 counts and percentages of the three groups.

Part 3

- To assess the observed bleeding in the HIV positive (on ARVs and not on ARVs) and the HIV negative groups of paediatric patients undergoing dental extractions.
- To compare the observed bleeding between the groups.

3.4 Location of the study

The study took place at the CMJAH, Gauteng Province, South Africa. CMJAH is an academic hospital associated with the University of the Witwatersrand. It is a tertiary hospital acting as a referral hospital for a number of smaller regional hospitals.

3.5 Ethical considerations

Ethical approval for the study was received from the HREC (Appendix 1) of the University of the Witwatersrand, Johannesburg, Gauteng Province, South Africa.

Approval for the study was verbally obtained from the authorities at CMJAH.

After discussion and explanation of the study and giving of an information packet, written informed consent was obtained from the parent or legal guardian of each patient prior to admission into the study. Assent was sought from the patients who were six years old or older. Appendices 2 to 8.

Patients and their guardians who had an HIV test for the purposes of this study received pre- and post-test counselling.

3.6 Study design

A prospective, contextual, descriptive research design was followed in this study.

***Prospective**:* A prospective study design was chosen, as this determines how the factors under study relate to outcome in the cohort over time. Evidence usually

ranks higher than that of retrospective studies, although prospective studies tend to be more time consuming and expensive (95, 96, 97).

***Contextual**:* This study is contextual as it was conducted within a specific context. De Vos (98) describes context as a small-scale world. This study was conducted at CMJAH on children undergoing dental extractions.

***Descriptive**:* A descriptive design was chosen. The primary purpose of this design is to develop a body of knowledge. This study method provides a picture of situations as they naturally happen. No manipulation of variables is involved; however, there is control over extraneous variables. The setting for a descriptive study is therefore natural but conducting the study involves a high degree of control (99, 100).

3.7 Study population

The study population was all paediatric patients presenting for dental extraction at CMJAH.

3.8 Study sample

3.8.1 Sample statement

In consultation with a biostatistician a sample size of 16 in each group was used. This sample had a 90% power to detect a difference in means of 4.0 (one standard deviation) assuming that the common deviation was 3.3 (reference) using a two group t-test with a 0.05 two-sided significance level (11, 12, 13).

3.8.2 Sampling method

A consecutive, convenience sampling method was used in this study.

The convenience sampling method was chosen because of the time constraints and the scope of the research. The most readily accessible paediatric patients presenting for dental extractions were included. It was acknowledged that a convenience sample cannot fully represent the study population (14).

The study further used a consecutive sampling method where every paediatric patient who presents for dental extraction was invited to take part in the study. Consecutive sampling was the most reliable form of convenience sampling as research bias was limited (14).

3.8.3 Inclusion criteria

The following inclusion criteria were used for the study:

- known HIV positive paediatric patients on antiretroviral treatment
- known HIV positive paediatric patients not on antiretroviral treatment
- HIV negative paediatric patients.

3.8.4 Exclusion criteria

The following exclusion criteria were used for the study:

- refusal to give consent
- HIV positive or negative paediatric patients' known to have a coagulation abnormality.

3.9 Procedure for data collection

The researcher identified potential patients to enrol in the study at the dental clinic. Known HIV positive patients were invited to take part in the study. If they agreed, informed, signed consent was obtained from the parent or legal guardian and assent from those paediatric patients over six years old. Potential HIV negative patients were invited to take part in the study. If they agreed a CMJAH HIV councillor counselled them. Consent for HIV testing was then obtained from the parent or legal guardian. A blood sample for a rapid HIV test was taken and sent to NHLS laboratory at CMJAH for analysis. Once the results were known the hospital councillor counselled the parents again. All paediatric patients found to be HIV positive during data collection for this research report were referred to the CMJAH virology clinic for follow up treatment. Signed informed consent was then obtained from the confirmed HIV negative patients' parents and assent was obtained from paediatric patients of appropriate age. See appendices two to eight for information given to the paediatric patients and their caregivers, as well as the consent and assent forms.

The anaesthetics for the paediatric patients enrolled in the study were given by the consultant and registrar staff of the Department of Anaesthesiology at CMJAH. The researcher was present at the induction of anaesthesia for all enrolled paediatric patients and collected all the blood specimens for the study. The paediatric patients received a general anaesthetic using the following standard anaesthetic technique:

- the child was assessed on arrival in theatre
- the parent accompanied the child into theatre for induction of anaesthesia

- standard anaesthetic monitors were applied to the patient (these were; an oxygen saturation probe, ECG monitoring, non-invasive blood pressure monitoring and a capnograph)
- anaesthesia was induced by the inhalational method using sevoflurane.

After anaesthesia was established, at the time of intravenous cannula insertion approximately 7 ml of blood was drawn from the child for the following tests: platelet count, CD4 count, CD4 percentage and TEG analysis.

The platelet, CD4 count and CD4 percentage specimens were sent to the NHLS laboratory at CMJAH for analysis. The blood specimen for the TEG was immediately handed to the anaesthesiology technician for analysis.

Specimens sent to the NLHS laboratory were analysed in a standard manner. The CMJAH NLHS laboratory is an accredited laboratory that adheres to Good Laboratory Research Practice.

TEG analysis was done as follows:

- 1 ml of whole blood was placed in a TEG kaolin tube
- 340 µl of this blood was placed in the TEG cup with 20 µl of calcium chloride within 4-6 minutes of venesection
- the cup was placed in the calibrated TEG machine and allowed to oscillate at 37℃
- the TEG was allowed to run until the parameters being monitored were available.

One anaesthesiology technician performed the TEG analysis. The TEG machine was calibrated every day before the commencement of surgery. The equipment (cups and pipette tips) required for analyses were all from the same batch and were stored in the department of anaesthesiology research office at room temperature. The kaolin tubes were stored at constant temperature in the refrigerator in the research office.

The following data was obtained and entered on a data capture sheet (Appendix 9):

- demographic data (name, hospital number, gender, and age)
- American Society of Anaesthetists (ASA) classification
- co-morbidities
- HIV status
- ARVs of HIV positive patients
- concurrent medications that patient is receiving
- blood results
- surgical complications including significant bleeding
- anaesthetic complications.

The data was entered onto an Excel spreadsheet.

The data collection procedure is illustrated in figure 3.1.

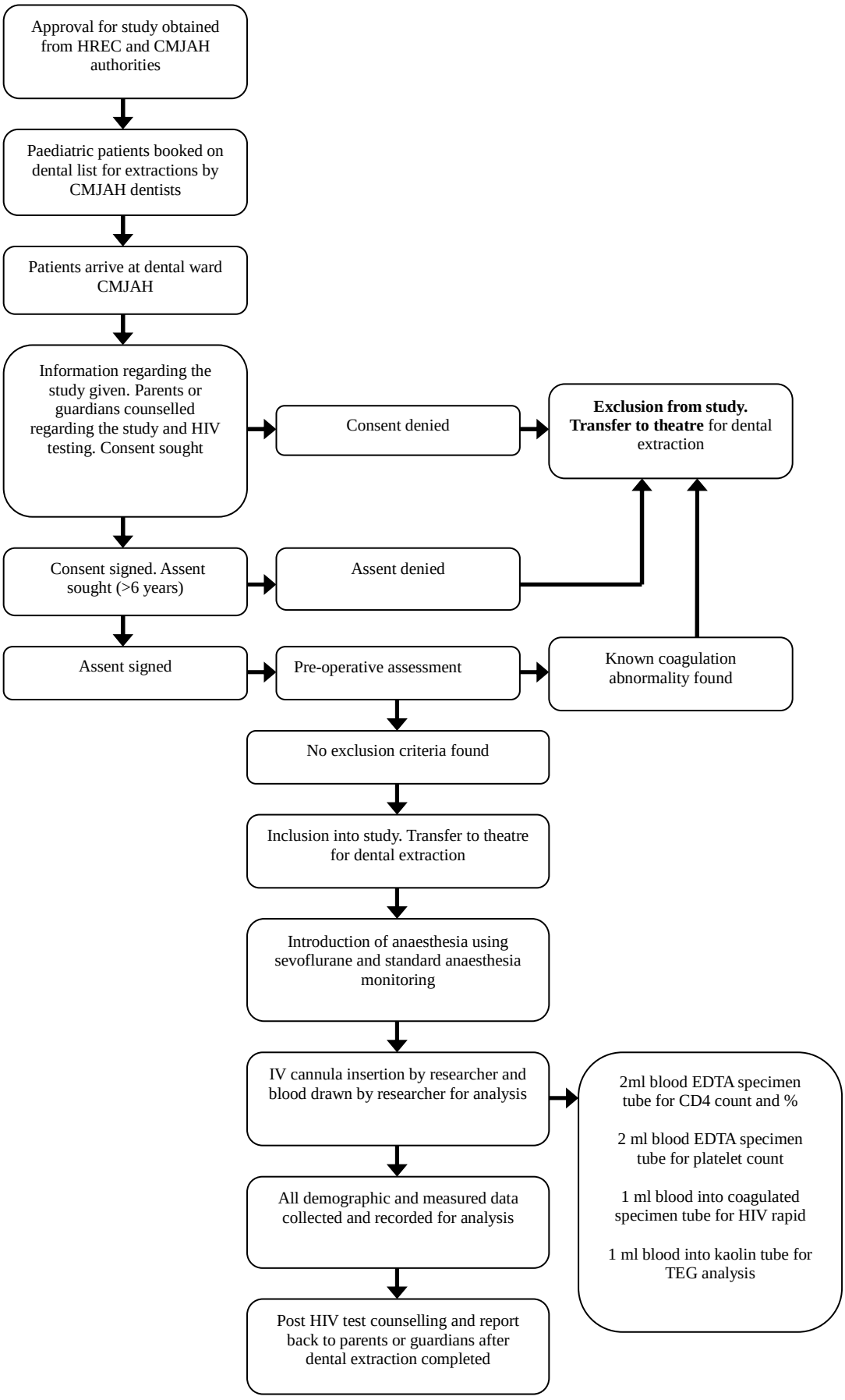


Figure 3.1 Flow diagram of the data collection procedure

3.10 Statistical analysis

Platelet counts, TEG parameters and CD4 counts and percentages are continuous and were summarized by patient group using mean, standard deviation and 95% confidence intervals. The three groups were compared with respect to the individual platelet counts, TEG parameters and CD4 counts and percentages using a one-way analysis of variance (ANOVA) followed by pair wise comparison using the Bonferroni adjustment to address multiplicity. To deal with big standard deviations and skewed data a one-way ANOVA for ranks was used to test for differences between the groups.

Patient groups were compared with respect to bleeding complications using the Fisher's exact test, while for CD4 counts the Welch t-test, adjusting for unequal variances, was employed. These results were also confirmed using the Wilcoxon rank-sum test.

Testing was done at the 0.05 level of significance.

3.11 Validity and reliability

The validity and reliability of the study were increased by the following measures.

One anaesthesiology technician trained in TEG analysis did the TEG analysis for this study. This was so that standard practices were adhered to and reliable results were obtained. A single TEG machine (TEG®) was used to limit variance that might occur between machines. The machine was calibrated to manufacturer

standards on each day that TEG analysis was performed to ensure accurate results were obtained.

Blood specimens drawn for HIV testing, platelet count and CD4 count and percentage were sent to the NHLS laboratory at CMJAH. In the laboratory Good Laboratory Research Practices are adhered to. This ensured reliable results from these tests.

The researcher, prior to analysis, collected all data. This ensured that data was collected in a standard manner from reliable sources.

Assessment of an increase in observed bleeding was subjective. The reliability of this observation was increased as only one senior member of the Department of Paediatric Dentistry assessed bleeding in all the paediatric patients who had dental extractions.

3.12 Conclusion

In this chapter a detailed description of the research methodology used in this study was presented.

CHAPTER 4: RESEARCH RESULTS

This chapter will present the results laid out by the objectives of the study and of the statistical analyses discussed in chapter three.

4.1 Sample realisation

Original consultation with the biostatistician recommended a sample size of 16 paediatric patients in each of the three study groups, HIV negative, HIV positive not on ARVs and HIV positive on ARVs. After two years of data collection only 12 patients had been recruited in the HIV positive not on ARVs group. These numbers were reviewed by the same biostatistician and found to be adequate for the purposes of this study.

Seventy six paediatric patients between 2 and 11 years of age were enrolled in the study. Of these children, 47 (61.84%) were HIV negative, 12 (15.79%) were HIV positive and not on ARVs and 17 (22.37%) were HIV positive and taking ARVs.

4.2 Demographic data

This study took place at CMJAH from November 2009 to November 2011.

Six of the children were two-year-olds, 15 were three-year-olds, 19 were four-year-olds, 14 were five-year-olds, eight were six-year-olds, three were seven-year-olds, six were eight-year-olds, three were nine-year-olds, one was 10 years old and one was 11 years old. Figure 4.1 shows the total number of paediatric patients

enrolled divided by age; each age group is then further divided into those children who were HIV negative, HIV positive and HIV positive on ARVs.

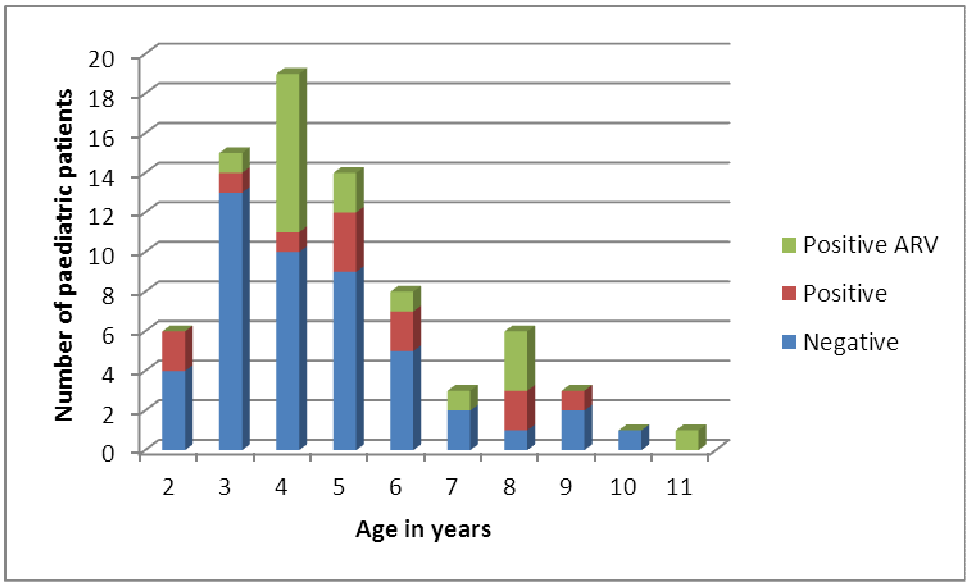


Figure 4.1 Ages of paediatric patients studied divided into HIV negative, HIV positive and HIV positive on ARVs categories

The mean age of the paediatric patients in this study was 4.86 years with the range being 2 to 11 years. There were an equal number of male and female paediatric patients enrolled.

Of the paediatric patients enrolled in the study 45 (59.2%) were assessed as being ASA category 1 patients, 22 (29.0%) being ASA category 2 patients and nine (11.8%) as ASA category 3 patients.

The mean number of teeth extracted from the paediatric patients was 9.67 (range 1-20). Of these teeth each patient had a mean of 4.55 single cusped teeth

(incisors and canines) extracted (range 0-12) and a mean of 5.12 multiple cusped teeth (molars) extracted (range 0-12).

The mean number of single cusped teeth extracted from the paediatric patients in the HIV negative group was 3.62 (standard deviation (SD) 2.58). The mean in the HIV positive group not on ARVs was 7.08 (SD 3.73) . The mean in the HIV positive group on ARVs was 5.35 (SD 2.96). Using one-way ANOVA for ranks with the Bonferroni adjustment to address multiplicity a p-value of 0.002 was obtained, indicating a statistically significant difference. Comparing the three groups by rank, the differences were found to be between the HIV negative group and the HIV positive group not on ARVs ($p = 0.006$) and between the HIV negative group and the HIV positive group on ARVs ($p = 0.048$).

The mean number of multiple cusped teeth extracted from the paediatric patients in the HIV negative group was 4.83 (SD 2.40). The mean in the HIV positive group not on ARVs was 6.00 (SD 3.10). The mean in the HIV positive group on ARVs was 5.94 (SD 2.51). Using one-way ANOVA for ranks with the Bonferroni adjustment to address multiplicity a p-value of 0.222 was obtained. The difference in number of multiple cusped teeth extracted between the groups was not statistically significant.

This data is summarised in table 4.1

Table 4.1 Summary of extracted teeth

	n	mean single-cusped teeth	SD single cusped teeth	mean multiple cusped teeth	SD multiple cusped teeth
HIV negative	47	3.62	2.58	4.83	2.40
HIV positive not on ARVs	12	7.08	3.73	6.00	3.10
HIV positive on ARVs	17	5.35	2.96	5.94	2.51
		p = 0.002		p = 0.222	

4.3 The coagulation profiles of the paediatric patients studied

Part 1 of the study objectives was to describe the coagulation profile of the HIV positive patients (on ARVs and not on ARVs) and the HIV negative patients and then to compare the coagulation profiles. This included platelet counts and TEG parameters (r-time, K-time, α-angle and MA).

4.3.1 Platelet count

None of the paediatric patients enrolled in this study had platelet counts less than the thrombocytopenia threshold defined in chapter 1 (150 $x10^9$/l).

The mean platelet count of the HIV negative patients was 375.87 $x10^9$/l, the SD 89.05 and the 95% confidence interval (95% CI) 350.41 to 401.33.

The mean platelet count of the HIV positive patients not on ARVs was 407.00 $x10^9$/l, the SD 117.30 and the 95% CI 340.63 to 473.37.

The mean platelet count of the HIV positive group on ARVs was 343.18 x10^9/l, the SD 87.58 and the 95% CI 301.54 to 384.81.

One-way analysis of variance for ranks, using the Bonferroni adjustment to address multiplicity, of the three groups did not show a statistically significant difference (p = 0.2087).

This data is summarised in table 4.2 below.

Table 4.2 Summary of description of platelet count results

Group	n	platelet count mean	platelet count SD	95% CI lower limit	95% CI upper limit
HIV negative	47	375.87	89.05	350.41	401.33
HIV positive not on ARVs	12	407.00	117.30	340.63	473.37
HIV positive on ARVs	17	343.18	87.58	301.54	384.81
					p = 0.2087

4.3.2 Thromboelastogram r-time

The mean TEG r-time of the HIV negative patients was 5.04 minutes, the SD 3.40 and the 95% CI 4.17 to 5.91.

The mean TEG r-time of the HIV positive patients not on ARVs was 5.51 minutes, the SD 2.73 and the 95% CI 3.96 to 7.05.

The mean TEG r-time of the HIV positive group on ARVs was 4.87 minutes, the SD 4.60 and the 95% CI 2.68 to 7.05.

One-way analysis of variance for ranks, using the Bonferroni adjustment to address multiplicity, of the three groups did not show a statistically significant difference (p = 0.4738).

This data is summarised in table 4.3 below.

Table 4.3 Summary of description of TEG r-time results

Group	n	TEG r-time mean	TEG r-time SD	95% CI lower limit	95% CI upper limit
HIV negative	47	5.04	3.40	4.17	5.91
HIV positive not on ARVs	12	5.51	2.73	3.96	7.05
HIV positive on ARVs	17	4.87	4.60	2.68	7.05
					p = 0.4738

4.3.3 Thromboelastogram K-time

The mean TEG K-time of the HIV negative patients was 1.53 minutes, the SD 0.98 and the 95% CI 1.25 to 1.81.

The mean TEG K-time of the HIV positive patients not on ARVs was 1.53 minutes, the SD 0.49 and the 95% CI 1.25 to 1.81.

The mean TEG K-time of the HIV positive group on ARVs was 1.72 minutes, the SD 1.35 and the 95% CI 1.08 to 2.36.

One-way analysis of variance for ranks, using the Bonferroni adjustment to address multiplicity, of the three groups did not show a statistically significant (p = 0.6967).

This data is summarised in table 4.4 below.

Table 4.4 Summary of description of TEG K-time results

Group	n	TEG K-time mean	TEG K-time SD	95% CI lower limit	95% CI upper limit
HIV negative	47	1.53	0.98	1.25	1.81
HIV positive not on ARVs	12	1.53	0.49	1.25	1.81
HIV positive on ARVs	17	1.72	1.35	1.08	2.36
					p = 0.6967

4.3.4 Thromboelastogram α-angle

The mean TEG α-angle of the HIV negative patients was 68.12 degrees, the SD 8.78 and the 95% CI 65.61 to 70.63.

The mean TEG α-angle of the HIV positive patients not on ARVs was 69.46 degrees, the SD 5.82 and the 95% CI 66.16 to 72.75.

The mean TEG α-angle of the HIV positive group on ARVs was 69.18 degrees, the SD 9.65 and the 95% CI 64.59 to 73.77.

One-way analysis of variance for ranks, using the Bonferroni adjustment to address multiplicity, of the three groups did not show a statistically significant difference (p = 0.7948).

This data is summarised in table 4.5 below.

Table 4.5 Summary of description of TEG α-angle results

Group	n	TEG α-angle mean	TEG α-angle SD	95% CI lower limit	95% CI upper limit
HIV negative	47	68.12	8.78	65.61	70.63
HIV positive not on ARVs	12	69.46	5.82	66.16	72.75
HIV positive on ARVs	17	69.18	9.65	64.59	73.77
					p = 0.7948

4.3.5 Thromboelastogram MA

The mean TEG MA of the HIV negative patients was 71.09 mm, the SD 8.41 and the 95% CI 68.69 to 73.50.

The mean TEG MA of the HIV positive patients not on ARVs was 72.78 mm, the SD 6.43 and the 95% CI 69.15 to 76.42.

The mean TEG MA of the HIV positive group on ARVs was 69.08 mm, the SD 8.42 and the 95% CI 65.07 to 73.08.

One-way analysis of variance for ranks, using the Bonferroni adjustment to address multiplicity, of the three groups did not show a statistically significant difference (p = 0.2982).

This data is summarised in table 4.6 below.

Table 4.6 Summary of description of TEG MA results

Group	n	TEG MA mean	TEG MA SD	95% CI lower limit	95% CI upper limit
HIV negative	47	71.09	8.41	68.69	73.50
HIV positive not on ARVs	12	72.78	6.43	69.15	76.42
HIV positive on ARVs	17	69.08	8.42	65.07	73.08
					p = 0.2982

4.4 The CD4 count and percentage of the study group

Part 2 of the study objectives was to determine and then compare the CD4 count and percentage of the paediatric patients enrolled in the study.

4.4.1 CD4 count

The mean CD4 count of the patients in the HIV negative group was 1227.64 $x10^6$/l, with a SD of 419.10. The 95% CI was 1107.82 to 1347.46.

The mean CD4 count of the patients in the HIV positive group not on ARVs was 686.08 x10^6/l, with a SD of 263.16. The 95% CI was 537.19 to 834.98.

The mean CD4 count of the patients in the HIV positive group on ARVs was 1234.71 x10^6/l, with a SD of 621.08. The 95% CI was 939.47 to 1529.95.

Comparing the three groups using one-way ANOVA for ranks and the Bonferroni adjustment to address multiplicity, a statistically significant difference was found (p = 0.0002).

These results are summarised in table 4.7.

Table 4.7 Summary of the description of results for CD4 count

Group	n	CD4 count mean	CD4 count SD	95% CI lower limit	95% CI upper limit
HIV negative	47	1227.64	419.10	1107.82	1347.46
HIV positive not on ARVs	12	686.08	263.16	537.19	834.98
HIV positive on ARVs	17	1234.71	621.08	939.47	1529.95
					p = 0.0002

A comparison for rank showed that the differences were between the HIV negative group and the HIV positive group not on ARVs (p = 0.000) and between the HIV positive group on ARVs and the HIV positive group not on ARVs (p = 0.004). The was no statistically significant difference between the HIV negative and HIV positive on ARVs groups (p = 1.0000).

4.4.2 CD4 percentage

The mean CD4 percentage of the patients in the HIV negative group was 30.55%, with a SD of 6.28. The 95% CI was 28.76 to 32.35.

The mean CD4 percentage of the patients in the HIV positive group not on ARVs was 15.83%, with a SD of 3.99. The 95% CI was 13.57 to 18.08.

The mean CD4 percentage of the patients in the HIV positive group on ARVs was 25.44%, with a SD of 6.31. The 95% CI was 22.44 to 28.44.

Comparing the three groups using one-way ANOVA for ranks and the Bonferroni adjustment to address multiplicity, a statistically significant difference was found (p = 0.0000).

These results are summarised in table 4.8.

Table 4.8 Summary of the description of results for CD4 percentage

Group	n	CD4 % mean	CD4 % SD	95% CI lower limit	95% CI upper limit
HIV negative	47	30.55	6.28	28.76	32.35
HIV positive not on ARVs	12	15.83	3.99	13.57	18.08
HIV positive on ARVs	17	25.44	6.31	22.44	28.44
					p = 0.0000

A comparison for rank showed that the differences were between the HIV negative group and the HIV positive group not on ARVs (p = 0.000), between the HIV positive group on ARVs and the HIV positive group not on ARVs (p = 0.001) and between the HIV negative group and the HIV positive group on ARVs (p = 0.013).

4.5 Assessment of bleeding

Part 3 of the study objectives was to assess the observed bleeding after dental extraction in the three groups of paediatric patients and then to compare the bleeding.

Twenty-two (28.95%) patients were considered by the dentist to have increased bleeding after dental extraction. None of the bleeding was significant in terms of the study definition.

Of the 22 patients with increased bleeding, ten were in the HIV negative group, six in the HIV positive group not on ARVs and six in the HIV positive group on ARVs.

This data is graphically represented in figure 4.2 below.

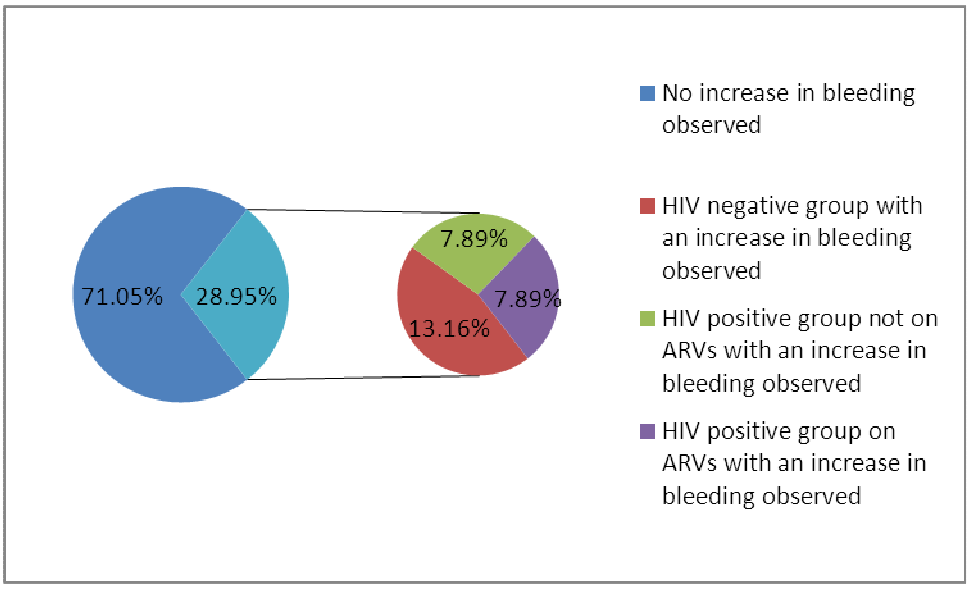


Figure 4.2 Pie chart of the number of patients assessed to have increased bleeding.

Using the Fisher's exact and one-sided Fisher's exact tests the numbers of bleeding patients in each group were found not to be statistically significant (p-values ranged from 0.054 to 0.101).

The group of HIV positive patients was then assessed as a whole for bleeding. The total number of HIV positive patients was 29. Of these 12 (41.38%) had an observed increase in the amount of bleeding (bleeders) and 17 (58.62%) did not (non-bleeders). These two groups (bleeders and non-bleeders) were then compared in terms of CD4 count.

The mean CD4 count of all the HIV positive paediatric patients was 1007.69 $x10^6$/l. The SD was 568.55. The 95% CI was 791.43 to 1223.95.

The mean CD4 count of the bleeders was 727.83 $x10^6$/l. The SD was 332.52. The 95% CI was 516.56 to 939.11.

The mean CD4 count of the non-bleeders was 1205.24 $x10^6$/l. The SD was 624.06. The 95% CI was 884.37 to 1526.10.

A two-sample Welsh t-test with unequal variances was used to show that those patients who bled had lower CD4 counts. This was statistically significant ($p = 0.0129$).

This data is summerised in table 4.9 below.

Table 4.9 Summary of data comparing HIV positive patients and CD4 count

Group (HIV positive)	n	CD4 count mean	CD4 count standard error	CD4 count SD	CD4 count 95% CI lower limit	CD4 count 95% CI upper limit
Increased bleeding observed	12	727.83	95.99	332.52	516.56	939.11
No increase in bleeding observed	17	1205.24	151.36	624.06	884.37	1526.10
All HIV positive paediatric patients	29	1007.69	105.58	568.55	791.43	1223.95
Difference		-477.40	179.23		-845.21	-109.60
						p = 0.0129

The non-parametric Wilcoxon rank-sum (Mann-Whitney) test also confirmed that the bleeding group had significantly lower CD4 counts ($p = 0.0335$).

4.6 Conclusion

The research results presented in this chapter describe the data collected and statistical analyses needed to satisfy the aims and objectives of the research.

CHAPTER 5: DISCUSSION OF RESEARCH RESULTS

In this chapter the results presented in the previous chapter will be interpreted and discussed in accordance with the problem statement, aims and objectives of the study.

5.1 Sample realisation

Original consultation with the biostatistician recommended a sample size of 16 paediatric patients in each of the three study groups, HIV negative, HIV positive not on ARVs and HIV positive on ARVs. After two years of data collection only 12 patients had been recruited in the HIV positive not on ARVs group. These numbers were reviewed by the same biostatistician and found to be adequate for the purposes of this study.

Possible reasons for the low numbers recruited in this group included:

- the prompt commencement for ARVs on diagnosis of HIV, thus limiting global numbers in this group
- fear of stigmatisation, thus non-disclosure of known status or refusal of consent to HIV testing
- overburdened caregivers not having the capacity to bring these children for dental treatment.

5.2 Demographic data

The age distribution of children in this study is in keeping with the 1999/2002 South African National Children's Oral Health Survey (6). In that survey it was

found that 51% of four to five years olds had dental caries. In this study most of the children (63%) were in the three to five year old age bracket.

The incidence of dental caries is higher in HIV positive children than in HIV negative children (78, 80). In this cohort, more single cusped teeth were extracted from both HIV positive groups when compared to the HIV negative group ($p = 0.006$) (although the difference in the number of multiple cusped teeth extracted was not statistically significant in this sample), showing that this group indeed followed the global trend, with the incidence of dental caries being higher in the HIV positive children.

According to Oswald et al. (50) there is no gender difference in ROTEM® profiles in children less than 16 years of age, thus the paediatric patients in this study were not divided into male and female groups for statistical analysis.

5.3 Part 1

Part 1 of the study aimed to determine if there was a difference in the coagulation profiles of HIV negative and HIV positive children (on ARVs and not on ARVs) undergoing dental extractions at CMJAH. A description and statistical analysis of the results of the platelet count and TEG profiles of these groups was presented in Chapter 4.

No statistically significant differences were found when comparing:

- platelet count
- TEG r-time
- TEG K-time
- TEG α-angle
- TEG MA.

Studies have shown that TEG abnormalities will only be apparent if thrombocytopenia exists (16, 27, 42). As no patients in this study had thrombocytopenia, this may explain why there were no statistically significant TEG changes between the HIV negative, HIV positive on ARVs and HIV positive not on ARVs groups. A previous study showed 21% of HIV positive patients to be thrombocytopenic; however, this was not the case in this study (90).

5.4 Part 2

Part 2 of the study aimed to compare the CD4 counts and percentages of the HIV positive paediatric patients on ARVs and those not on ARVs and that of HIV negative paediatric patients undergoing dental extractions at CMJAH. Here a statistically significant difference was found.

The differences in CD4 count between the HIV negative and HIV positive not on ARVs, and the HIV positive on ARVs and HIV positive not on ARVs groups, were both found to be statistically significant ($p = 0.000$ and 0.004).

The difference between the mean CD4 count of the HIV negative group and the HIV positive on ARVs group was 7.068 (p = 1.000). This was not statistically significant.

The use of ARVs in the HIV positive on ARVs group is thus seen to increase the CD4 count to the same mean as the HIV negative group, indicating the effectiveness of the treatment modality in the group. This is consistent with worldwide indicators of clinical success and normalisation of prognosis to that of HIV negative individuals (67).

5.5 Part 3

The aim of Part 3 of the study was to compare observed bleeding of the HIV positive patients (on ARVs and not on ARVs) with that of the HIV negative paediatric patients undergoing dental extractions at CMJAH.

On initial analysis of the data and comparison only between the three stipulated groups, no statistically significant difference was found to exist between the HIV negative, HIV positive on ARVs and the HIV positive not on ARVs groups. However, on further analysis, taking the observed bleeding into account and comparing it to the CD4 count in the entire HIV positive group (regardless of whether the paediatric patient was taking ARVs or not), it was found that those patients with a lower CD4 count bled more than those with a higher CD4 count (p = 0.0129 and 0.0335).

From the data collected in the study and the analysis thereof, it can be seen that in the group of paediatric patients (divided into HIV negative and HIV positive on ARVs and not on ARVs) there was no statistical significance in the difference between the platelet count and TEG profile. As was expected, there was a statistically significant difference in the CD4 count between the HIV negative and HIV positive group not on ARVs and also between the HIV positive not on ARVs group and the HIV positive on ARVs group. There was no statistically significant difference in the bleeding observed between these three study groups, but within the whole HIV positive group more bleeding was observed in those patients with lower CD4 counts. The answer to the question, “why do those with lower CD4 counts bleed more?” begs further analysis of the data which was not within the scope of this research report and further study.

In commenting on the problem statement for this research report, it can be stated that not all HIV positive paediatric patients bleed more than those with known negative or unknown status. Those HIV positive paediatric patients with lower CD4 counts bleed more than those whose CD4 counts are the same as or approaching that of HIV negative patients. The data and the analysis thereof done within the scope of this research report, does not answer the question whether the HIV positive paediatric patients with lower CD4 counts had abnormal coagulation profiles. All patients had platelet counts within the normal range for the laboratory and there was no statistically significant difference between the TEG profiles of the three study groups.

In conclusion, HIV positive children with lower CD4 counts are observed to bleed more than those with higher and normal CD4 counts. That is those with progression of HIV/AIDS disease bleed more. Further research is needed to determine the reason for the bleeding.

5.6 Conclusion

In this chapter a discussion and interpretation of the results presented in chapter 4 was provided.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

This chapter will summarise and conclude the research report, in doing so it will refer to the problem statement, aims and objectives of the study. Reference to the limitations of the study and recommendations for further research will then be made.

6.1 Summary

Paediatric HIV/AIDS remains a significant health care challenge in South Africa. Oral health and coagulation are only two of the many problems experienced by HIV positive paediatric patients.

This research report began with an observation that known HIV positive paediatric patients bled more than known HIV negative paediatric patients or those with unknown HIV status while undergoing dental extractions at CMJAH. The observation prompted a prospective, contextual, descriptive study looking at the coagulation profile (platelet count and TEG profile (r-time, K-time, α-angle and MA), CD4 counts and percentages and observed clinical bleeding in HIV negative, HIV positive not on ARVs and HIV positive on ARVs paediatric patients presenting for dental extraction.

Over a two year period 47 HIV negative, 12 HIV positive not on ARVs and 17 HIV positive on ARVs paediatric patients were enrolled in the study using a consecutive, convenience sampling method. Each paediatric patient was given a standard inhalational general anaesthetic using sevoflurane and during

intravenous cannulation the researcher drew blood from each child for analysis. A senior dentist from the Department of Paediatric Dentistry assessed bleeding in all cases.

The data obtained for each of the three study groups was compared using a one-way ANOVA followed by pair wise comparison using the Bonferroni adjustment to address multiplicity. To deal with the big standard deviations and skewed data a one-way ANOVA for ranks tested for differences between the groups. No statistically significant differences were found when comparing the groups for platelet count ($p = 0.2087$), TEG r-time ($p = 0.4738$), TEG K-time ($p = 0.6967$), TEG α-angle ($p = 0.7948$) or TEG MA ($p = 0.2982$). There was a statistically significant difference between the HIV negative and HIV positive not on ARVs groups ($p = 0.000$ and 0.004) and HIV positive on ARVs and HIV positive not on ARVs groups ($p = 0.000$ and 0.001) when comparing CD4 count and percentage.

Patient groups were compared with respect to bleeding complications using the Fisher's exact test. There was no statistically significant difference in observed bleeding between the three groups of paediatric patients. The entire HIV positive group was then compared for bleeding, and using the Welch t-test, adjusting for unequal variances it was found that there was statistically significantly more bleeding in the HIV positive children with lower CD4 counts regardless of treatment with ARVs ($p = 0.0129$). These results were also confirmed using the Wilcoxon rank-sum test ($p = 0.0335$).

Although this study showed a statistically significant difference between bleeding in the HIV positive paediatric patients with lower CD4 counts (when compared to HIV positive paediatric patients with higher or normal CD4 counts), the tests of coagulation used in the study do not show why this bleeding occurs. Further research into coagulation in HIV positive paediatric patients is needed.

6.2 Conclusions

This research report began with an observation. The clinical observation was made that know HIV positive paediatric patients bled more than those with known negative or unknown status. After data analysis this observation, in this group of patients has been found to be invalid. HIV positive children do not bleed more than HIV negative children.

Within the HIV positive group, however, those children with more advanced disease, as evidenced by a lower CD4 count, are observed to bleed more than those with early or treated disease.

In answering part 1 of the aims and objectives of the study, no statistically significant difference was found in the coagulation profiles of the three groups of patients.

A statistically significant difference in the CD4 count of the HIV negative and HIV positive on ARVs group versus the HIV positive group not on ARVs was found. This satisfies part 2 of the study aims and objectives.

No statistically significant difference was found when comparing the observed bleeding between the HIV positive patients on ARVs with those not on ARVs and the HIV negative paediatric patients. This fulfils part 3 of the aims and objectives of the study.

Further analysis of the data for observed bleeding found that within the entire group of HIV positive patients, the children with a lower CD4 count bleed more than those with a higher or normal count. The analysis done (within the scope of this research report) on the data collected for this study does not give a biochemical explanation for this. The TEG does not test for specific factor deficiencies which may be present in more advanced disease as a result of bone marrow suppression, liver disease or opportunistic infection amongst other causes. Vascular and collagen function were not considered as causes for increased bleeding in this group in this study.

6.3 Limitations

The study was done contextually at CMJAH. This context may not allow for generalisation of the results of the study, however, it was an important study to be done in that context as it addressed an observed problem.

The limited number of study subjects in this research report could affect the ability to generalise the study results. A biostatistician was consulted regarding whether the small number of study subjects had adequate power to detect a difference in means and a 90% power was found. Even though the sample size was small in this negative study, the study may provide a baseline to direct further research.

A further limitation of this small study is that randomisation and sample selection could be biased. For this reason a consecutive sampling method was used and the bias was limited.

The assessment of clinical bleeding was subjective; however, one, blinded, senior dentist from the Department of Paediatric Dentistry at CMJAH assessed all the paediatric patients for bleeding. In this way the subjectivity of this observation, and the bias of the dentist, was reduced and standardised.

Dental caries is more prevalent in HIV positive children than in HIV negative children. Although there was no statistically significant difference in the number of multiple cusped teeth extracted from each group, there were statistically significantly more single cusped teeth extracted from both HIV positive groups when compared to the HIV negative group. The extraction of more teeth may have led to more bleeding; a possible limitation to the study. However, as there is more caries in HIV positive children, it may not be possible to adjust for this limitation in any study.

6.4 Recommendations

For the purposes of this research report, the platelet count and TEG profile were chosen to assess coagulation. Future research may need to include individual coagulation factors as part of the coagulation profile and perhaps also look at vascular function and collagen. This may show the reason for the observed increase in bleeding in HIV positive paediatric patients.

Further statistical analysis of this data comparing each individual HIV positive patient regardless of treatment with ARVs (for platelet count and TEG parameters) may reveal why those with lower CD4 counts bled more.

A more in depth look at the coagulation profile of each individual child with increased bleeding is recommended. This analysis should include a review of any co-morbid medical conditions in these children as well as scrutinising any drug therapies they may be on, including ARVs.

Those patients with more advanced disease, as evidenced by the lower CD4 count, may have other co-morbid disease which affects platelet function (not assess by the TEG) or involvement of the liver in a process which affects clotting factor production. These questions are not answered by the limited scope of this study.

The collection of data was protracted in this study for a number of reasons. The most striking of which was the lower numbers of HIV positive patients than expected. Reasons were postulated, some being the effective use of ARVs to

prevent mother to child transmission of HIV (a number of positive mothers had children who tested negative), the stigma attached to disclosing HIV status and the fear of testing for HIV in a society where the prevalence of HIV is so high. It was observed that parents and guardians of paediatric patients already taking ARVs we most eager to give consent for admission into the study. Future studies should include investigation into the social circumstances of the paediatric patients as this may well answer some of the questions related to the low number of HIV positive children not on ARVs recruited into the study.

The researcher agrees with the findings of Moodley et al. (94) that disclosure of HIV status and destigmatisation of the disease may well improve the life expectancy and quality of life of children living with HIV.

This study and research report should be viewed as a pilot study for further research into coagulation in HIV positive children.

6.5 Conclusion

This chapter has summarised and concluded the study. It then made reference to the limitations of the study. Finally recommendations for further analysis of the collected data and future research were made.

Appendix 1: HREC approval

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Zeijlstra

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER M080908**
PROJECT	Coagulation Profiles of HIV Positive and Negative Paediatric Patients Undergoing Dental Extractions at the Johannesburg Hospital
INVESTIGATORS	Dr AE Zeijlstra
DEPARTMENT	Department of Anaesthesia
DATE CONSIDERED	08.09.26
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 08.09.29 **CHAIRPERSON** ..... (Professor P E Cleaton Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Mrs J Scribante

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 2: Information packet – parent/caregiver (HIV positive)

Dear parent/caregiver

Hello, my name is Anne Zeijlstra and I am a doctor at Johannesburg Hospital. I make people sleep and pain free for operations. As part of my Master's degree in anaesthetics I am studying bleeding and blood clotting in children. I want to compare clotting in HIV positive children and HIV negative children when they have teeth taken out under anaesthetic. The anaesthetic is a deep sleep during which the surgery is done. During this sleep your child will not feel any pain.

HIV is short of Human Immunodeficiency Virus. HIV can infect children. Most children get infected with HIV when they are still in their mothers' womb, during birth, or from breastfeeding. Once HIV is in the body it attacks the immune system, which fights infection. Antiretroviral drugs are medicines that can slow this attack down. The virus and these medicines can change the way the blood clots. It is this change that I want to study.

If your child takes part in this study he/she will be made to sleep for the operation, just as we make children sleep for operations every day, by breathing an anaesthetic gas. Every child who is asleep for an operation has a drip put in his/her arm or hand. This will be no different for your child. At the time that the drip is put in, one-and-a-half teaspoons of blood will be taken from your child for testing. This blood will have three tests done on it:

1. A test for the number of cells that make the blood clot (platelet count)

2. A test for cells that are part of the immune system that fights infection (CD4 count)

3. A test for how well the blood clots (Thromboelastogram).

Your child's operation will carry on in the same way, as it would have even if your child were not part of this study. If you do not want your child to be part of the study, the treatment and care your child gets will not change in any way.

The study will be anonymous. Confidentiality will be maintained. No names will be put in any of the results of the study.

If you are willing to allow your child to participate in the study, you will need to sign a CONSENT FORM. If your child is 6 years of age or more I will also ask his/her permission and he/she will be asked to sign an ASSENT FORM.

Please understand that you can withdraw from the study any time without having to give a reason. The study is completely voluntary and not taking part in it or withdrawing from it, carries no penalty or repercussion of any sort.

Before giving permission please be sure that you understand what we are testing. If you are unsure we will make sure that someone is available to answer your questions in your home language. Please feel free to ask any questions at any time during the process.

Thank you.

Anne Zeijlstra
Anaesthetic Doctor

Appendix 3: Information packet – parent/caregiver (HIV unknown/HIV negative)

Dear parent/caregiver

Hello, my name is Anne Zeijlstra and I am a doctor at Johannesburg Hospital. I make people sleep and pain free for operations. As part of my Master's degree in anaesthetics I am studying bleeding and blood clotting in children. I want to compare clotting in HIV positive children and HIV negative children when they have teeth taken out under anaesthetic. The anaesthetic is a deep sleep during which the surgery is done. During this sleep your child will not feel any pain.

HIV is short of Human Immunodeficiency Virus. HIV can infect children. Most children get infected with HIV when they are still in their mothers' womb, during birth, or from breastfeeding. Once HIV is in the body it attacks the immune system, which fights infection. Antiretroviral drugs are medicines that can slow this attack down. The virus and these medicines can change the way the blood clots. It is this change that I want to study.

If your child takes part in this study he/she will be made to sleep for the operation, just as we make children sleep for operations every day, by breathing an anaesthetic gas. Every child who is asleep for an operation has a drip put in his/her arm or hand. This will be no different for your child. At the time that the drip is put in, one-and-a-half teaspoons of blood will be taken from your child for testing. This blood will have three tests done on it:
A test for the number of cells that make the blood clot (platelet count)
A test for cells that are part of the immune system that fights infection (CD4 count)
A test for how well the blood clots (Thromboelastogram).

A group of children whom we know are HIV positive are taking part in this study, but in order to know whether there is a difference between HIV positive and HIV negative children we need to test a group of children who are negative for HIV to be sure that they are HIV negative. It is for this reason that I need your permission to test your child's blood to make sure that he/she can be included in the HIV negative group of children.

Your child's operation will carry on in the same way, as it would have even if your child were not part of this study. If you do not want your child to be part of the study, the treatment and care your child gets will not change in any way.

The study will be anonymous. Confidentiality will be maintained. No names will be put in any of the results of the study.

If you are willing to allow your child to participate in the study, you will need to sign a CONSENT FORM. If your child is 6 years of age or more I will also ask his/her permission and he/she will be asked to sign an ASSENT FORM.

Please understand that you can withdraw from the study any time without having to give a reason. The study is completely voluntary and not taking part in it or withdrawing from it, carries no penalty or repercussion of any sort.

Before giving permission please be sure that you understand what we are testing. If you are unsure we will make sure that someone is available to answer your questions in your home language. Please feel free to ask any questions at any time during the process.

Thank you.

Anne Zeijlstra
Anaesthetic Doctor
Telephone number: 011 488 4397

Appendix 4: Consent form

Research Title: Coagulation profiles of HIV-positive and -negative paediatric patients undergoing dental extractions at the Johannesburg Hospital.

I _____ understand what this study is about and give consent of my child/the child I care for to participate in this study. I have read and understand the information sheet and my questions have been answered. I am aware that the procedures will not harm the child in any way. I am aware that the child may withdraw from the study at any time without any prejudice toward the child or me. I understand that my name and that of my child will not appear in any of the results of the research.

_____
Parent/Caregiver name

_____
Parent/Caregiver signature

_____
Date

_____
Researcher name

_____
Researcher signature

_____
Date

Appendix 5: Consent form (HIV testing)

Research Title: Coagulation profiles of HIV-positive and -negative paediatric patients undergoing dental extractions at the Johannesburg Hospital.

Declaration of consent to the HIV diagnostic test.

I _____ declare that I have received pre-test counselling and education on HIV/AIDS. I understand the reasons to be tested and the implications of knowing my child's HIV status. I am willing to have my child tested to see if he/she is HIV positive or negative, and consent to the authorized person taking a sample of blood for this purpose.

_____
Parent/Caregiver name

_____
Parent/Caregiver signature

_____
Date

_____
Researcher name

_____
Researcher signature

_____
Date

Appendix 6: Information packet – child (HIV unknown/HIV negative)

Hello

My name is Anne Zeijlstra. I work as a doctor here at the Johannesburg Hospital. I am going to help make you sleep for your operation.

Do you know what HIV is? It is an infection that some children in this country and the world have. This infection can get into the blood of children when they are still in their Mommy's tummies before they are even born. Sometimes it gets into the blood from other blood that has the infection in it. Remember, HIV can't get into your blood from touching other people or things. HIV attacks the soldiers of the body so that the body can't fight infection properly. We can give medicine that helps the body to fight back.

When you fall and hurt yourself, have you noticed that your body stops the bleeding all by itself? This is called blood clotting. Well, HIV can change the way the body stops bleeding.

I want to see how this bleeding is different in children with HIV and children without HIV. I also want to see if the medicine we give for HIV changes the bleeding.

This is where you can help us to understand more about blood clotting. When you are fast asleep for your operation you will get a drip, like all children who have operations. I don't want you to worry because you will be so fast asleep that you will not feel it at all. At the same time as the drip is put in, I am going to take one-and-a-half teaspoons of blood from you so that I can test it to see how it clots; a bit like making a scab to stop bleeding if you hurt yourself.

Your treatment will be exactly the same whether you say yes and decide to be a part of the study or whether you say no. You can also decide that you don't want to be part of the study at any time, and nothing bad will happen if you do.

Nobody will know that you have been a part of this study, because I will keep your name a secret.

If you agree to be a part of the study, please write your name on the ASSENT FORM, the piece of paper that you got with this letter.

Before you write your name on that form, I want you to be sure that you know what I am going to do and why I am doing it. If there is anything that you don't understand, or if you have any questions, please don't be scared to ask questions at any time.

Thank you.

Anne Zeijlstra
Anaesthetic Doctor

Appendix 7: Information packet – child (HIV positive)

Hello

My name is Anne Zeijlstra. I work as a doctor here at the Johannesburg Hospital. I am going to help make you sleep for your operation.

When you fall and hurt yourself, have you noticed that your body stops the bleeding all by itself? This is called blood clotting. I want to see how this bleeding is different in different children.

This is where you can help us to understand more about blood clotting. When you are fast asleep for your operation you will get a drip, like all children who have operations. I don't want you to worry because you will be so fast asleep that you will not feel it at all. At the same time as the drip is put in, I am going to take one-and-a-half teaspoons of blood from you so that I can test it to see how it clots; a bit like making a scab to stop bleeding if you hurt yourself.

Your treatment will be exactly the same whether you say yes and decide to be a part of the study or whether you say no. You can also decide that you don't want to be part of the study at any time, and nothing bad will happen if you do.

Nobody will know that you have been a part of this study, because I will keep your name a secret.

If you agree to be a part of the study, please write your name on the ASSENT FORM, the piece of paper that you got with this letter.

Before you write your name on that form, I want you to be sure that you know what I am going to do and why I am doing it. If there is anything that you don't understand, or if you have any questions, please don't be scared to ask questions at any time.

Thank you.

Anne Zeijlstra
Anaesthetic Doctor
Telephone number: 011 488 4397

Appendix 8: Assent form

Research Title: Coagulation profiles of HIV-positive and -negative paediatric patients undergoing dental extractions at the Johannesburg Hospital.

I _____ am happy to participate in this study. I understand that my blood will be taken, for testing, once I am asleep and know that this will not hurt me. I understand what the study is about and my questions have been answered. I know that I can say that I don't want to be part of this study at any time. I know that nobody will see my name and know that I was part of the study when it is finished.

_____
Subject name

_____
Subject signature

_____
Date

_____
Researcher name

_____
Researcher signature

_____
Date

Appendix 9: Data collection sheet

DENTAL EXTRACTION AND COAGULOPATHY

Study number	
Patient name	
Hospital number	

Age	
Gender	

ASA	1	
	2	
	3	
	4	
	5	

Co-morbid disease	

Is the patient HIV positive?	Yes	
	No	

Platelet count		
CD4 count		
TEG	r time	
	K time	

	α angle	
	MA	

Relevant bleeding	>10% blood volume loss	
	Haemodynamic instability	
	Admission for bleeding	
	Needed blood transfusion	

Surgical complications	

Anaesthetic complications	

Anti-viral medication	Dose	Frequency	Route	Date Started
Other medication	Dose	Frequency	Route	Date Started

REFERENCE LIST

1. Evron S, Glezerman M, Harow E, Sadan O, Ezri T. Human Immunodeficiency Virus: Anesthetic and Obstetric Considerations. Anesthesia and Analgesia 2004;98:503-511.

2. Sapa. SA HIV estimated 5,6 million. 7 May 2008. http://www.polity.org.za/article.php?a_id=132696. (Accessed 2008.05.31).

3. Lehohla P. Inside Statistics: Data that don't grab headlines no less important. 26 August 2004. http://www.statssa.gov.za/news_archive/26aug2004_1.asp. (Accessed 2008.05.31).

4. Leelanukrom R, Pancharoen C. Anesthesia in HIV-infected children. Pediatric Anesthesia 2007;17:509-519.

5. Tickle M, Threlfall AG, Pilkington L, Milsom KM, Duggal MS, Blinkhorn AS. Approaches taken to the treatment of young children with carious primary teeth: a national cross-sectional survey of general dental practitioners and paediatric specialists in England. British Dental Journal 2007;203(E4):1-6.

6. Postma TC, Ayo-Yusuf OA, Van Wyk PJ. Socio-demographic correlates of early childhood caries prevalence and severity in a developing country – South Africa. International Dental Journal 2008;58:91-97.

7. Foster H, Fitzgerald J. Dental disease in children with chronic illness. Archives of Disease in Childhood 2005;90:703-708.

8. Spiess BD. Coagulation Monitoring in the Perioperative Period. International Anaesthesiology Clinics 2004;42(2):55-71.

9. Miguez-Burbano MJ, Jackson Jr. J, Hadrigan S. Thrombocytopenia in HIV Disease: Clinical Relevance, Pathophysiology and Management. Current Medical Chemistry – Cardiovascular and Hematological Agents 2005;3:365-376.

10. Thachil J, Hall GW. Is this immune thrombocytopenic purpura? Archives of Disease in Childhood 2008;93:76-81.

11. Dixon WJ, Massey FJ. Introduction to Statstical Analysis. 4th ed.: McGraw-Hill, 1983.

12. O'Brien RG, Muller KE. Applied Analysis of Variance in Behavioral Science. New York: Marcel Dekker, 1983:297-344.

13. StrataCorp 2006. Strata Statistical Software: Release 10.0. College Station, TX: Strata Corporation.

14. Endacott R, Botti M. Clinical research 3: sample selection. Intensive and Critical Care Nursing 2005;21:51-55.

15. Declaration of Helsinki (1964). http://www.cirp.org/library/ethics/helsinki/. (Accessed 2008.07.26).

16. Oshita K, Az-ma T, Osawa Y, Yuge O. Quantitative Measurement of Thromboelastography as a Function of Platelet Count. Anesthesia and Analgesia 1999;89:296-299.

17. Rochon AG, Shore-Lesserson L. Coagulation Monitoring. Anesthesiology Clinics 2006;24:839-856.

18. Mann KG. Thrombin Formation. Chest 2003;124:4S-10S.

19. Hopley L, Van Schalkwyk J. Assessing Coagulation. 26 July 2006. http://www.anaesthetist.com/icu/organs/blood/coags/coag.htm. (Accessed 2008.08.14).

20. Ganong WF. Review of Medical Physiology. 22nd ed.: Lange Medical Books/McGraw Hill, 2005.

21. Watson HG, Greaves M. Can We Predict Bleeding? Seminars in Thrombosis and Hemostasis 2008;34:97-103.

22. Barrowcliffe TW, Cattaneo M, Podda GM, Bucciarelli P, Lussana F, Lecchi A, et al. New approaches for measuring coagulation. Haemophilia 2006;12(suppl.3):76-81.

23. Shah U, Ma AD. Tests of platelet function. Current Opinion in Hematology 2007;14(5):432-437.

24. Ganter MT, Hofer CK. Coagulation Monitoring: Current Techniques and Clinical Use of Viscoelastic Point-of-Care Coagulation Devices. Anesthesia and Analgesia 2008;106(5):1366-1375.

25. Luddington RJ. Thromboelastography/thromboelastometry. Clinical and Laboratory Haematology 2005;27:81-90.

26. Sharma SK, Vera RL, Stegall WC, Whitten CW. Management of a Postpartum Coagulopathy Using Thromboelastography. Journal of Clinical Anesthesia 1997;9:243-247.

27. Orlikowski CEP, Rocke DA, Murray WB, Gouws E, Moodley J. Thromboelastographic changes in pre-eclampsia and eclampsia. British Journal of Anaesthesia 1996;77:157-161.

28. Hadley GP, McGarr P, Mars M. The role of thromboelastography in the management of children with snake-bite in southern Africa. Transactions of the Royal Society of Tropical Medicine and Hygiene 1999;93:177-179.

29. Pivalizza EG. Perioperative Use of the Thromboelastograph® in Patients with Inherited Bleeding Disorders. Journal of Clinical Anesthesia 2003;15:366-370.

30. Osthaus WA, Boethig D, Johanning K, Rahe-Meyer N, Theilmeier G, Breymann T, Suempelmann R. Whole blood coagulation measured by modified thrombelastography (ROTEM®) is impaired in infants with congenital heart diseases. Blood Coagulation and Fibrinolysis 2008;19(3):220-225.

31. Rajwal S, Richards M, O'Meara M. The use of recalcified citrated whole blood – a pragmatic approach for thromboelastography in children. Pediatric Anesthesia 2004;14:656-660.

32. Twedell D. Incorporating the Use of Thromboelastographic Studies When Assessing Bleeding Patients. The Journal of Continuing Education in Nursing 2008;39(1):14-15.

33. Heaney M, Looney Y, McKinstry C, O'Hare B. Sequential clot strength analyses following diclofenac in pediatric adenotonsillectomy. Pediatric Anesthesia 2007;17:1078-1082.

34. Sharma SK, Philip J, Wiley J. Thromboelastographic Changes in Healthy Parturients and Postpartum Women. Anesthesia and Analgesia 1997;85:94-98.

35. Yee DL, Edwards RM, Mueller BU, Teruya J. Thromboelastographic and Hemostatic Characteristics in Pediatric Patients with Sickle Cell Disease. Archives of Pathology and Laboratory Medicine 2005;129:760-765.

36. Goobie SM, Soriano SG, Zurakowski D, McGowen FX, Rockoff MA. Hemostatic Changes in Pediatric Neurosurgical Patients as Evaluated by Thrombelastograph®. Anesthesia and Analgesia 2001;93:887-892.

37. Chitlur M, Warrier I, Rajpurkar M, Hollon W, Llanto L, Wiseman C, Lusher JM. Thromboelastography in children with coagulation factor deficiencies. British Journal of Haematology 2008;142:250-256.

38. Kettner SC, Pollak A, Zimpfer M, Seybold T, Prusa AR, Herkner K, Kuhle S. Heparinase-Modified Thromboelastography in Term and Preterm Neonates. Anesthesia and Analgesia 2004;98:1650-1652.

39. Valeri CR, Ragno G. In vitro testing of platelets using the thromboelastogram, platelet function analyser, and the clot signature analyser to predict the bleeding time. Transfusion and Apheresis Science 2006;35:33-41.

40. Stoelting RK, Hiller SC. Pharmacology and Physiology in Anesthetic Practice. 4th ed. Philadelphia: Lippincott Williams and Wilkins, 2006.

41. Jackson GNB, Ashpole KJ, Yentis SM. Head-to-Head The TEG® vs the ROTEM® thromboelastography/thromboelastometry systems. Anaesthesia 2009;64:212-215.

42. Bowbrick VA, Mikhailidis DP, Stansby G. The Use of Citrated Whole Blood in Thromboelastography. Anesthesia and Analgesia 2000;90:1086-1088.

43. Miller BE, Tosone SR, Guzzetta NA, Miller JL, Brosius KK. Fibrinogen in Children Undergoing Cardiac Surgery: Is It Effective? Anasthesia and Analgesia 2004;99:1341-1346.

44. Ng KFJ. Changes in Thromboelasograph™ Variables Associated with Aging. Anesthesia and Analgesia 2004;99(2):449-454.

45. Haizinger B, Gombotz H, Rehak P, Geiselseder G, Mair R. Activated thromboelastogram in neonates and infants with complex congenital heart disease in comparison with healthy children. British Journal of Anaesthesia 2006;97(4):545-552.

46. Miller BE, Bailey JM, Mancuso TJ, Weinstein MS, Holbrook GW, Silvey EM, et al. Functional Maturity of the Coagulation System in Children: An Evaluation Using Thromboelastography. Pediatric Anesthesia 1997;84:745-748.

47. Chan K-L, Summerhayes RG, Igjatovic V, Horton SB, Mongale PT. Reference Values for Kaolin-Activated Thromboelasography in Healthy Children. Anesthesia and Analgesia 2007;105(6):1610-1613.

48. Haas T, Preinreich A, Oswald E, Pajk W, Berger J, Kuehbacher G, Innerhofer P. Effects of albumin 5% and artificial colloids on clot formation in small infants. Anaesthesia 2007;62:1000-1007.

49. Edwards RM, Naik-Mathuria BJ, Gay AN, Olutoye OO, Teruya J. Parameters of Thromboelastography in Healthy Newborns. American Journal of Clinical Pathology 2008;130:99-102.

50. Oswald E, Stalzer B, Heitz E, Weiss M, Schmugge M, Strasak A, et al. Thromboelastometry (ROTEM®) in children: age-related reference ranges and correlations with standard coagulation tests. British Journal of Anaesthesia 2010;105(6):827-835.

51. Pivalizza EG, Pivalizza PJ, Gottschalk LI, Kee S, Szmuk P, Abramson DC. Celite-Activated Thromboelastography in Children. Journal of Clinical Anesthesia 2001;13:20-23.

52. Hughes SC. HIV and anesthesia. Anesthesiology Clinics of North America 2004;22:379-404.

53. Fauci AS. 25 years of HIV. Nature 2008;453:289-290.

54. Weiss RA. Special Anniversary Review: Twenty-five years of human immunodeficiency virus research: successes and challenges. Clinical and Experimental Immunology 2008;152:201-210.

55. Evans RH, Scadden DT. Haematological aspects of HIV infection. Baillière's Clinical Haemotology 2000;13(2):215-230.

56. Petersen P-E. Policy for Prevention of Oral Manifestations in HIV/AIDS: The Approach of the WHO Global Oral Health Program. Advances in Dental Research 2006;19:17-20.

57. Burns DN, Mofenson LM. Paediatric HIV-1 Infection. Lancet 1999;354(suppl II):1-6.

58. Little K, Thorne C, Luo C, Bunders M, Ngongo N, McDermont P, Newell M-L. Disease Progression in Children with Vertically-Acquired HIV Infection in Sub-Saharan Africa: Reviewing the Need for HIV Treatment. Current HIV Research 2007;5:139-153.

59. WHO, UNICEF, UNAIDS. GLOBAL HIV/AIDS RESPONSE: Epidemic update and health sector progress towards Universal Access, Progress Report 2011 p.162. http://www.who.int/hiv/topics/paediatric/data/en/index1.html 9 (Accessed 2012.02.16).

60. Scadden DT, Zon LI, Groopman JE. Pathophysiology and management of HIV-associated hematologic disorders. Blood 1989;74:1455-1463.

61. Rieg G, Yeaman M, Lail AE, Donfield SM, Gomperts ED, Daar ES, Hemophilia Growth Development Study. Platelet Count Is Associated with Plasma HIV Type 1 RNA and Disease Progression. AIDS Research and Human Retroviruses 2007;23(10):1257-1261.

62. Karpatkin S, Nardi M, Green D. Platelet and Coagulation Defects Associated with HIV-1-Infection. Thrombosis and Haemostasis 2002;88:389-401.

63. Fantoni M, Del Borgo C, Autore C. Evaluation and management of metabolic and coagulative disorders in HIV-infected patients receiving highly active antiretroviral therapy. AIDS 2003;17(suppl. 1):S162-S169.

64. Saif MW, Greenberg B. HIV and Thrombosis: A Review. AIDS patient care and STDs 2001;15(1):15-24.

65. Saber AA, Aboolian A, LaRaja RD, Baron H, Hanna K. HIV/AIDS and the Risk of Deep Vein Thrombosis: A Study of 45 Patients with Lower Extremity Involvement. The American Surgeon 2001;67(7):645-647.

66. Levine AM, Vigen C, Gravink J, Mack W, Watts CH, Liebman HA. Progressive Prothrombotic State in Women with Advancing HIV Disease. Journal of Acquired Immune Deficiency Syndromes 2006;42(5):572-577.

67. Kelley CF, Kitchen CMR, Hunt PW, Rodrigues B, Hecht FM, Kitahata M, Crane HM, Willig J, Mugavero M, Saag M, Martin JN, Deeks SG. Incomplete Peripheral $CD4^+$ Cell Count Restoration in HIV-Infected Patients Receiving Long-Term Antiretroviral Treatment. Clinical Infectious Diseases 2009;48:787-794.

68. Division of Pharmacology, Faculty of Health Sciences, University of Cape Town. South African Medicines Formulary. 7^{th} ed.: South African Medical Association, 2005.

69. Yazdanpanah Y, Viget N, Cheret A, Guerroumi H, Gerard Y, Ajana F, et al. Increased bleeding in HIV-positive haemophiliac patients treated with lopinavir-ritonavir. AIDS 2003;17(16):2397-2399.

70. Wilde JT. Protease inhibitor therapy and bleeding. Haemophilia 2000;6:487-490.

71. Racoosin JA, Kessler CM. Bleeding episodes in HIV-positive patients taking protease inhibitors: a case series. Haemophilia 1999;5:266-269.

72. Neilsen H. Hypermenorrhoea associated with ritonavir. The Lancet 1999;353(9155):811.

73. Lorenzi P, Spicher VM, Laubereau B, Hirschel B, Kind C, Rudin C, Irion O, Kaiser L, the Swiss HIV Cohort Study, he Swiss Collaborative HIV and Pregnancy Study, the Swiss Neonatal HIV Study. Antiretroviral therapies in pregnancy: maternal, fetal and neonatal effects. AIDS 1998;12(18):F241-F247.

74. Koppel K, Bratt G, Schulman S, Bylund H, Sandstrom E. Hypofibrinolytic State in HIV-1-Infected patients Treated with Protease Inhibitor-containing Highly Active Antiretroviral Therapy. Journal of Acquired Immune Deficiency Syndromes 2002;29(5):441-449.

75. Barbaro G. Highly Active Therapy-associated Metabolic Syndrome: Pathogenesis and Cardiovascular Risk. American Journal of Therapeutics 2006;13(3):248-260.

76. Barbaro G. HIV infection, highly active antiretroviral therapy and the cardiovascular system. Cardiovascular Research 2003;60:87-95.

77. De Andrade CM, Duarte G, Quintana SM, Montes MBA, Toloi MRT. Effect of antiretroviral therapy on hemostasis in Brazilian pregnant women with HIV infection. Blood Coagulation and Fibrinolysis 2007;18(8):769-774.

78. Silva-Boghossian C, Castro GF, Teles RP, De Souza IPR, Colombo, APV. Salivary microbiota of HIV-positive children and its correlation with HIV status, oral diseases, and total secretory IgA. International Journal of Paediatric Dentistry 2008;18(3):205-216.

79. Robinson PG. Implications of HIV Disease for Oral Health Services. Advances in Dental Research 2006;19:73-79.

80. Ranganathan K, Hemalatha R. Oral Lesions in HIV Infection in Developing Countries: an Overview. Advances in Dental Research 2006;19:63-68.

81. Johnson NW, Glick M, Mbuguye TNL. (A2) Oral Health and General Health. Advances in Dental Research 2006;19:118-121.

82. Arendorf TM, Bredekamp B, Cloete CA, Sauer G. Oral manifestations of HIV infection in 600 South African patients. Journal of Oral Pathology and Medicine 1998;27(4):176-179.

83. Lyles AN. What the Dentist Should Know About a Patient with HIV/AIDS. Journal of the Californian Dental Association 2001;29(2)158-169.

84. WHO. Taking stock: HIV in children. 2006. www.who.int/hiv/toronto2006/takingstockchildren.pdf (Accessed 2012.02.06).

85. Meira DG, Lorand-Metza I, Toro ADC, Silva MTN, Vilela MMS. Bone Marrow Features in Children with HIV Infection and Peripheral Blood Cytopenias. Journal of Tropical Pediatrics 2005;51(2)114-119.

86. Van Rossum AMC, Fraaij LA, De Groot R. Efficacy of highly active antiretroviral therapy in HIV-1 infected children. The Lancet Infectious Diseases 2002;2:93-102.

87. Handforth J, Sharland M. Triple Nucleoside Reverse Transcriptase Inhibitor Therapy in Children. Pediatric Drugs 2004;6(3):147-159.

88. WHO. Antiretroviral therapy for HIV infection in infants and children: Towards universal access. 2010. http://www.who.int/hiv/pub/paediatric/paed-prelim-summary.pdf (Accessed 2012.02.16).

89. Eley B, Nuttall J. Antiretroviral therapy for children: challenges and opportunities. Annals of Tropical Paediatrics 2007;27:1-10.

90. Consolini R, Bencivelli W, Legitimo A, Galli L, Tovo P, Gabiano C, De Martino M. A Pre-HAART Follow-up Study of the Hematologic Manifestations in Children With Perinatal HIV-1 Infection: Suggestions for Reclassification of Clinical Staging. Journal of Pediatric Hematology/Oncology 2007;29(6):376-383.

91. Adetifa IMO, Temiye EO, Akinsulie AO, Ezeaka VC, Iroha EO. Haematological abnormalities associated with paediatric HIV/AIDS in Lagos. Annals of Tropical Paediatrics 2006;26:121-125.

92. Hoernle EH, Reid TE. Human immunodeficiency virus infection in children. American Journal of Health-system pharmacy 1995;52(9):961-979.

93. Ellaurie M. Thrombocytosis in Pediatric HIV infection. Clinical Pediatrics 2004;43(7):627-629.

94. Moodley K, Myer L, Michaels D, Cotton M. Paediatric HIV disclosure in South Africa-caregivers' perspectives on discussing HIV with infected children. South African Medical Journal 2006;96(3):201-203.

95. Vandenbroucke JP. Prospective or Retrospective: What's in a name? British Medical Journal 1991;302(6771):249-250.

96. Manolio TA, Bailey-Wilson JE, Collins FS. Genes, environment and the value of prospective cohort studies. Nature Review Genetics 2006;7(10):812-820.

97. Euser AM, Zoccali C, Jager KJ, Dekker FW. Cohort Studies: Prospective versus Retrospective. Nephron Clinical Practice 2009;113(3):214-217.

98. De Vos AS. Research at Grass Roots. Pretoria: Van Schick, 2001

99. Burns N, Grove SK. The practice of Nursing Research: Conduct, Critique and Utilization. 4th ed. Philedelphia: WB Saunders, 2003.

100. Sim J, Wright C. Research in Health Care Concepts, Designs and Methods. Cheltenham: Stanley Thomas, 2001.