Coagulation in the HIV- positive pregnant patient: a thromboelastographic study

by

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Supervisor: Professor Robert A. Dyer

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DECLARATION

I, *Dr Slindile Mayeza*, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Signature: Signed by candidate

Date: 10 December 2019

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Acknowledgements

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I acknowledge the technical assistance of Mrs Lizel Loo in the performance of thromboelastography.

Finally, the staff and patients of Mowbray Maternity Hospital are also acknowledged for their patience and support.

List of Abbreviations

AIDS	Acquired immunodeficiency syndrome
ART	Anti-retroviral therapy
CS	Caesarean section
HAART	Highly active anti-retroviral therapy
HIV	Human immunodeficiency virus
MA	Maximum amplitude
TEG	Thromboelastography

Title page

Coagulation in the HIV positive pregnant patient: a thromboelastography study

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Key words

HIV, coagulation, pregnancy, thromboelastography, anaesthesia

Abstract/Summary

Human immunodeficiency virus (HIV) infection is associated with haematological changes, including thrombocytopaenia. Pregnancy induces a hypercoagulable state. There are limited data on the coagulation status of women with term pregnancy and HIV receiving anti-retroviral medication. Regional anaesthesia is the technique of choice for caesarean section, and is contraindicated in a hypo-coagulable state. We therefore investigated the coagulation status of term pregnant women with HIV, presenting for elective caesarean section (CS). This was a single-centre cross-sectional observational study, using thromboelastography, comparing the coagulation status of HIV negative and -positive women with no other comorbidities, in pregnancy at term. A blood sample was taken immediately prior to spinal anaesthesia, and thromboelastography was performed within 4 minutes. In addition, platelet count, haemoglobin, and fibrinogen level were measured. Blood samples were obtained from 75 patients. There were no between-group differences in obstetric and demographic data, and no difference in platelet count. The mean (SD) fibrinogen level was higher in HIV positive women (3.9 [1.5] vs 3.5 [0.7] g/L) respectively, p=0.04. There were no significant differences in the r-time, alpha-angle, k-time, MA, or LY-30. The results of this thromboelastography study show that in asymptomatic HIV positive pregnant patients on anti-retroviral treatment, there are no significant differences in coagulation parameters when compared with HIV negative patients. This suggests that routine assessment of coagulation is unnecessary before spinal anaesthesia in patients without other co-morbidities. Further studies could demonstrate the incidence of abnormalities in coagulation or platelet function in patients with AIDS defining disease or HIV positive patients with other co-morbidities.

Publication-ready manuscript

Coagulation in the HIV positive pregnant patient: a thromboelastography study

Introduction

In 2017, South Africa was estimated to have a prevalence of human immunodeficiency virus (HIV) infection in adults aged 15-49 years, of 18.8% [1]. The 2015 National Antenatal Sentinel HIV and Syphilis Survey in South Africa estimated a prevalence of 30.8% amongst women presenting for antenatal care, the lowest being in the Western Cape Province (18.9%) [2].

HIV infection is known to be associated with haematological changes. The most common is anaemia, found in 95% of patients in the course of their disease. Thrombocytopaenia is also a feature, the most common cause of which is immune thrombocytopaenia [3]. The changes in the coagulation system could collectively represent a hypocoagulable state. Pregnancy is well known to induce a hypercoagulable state; other haematological features include a dilutional anaemia and relative thrombocytopaenia [4].

Regional anaesthesia is the technique of choice for CS in the parturient in the absence of contraindications, and has been shown to decrease morbidity and mortality in HIV positive patients undergoing elective CS [5,6]. Thromboelastography (TEG) has been extensively utilised in previous studies to demonstrate the coagulation status of pregnant patients [7,8], and the measured parameters influence the decision to perform regional or general anaesthesia for CS.

There are limited studies examining the effect of HIV infection and anti-retroviral medication on coagulation in pregnancy [6,9]. We therefore performed a cross-sectional study comparing the coagulation profile (as assessed by TEG) of HIV positive versus -negative patients at term. Secondary outcomes included a comparison of TEG variables in patients in whom anti-retroviral therapy (ART) had been commenced before or during pregnancy. In addition, between-group comparisons were performed of platelet count, fibrinogen level, and blood loss during CS.

Methods

This was a single centre observational study, which was approved by the Human Research Ethics Committee of the Faculty of Health Sciences of the University of Cape Town (HREC Ref: 241/2013). Patients were recruited prior to elective CS at Mowbray Maternity Hospital, Cape Town, South Africa, from August 2013 to April 2014. Informed consent was obtained from all patients by the principal investigator at least 12 hours prior to CS. The standard of care at this hospital is for all patients to be screened for HIV infection as part of the antenatal assessment. All blood specimens drawn during the study were labelled with study- specific labels, rendering patients anonymous. The results of the study had no effect on the quality of care received by the participants.

Immediately prior to spinal anaesthesia, a 16- or 18-gauge intravenous cannula was inserted. A blood sample of 10 mL was taken from the cannula. The initial 8 mL was placed in an EDTA tube (BD Vacutainer ® K3E 7.2 mg) for measurement of platelet count; haemoglobin (Hb) level and CD4 count, if the latter had not been performed within six months of participation in the study. The remaining 2 mL of the specimen was placed in a citrated tube (VACUETTE ® 9NC Coagulation Sodium Citrate 3.2%) for the assessment of the fibrinogen level. A separate syringe was used to withdraw a further 2 mL of blood for TEG measurements after the initial sampling, thus eliminating contamination by tissue factors that could affect the results. An aseptic venipuncture was performed if there was difficulty in obtaining a satisfactory blood sample from the cannula.

All TEGs were performed on site within four minutes of sampling by a qualified technologist using the TEG ®5000 Haemostasis Analyzer System (Haemonetics ® Corp, Braintree, MA, USA; Software version 4.2). Regular quality control and calibrations were performed according to manufacturer's recommendations. One mL of native blood was activated using kaolin, and 360 µL pipetted into a cup that had been pre-warmed to 37 degrees Celsius. The same technologist performed all the TEGs, thus eliminating inter-individual variability in performing the test. The samples ran for a period of 60 minutes. The mean (SD) r time (time taken for clot formation to start), k time (time from end of r time until the clot reaches 20 mm), α angle (the tangent of the curve when the k time is reached), maximum amplitude (MA), indicative of platelet function and fibrinogen level, and a marker of clot

strength, and LY30 (percentage decrease in MA after 30 minutes) [10] were recorded and compared between HIV-positive and -negative patients.

The platelet count, Hb level, CD4 count and fibrinogen levels were all performed by the National Health Laboratory Service (NHLS) at Groote Schuur Hospital. Reference ranges were applied as per NHLS standards. Estimated blood loss was recorded by the attending senior anaesthetist, using observation of swabs, measurement in suction bottles, and assessment of surgical field losses.

Demographic data is presented as mean ± SD. TEG data was analysed using the paired *t* test and Wilcoxon signed rank test. The required sample size was estimated for this comparison of TEG parameters between HIV-positive and-negative women, using similar methodology to that of Butwick et al [11]. A 20% difference in TEG ® parameters was regarded as a clinically relevant endpoint for the study. Assuming an r-time estimate for the normal pregnant population of 6.5 minutes, with a standard deviation (SD) of 2.5 minutes, 32 patients would be required in each group to detect a 1.5 minute difference in the HIV-positive group greater or less than the r-time in the control group, with 80% power and alpha error 0.05. Seventy five patients were recruited, to account for possible errors in the processing of samples. The statistical programme used was Statistica Version 11 (StatSoft Inc, Tulsa, USA).

Results

Blood samples were obtained from 75 patients, of whom 38 were HIV-positive and 37 HIV-negative. Obstetric and demographic data is presented in Table 1.

There were no significant between-group differences. Laboratory data are presented in Table 2. Four specimens were discarded by the laboratory due to inadequate volume for analysis. The fibrinogen level in the HIV positive patients was higher than in the control group (p=0.04). The TEG values for the two groups are presented in Table III. There was no significant difference in the r-time, alpha-angle, k-time, MA, or LY-30. The results were not influenced by the timing of commencement of anti-retroviral therapy (ART), before or during pregnancy.

Discussion

This cross-sectional comparison of TEG parameters in pregnant women close to term, showed no significant differences between HIV-positive and -negative patients, except for a clinically insignificantly higher fibrinogen level in HIV positive women. Clot strength as evidenced by the MA was also not significantly different. The LY-30 in both groups showed no evidence of fibrinolysis. As all of the patients in the HIV cohort were on ART during the time of this study, no conclusions can be drawn as to the effect of ART on coagulation parameters. There were also no between-group differences in platelet count, fibrinogen level, or blood loss at caesarean delivery.

Regional anaesthesia is the preferred method in pregnancy, where epidural anaesthesia is the gold standard for labour analgesia, and spinal anaesthesia predominates for CS, avoiding the risks of general anaesthesia, such as failed tracheal intubation. The presence of coagulation abnormalities influences the decision to perform regional anaesthesia in pregnancy. The high prevalence of HIV infection in the South African population and the increasing rate of CS necessitates the understanding of haematological changes and the implications for regional anaesthesia.

HIV causes endothelial dysfunction with activation of the coagulation system. This is evidenced by the finding of elevated D-dimer- and thrombin-anti thrombin complex levels [9,12], a progressive increase of Factor VIII levels, and a decline in functional Protein S and Protein C levels. These changes are more pronounced with advancing disease [3,13]. The presence of lupus anticoagulant and antiphospholipid antibodies, which are associated with a prothrombotic state, may also contribute to HIV infection- associated thrombosis [3,13], although this is controversial [13,14].

Pregnancy is associated with an increase in pro-coagulant factors (II, VII, VIII, X and XI) and a decrease in anti-coagulant factors (Protein C and S, and Antithrombin III) [4,9]. Fibrinogen levels rise from 28 weeks' gestation, and D-dimer levels are raised throughout pregnancy. These haematological occurrences are thought to be a physiological adaptation to minimise blood loss during childbirth [4]. Karlsson et al showed increased coagulation in Scandinavian women [15], as did Gorton et al in the United Kingdom [16].

The maximal amplitude (MA) on the TEG tracing is a measure of clot strength, and is a direct indication of fibrinogen and platelet interaction [17]. The MA has therefore been found to correlate well with platelet count [18]. The increased fibrinogen level in pregnancy may reduce the effect of thrombocytopaenia on the TEG tracing, as both contribute to the MA [19]. Thrombocytopaenia is one of the earliest manifestations of HIV infection [20] and immune thrombocytopaenia is the most common cause [3]. Two previous studies in healthy patients found MA (mean) values of 66.4 [7] and 75.4 [21]. The latter study found fibrinogen levels of 5.0 g/L (3.7-6.4). These values are similar to those found in our study (MA 74.1 in the HIV negative- and 72.7 in HIV positive patients), although fibrinogen levels in our population were lower. These results indicate that asymptomatic HIV positive parturients remain within normal limits for this parameter, as suggested by previous studies. The findings in one study in HIV positive patients in the non-obstetric population, have suggested defects in platelet function. There was reduced aggregation in response to thrombin receptor-activating peptide (TRAP), adenosine phosphate (ADP) and collagen. In addition, an increased response to epinephrine (EPI) was shown using a platelet aggregation assay, when comparing HIV positive-, 80% of whom were on ART, and -negative patients [22].

A study by Arildsen et al, which compared haematological changes in HIV positive patients before and after highly active anti-retroviral therapy (HAART), found endothelial dysfunction, lower fibrinogen levels and higher D-dimer levels prior to treatment. The raised D-dimers did not fully correct following a treatment period of six months [12]. Haugaard et al also found increased D-dimer levels in untreated HIV positive patients, but still found that these patients were relatively hypercoagulable as assessed by TEG [23].

A further study found that HIV patients on ART had increased levels of tissue plasminogen activator antigen (t-PA) and plasminogen activator inhibitor type 1 (PAI-1), which suggests increased fibrinolysis [9]. Our study found no between-group difference in fibrinolysis as assessed by the LY30, which may related to the fact that our patients were receiving ARV therapy [12].

The findings of our study, comparing the coagulation status using TEG of HIV positive and -negative patients, suggests that in asymptomatic HIV positive patients, routine laboratory or point of care coagulation studies are not indicated before regional anaesthesia. Our results are in keeping with a recent study on predictors of

thrombocytopaenia in asymptomatic patients scheduled for caesarean delivery, which found that only preeclampsia was predictive of mild thrombocytopaenia. A sub-analysis in that study showed that HIV status was not independently associated with moderate thrombocytopaenia, and that all asymptomatic patients had a platelet count >70 000/μL. The authors concluded that on this basis a routine full blood count is indicated in preeclampsia, but not in patients who are HIV positive, unless there are other comorbidities [24].

Davies et al evaluated differences in healthy pregnant- versus preeclamptic women using the Platelet Function Analyzer (PFA-100) and TEG. This study found a mean MA of 73 mm, and that despite an abnormal clotting time in the preeclampsia cohort, the MA remained within the normal range. This indicates that TEG may not detect abnormalities in primary haemostatic function [19].

A limitation of our study was that we did not study platelet function, or the effect of ART, since most of our patients were receiving this medication at the time of the study.

In conclusion, the results of this thromboelastography study show that in asymptomatic HIV positive pregnant patients on ARV treatment, there are no significant differences in coagulation parameters when compared with HIV negative patients. This suggests that routine assessment of coagulation is unnecessary before spinal anaesthesia in patients without further co-morbidities. Further studies could demonstrate the incidence of abnormalities in coagulation or platelet function in patients with AIDS defining disease or HIV positive patients with other co-morbidities.

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We acknowledge Professor M James for assistance with statistical analysis, and thank the staff of the Mowbray Maternity Hospital for their professional support.

Competing interests

The authors declare that there were no conflicts of interest.

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Tables:

Table 1. Obstetric and demographic data presented as mean (standard deviation)

	HIV positive (n=38)	HIV negative (n=37)
Age (years)	32.18 (4.61)	30.40 (5.17)
Weight (kg)	88.03 (14.17)	88.19 (16.87)
Gestational age (weeks)	38.92 (0.88)	39.27 (0.87)
CD4 count (cells/μL)	546 (239)	N/A
Estimated blood loss (mL)	516 (255)	469 (280)

HIV: human immunodeficiency virus

Table 2. Laboratory data

	HIV positive	HIV negative	p-value
Haemoglobin (g/dL)	11.7 (1.3)	11.8 (1.4)	0.62
Platelet count ($x10^9$/L)	246.8 (59.0)	261.7 (64.4)	0.29
Fibrinogen (g/L)	3.9 (1.5)	3.5 (0.7)	0.04

Table 3. The TEG values for both groups

	HIV positive	HIV negative	p-value
r time (minutes)	4.83 (1.66)	4.21 (1.51)	0.09
k time (minutes)	1.50 (0.39)	1.41 (0.65)	0.49
α angle (degrees)	67.66 (1.66)	69.47 (6.96)	0.23
maximum amplitude (mm)	72.69 (3.78)	74.10 (6.57)	0.25
LY30 (%)	0.86 (1.15)	2.21 (7.88)	0.29

Appendices:

1: HREC approval

UNIVERSITY OF CAPE TOWN

Faculty of Health Sciences
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Room E52-24 Groote Schuur Hospital Old Main Building
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Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
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Website address: http://www.health.uct.ac.za/research/humanethics/forms/

20 May 2013

HREC REF: 241/2013

Dr S Mayeza
Anaesthesia
D24
NGSH

Dear Dr Mayeza

PROJECT TITLE: COAGULATION IN THE HIV POSITIVE PREGNANT PATIENT: A THROMBOELASTOGRAPHIC STUDY

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee.

It is a pleasure to inform you that the Ethics Committee has **formally approved** the above-mentioned study.

Approval is granted until 28 May 2014.

Please submit to the HREC a Progress Report Form if the study continues beyond the approval period. Please submit a Closure Report Form on completion of the study. (Forms can be found on our website: http://www.health.uct.ac.za/research/humanethics/forms/

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the REC. REF in all your correspondence.

Yours sincerely

PROFESSOR MARC BLOCKMAN
CHAIRPERSON, FHS human research ethics committee

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

Lemjedi

Appendix 2:

Informed Consent Form

TITLE: Coagulation in the HIV positive pregnant patient: A Thromboelastographic study

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You are being invited to participate in a research study. Before you agree to participate, it is important to know what the study is about, why it is being done and what will be involved. Please read the information below carefully and ask the investigator any questions you may have or for more information.

Background

Pregnant women are known to be hypercoagulable. This means that they form clots in their blood much more effectively than women who are not pregnant. This helps to lessen the blood lost when they give birth. Unfortunately, it also means that they are at risk of forming clots that are not needed and could be dangerous to their health. Only a small number of pregnant patients will suffer this complication.

Patients with HIV also have a higher risk of forming clots that are not needed because they are also hypercoagulable. The risk increases as the disease gets worse. HIV also causes other abnormalities in the blood such as anaemia (low haemoglobin) and thrombocytopenia (low platelets).

There are numerous tests that can test coagulation (clotting) in any person. Thromboelastography (TEG) tests whole blood coagulation. It gives a more complete picture of clotting from when blood starts to clot to when the clot starts to break down.

Purpose of the study:

The reason for this study is to compare clotting in pregnant women with HIV to those who do not have HIV. We will be using the TEG to find any differences.

At the time of insertion of a drip for your Caesarean Section, we will take blood specimens to a total of 10ml blood (a tablespoon). This will be used in tests to assess and compare how well your clotting system is working. If we cannot get enough blood from the cannula, we may need to take the blood separately.

Risks to participant

There are no extra risks to you as a participant. We will only be taking 10ml of blood. The intravenous line (drip) is standard procedure for all patients having a Caesarean Section. It will be inserted by a qualified member of the anaesthetic staff who is part of the investigating team. Should we need to take blood separately, this will also be done by a member of the team.

Benefits to the participant

There will be no direct benefits to you as a participant. We however hope to learn more about clotting in patients with HIV during their pregnancies. This will hopefully give us more information to improve our quality of care in the future.

Voluntary Participation

Your participation is entirely voluntary. You may decline to participate in this study. If you agree to participate, you will be asked to sign this consent form. You may withdraw your consent at any time and without giving a reason.

Your treatment and care is in no way dependent on your participation. You will receive the same level of care whether you participate or not and there will be no changes to your treatment.

Costs to participant

There are no costs to the participant.

Compensation to the participant

There is no compensation to you for participation in this study

Results of the participant

If we find anything not normal in your results we will inform you of these as well as how your future pregnancies may be affected. Any questions you have will be answered.

Consent:

I, _____ (patient's name and surname), confirm that I have read and understood the information and have had the opportunity to ask questions. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason and without cost. I understand that I will be given a copy of this consent form.

Signature _____ Date _____

Witness _____(Name and Surname)

_____(Signature)

PATIENT STICKER

Should you have any questions or complaints about the research or any related matters, please contact:

Dr S Mayeza

(Principal investigator)

021 404 5001

Dr MJ Arcache

(Supervisor)

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Lizel Immelman

(Sr Medical Tech at Dept Anaesthesia Coagulation Research lab)

021 406 6144

Prof M Blockman

(Chairperson UCT HSF Human Ethics)

021 406 6492

Appendix 3:

Author Guidelines

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Correspondence to: Dr Corresponding Author (full postal address including e-mail address)

**footnote if presented in part at any national or international meetings, with details including location and date.*

- The title should describe the purpose and content of the paper; in general, this should not exceed 20 words. Include relevant words such as 'randomised controlled trial', 'prospective', 'observational', etc.
- The maximum number of authors is nine; if there are additional contributors, the journal will acknowledge them in an Appendix to the published paper and their names will be indexed appropriately on PubMed.
- Please do not include authors' qualifications. Note that statements such as 'Author XX and Author YY both contributed equally to this work' must not be used.

Short title of up to 60 characters suitable for a running header

Keywords
Each manuscript should have 3-5 keywords on the title page. Our preference is for keywords from **this list**; however we do allow authors to choose their own based on making searching for articles on the internet etc as easy as possible.

Summary
The Summary should follow the sequence of the main body of the text, i.e. Introduction, Methods, Results, Discussion, but should not be structured. It should briefly state the purpose of the study or investigation; basic procedures; important results (giving numbers studied, values for results with p values) including relevant findings from Results, Tables and Figures; and principal conclusions.

Use the same sequence when presenting the methods and results as in the main body of the text, always mention the groups in the same order, and ensure the numbers in the Summary exactly match those in the main body; it may be preferable to write the summary after having finished writing the main paper in order to ensure that these features match. Abbreviations should not be used except for units of measurement.

Introduction
The Introduction should give a concise account of the subject's background. Previously published work should only be quoted if it has a direct bearing on the present study. The Introduction should clearly and explicitly state the aims of the project.

Methods
A statement confirming Local Research Ethics Committee approval and written informed consent should be at the beginning of this section (see Ethical Considerations, below).

The Methods section must describe in sufficient detail the techniques and processes used so that the investigation can be interpreted and repeated by readers. Any modification of previously published methods should be described and appropriate reference given. If the methods are commonly used, only a reference to the original source is required. If special equipment is used, then the manufacturer's details (including town and country) should be given in parentheses. Drugs should be identified by their recommended international non-proprietary names (NB adrenaline and noradrenaline are used in preference to epinephrine and norepinephrine). Label groups in a way that is easy to follow; thus 'propofol group' and 'thiopental group' instead of 'Group P' and 'Group T'. (Occasionally, abbreviated group titles may be better, e.g. 'Group BLAB' instead of 'bupivacaine-lidocaine-adrenaline-bicarbonate group'). Remember to include inclusion/exclusion criteria and a justification of sample size. For randomised controlled trials, sufficient detail should be given on the following to allow readers to properly judge the risk of bias in the study: random sequence generation, allocation concealment, blinding (of patients, investigators, clinical staff, observers/assessors as appropriate) and handling of dropouts/withdrawals (intention to treat principle). Selective reporting bias will be assessed by comparison of the report with its protocol/trial registry entry (see above). The statistical methods used to investigate data should be given at the end of the Methods section (see below).

Results
Express results as mean (SD), median (IQR [range]) or number (proportion) as appropriate. Results (including actual p values) must be presented for all measurements detailed in the Methods section, and in the same order. Results should not be repeated unnecessarily. For example, if a graph is used, do not also present the same information in the text or in a Table. Results should not be given to an unwarranted number of decimal places and 95% confidence intervals should be used where possible (see Statistics, below).

In randomised trials, baseline data (age, ASA physical status, duration of operation, etc.) should not be subjected to statistical comparison, since it is already known that the subjects were randomly allocated and that any difference is therefore due to chance. Describe characteristics and, if possible, allow for differences in the analysis and discussion.

When reporting the effect of an intervention, absolute risk (AR), relative risk (RR) and 'number needed to treat' (NNT) are more easily understood by readers and may be preferable to odds ratio (OR).

Graphs and Tables should be appropriate for the data to be displayed. Tables usually convey more precise numerical information; graphs should be reserved for highlighting changes over time or between treatments.

Report actual p values, rather than ranges or limits (e.g. p = 0.032, rather than p < 0.05).

- Use 'survival' curves for outcomes that are time, e.g. 'time to extubation' or 'time to hospital discharge', particularly if it is the primary outcome
- Means should be expressed to a sufficient precision that they are different, with a minimum of three significant digits e.g. 372, 37.2, 3.72, 0.372 etc.
- Means should be followed by the standard deviation, not the standard error.
- Standard deviations do not need to be different and should be a minimum of two significant digits.
- Rates should be followed by proportion if the denominator exceeds 100, e.g. 90/200 (45%) but 9/20.

Discussion
The Discussion should present an interpretation of the results against a background of existing knowledge. Any conclusions must be warranted by the results. Avoid a paragraph headed 'Conclusions' that merely repeats a summary of the results.

Acknowledgments
The authors should acknowledge those who have made substantial contributions to the study or preparation of the manuscript but whose contributions do not fulfil the requirements for authorship (see above). The trial registration site and number should be included in this section.

Competing interests
A statement should be made at the end of all manuscripts stating any funding obtained and any potential competing interests. For example: 'No external funding and no competing interests declared' or 'Funded by the XXXX Association, grant no. yyyy. Author AB has received payments from ZZZZ Ltd for consultancy work', etc. as appropriate.

Appendices
Information or data not directly a result of the study but necessary for the reader to understand the manuscript should be included as an Appendix. Examples might include copies of questionnaires, recognised mathematical processes used to generate results or previously published and validated classification systems. All should be appropriately referenced and authors must obtain permission from the copyright holders if the contents have been previously published.

References
References must be numbered sequentially as they appear in the text. References cited in Figures or Tables (or in their legends and footnotes) should be numbered according to the place in the text where that Table or Figure is first cited. Reference numbers in the text should be inserted after one space and before punctuation, e.g. [6].

Where more than one reference is cited, these should be separated by a comma, e.g. [1, 4, 39]. For sequences of consecutive numbers, give the first and last number of the sequence separated by a hyphen, e.g. [22-25].

Abstracts may be quoted as references as long as they have been published in peer-reviewed journals. Internet sites may be quoted as references by listing them in the normal way in the text. Unpublished observations, personal communications and abstracts published only in proceedings of meetings should be quoted within the text of

the manuscript in parentheses. Information from manuscripts submitted but not yet accepted should be cited in the text as unpublished observations. References cited for the first time in Tables or Figures should be numbered in the sequence established by the first mention of the particular Table/Figure in the text. All references (including those in press) should be listed at the end of the text in the order they are quoted. For internet sites, please include the date accessed in parentheses. List all authors unless there are seven or more, in which case give the first three followed by ', et al.' Journal titles should be written in full and in italics, followed by a semi-colon then a space followed by the volume number **in bold** (issue numbers are not required) then a colon then a space and finally the page numbers. The *Anaesthesia* **Endnote template may be found here**.

Examples:

1. Author AB, Author CD. Title of paper. *Journal Title Written Out in Full in Italics* 2019; **12**: 123-4.
2. Author AB, Author CD. Title of paper published as 'ePub ahead of print'. *Journal Title Written Out in Full in Italics* 2019. Epub ahead of print 15 Dec; doi xx.xxxx/xxx.xxxxxx.
3. Author AB, Author CD, Author EF, et al. Seven or more authors – what's the point? (chapter title). In: Editor GH, Editor IJ, eds. *Title of Book*. Place: Publisher, 2019: 345-67.
4. Author AB. *Book Title*, 5th edn. Place: Publisher, 2019.
5. Author(s) of website. Title of document/page, 2019. www.URL.co.uk/link.pdf (accessed 01/01/2019).

The **International Committee of Medical Journal Editors** has stated that: "Authors are responsible for checking that none of the references cite retracted articles except in the context of referring to the retraction. For articles published in journals indexed in MEDLINE, the ICMJE considers PubMed the authoritative source for information about retractions. "Retracted articles can be identified by using the following search strategy in PubMed, e.g. for an author J. Smith enter "Smith*J AND retracted publication[pt]".

Tables

Include Tables in the same file as the text, but after the References. Each Table should be on a separate page. Number the Tables consecutively with Arabic numerals. Each Table should have a brief caption immediately above it; this should provide enough information for readers to follow the Table without having to look through the text (e.g. 'Characteristics of patients receiving vecuronium or rocuronium for caesarean section' rather than just 'Patients' characteristics'). The caption should explain whether the values refer to mean (SD), number (proportion), etc. Abbreviations should not be mentioned in the caption without explanation. Abbreviations used in the body of the Table should be explained as footnotes in the order in which they are first mentioned.

For adults: age, weight, height and BMI should be expressed as mean (SD). For children: age, weight, height and BMI should be expressed as median (IQR [range]). Study groups should form the columns rather than the rows. If statistical comparisons are being made, a separate column with exact p values should appear.

Figures

Please supply each Figure as a separate file and not embedded within the Word document. We ask that Figures are supplied at a resolution of 300 pixels per inch for photographs and 600 pixels per inch for line art or a combination of photograph and labelling. Please do not send image files larger than 10MB. Further guidance can be found **here**.

Please ensure related graphs have the same format (fonts, use of symbols, etc.), and that the groups are presented in the same order in each graph (and in the same order as in the rest of the manuscript). The same requirements for abbreviations and units apply as for those in the text and Tables. There should be not titles, plot frames,

gridlines or legend boxes within the graphs, and symbols and error bars should be explained in the caption. Avoid the use of 3-D unless absolutely necessary. Colour figures (e.g. photographs, complex flow diagrams, etc.) may be used without charge.

Captions for Figures
Each Figure caption should include an explanation of the symbols used to provide enough information for readers to follow it without having to look through the text.

Supporting Information (online only)
Additional material such as video clips, lengthy Appendices (e.g. extensive reference lists or mathematical formulae/calculations), etc. which are relevant to a particular article but not suitable or essential for the print edition of the Journal, may also be considered for publication. Please refer to all supporting information in the manuscript using Table S1, Figure S1, etc. and supply as separate files. Further information on suitable file formats may be found **here**.

Language
Anaesthesia uses UK English spelling e.g. “ise” not “ize”, “anaesthesia” not “anesthesia”, etc. Please avoid long, complicated sentences and the passive voice when the active is more appropriate (e.g. ‘We chose epidural anaesthesia because...’ instead of ‘Epidural anaesthesia was chosen by the authors because...’). Focus on the actual message of each sentence; thus ‘Hypotension is important because...’ instead of ‘It would be remiss of us not to mention hypotension because...’. Remember that lungs are ventilated, not patients (nor are they intubated – their tracheas are).

Similarly, patients are not induced – anaesthesia is – or put on ventilators. Correct terms are tracheal (not **endo**tracheal) tube and neuromuscular blocking drugs (not muscle relaxants). Please refer to recent issues of the Journal for preferred wording/spelling.

The abbreviation LMA is only to be used if referring to a specific device made by The Laryngeal Mask Company Ltd, and with the first mention in the Summary and in the main text highlighted by (R) and ‘LMA is a registered trademark of The Laryngeal Mask Company Ltd, an affiliate of Teleflex Incorporated’ as a footnote. If used, the correct format is ‘LMA® laryngeal mask’ for the first mention (n.b. not just ‘LMA®’) and ‘LMA laryngeal mask’ thereafter. The same to apply to LMA® Classic, LMA® Flexible, LMA® Fastrach (n.b. previously labelled ILMA®), LMA® ProSeal, LMA® Supreme, LMA® Unique (n.b. ‘cLMA’ not to be used). The generic term of ‘laryngeal mask’ should be used for describing inflatable-cuff supraglottic airways in general.

Abbreviations
The Journal does not encourage the use of abbreviations, especially in the Summary, since their frequent use makes papers difficult to read. We will accept abbreviations in the following circumstances:

- Universal abbreviations that do not need to be written out in full when first mentioned in the text, e.g. ASA, BMI, ECG, ICU, HDU, SD, SEM, 95% CI, IQR, ANOVA, S_pO_2, F_IO_2, pH.
- Acceptable common abbreviations should be written out in full at their first mention, e.g. CNS, CSF, HME, PEEP, PCA, SCBU, CTG, EEG, BIS, CVP, PAP, PCWP, ECT – unless they are only mentioned a few times, in which case please spell them out throughout. Please do not use abbreviations that are clumsy or will be unfamiliar to the majority of readers, e.g. DI (difficult intubation), TTFB (time to first breath), etc.
- Acceptable abbreviations whose use should be restricted to situations where space is limited, such as in formulae or in Tables and Figures, e.g. O_2, CO_2, N_2O, HCO^{3-}, Na^+, K^+, Mg^{2+}.

Numbers and units
Numbers should be spelled out in full when they start a sentence, and when they are less than 10 (unless they are followed by units of measurement). Thus: ‘Thirteen days

later, five patients each received 7 ml solution...' Commas are used to indicate thousands above 10,000: thus, 2000 and 20,000. Please give costs in sterling (£) with equivalent Euros and US dollars (€/$) in brackets.

Use the format $mg.kg^{-1}$ not mg/kg for all units. Use SI units throughout the text except for vascular pressure measurements (mmHg or cmH_2O) and haemoglobin concentration ($g.l^{-1}$). Litres are indicated by lower case 'l'. Use the 24-hour clock for times.

Ethical considerations

Manuscripts will only be considered for publication in *Anaesthesia* if they adhere to the highest ethical standards. These are detailed in two editorials published in the journal, that are available **here** and **here** and which potential authors are strongly advised to consult.

The Editorial Board takes all cases of possible publication misconduct seriously and will investigate these according to the recommendations of the **Committee on Publication Ethics (COPE).** Further guidance can be found in our **Editorial Policies**.

All clinical trials that prospectively assign human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome should be registered before the time of first recruitment. There are several public registries now available which meet the requirements of the ICMJE and these are listed on the **WHO International Clinical Trials Registry Platform.** The registry, registration number and date of registration must be stated in the Acknowledgements section of the manuscript. This should have been done before patient recruitment commenced. Reports of original research that were not registered before the study was carried out should include separate submission of the original protocol for the study. If the submitted report differs from the protocol, an explanation of the reason for this should be provided. Authors should be willing and able to submit their raw study data to the journal, if requested, after submission.

Anaesthesia supports and encourages the use of the **EQUATOR (Enhancing the QUAlity and Transparency Of health Research) Network guidelines** to ensure the transparent and accurate reporting of research studies. The authors of clinical intervention studies are advised to review the **CONSORT statement** regarding the reporting of randomised trials prior to manuscript submission.

We strongly encourage authors to register systematic review protocols on a similar database (for instance, PROSPERO **http://www.crd.york.ac.uk/PROSPERO/**).

All clinical trails should be conducted in accordance with the ethical principles as set out in the Declaration of Helsinki. In brief, the minimum ethical standards for *Anaesthesia* include:

- Approval by a Research Ethics Committee (REC) or equivalent Institutional Review Board (IRB) must be obtained prospectively for all studies on human subjects, including studies in which participants' skills are tested using manikins. Some studies involving audit and epidemiological surveys, assessments of medical equipment or analysis of previously collected, non-identifiable information from a database may be exempt from this stricture if participants are appropriately protected against coercion and there is due regard to confidentiality. Publication of the results, however, would usually still require informed consent and assurances regarding confidentiality (including approval by the Caldicott Guardian or equivalent for patient data and the relevant Research and Development department), even if the REC/IRB has indicated that formal submission is unnecessary.
- While an essential preliminary step, REC/IRB approval does not guarantee the ethical standards of a study will meet the requirements of the Editorial Board of Anaesthesia. If authors have any concerns that ethical issues might compromise

publication, they are invited to contact the Editor-in-Chief before embarking on the study.

- The Editorial Board supports the view of the ICH Harmonised Tripartite Guideline for Good Clinical Practice that full prospective written informed consent should be obtained from all subjects of clinical trials, including participants in manikin studies (see above). This would normally comprise provision of written information to potential research participants, allowance of adequate time for them to consider their involvement and ask questions, and the use of specific consent forms (for the study, not just for routine surgery/anaesthesia) that should be signed by the participants to indicate their consent and stored in case they require examination later.
- Studies of novel treatments, in particular drug studies where the agent used is given via unlicensed routes (especially neuraxial or perineural), may have received approval from the REC/IRB, but the Editorial Board is likely to reject such studies if it considers the risks posed outweigh the potential benefits. Such a conclusion is more likely to be reached if the drug in question is not widely used in routine practice (as evidenced by inclusion in standard textbooks), if the study participants are especially vulnerable (e.g. children, women in labour), if there are questions over consent, or if only modest improvements in outcome are expected where other, well established methods already exist.
- Animal studies will only be considered for publication if they have ethical and governmental approval, and have been conducted under appropriate standards of care. Researchers will be expected to follow the ARRIVE guidelines for experimentation in animal research.

Statistics

The following guidelines may help authors present their work in a better and more rigorous way that avoids common statistical errors that frequently lead to rejection. This is not an exhaustive list and, of course, the Editorial Board and reviewers of manuscripts may ask authors for revisions that are not detailed here. Authors are advised that it is a condition of submission that they be prepared to send individual patient data, or other data, to be checked.

Methods

Randomisation methods should be made explicit (e.g. coin toss, random numbers, etc.). Please describe if stratification of the allocation system in a randomised controlled trial is performed (e.g. by age or recruiting centre) or block (permuted sequence or otherwise). For instance, most anaesthetic randomised controlled trials have exactly the same number of patients in each group but do not mention any blocking method (which would include putting equal numbers of folded pieces of paper for each group in an urn). Blinding must be as good as possible within constraints of clinical practice.

Where there are several outcomes to be reported, the most important (primary) outcome should be clearly stated, along with any secondary outcomes. Beware of reporting as ‘significant’ or ‘important’ a positive result of a secondary outcome, when the study was in fact powered (sample sized) to a different primary outcome.

Power analysis

Some justification of sample size is always necessary for all observational studies, randomised or non-randomised controlled trials, or other types of study. Justification may be quantitative or qualitative (e.g. a ‘convenience sample’), although the latter may be regarded as weaker than the former. Details provided (for continuous variables) should include the power level; the significance level at which a result is sought; and the expected control and study group proportions or mean and pooled SD, in order to allow reviewers and readers to follow the calculation. The method used to justify power should be referenced and enough detail provided, so that the calculation can be repeated by readers. Conventionally, the power of study should be at least 80% but where different this should be stated and justified. The ‘clinically important difference’ that the study is

designed to detect should indeed be clinically relevant. Beware of setting an unreasonably large 'clinically important difference' to justify small sample size, as reviewers will recognise this is done simply to facilitate a small study.

General rules:

- Use mean (SD) unless data are discrete (e.g. Apgar scores, sedation scores) or grossly non-normally distributed: use median (IQR [range]) or you are interested in the 'true' value for the population (use SEM).
- Visual analogue scores (VAS) for pain may be treated as continuous data and be subjected to parametric tests as long as the sample size is large (> 50) and the data appear normally distributed. VAS for other modalities (nausea, drowsiness) have not been so extensively validated and are best treated as ordinal data.
- Scales of measurement can be problematic (e.g. Cormack-Lehane scale, VAS, etc.) because a value of say 2 on the scale does not imply something twice the value of 1, etc. So they cannot logically be regarded as linear, continuous scales. It is safer to regard them as ordinal scales. However, for some scales such as VAS for pain it appears established norm that this may be regarded as continuous, especially for large sample sizes (e.g. >50).

Inferential statistics

- Use simple statistical tests where possible.
- Avoid multiple comparisons, or correct for them if used.
- Reference unusual tests; and assume that the more unusual the test, the more likely it is that a specialist statistical referee will review the paper.
- Include details of any computer package/version used.
-
- When looking for relationship between variables, use correlation to describe a simple descriptive association between two variables.
- Use regression to describe a quantitative relationship between two or more variables, especially where one is predictive and other(s) dependent. Non-linear regression may be appropriate. Regression methods yield a formula to relate the variables being described.
- Use the Bland-Altman method to describe the performance of two different methods used in measurement, analysis or diagnosis.

Conclusions

All conclusions should be warranted by the results and not extend beyond the confines of the study conditions. A negative result does not mean that there is definitely no difference (confidence in the conclusion is dependent upon the power of the study), and a positive result does not mean that there definitely is a difference (confidence in the conclusion is dependent upon the alpha error). The journal has a very useful **statistics section** containing articles which authors may find useful in preparing their manuscripts for statistical content.