

**ANTIPHOSPHOLIPID SYNDROME SCREENING RESULTS AND ASSOCIATED
OBSTETRIC OUTCOMES AT RAHIMA MOOSA MOTHER AND CHILD HOSPITAL**



Simamkele Siviwe Hela

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 Obstetrics and Gynaecology

Johannesburg 2024

DECLARATION

I, Simamkele Siviwe Hela, declare that this research report is my own unaided work. It is being submitted for the degree of Master of Medicine (M.Med) at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.



December 2024

DEDICATION

I dedicate this MMed to my lovely wife Melanie Hela, and to my late parents,
Mzwandile and Bukelwa Hela

PRESENTATION

Poster presentation at the South African Society for Obstetricians and Gynaecologists (SASOG) 2022 26-30 November 2022, Cape Town.

Presentation at Wits O&G Women's Health Symposium 2023 on the 1st of December 2023

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to Dr A. Wise, my supervisor, for her patient guidance and enthusiastic and useful critiques of this research work. I also thank the National Health Laboratory Services (NHLS) for granting the patient data.

I thank the department of Obstetrics and Gynaecology at RMMCH for all their support towards the study, and Dr Admire Chikandiwa for his statistical support.

TABLE OF CONTENTS

DECLARATION.....	ii
DEDICATION.....	iii
PRESENTATION.....	iv
ACKNOWLEDGEMENTS.....	v
LIST OF TABLES.....	vii
LIST OF ABBREVIATIONS.....	ix
JOURNAL ARTICLE.....	0
ABSTRACT.....	1
INTRODUCTION.....	2
METHODS.....	3
RESULTS.....	4
DISCUSSION.....	8
LIMITATIONS.....	8
RECOMMENDATIONS.....	9
CONCLUSION.....	9
ACKNOWLEDGEMENTS.....	9
REFERENCES.....	9
APPENDICES.....	13
Appendix A: Approved Research Protocol.....	13
Appendix B: Data collection sheet.....	29
Appendix C: Permission to conduct study.....	33
Appendix D: Certificate: Introduction to Research Ethics.....	34
Appendix E: Adherence to HREC.....	35
Appendix F: Ethics clearance certificate.....	36
Appendix G: Permission letter from NHLS.....	37
Appendix H: Journal guidelines for SAMJ.....	39
Appendix I: Turnitin plagiarism report.....	54

LIST OF TABLES

Table 1: Results of ACLA, ANA, B2GP1 and LA testing.....	6
Table 2: Thrombotic events.....	8
Table 3: Demographics and history of participants.....	9
Table 4: Total number of patients tested for APS and their results.....	10
Table 5: Obstetric outcomes of participants.....	11
Table 6: Short-term neonatal outcomes.....	13

LIST OF ABBREVIATIONS

aCL	Anticardiolipin
ALUGEN	African Lupus Genetics Network
aB2GP1	Antibeta2Glycoprotein1
ANA	Antinuclear Antibodies
aPLs	Antiphospholipid Antibodies
APS	Antiphospholipid Syndrome
CAPS	Catastrophic Antiphospholipid Syndrome
EULAR	European League Against Rheumatism
FGR	Fetal Growth Restriction
HELLP	Haemolysis, Elevated Liver enzymes, Low Platelets
LA	Lupus Anticoagulant
LMWH	Low Molecular Weight Heparin
NHLS	National Health Laboratory Service
NNW	Neonatal ward
NICU	Neonatal Intensive care unit
RPL	Recurrent Pregnancy Loss
SGA	Small for Gestational Age
SLE	Systemic Lupus Erythematosus
UFH	Unfractionated Heparin

JOURNAL ARTICLE (SAMJ)

ANTIPHOSPHOLIPID SYNDROME SCREENING RESULTS AND ASSOCIATED OBSTETRIC OUTCOMES AT RAHIMA MOOSA MOTHER AND CHILD HOSPITAL

Hela SS, MBChB

Lombaard HN, MBChB (Pret) , MMed(OetG)(Pret), PgDip(HSE)(Wits)

Wise AJ, MBBCh, FCOG, MMed, Dip HIV Man, Cert Maternal and Fetal Medicine, MSc

Study setting:

Rahima Moosa Mother and Child Hospital, Corner of Fuel Road and Oudtshoorn Street, Coronationville, Johannesburg, South Africa

Department affiliations:

Department of Obstetrics and Gynaecology, University of the Witwatersrand

Conflict of interest:

The authors have no conflicts of interest to declare.

Simamkele Siviwe Hela^a, Amy Wise^b, Hennie Lombaard^c

^a University of the Witwatersrand, Rahima Moosa Mother and Child Hospital

^b Specialist Obstetrician and Gynaecologist, Subspecialist Maternal and Fetal medicine, MBBCh(WITS), MMed (O&G), Rahima Moosa Mother and Child Hospital, University of the Witwatersrand.

^c Professor Obstetrician and Gynaecologist, Subspecialist Maternal and Fetal Medicine, MBChB (Pret), MMED (OetG)(Pret), PgDip (HSE), Pavilion for Women, Texas Children's Hospital

Corresponding author email: simahela@gmail.com

ABSTRACT

Background

Antiphospholipid syndrome (APS) is an autoimmune disease associated with venous and arterial thrombosis and adverse pregnancy outcomes that include recurrent early miscarriage, fetal death, early onset preeclampsia and placental insufficiency. However, there is limited data on APS prevalence and its impact in Africa.

Objectives

This study aimed to investigate the prevalence of APS by laboratory criteria and its obstetric outcomes among pregnant women accessing care at Rahima Moosa Mother and Child Hospital (RMMCH) in Johannesburg, South Africa.

Methods: Medical records of patients who were tested for at least two of the following – ACLA, LA, aB₂GP₁ and ANA between July 1, 2019, and December 31, 2019.

Results: There were 511 patients who were tested for APS during the study period and 123 records were available for analysis. Only 2 (1.6%) met the diagnostic criteria for APS in the 123 records and 5 (0.84%) of the 511 patients met the diagnostic criteria.

Conclusion: Despite the challenges associated with diagnosing APS, this study emphasises on the importance of screening for other conditions that may result in poor fetal and neonatal outcomes other than antiphospholipid syndrome,

Keywords: Antiphospholipid syndrome, Neonatal death, Antibodies

INTRODUCTION

Pregnancy and the postpartum period inherently pose an increased risk of hypercoagulability, a state further accentuated by any underlying pathology affecting the endothelial system or the coagulation cascade. Amongst these conditions, antiphospholipid syndrome (APS) stands as a notable concern with the presence of persistent antiphospholipid antibodies (aPLs), lupus anticoagulant (LA), anti- β 2-glycoprotein 1 (aB2GP1) and anticardiolipin (aCL) (1). APS is an autoimmune disorder characterised by its association with venous and arterial thrombosis, as well as adverse pregnancy outcomes, including recurrent early miscarriages, fetal death, early onset preeclampsia, and placental insufficiency (2). This syndrome is defined by the presence of antiphospholipid antibodies, which trigger complex thrombotic and inflammatory mechanisms, leading to a spectrum of vascular and obstetric manifestations .(3)

Research from the USA has shown that pregnant women with aPLs are at a 24% higher risk of thrombotic events during pregnancy, particularly venous thromboembolism (VTE), compared to the general population (4). Furthermore, APS can be classified into primary APS (occurring independently) or secondary APS (occurring in conjunction with other autoimmune diseases, most commonly systemic lupus erythematosus [SLE]) (5). Additionally, a rare but life-threatening form known as Catastrophic APS (CAPS) leads to multiorgan thrombosis (6). The Sydney classification serves as a valuable tool in diagnosing APS, by combining clinical criteria such as thrombotic events and pregnancy-related complications, with laboratory criteria including specific antibody tests, including the detection of LA, aCL and aB2GP1 antibodies(7). Even though it is not part of testing criteria, ANA is tested for because patients with ANA-positive APS have been found to have more triple aPL

positivity and more thrombosis and obstetrical recurrences in comparison with ANA-negative APS patients (8).

In the United States, women make up approximately 80% of APS cases and 24% of thrombotic events in women with APS occur during pregnancy or the puerperium period (9)

In 2023, the American College of Rheumatology (ACR) have revised the criteria for testing for APS, currently it involves a weighted and scoring system which has a very high specificity of 99% compared to the 86% of the Sydney criteria (10) The diagnosis of sero-negative APS (SNAPS) can be considered in some of our patients who did not meet the criteria for APS, SNAPS is a diagnosis of exclusion, both hereditary and acquired causes of thrombophilia need to be ruled out before the diagnosis can be made(11).

APS predominantly affects women of reproductive age (15-45 years), and data from the Royal College of Obstetricians and Gynaecologists (RCOG) in the UK indicate that 15% of women with aPLs experience recurrent pregnancy loss, with a dismal live birth rate as low as 10% in the absence of pharmacological intervention (12). Despite the global impact of APS, epidemiological data in Africa remain limited, primarily due to challenges associated with underdiagnosis and underreporting of APS cases. This scarcity of data extends to autoimmune diseases in Africa as a whole, with SLE serving as a prime example. The African Lupus Genetics Network (ALUGEN) initiated a database in 2015 to address this deficiency and stimulate prospective studies focusing on Africans with SLE (13). Reports have highlighted a higher incidence of severe SLE features in people of African descent living in developed countries, but in sub-Saharan African countries, the burden remains largely unexplored due to resource constraints and the absence of universal health coverage (14).

Given the limited understanding of APS and its impact on pregnancy outcomes in Africa, this study aims to help fill this crucial knowledge gap by investigating the prevalence of APS and its obstetric outcomes among pregnant women with risk factors for APS, accessing care at a regional hospital in Johannesburg, South Africa. Through this research, we seek to enhance our understanding of APS within the context of healthcare in South Africa.

METHODS

Study Design

This was a cross-sectional study with a clinical audit component, aiming to describe the diagnosis, treatment and outcomes of obstetric patients who were investigated for APS accessing care at an academic hospital in Johannesburg, South Africa. Patients were tested during pregnancy and immediately postpartum in response to prior history or complications during the index pregnancy or post-partum.

Study site

The study site is a 338-bed regional hospital that provides maternal and paediatric care to the surrounding communities. In 2019 there were nearly 14 000 deliveries. Maternal hypertensive disorders were recorded in approximately 5% of the pregnancy women who delivered (15). The National Health Laboratory Service (NHLS) conducts an average of 65-70 antiphospholipid syndrome (APS) tests monthly.

Study population

Our study focused on pregnant women who sought healthcare services at RMMCH during the period from 1 July 2019 to 31 December 2019 and were tested for APS. They had to have tested for at least two of the following antibodies: anticardiolipin antibodies (ACLA), beta-2 glycoprotein 1 antibodies (B2GP1), lupus anticoagulant (LA), and antinuclear antibodies (ANA) during the specified time frame.

Sampling and sample size:

There were 593 people tested for antiphospholipid syndrome during the six-month period, 123 maternity files available for review. Indications for testing included prior medical and obstetric history suggestive of APS, as well as events in the current pregnancy such as delivering a stillborn.

Analysis

We conducted descriptive analysis to summarize the demographic characteristics of the study participants, including age, race, and parity. Median and interquartile range (IQR) were used for continuous variables, while frequency distributions were used for

categorical variables. We assessed the prevalence of comorbidities using frequency distributions to provide a comprehensive overview of these health conditions among the participants. The evaluation of the Sydney classification criteria for APS focused on the prevalence of specific antibodies, thrombotic event incidence, and pregnancy-related complications.

We analysed maternal outcomes, including the mode of delivery and postnatal complications. Fetal outcomes focused on the fetal delivery outcomes (live births or stillbirths), neonatal Apgar scores, and the requirement for neonatal resuscitation.

Data management:

A data sheet was used to record the following: demographics, obstetric, medical and surgical history, serology results, treatment received and short-term maternal and neonatal outcomes. The data was then captured into the REDcap database. For statistical analysis, an anonymised dataset was exported into STATA format and analysed using STATA version 16. To ensure data security and confidentiality, all data was stored on a password-protected device, and access was restricted to the research team.

Ethical considerations:

This study adhered to strict ethical guidelines and obtained necessary permissions for its execution. Formal approval was granted by the Heads of Obstetrics and Gynaecology and National Health Laboratory Service (NHLS), which provided access to the laboratory results database. Additionally, ethical clearance was secured from the Human Research Ethics Committee (HREC-Medical) under Ethics No. M210554. Permission was also sought and granted by the CEO of Rahima Moosa Mother and Child Hospital. The study was registered with National Health Research Database.

RESULTS

Study participant characteristics

Table 1 Study participants characteristics (N=123)

Characteristic	Metrics
Median (IQR) Age -Years	29 (25-35)
Race, n (%)	
Black	107 (87)
Asian	4 (3.3)
Coloured	7 (5.7)
White	3 (2.4)
Missing	2 (1.6)
Parity, n (%)	
0	35 (28.5)
1	33 (26.8)
2	35 (28.5)
3	14 (11.4)
≥4	6 (4.9)
Gravidity, n (%)	
1	22 (17.9)
2	22 (17.9)
3	32 (26)
4	28 (22.8)
≥5	16 (13)
Missing	3 (2.4)
HIV status, n (%)	
Positive	18 (14.6)
Negative	100 (81.3)
Missing	5 (4.1)
Rhesus factor, n (%)	
Rhesus positive	112 (91.1)
Rhesus negative	3 (2.4)
Missing	8 (6.5)
Median Haemoglobin (g/dL)	12.0 (10.8-13)
Comorbidities, n (%)	
Chronic Hypertension	48 (39)
Diabetes Mellitus	4 (3.3)
Epilepsy	1 (0.8)
Asthma	2 (1.6)
None	68 (55.3)

Table 1 presents the demographic characteristics and obstetric history of our study participants (N=123). The median age [interquartile range (IQR)] of these pregnant women was 29 years, (IQR: 25-35). The majority identified as Black, 107 (87%), while smaller proportions belonged to Asian, 4 (3.3%), Coloured, 7 (5.7%), and White, 3 (2.4%) racial categories. A few participants, 2 (1.6%) had unknown race information.

A balanced parity distribution was observed, with approximately 33 (27.5%) of participants having parity values of 0 or 1, and 34 (28.3%) having a parity of 2. For those with higher parity values, 14 (11.7%) had a parity of 3, and 6 (4.9%) had four or more children. Gravidity exhibited a more varied pattern, with 32 (26.0%) of participants falling into the category of gravidity 3.

Regarding comorbidities, hypertension was the most prevalent with 48 (39%), followed by diabetes mellitus (all forms) 0, 4 (3.3%). A significant proportion of 68 (55.3%) participants reported no comorbidities. HIV status data indicated 18 (14.6%) of the women were HIV-positive and all on treatment, while 100 (81.3%) were HIV-negative, but data for 5 (4.1%) were missing. Most participants [112 (91.1%)] were Rhesus positive, while a smaller fraction was Rhesus negative, 3 (2.4%). Data for Rhesus factor was missing for 8 (6.5%) of participants, underlining the importance of data completeness. The median haemoglobin level at booking was 12g/dL (IQR: 10.8-13.), 29 (23.6%) were anaemic with haemoglobin level of less than 11g/dL.

Sydney Classification Criteria Evaluation

The study participants were evaluated for antiphospholipid syndrome (APS) based on both the clinical and laboratory criteria of the Sydney Classification (16). The clinical criteria are presented in Table 2, highlighting indications related to thrombosis history and pregnancy-related complications. For thrombosis history, 2 (1.6%) of participants had a previous history of thrombosis, and one had a thrombosis in the index pregnancy. In terms of pregnancy-related complications, 17 (13.8%) experienced early onset preeclampsia, while 19 (15.4%) had early onset preeclampsia combined with intrauterine fetal death. Furthermore, 20 (16.3%) had a history of two or more first-trimester miscarriages, and 30 (24.4%) had at least one episode of preeclampsia leading to pre-term birth. Additionally, 29 (23.6%) had experienced fetal death after 10 weeks of gestation. There were 8 cases (6.5%) with missing data.

The laboratory criteria for APS based on specific antibody testing are outlined in Table 3. Among the participants, 6 (5%) tested positive for LA. Additionally, aCL testing revealed that 2 (1.6%) were positive for IgM antibodies, while 1 (0.8%) were positive for IgG antibodies. In the case of aB2GP1 antibody testing, 3 (2.4%) were positive for IgM antibodies, and 2 (1.6%) were positive for IgG antibodies. ANA positivity was observed in 2 (1.6%) of the participants.

All participants were yet to be tested for APS the second time by the time the study was commenced, and only two participants met the criteria according to the Sydney laboratory criteria.

Timing of the testing – 1st/2nd/3rd trimester or Peri-partum

Those peri-partum – testing indicated due to stillbirth? 2nd/3rd trimester?

Table 2 Sydney classification clinical criteria of APS (N=123)

Indication	Metrics
Thrombosis history n (%)	
Previous	2 (1.6)
Current	1 (0.8)
None	120 (97.5)
Pregnancy-related complication n (%)	
Early Onset Preeclampsia	17 (13.8)
Early onset Preeclampsia with intrauterine fetal death	19 (15.4)
≥2 First trimester miscarriages	20 (16.3)
≥1 Preeclampsia or placental insufficiency with pre-term birth	30 (24.4)
≥1 fetal death after 10 weeks gestation	29 (23.6)

Table 3 Sydney classification laboratory criteria of APS (N=123)

Lab test	n (%)
----------	-------

Lupus Anti-coagulant (LA)

Positive	6 (5.0)
Negative	114 (92.7)
Missing	3 (2.3)

Anti-cardiolipin antibodies

IgM positive	2 (1.6)
IgM negative	121 (98.4)
IgG positive	1 (0.8)
IgG Negative	122 (99.2)

**Anti-B2 Glycoprotein 1
antibodies**

IgM positive	3 (2.4)
IgM negative	120 (97.6)
IgG positive	2 (1.6)
IgG Negative	121 (98.4)

Antinuclear antibody (ANA)

Positive	2 (1.6)
Negative	104 (84.6)
Missing	17 (13.8)

Table 4 depicts the total number of patients who were tested for APS, this includes those whose medical files could not be retrieved. A total of 572 were tested according to NHLS, of these, 511 (89.3%) were tested for at least two of the four (ACLA, ANA, aB₂GP₁, LA). Only 5(0.87%) met the initial criteria for APS diagnosis, having been tested once, they would still need a second set to confirm APS. ACL IgG was positive in all of the patients, 3 patients had medium titres of 61 GPL, 43 GPL and 45 GPL while the other 2 patients had high titres of 105GPL and 109GPL. A total of 345 were tested for ANA, 40 (11.6%) were positive, 32 (9.28) had high titres of $\geq 1/80$.

Table 4 Total number of patients tested for APS and their results

		Negative n(%)	Equivocal n(%)	Positive n(%)	Total n(%)
B2GP1	IgM	526 (96.87)	13 (2.39)	4 (0.74)	543 (100)
	IgG	536 (97.10)	7 (1.27)	9 (1.63)	552 (100)
ACLA	IgM	500 (90.58)	52 (9.42)	0 (0)	552 (100)
	IgG	544 (95.10)	23 (4.02)	5 (0.87)	572 (100)
LA	-	478 (93.54)	-	33 (6.46)	511 (100)
		Negative	Positive <1/80	Positive ≥1/80	
ANA	-	305 (88.4)	8 (2.31)	32 (9.28)	345 (100)

Maternal Outcomes

In our study, we examined the maternal outcomes of the pregnant women who underwent APS screening. The outcomes are summarised in Tables 4 and 5. In table 5, the 123 participants, 79 (64.2%) had a normal vaginal delivery (NVD), while 39 (28.5%) underwent a caesarean section. However, 9 participants (7.3%) had missing data regarding the mode of delivery.

A small proportion of participants experienced post-natal complications. These complications included one case (0.8%) of retained placenta, three cases (2.4%) of primary postpartum haemorrhage (PPH), one case (0.8%) requiring evacuation in the operating theatre, one patient (0.8%) required a relook laparotomy for PPH, and one (0.8%) developed HELLP syndrome. The majority of participants, 116 (94.4%) did not experience any post-natal complications.

Regarding the overall maternal outcome, 118 (96%) of patients were discharged from the hospital in stable condition. Two patients (1.6%) were transferred to another facility for further care, and one participant (0.8%) refused hospital treatment. However, there were two cases (1.6%) with missing data related to the overall maternal outcome

Table 5: Maternal outcomes among pregnant women undergoing APS screening (N=123)

Description	n (%)
-------------	-------

Mode of delivery

Normal vaginal delivery	79 (64.2)
Caesarean section	39 (28.5)
Missing	9 (7.3)

Post-natal complications

Retained placenta	1 (0.8)
Primary PPH	3 (2.4)
Evacuation in theatre	1 (0.8)
Relook laparotomy for PPH	1 (0.8)
HELLP syndrome	1 (0.8)
No complication	116 (94.4)

Overall maternal outcome

Discharged	118 (96.0)
Transferred	2 (1.6)
Refusal of hospital treatment	1 (0.8)
Missing	2 (1.6)

Fetal outcomes

Patients who delivered stillbirths had presented with the diagnosis and this served as an indication for testing for APS, as part of the workup for intrauterine fetal death. Among the newborns, 70 (56.9%) were born alive, 15 (12.2%) were fresh stillbirths (FSB), and 35 (28.5%) were macerated stillbirths (MSB). There were three cases (2.4%) with missing data regarding the fetal outcome.

Seven (14.0%) of the stillbirths (6 MSB, 1 FSB) were associated with at least one positive APLs result, aB2GP1 was positive in five (4%) patients with MSB. The patient

who delivered the FSB had a positive lupus anticoagulant. There were 2 (1.6%) who had one or more positive APLs, one of the patients had both ANA and aCL (FSB), whilst the other had aB2GP1 and LA (MSB).

Apgar scores, assessed at 1, 5 and 10 minutes after birth, showed generally favourable results with median scores of 8 (IQR: 7-9) at 1 minute, 9 (IQR: 8-9) at 5 minutes, and 9 (IQR: 8-9) at 10 minutes. Four neonates had APGAR score below 7 at 5 minutes but they all responded well to bag mask ventilation and did not require invasive ventilation. Chest compressions were necessary in 1 (0.8%) neonate.

Newborns were admitted to various units based on their condition. Most were admitted to the postnatal ward with their mothers 62 (50.4%), while a smaller proportion of 2 (1.6%) and 6 (4.9%) were admitted to the neonatal admissions ward and the neonatal high care unit/neonatal intensive care unit (NICU/HCU) respectively.

Table 6 Fetal outcomes among pregnant women undergoing APS screening (N=123)

Description	n (%) or median (IQR; range)
Fetal outcome	
Alive	70 (56.9)
FSB	15 (12.2)
MSB	35 (28.5)
Missing	3 (2.4)
Apgar score (median, (n=70) (IQR))	
At 1 min	8 (7-9)
At 5 min	9 (8-9)
At 10 min	9 (8-9)
Neonatal resuscitation	
Bag and Mask	6 (4.9)
CPR	16 (13)
Place of admission (70 live births)	
Postnatal ward with mother	62 (88.6)

Neonatal admissions ward	2 (2.85)
Neonatal high care unit/Neonatal ICU	6 (8.6)
Missing	3(4.3)

Reasons for admission to NNW or NICU/HCU

LBW	6 (4.9)
Prematurity	1 (0.8)
Required oxygen	1 (0.8)

Management and Outcomes of APS-Positive Patients

Two pregnant women were diagnosed by the attending clinicians with APS based on positive anticardiolipin IgG antibody above 40 GPL-u/ml, they also tested positive for antinuclear antibody and one had a positive Lupus anticoagulant test as well. To manage their condition, both patients were prescribed low molecular weight heparin (LMWH) and aspirin. One patient had a stillbirth, the other a live birth; both mothers were discharged in a stable condition.

Discussion

Our study aimed to describe the testing, treatment and outcomes of pregnant patients who were investigated for APS at Rahima Moosa Mother and Child Hospital. We reported on women who had been tested for APS as they had a risk factor which prompted screening . The results of our study highlight the importance of also 13

investigating other causes of poor obstetrics outcomes since the prevalence amongst women with risk factors was low. We should consider revising our indications for testing for APS. APS is a condition that poses significant risks to both maternal and fetal health(17).

The number of patients with APS in this study population was two as per the treating clinicians out of 123 (1.6%) pregnant women which is slightly compared to data from America and UK where the incidence is 1.1-1.5 per 100 00 and 1.8-2.9 per 100 000 people, respectively. Out of the total number tested LA was the most commonly positive antibody (6.46%), this would need to have a positive repeat test after 12w to confirm APS. APS is a challenging condition to diagnose due to its heterogeneous clinical presentation and the need for repeated testing for confirmation (18). The limited number of study patients meeting the diagnostic criteria in this study highlights the need to follow-up patients and do the repeat testing at 12 weeks.

The clinical and laboratory criteria for APS were evaluated in detail among the study participants. Thrombosis history and pregnancy-related complications were assessed as part of the Sydney classification clinical criteria. Thrombosis history was relatively uncommon, with only a small percentage of patients reporting prior 2 (1.6%) or current 1 (0.8%) thrombotic events. However, a significant proportion of pregnant women had experienced pregnancy-related complications, including early onset preeclampsia, first-trimester miscarriages, and fetal death after 10 weeks gestation. These findings were consistent with Sydney classification clinical criteria, suggesting that APS laboratory testing was warranted (16).

Laboratory criteria for APS were assessed based on specific antibody testing, including LA, ACLA, aB2GP1 and ANA. The results showed a low prevalence of these antibodies among the study patients, with only a small percentage testing positive for LA, ACLA, aB2GP1, or ANA. (19)Therefore, the prevalence observed in this study does not represent the true prevalence of APS in the population. Of the 40 (11.6%) patients that had a positive ANA, 32 (9.28%) had titres of above 1/80, according to the 2019 EULAR/ACR classification of SLE such patients meet a part of the immunological criteria for diagnosis of SLE(20).The most common pattern was the speckled pattern which was in 38 patients, the other 2 patients had a nucleolar pattern.

The maternal outcomes of the pregnant women who underwent APS screening were generally favourable, with two patients getting transferred to the neighbouring Helen Joseph Hospital for further management of acute kidney injury. While a small proportion of patients experienced post-natal complications, such as retained placenta and primary postpartum haemorrhage, the overall maternal outcomes were reassuring.

The presence of ACL IgG and aB2GP1 IgG antibody, is in keeping with international studies that have shown that it is associated with a 3-fold increase in adverse pregnancy outcomes(21)

Fetal outcomes among the women screened for APS showed a diverse range of findings reflecting the indication for APS testing, a large proportion were stillbirths. In this study, antiB2GP1 IgG was the marker associated with the most stillbirths. This is a similar finding in a study by Silver et al, where they found that elevated antibeta2glcoprotein1 and cardiolipin levels were associated with threefold increased odds of stillbirth(22). Apgar scores, a measure of newborn health and vitality, demonstrated favourable results in general, indicating that most newborns were in good condition at birth.

This study has several limitations. First, the retrospective nature of the study and reliance on medical records resulted in missing or incomplete data. Second, the study focused on a specific population of pregnant women at a single healthcare facility, limiting the generalisability of the findings to broader populations. The study did not explore long-term maternal and neonatal outcomes, and further research is needed to assess the impact of APS on health beyond the immediate postpartum period.

Conclusion

Despite the challenges associated with diagnosing APS, this study emphasises the importance of screening for other conditions that may result in poor fetal and neonatal outcomes other than antiphospholipid syndrome. Preconceptual testing for APS should be advised for all those patients with positive aPLs but did not meet the criteria for diagnosis, such patients should be on prophylactic low dose aspirin to decrease the risk of thrombosis. Confirmatory testing outside of pregnancy is important as it would help diagnose or rule out APS. Further testing and assessment

to rule out SLE could have had longterm benefits for the patients who tested ANA positive.

REFERENCES

1. Mezhev V, Segan JD, Tran H, Cicuttini FM. Antiphospholipid syndrome: a clinical review. Vol. 211, Medical Journal of Australia. John Wiley and Sons Inc.; 2019. p. 184–8.
2. Arslan E, Branch DW. Antiphospholipid syndrome: Diagnosis and management in the obstetric patient. Vol. 64, Best Practice and Research: Clinical Obstetrics and Gynaecology. Bailliere Tindall Ltd; 2020. p. 31–40.
3. Alijotas-Reig J, Esteve-Valverde E, Anunciación-Llunell A, Marques-Soares J, Pardos-Gea J, Miró-Mur F. Pathogenesis, Diagnosis and Management of Obstetric Antiphospholipid Syndrome: A Comprehensive Review. Vol. 11, Journal of Clinical Medicine. MDPI; 2022.
4. Unlu O, Zuily S, Erkan D. The clinical significance of antiphospholipid antibodies in systemic lupus erythematosus. Eur J Rheumatol. 2016 Oct 6;3(2):75–84.
5. Fischer MJ, Rauch J, Levine JS. The Antiphospholipid Syndrome. Semin Nephrol. 2007 Jan;27(1):35–46.
6. Nayer A, Ortega LM. Catastrophic antiphospholipid syndrome: A clinical review. Vol. 3, Journal of Nephropathology. Society of Diabetic Nephropathy Prevention; 2014. p. 9–17.
7. Gardiner C, Hills J, MacHin SJ, Cohen H. Diagnosis of antiphospholipid syndrome in routine clinical practice. Lupus. 2013 Jan;22(1):18–25.
8. Clinical and prognostic significance of antinuclear antibodies in primary [Internet]. 2021. Available from: <https://www.elsevier.com/open-access/userlicense/1.0/>
9. Soh MC, Nelson-Piercy C. Antiphospholipid syndrome in pregnancy. Vol. 5, Expert Review of Obstetrics and Gynecology. 2010. p. 741–61.
10. Barbhaiya M, Zuily S, Naden R, Hendry A, Manneville F, Amigo MC, et al. The 2023 ACR/EULAR Antiphospholipid Syndrome Classification Criteria. Arthritis and Rheumatology. 2023 Oct 1;75(10):1687–702.
11. Pignatelli P, Ettorre E, Menichelli D, Pani A, Violi F, Pastori D. Seronegative antiphospholipid syndrome: Refining the value of “non-criteria” antibodies for diagnosis and clinical management. Vol. 105, Haematologica. Ferrata Storti Foundation; 2020. p. 562–72.
12. The Investigation and Treatment of Couples with Recurrent First-trimester and Second-trimester Miscarriage Green-top Guideline No. 17. 2011.
13. Hodkinson B, Mapiye D, Jayne D, Kalla A, Tiffin N, Okpechi I. The African Lupus Genetics Network (ALUGEN) registry: Standardized, prospective follow-up studies in African patients with systemic lupus erythematosus. Lupus. 2016 Mar 1;25(3):325–30.
14. Essouma M, Nkeck JR, Endomba FT, Bigna JJ, Singwe-Ngandeu M, Hachulla E. Systemic lupus erythematosus in Native sub-Saharan Africans: A systematic review and meta-analysis. J Autoimmun. 2020 Jan 1;106:102348.

15. Bera. Maternal Mortality and Morbidity Statistics. Johannesburg: Rahima Moosa Mother and Child Hospital; 2020.
16. Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *Journal of Thrombosis and Haemostasis*. 2006 Feb;4(2):295–306.
17. Khangura RK, Cooper S, Luo GY. Antiphospholipid antibody syndrome: Pathogenesis, diagnosis, and management in pregnancy. Vol. 1, *Maternal-Fetal Medicine*. Wolters Kluwer Health; 2019. p. 38–42.
18. Dabit JY, Valenzuela-Almada MO, Vallejo-Ramos S, Duarte-García A. Epidemiology of Antiphospholipid Syndrome in the General Population. 1926; Available from: <https://doi.org/10.1007/s11926-021-01038-2>
19. Vandavelde A, Devreese KMJ. Laboratory Diagnosis of Antiphospholipid Syndrome: Insights and Hindrances. Vol. 11, *Journal of Clinical Medicine*. MDPI; 2022.
20. Aringer M, Costenbader K, Daikh D, Brinks R, Mosca M, Ramsey-Goldman R, et al. 2019 European League Against Rheumatism/American College of Rheumatology Classification Criteria for Systemic Lupus Erythematosus. *Arthritis and Rheumatology*. 2019 Sep 1;71(9):1400–12.
21. Poulton K, Ripoll VM, Pericleous C, Meroni PL, Gerosa M, Ioannou Y, et al. Purified IgG from patients with obstetric but not IgG from non-obstetric antiphospholipid syndrome inhibit trophoblast invasion. *American Journal of Reproductive Immunology*. 2015 May 1;73(5):390–401.
22. Silver RM, Parker CB, Reddy UM, Goldenberg R, Coustan D, Dudley DJ, et al. Antiphospholipid antibodies in stillbirth. *Obstetrics and Gynecology*. 2013 Sep;122(3):641–57.

APPENDICES

Appendix A: Approved Research Protocol

Antiphospholipid syndrome screening results and associated obstetric outcomes at Rahima Moosa Mother and Child Hospital

Dr S S Hela

2427501

MMed (Obstetrics and Gynecology)

University of the Witwatersrand

Supervisor: Dr A Wise

Senior Consultant O&G

Sub-Specialist Maternal-Fetal Medicine

Research Co-Ordinator MMed O&G

Department of Obstetrics and Gynecology

Rahima Moosa Mother and Child Hospital

University of the Witwatersrand

Hennie Lombaard

MBChB(Pret) MMed(OetG)(Pret) PgDip(HSE)(Wits)

Professor

Maternal and Fetal Medicine

Department of Obstetrics and Gynecology
Pavilion For Women
Texas Children's Hospital

<u>Contents:</u>	Page
List of abbreviations	iii
1. Introduction	1
2. Aim	8
3. Study Objectives	8
4. Methods	9
5. Data analysis	10
6. Ethics	10
7. Timing	11
8. Funding	11
9. Problems	12
10. References	12

List of Abbreviations

aCL- Anticardiolipin

aGLP1- Antibeta2Glycoprotein1

ALUGEN- African Lupus Genetics Network

ANA- Antinuclear Antibodies

APS- Antiphospholipid syndrome

aPLs- Antiphospholipid Antibodies

CAPS- Catastrophic Antiphospholipid Syndrome

EULAR- European League Against Rheumatism

FGR- Fetal Growth Restriction

HELLP – Haemolysis, Elevated Liver enzymes, Low Platelets

LA- Lupus Anticoagulant

LMWH- Low Molecular Weight Heparin

NHLS – National Health Laboratory Service

RPL- Recurrent Pregnancy Loss

SGA- Small for Gestational Age

SLE- Systemic Lupus Erythematosus

UFH- Unfractionated Heparin

1. INTRODUCTION

Hypercoagulability is greatly increased during pregnancy and the puerperium; this is further exacerbated by any pathology that effects the endothelial system or coagulation cascade¹. Antiphospholipid syndrome (APS) is an autoimmune disease associated with venous and arterial thrombosis, adverse pregnancy outcomes that include recurrent early miscarriage, fetal death, early onset preeclampsia, and placental insufficiency². It is characterized by the presence of antiphospholipid (aPL) which are responsible for multiple thrombotic and inflammatory mechanisms which result in a wide range of vascular and obstetric manifestations³. A study on women with aPLs showed that the risk of thrombotic events increased by 24% during pregnancy and that pregnant patients with aPLs were at increased risk of developing venous thromboembolism (VTE) compared to the general population (5% vs 0.025-0.1%)⁴.

Antiphospholipid Syndrome can be classified as either primary (no concomitant autoimmune disorder) or secondary depending on the associated aetiology, a third subtype referred to as catastrophic APS (CAPS) also exists. Secondary APS occurs in association with other autoimmune diseases, most commonly systemic lupus erythematosus (SLE). Catastrophic Antiphospholipid Syndrome (CAPS) although rare is a life-threatening form resulting in multiorgan thrombosis. In the USA, women are reported to account for approximately 80% of patients with APS⁵. In women who have been diagnosed with APS, 24% of thrombotic events occurred during pregnancy or the puerperium period.

Antiphospholipid Syndrome is predominantly diagnosed in women of reproductive age (15-45)⁶. The RCOG data from the UK have shown women who have aPL, 15% are also found to have recurrent pregnancy loss and the live birth rate with no

pharmacological intervention has been reported to be as low as 10%⁷.

Epidemiological data of APS in Africa are limited, this is mostly due to under diagnosis and under reporting of APS due to multiple challenges. In general autoimmune diseases in Africa are not as widely researched and published. Systemic lupus erythematosus which is amongst one of the most recognized autoimmune diseases serves as a typical example of this. In 2015, the African Lupus Genetics Network (ALUGEN) designed a database looking at standardized, prospective studies on Africans with SLE over a period of at least 5 years. A higher incidence of SLE with severe features has been reported in people of African descent living in developed countries⁸. Studies also report a high incidence of severe SLE on the African continent itself and so the ALUGEN database with all its resources will inspire further academic curiosity into SLE in Africa⁹. The burden remains unknown in sub-Saharan countries, management is also challenging as there is no universal health coverage, thus making the process of investigating too expensive¹⁰.

The most common clinical presentations of APS include venous thromboembolism, stroke, recurrent early miscarriages (1st trimester) and late pregnancy losses (2nd & 3rd trimester). APS sometimes causes thromboses in areas that are not frequently affected by prothrombotic states such as the adrenal gland, retina and skin. In utero it affects the placenta leading to a deficient vascular bed and abnormal placentation with resultant reduced placental flow, fetal growth restriction (FGR) and HELLP syndrome. The renal system gets affected secondary to occlusion of renal blood vessels and immune complex deposition from SLE in secondary APS. The commonest feature of arterial thrombosis is transient ischemic attack, a survey in the USA showed that the rate of cerebrovascular accidents was 34.2 per 100,000 deliveries¹¹.

The link between aPL and preeclampsia is not yet fully understood, studies have shown 32-50% of patients with SLE and previous thrombosis do develop gestational hypertension/preeclampsia¹¹. Preeclampsia in patients with APS generally occurs early (<34weeks) and is more severe, this is associated with the presence of aCL and LA ¹². The risk of developing pregnancy induced hypertension and preeclampsia are greatly increased when there's more than one type of aPL present¹³. A syndrome of Haemolysis, Elevated Liver enzymes, Low Platelet count (HELLP syndrome) is also reported to occur more in patients with APS, the onset is usually in the second trimester and may worsen for up to three weeks postpartum.

Fetal loss is the commonest early complication related to the fetus, it results from impaired trophoblastic development and poor development of the fetoplacental circulation. Due to damaged uteroplacental vasculature and placental abruption, an increased incidence of intrauterine death and stillbirth has been reported in patients with APS .

The Global Antiphospholipid Syndrome Score is used to profile aPLs according type, titer, number aPLs present and persistence, this helps to determine the risk of thrombosis and obstetric adverse events, and subsequently the intensity of treatment. However, the presence of aPLs does not confirm diagnosis of APS but it does increase the risk thrombosis and, morbidity during pregnancy depending on the aPLs profile. Lupus anticoagulant is strongly associated with late fetal loss (>10weeks gestation)²³. Another study also showed that positive anticardiolipin was associated with first trimester losses and fetal growth restriction (FGR)¹⁴. A study by Yamada et al demonstrated that aPLs were associated with low birth weight and preterm labour, the risk increases with the number and types of aPLs present¹³.

Antiphospholipid antibodies are common in patients with SLE and are associated with 50% risk of thromboembolism, hence why in most institutions ANA (Antinuclear antibodies) are also tested when APS screen is being done. Antinuclear antibodies are also associated with adverse pregnancy outcomes including recurrent pregnancy loss, endometrioses, infertility, IVF failure and ovarian dysfunction¹⁵. A study in France has shown that both aPL and ANA have also been found in patients undergoing IVF for infertility, but it is still debatable whether they are responsible for the actual infertility¹⁶.

In 2006, an International Consensus Statement was made on an update of classification criteria for definite APS, from the previously used Sapporo Classification¹⁷. The committee concluded that APS patients should be classified according to whether inherited or acquired causes of thrombosis were present or not. Researchers should not classify APS if the period separating the clinical event and the positive laboratory results is more than 5 years. Thus, the widely used Sydney Classification came into effect. Antiphospholipid syndrome is present if at least one of the clinical criteria and one of the laboratory criteria is met.

Clinical Criteria	Laboratory Criteria
1. Vascular thrombosis 1 or more clinical episodes of thrombosis confirmed by imaging studies or histopathology. 2. Pregnancy morbidity (a) 1 or more unexplained deaths of a morphologically (by u/s or clinical evaluation) normal fetus at or beyond the 10th week of gestation. (b) 1 or more premature births of a morphologically normal neonate <34th week of gestation due to: (i)	1. LA present in plasma, on two or more occasions at least 12 weeks apart 2. aCL antibody of IgG and/or IgM isotype in serum or plasma, present in medium or high titer (i.e. >40 GPL or MPL, or >the 99th percentile), on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA 3. Anti-b2 glycoprotein-I antibody of IgG and/or IgM isotype in serum or plasma (in titer >the 99th percentile), present on two or more occasions, at

eclampsia or severe preeclampsia (ii) recognized features of placental insufficiency (c) 3 or more unexplained consecutive spontaneous abortions <10th week of gestation, with all other maternal or paternal anatomic, hormonal chromosomal causes excluded.	least 12 weeks apart, measured by a standardized ELISA ^{xviii} .
--	---

Preconceptual counselling is of vital importance when managing patients with APS, they require extensive education and monitoring by a multidisciplinary team. The counselling should include the risk of fetal loss, the risk of preeclampsia and HELLP syndrome, risk of thromboses, the need for anticoagulation, the risk of giving birth to a SGA or FGR and the likelihood of preterm delivery¹⁸. Patients with a previous obstetric APS should be reassured that the recurrence risk is very low with treatment and close surveillance. Thrombotic APS appears to have a poorer prognosis compared to obstetric APS with a higher incidence of preterm deliveries, SGA and preeclampsia, with thromboembolic events occurring in 4.9% patients¹⁹. Those who have had a previous catastrophic APS should be advised not to fall pregnant again even though there is no data about the risk of recurrent disease. Those with a concurrent active SLE should also be advised against conceiving until the disease has been inactive for at least six months¹⁹. Systemic lupus erythematosus, even despite advances in treatment is still associated with a mortality rate 2,4 -5,9%²⁰. Studies have shown that such patients may benefit from antimalarial drugs like chloroquine as it may reduce flares and the risk of congenital heart block²¹.

Patients with APS should be seen at least every 4–6 weeks for preeclampsia screening, fetal growth monitoring after 20 weeks and maternal monitoring. Early

ultrasound and fetal anomaly scans are recommended, those who have a notched waveform on the uterine artery on sonar should have closer surveillance with regular growth scans. Patients receiving anticoagulants must be assessed for both thrombotic and bleeding problems²². Such patients should also be made aware of the teratogenicity of warfarin and importance of monitoring their menstrual cycle. With the current recommended guidelines, the likelihood of a successful pregnancy in a woman diagnosed with APS is about 70%. The combination of a heparin agent and low dose aspirin (LDA) is the current recommended treatment because it provides thromboprophylaxis and improves pregnancy outcomes. LDA should be started preconceptionally because of the positive effects on implantation, as well as its antiplatelet properties, a baseline platelet count must be sought before commencing. Heparin is usually begun early first trimester after confirming the viability of the pregnancy. Most patients with APS are maintained on long-term anticoagulation, frequently warfarin, so to minimize the risk of warfarin embryopathy, these patients should be transitioned from warfarin to a heparin either prior or very early in pregnancy²⁵. Women with APS diagnosed on the basis of an obstetric criteria but with no history of thrombosis should be on prophylactic dose heparin and LDA. There is controversy surrounding the use of heparin in pregnancy, four heparin trials were conducted in women diagnosed with APS, in two of these the proportion of successful pregnancies substantially improved with the addition of unfractionated heparin (UFH) to LDA. The other two trials used low molecular weight heparin (LMWH) and did not find a significant benefit when combined with LDA, this is primarily because the live birth rates with LDA was already good (70-75%)²⁶. Another two studies compared UFH and LMWH, each paired with LDA and found no difference in outcomes. Treatment to prevent adverse pregnancy outcomes in with

APS and fetal death (>10 weeks gestation) or previous early delivery (<34 weeks) due to severe preeclampsia or placental insufficiency has not been established, although heparin is initiated on such patients, professional guidelines have been reluctant in recommending its use for prevention of these later adverse pregnancy outcomes. Women with repeatedly positive aPLs with no prior history of thrombosis are at a high risk of developing pregnancy associated thrombosis so prophylactic UFH or LMWH is recommended in such cases. Also, in the absence of thrombosis in women with history of RPL, fetal death or early preterm birth due to severe preeclampsia or placental insufficiency should be given a choice in terms of treatment since prophylactic UFH or LMWH regimens are rarely associated with serious side effects such as osteopenia, bleeding or heparin induced thrombocytopenia. Postpartum thromboprophylaxis with UFH, LMWH or warfarin should be strongly considered in all women with APS and is absolutely indicated in women with prior thrombosis, most of whom will restart their long-term anticoagulation regimen²⁷. In those with no previous thrombosis, treatment is for six weeks post-delivery, both heparin and warfarin are safe for breastfeeding mothers. In patients with obstetric APS and without prior thrombosis, data suggests that long term LDA may decrease the risk of an initial thrombotic event¹. Low dose aspirin and LMWH could also benefit those patients with a specific autoimmunity i.e., those with positive autoantibodies without clinical or biological criteria for defined disease²⁸.

2. AIM

The aim of this study is to describe the diagnosis, treatment and outcomes of patients who were investigated for APS at Rahima Moosa Mother and Child Hospital, Johannesburg, South Africa between 1 July 2019 and 31 December 2019

3. STUDY OBJECTIVES

1. To determine the number of patients that had bloods drawn for at least two or more of ACLA, ANA, B2GP1 and LA between 1 July 2019 and 31 December 2019 at RMMCH
2. To describe the demographics and history of the included patients
3. To determine the number of women of the sample who have APS according to the Sydney criteria
4. To describe the obstetric outcomes of those who have been investigated
5. To describe the short-term neonatal outcomes (from birth until discharge from the Hospital) of those included in the sample
6. To describe the treatment regimens received by patients

4. METHODS

Type: Retrospective case control study

Site: Rahima Moosa Mother and Child Hospital, located in Coronationville, Johannesburg is a 146-bed provincial Hospital, it serves the communities of Newclare, Noordgesig, Sophiatown, Cosmo City, Coronation Ville and Diepsloot with both maternal and paediatric care. In 2019, there were a total of 13 957 deliveries of 14 199 neonates, about 5% of the patients who delivered had a hypertensive disorder. The NHLS at RMMCH conducts on

average 65-70 APS tests a month, almost all of these are conducted on new patients.

Inclusion criteria: All women who were tested for at least two of the following: ACLA, B2GP1, LA and ANA and who were pregnant in the time period 1 July 2019 to 31 December 2019.

Exclusion criteria: Nil

Sample size: the expected sample is 180 patients over a period six months

Data collection: the process will commence as soon as the proposal has been approved by the ethics committee. A list of all patients who fit the inclusion criteria will be requested from the NHLS. These files will be collected and analyzed as per the objectives. A data sheet will be used to record the following: demographics, obstetric, medical and surgical history, serology results, treatment received and short-term maternal and neonatal outcomes. The data will be entered into REDcap database, PID 13812.

Bias: Selection bias may occur as the study is based on one institution that serves patients of a specific demographic and socio-economic status. The testing may not have been uniform according to the Sydney criteria and may reflect the individual clinician's approach.

5.DATA ANALYSIS

Objective 1: The frequency of each test with the number of viable deliveries as a denominator

Objective 2: The demographics and history will be described with frequencies and percentages and if appropriate associations will be looked for between those with and without confirmed APS.

Objective 3: Number of women from the total sample with APS per the Sydney criteria expressed as a percentage

Objective 4: The obstetric outcomes of the sample will be described as means and percentages. The outcomes of those who were diagnosed with APS will be compared to those who were negative by means of a T-test or Fishers exact test.

Objective 5: The neonatal outcomes will be described as means and percentages, and the outcomes of neonates of mothers with and without confirmed APS will be compared with 2x2 tables.

Objective 6: The varying regimens will be described as percentages and means, the association with the outcomes will be described if possible, with 2x2 tables.

6.ETHICS AND PERMISSIONS

Permission will be requested from the NHLS to access the results for that time period. Permission from the CEO at RMMCH will be sought to conduct the study. The study will be registered with the NHRD.

Ethics submission to the WITS HREC will be done as soon as the proposal is approved Post Graduation Committee. All personal information or potential patient identifiers will be removed prior to analysis. A separate patient log will be kept to maintain anonymity.

7. Timing

	Sept 2020	Oct 2020	Nov2020	Dec 2020	Jan 2021	Feb 2021	Mar 2021	Apr 2021	May 2021	Jun 2021	Jul 2021	Aug 2021	Sep 2021	Oct 2021	Nov 2021	Dec 2021	Jan 2022
Literature review																	
Preparing protocol																	
Protocol assessment																	
Ethics application																	
Collecting data																	
Data analysis																	
Writing up																	
Submission																	

8. FUNDING

Transport	R1000 from my own budget as I will be based in another institution during data collection
Stationery	Available (pens, paper)
Laptop and internet	Available, Wi-Fi and a laptop at home
Publishing	R6000 on a reputable journal
Data Analysis	A statistician will be needed to help with data analysis and interpretation
Conference costs	R7000

REFERENCES:

- May Ching Soh, Catherine Nelson -Percy. 2010. Antiphospholipid Syndrome in Pregnancy. *Expert Rev. Obstet. Gynecol.* 5(6): 741-761. DOI:10.1586/eog.10.57 (Accessed 22/10/2020)
- ² Gabbe S G, Niebyl J R, Simpson J L, Landon M, Galan H, Janiaux E et al. *Obstetrics: Normal and Problem Pregnancies*. 7th edition. Philadelphia, PA. Elsevier. 2017 (Accessed – 14/09/2020)
- ³ Michal J S, Mordechai d, Rofo G, Shani H, Langevitz P, Schiff E et al. 2011. High Positive antibody titers in women with Antiphospholipid Syndrome. *ACTA Obstetrics et Gynaecologica*. 1428-1433. DOI: 10.1111/j.1600-0412.2011.01236 (Accessed – 01/11/2020)
- ⁴ Tektonidou M.G, Andreolli L, Limper M, Amoura Z, Cervera R, Cuadrado M.J et al. 2019. EULAR recommendations for the management of antiphospholipid syndrome in adults. *Ann Rheum Dis* 2019, 78:1296-1304. DOI: 10.1136 (Accessed – 21/10/2020)
- ⁵ Lockshin MD. Antiphospholipid antibody. Babies, blood clots, biology. *JAMA*. 1997 May 21;277(19):1549-51. doi: 10.1001/jama.277.19.1549. PMID: 9153370 (Accessed - 17/09/2020)
- ⁶ Teresa G Berg 2020. Antiphospholipid Syndrome and Pregnancy. eMedicine Medscape article 261691. <https://emedicine.medscape.com/article/261691-overview> (Accessed - 26/09/2020)
- ⁷ Regan L, Backos M, Rai R. 2011 The Investigations and treatment of Couples with Recurrent First trimester and second-trimester Miscarriage. RCOG Green-Top Guideline No. 17 (Accessed – 21/10/2020)
- ⁸ Lau C S, Yin G, Mok MY.2006. Ethnic and geographical differences in systemic lupus erythematosus: An overview. *Lupus*. 15: 715-719 DOI: 10.1177/0961203306072311 (accessed – 26/09/2020)
- ⁹ Hodkinson B, Mapiye D, Jayne D, Kalla A, Tiffin N, Okpechi I. 2016. The African Lupus Genetics Network registry: standardized, prospective follow-up studies in African patients with systemic lupus erythematosus. *Lupus* 25:325-330. DOI 10.1177/0961203315606984 (accessed – 05/11/2020)
- ¹⁰ Becker A C, Libhaber E, Sliwa K, Singh S. 2011. Antiphospholipid antibodies black south africans with HIV and Acute coronary syndrome: prevalence and clinical correlates. *BMC Research Notes*. 4:379. DOI:10.1186/1756-0500-4-379 (accessed – 12/09/2020)
- ¹ Branch D, Khamashta M. 2003. Antiphospholipid syndrome: obstetric diagnosis, management and controversies. *Obstet. Gynecol.* 101(6), 1333-1344. DOI: 10.1016/s0029-7844(03)00363-6 (accessed – 02/07/2020)
- ¹² Galli M, Luciani D, Bertolini G, Barbui T. 2003. Lupus anticoagulants are stronger risk factors for thrombosis than anticardiolipin antibodies in the antiphospholipid syndrome: a systemic review. *Blood* 101(5),1827-1832. DOI:10.1182/blood-2002-02-0441 (accessed – 06/08/2020)
- ¹³ Yamada H, Atsumi T, Kobashi G, Chikako O, Kato E, Tsuruga N et al.2009. Antiphospholipid antibodies increase the risk of hypertension and adverse pregnancy outcomes. *J. Reprod. Immunol.* 79(2), 188-195. DOI: 10.1016/j.jr.2008.11.001 (accessed - 21/06/2020)
- ¹⁴ Opatryn L, David M, Kahn SR, Shrier I, Rey E.2006. Association between antiphospholipid antibodies and recurrent fetal loss in women without autoimmune disease: a metanalysis. *J. Rheumatol.* 33(11), 2214-2221 (accessed – 05/06/2020)
- ¹⁵ Zhu Q, Wu L, Xu B, Hu MH, Tong XH, Ji JJ et al. 2013. A retrospective study on IVF and ICSI outcomes in patients with antinuclear antibodies: The effects of prednisone plus low dose aspirin adjuvant treatment. *Reproductive biology and Endocrinology Journal*. 11:18. doi: 10.1186/1477-7827-11-98 (accessed – 03/09/2020)

- ¹⁶ Deroux A. 2016. Female infertility and serum autoantibodies: A systemic Review. *Clinical Reviews in Allergies and Immunology*. 53:78-86. DOI: 10.1007/512016-016-8586-2 (accessed – 05/08/2020)
- ¹⁷ Miyakis S, Lockshin D, Atsumi T, Branch D W, Brey R L, Cervera R, et al. 2006. International Consensus statement on an update of the classification criteria for definite antiphospholipid syndrome. *Journal of Thrombosis and Haemostasis*. 4: 295-396 DOI: 10.1111/j.1538-7836.2006. 01753.x (accessed – 21/09/2020)
- ¹⁹ Schreiber K, Beverley J. 2016. Pregnancy and Antiphospholipid Syndrome. *Seminars in Thrombosis and Haemostasis*. 42(07):780-788 DOI: 10.1055/s-0036-1592336 (accessed – 12/10/2020)
- ²⁰ Durcan L. 2019. Management strategies and future directions of Systemic Lupus Erythmatosus. *The Lancet*. 393: 2332-43. doi: 10.1016/S0140-6736(19)30237-5 (accessed -11/10/2020)
- ²¹ Hodkinson B, Makda M. 2015. Evidence based treatment of Systemic Lupus Erythmatosus and its complications. *SAMJ*. 105 (12): 1075 DOI 10.7196 (accessed – 16/10/2020)
- ²² Honer H. 2018. Modern Management of recurrent miscarriage. *Australia and New Zealand Journal of Obstetrics and Gynaecology*. 59:36-44. DOI:10111/ajo.1290 (accessed – 02/11/20200)
- ²³ Favaloro EJ, Wong RC, Silvestrini R, McEvoy R, Jovanovich S, Roberts-Thomson P. 2005. A multilaboratory peer assessment quality assurance program-based evaluation of anticardiolipin antibody, and b2- glycoprotein I antibody testing. *Semin. Thromb. Hemost.* 31(1), 73–84. (accessed – 11/11/2020)
- ²⁴ Shehata HA, Nelson-Piercy C, Khamashta MA. 2001. Management of pregnancy in antiphospholipid syndrome. *Rheum. Dis. Clin. North Am.* 27(3), 643–659. (accessed -21/11/2020)
- ²⁵ Caloris SD, Botha A, Santucci S, Garofalo S, Martino C, Perrelli A et al. 2010. Predictors of pregnancy outcome in antiphospholipid syndrome: A Review. *Clin Rev Allerg Immunol*. 38 116-124. DOI 10.1007/s12016-009-8144 (accessed – 05/11/2020)
- ²⁶ Appenzeller S, Souza F, Wagner A, Shoenfeld Y, Carvalho J. 2011. HELLP syndrome and its relationship with antiphospholipid syndrome and antiphospholipid antibodies: A Review. *Semin Arthritis Rheum*. 41 (3): 517-23. DOI 10.1016/j.semarthrit.2011.05.007 (accessed- 20/11/2020)
- ²⁷ Munoz-Rodrigues F, Font J, Cervera R, Reverter J C, Tassies D, Espinosa G et al. 1999. Clinical study and follow up of 100 patients with Antiphospholipid Syndrome. *Semin Arthritis Rheum*. 29 (3): 182-90. DOI 10.1016/s0049-0172(99)80029-8 (accessed- 04/11/2020)
- ²⁸ Bates S M, Middeldorp S, Roger M, James A H, Greer I. 2016. Guidance for the treatment and prevention of obstetric associated venous thromboembolism. *J Thromb Thrombolysis*. 41 (60): 92-128. DOI 10.1007/s11239-015-1309-0 (accessed 21/11/2020)

Appendix B: Data collection sheet

DATA COLLECTION SHEET

First Names	_____
Last Name	_____
Hospital Number	_____
Date of birth	_____
Age (years)	_____
Race:	Black Asian Colored Mixed Race White Other Unknown Not Reported
Booking Clinic:	RMMCH Diepsloot South Westbury Bosmont OR Tambo Randburg Parkhurst Other

History

Booking date _____

Parity

Gravidity

Obstetric outcome history

1stT

2ndT IUD<26

2ndT live<26

IUD>26

END>26

LND>26

live26-37

live>37

Medical history

Any medical condition: Yes/No

Medical condition: Diabetes
Epilepsy
Asthma
Cardiac
TB
Hypertension
Connective tissue disorder
Thyroid
Other

Current hypertension diagnosis: Chronic hypertension

Current Antihypertensive medication	Methyl-dopa	Amlodipine	Other

Lupus Anticoagulant Present: Yes/No

Anticardiolipin: IgM/ IgG or both

Titer-

AntiBeta2Glycoprotein: IgM /IgG or both

Titer-

Indication for testing

Treatment

ASA: Yes/ No

If yes, dosage

LMWH: Yes/No

If yes, dosage

Chloroquine: Yes/No

If yes, dosage

Other

Delivery

Date of Delivery

Mode of Delivery NVD

C/S

Other

Maternal post-natal details

Were there any post-natal complications Yes/No

Post-natal complications Retained placenta
 Genital tract trauma
 PPH < 24h post-delivery
 PPH>24h post-delivery
 Evacuation in theatre
 Laparotomy
 Subtotal hysterectomy
 Hysterectomy
 Genital tract sepsis
 Non-genital tract sepsis
 Other

Was the mother: discharged
 transferred
 signed refusal of hospital treatment

demised

When was that? _____

Follow up plan for mom _____

Baby's outcome

Time of delivery _____

At delivery baby was : alive
 FSB
 MSB

APGAR Scores _____

Did the baby receive: No resuscitation required Stimulation
 Bag mask ventilation
 Chest compressions
 Intubation
 Ventilation
 Other

If baby received 'other' resuscitation - specify _____

Cord gas (arterial)

Was a cord blood gas done Yes/No

pH _____

pO₂ _____

(mmHg)

pCO₂ _____

(mmHg)

Lactate _____

HCO₃ _____

(mmol/l)

BE _____

(mmol/l)

Hb _____

(g/dl)

glucose _____

(mmol/l)

After delivery baby was sent to: the post-natal ward with mum
 neonatal ward as lodger
 neonatal ward 16B
 neonatal high care/neonatal icu

Reason for admission (highest level in 1st 24h) : went with mum
 Low Birth Weight - for weight gain
 Suspected sepsis
 Premature
 Needed nasal prong O₂
 Needed C-PAP
 Needed Intubation and Ventilation
 Inotropic support
 Cooling
 Other

Specify other reason for admission _____

Appendix C: Permission to conduct study



OBSTETRICS AND GYNAECOLOGY
School of Clinical Medicine

11-11-2021

To whom it may concern

Dr Hela has the support of the department and myself to do his study entitled 'Antiphospholipid syndrome screening results and associated obstetric outcomes at Rahima Moosa Mother and Child Hospital.

Regards

A handwritten signature in black ink, appearing to read 'Amy Wise', with a horizontal line underneath.

Dr Amy Wise

Acting HOD Obstetrics and Gynaecology

Rahima Moosa Mother and Child Hospital

Senior Lecturer

University of the Witwatersrand

Faculty of Health Sciences

Johannesburg Hospital | Private Bag X39, Johannesburg, South Africa | T +27 11 488 3179 | F +27 11 643 2522 | www.wits.ac.za

Appendix D: Certificate: Introduction to Research Ethics



Zertifikat Certificat

Certificado Certificate

Promouvoir les plus hauts standards éthiques dans la protection des participants à la recherche biomédicale
Promoting the highest ethical standards in the protection of biomedical research participants



Certificat de formation - Training Certificate

Ce document atteste que - this document certifies that

Simamkele Hela

a complété avec succès - has successfully completed

Introduction to Research Ethics

du programme de formation TRREE en évaluation éthique de la recherche
of the TRREE training programme in research ethics evaluation

Release Date: 2021/04/07
CID : 6wPPyjmz



Professeur Dominique Sprumont
Coordinateur TRREE Coordinator



Continuing Education Program (5 Credits)
Programme de Formation continue (5 Crédits)



Continuing Education Program
Programmes de formation continue

Ce programme est soutenu par - This program is supported by :

European and Developing Countries Clinical Trials Partnership (EDCTP) (www.edctp.org) - Swiss National Science Foundation (www.snf.ch) - Canadian Institutes of Health Research (<http://www.cihr-irsc.gc.ca/2091.html>) - Swiss Academy of Medical Science (SAMS/ASSMSAMW) (www.sams.ch) - Commission for Research Partnerships with Developing Countries (www.kdpc.ch)

[REV : 20170310]

Appendix E: Adherence to HREC

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG



HUMAN RESEARCH
ETHICS COMMITTEE
(MEDICAL)

DECLARATION:

Adherence to HREC (Medical) Ethics Application Terms and Conditions

I, the undersigned, hereby declare that I have not collected data/ done secondary data analysis or any other form of research, prior to obtaining clearance certificate from the HREC (Medical) for study no: M210554.

I have read and understood the terms and conditions on section 9 of HREC (Medical) application form. I confirm that it is my responsibility to ensure that I have received final HREC (Medical) Clearance before commencing my research.

Simamkele Siviwe Hela
2427501
11/11/2021
Name, Surname and Signature
Student/Staff no if applicable: _____

Date: _____

Dr Amy Wise
Name, Surname and
Signature Supervisor (if
applicable)
11-11-2021
Date: _____

Appendix F: Ethics clearance certificate

A

Appendix G: Permission letter from NHLS



Academic Affairs and Research
Modderfontein Road, Sandringham, 2031
Tel: +27 (0)11 555 0367
Fax: +27 (0)11 386 6296
Email: academic.research@nhls.ac.za
Web: www.nhls.ac.za

25 October 2021

Applicant: Simamkele Hela
Institution: University of the Witwatersrand
E-mail Address: simahela@gmail.com
Tel: 0635431939 **Cell:** 0635431939

CC:

Project Title: Antiphospholipid syndrome screening results and associated obstetric outcomes at Rahima Moosa Mother and Child Hospital

Reference Number: PR2116810

Application Type(s):

1. Request for Data

RE: APPROVAL LETTER: REQUEST TO CONDUCT RESEARCH

This letter serves to advise that an application requesting permission to conduct research has been reviewed and was **"Approved"**. Please note that approval is granted on your compliance with the NHLS conditions of service and that the study can only be undertaken provided that the following conditions have been met.

1. All material and data requested should be in accordance with the research protocol submitted and as approved by the relevant Health Research Ethics Committee (HREC) in South Africa.
2. Access to NHLS material and/or data will be limited to the minimum required for successful completion of the approved study and will be made available to you **without patient names**.
3. Material and/or data obtained from the NHLS shall be used anonymously and shall not for any reason be used to track or recruit patients, as no pre-approval/consent is obtained from patients.
4. Processes are discussed with the relevant NHLS departments (i.e. Corporate Data Warehouse (CDW), NHLS Laboratory Management, and Operations Office, etc.) and are agreed upon.
5. Any changes and extensions to the study requirements should be submitted for approval by an approved HREC an application submitted on the AARMS system – <https://aarms.nhls.ac.za>.
6. Confidentiality is maintained at participant and institutional level and there is no disclosure of personal information or confidential information as described by NHLS policies.
7. The NHLS is indicted as a source of material and/or data requested in the original protocol that was approved by the (HREC) as NHLS does not have an institutional HREC.
8. A final report of the research study and any published paper resulting from this study are submitted to the NHLS AARMS or by email to academic.research@nhls.ac.za and that the NHLS has been acknowledged appropriately.

Please note that this letter constitutes approval by the NHLS Academic Affairs and Research Office. Data related queries may be directed to NHLS CDW, email: zarina.sabat@nhls.ac.za; contact number: 011 386 6074 and sample related queries (if applicable) shall be directed to the relevant business manager.

Physical Address: 1 Modderfontein Road, Sandringham, Johannesburg, South Africa
Postal Address: Private Bag X8, Sandringham, 2131, South Africa
Tel: +27 (0) 11 386 6000/ 0860 00 NHLS(6457) www.nhls.ac.za
Chairperson: Prof Eric Buch CEO: Dr Karmani Chetty
Practice number: 5200296



**NATIONAL HEALTH
LABORATORY SERVICE**

Academic Affairs and Research

Modderfontein Road, Sandringham, 2031

Tel: +27 (0)11 555 0367

Fax: +27 (0)11 386 6296

Email: academic.research@nhls.ac.za

Web: www.nhls.ac.za

Yours sincerely,

Dr Babatyi Malope-Kgokong
National Manager: AAR

Chairperson: Prof Eric Buch CEO: Dr Karmani Chetty
Physical Address: 1 Modderfontein Road, Sandringham, Johannesburg, South Africa Postal Address: Private Bag X8, Sandringham, 2131, South Africa
Tel: +27 (0) 11 386 6000/ 0860 00 NHLS(6457) www.nhls.ac.za
Practice number: 5200296

Appendix H: Journal guidelines for SAMJ

Author Guidelines

If authors' names are added or deleted after submission of an article, or the order of the names is changed, all authors must agree to this in writing.

Please note that co-authors will be requested to verify their contribution upon submission. Non-verification may lead to delays in the processing of submissions.

Author contributions should be listed/described in the manuscript.

Conflicts of interest

Conflicts of interest can derive from any kind of relationship or association that may influence authors' or reviewers' opinions about the subject matter of a paper. The existence of a conflict – whether actual, perceived or potential – does not preclude publication of an article. However, we aim to ensure that, in such cases, readers have all the information they need to enable them to make an informed assessment about a publication's message and conclusions. We require that both authors and reviewers declare all sources of support for their research, any personal or financial relationships (including honoraria, speaking fees, gifts received, etc) with relevant individuals or organisations connected to the topic of the paper, and any association with a product or subject that may constitute a real, perceived or potential conflict of interest. If you are unsure whether a specific relationship constitutes a conflict, please contact the editorial team for advice. If a conflict remains undisclosed and is later brought to the attention of the editorial team, it will be considered a serious issue prompting an investigation with the possibility of retraction.

Research ethics committee approval

Authors must provide evidence of Research Ethics Committee approval of the research where relevant. Ensure the correct, full ethics committee name and reference number is included in the manuscript.

If the study was carried out using data from provincial healthcare facilities, or required active data collection through facility visits or staff interviews, approval should be sought from the relevant provincial authorities. For South African authors, please refer to the guidelines for submission to the National Health Research Database. Research involving human subjects must be conducted according to the principles outlined in the Declaration of Helsinki. Please refer to the National Department of Health's guideline on Ethics in Health research: principles, processes and structures to ensure that the appropriate requirements for conducting research have been met, and that the HPCSA's General Ethical Guidelines for Health Researchers have been adhered to.

Clinical trials

As per the recommendations published by the International Committee of Medical Journal Editors (ICMJE), clinical trial research is any research that assigns individuals to an intervention, with or without a concurrent comparison/control group to study the cause-and-effect relationship between the intervention and health outcomes. All clinical trials should be registered with the appropriate national clinical trial registry (or any international primary register, if relevant), and the trial registration number should be cited at the end of the abstract. All clinical trial reports must also contain a data sharing statement as per the recommendations of the ICMJE. Statements are to indicate:

whether individual deidentified participant data will be shared;

what data in particular will be shared; whether additional, related documents will be available;
when the data will become available and for how long; by what access criteria data will be shared.

Please see the ICJME announcement for further details and illustrative examples of data sharing statements: [ICMJE Data Sharing Statements for Clinical Trials](#)

Since 1st December 2005, all clinical trials conducted in South Africa have been required to be registered in the South African National Clinical Trials Register. The SAMJ therefore requires that clinical trials be registered in the relevant public trials registry at or before the time of first patient enrollment as a condition for publication. The trial registry name and registration number must be included in the manuscript.

Please refer to the general guidelines for all papers at the top of this article for additional requirements with respect to ethics approval, funding, author contributions, etc. The format of original research articles should be followed for reporting of clinical trial results.

Patient Consent

Information that would enable identification of individual patients should not be published in written descriptions, photographs, and pedigrees unless the information is essential for scientific purposes and the patient (or parent or guardian) has given informed written consent for publication and distribution. We further recommend that the published article is disseminated not only to the involved researchers but also to the patients/participants from whom the data was drawn. Refer to Protection of Research Participants. The signed consent form should be submitted with the manuscript to enable verification by the editorial team.

Other individuals

Any individual who is identifiable in an image must provide written agreement that the image may be used in that context in the *SAMJ*.

Copyright notice

Copyright remains in the Author's name. The work is licensed under a Creative Commons Attribution - Noncommercial Works License.

Material submitted for publication in the *SAMJ* is accepted provided it has not been published or submitted for publication elsewhere. Please inform the editorial team if the main findings of your paper have been presented at a conference and published in abstract form, to avoid copyright infringement. All research already published as 'Conference proceedings' needs to be substantially re-written, with a new title, a new abstract and new and important results to back up any study before it will be considered for a new publication. The *SAMJ* does not hold itself responsible for statements made by the authors.

Previously published images

If an image/figure has been previously published, permission to reproduce or alter it must be obtained by the authors from the original publisher and the figure legend must give full credit to the original source. This credit should be accompanied by a letter indicating that permission to reproduce the image has been granted to the author/s. This letter should be uploaded as a supplementary file during submission.

Privacy statement

The *SAMJ* is committed to protecting the privacy of its website and submission system users. The names, personal particulars and email addresses entered in the website or submission system will not be made available to third parties without the user's permission

or due process. By registering to use the website or submission system, users consent to receive communication from the *SAMJ* or its publisher SAMA on matters relating to the journal or associated publications. Queries with regard to privacy may be directed to publishing@hmpg.co.za.

Ethnic/race classification

Use of racial or ethnicity classifications in research is fraught with problems. If you choose to use a research design that involves classification of participants based on race or ethnicity, or discuss issues with reference to such classifications, please ensure that you include a detailed rationale for doing so, ensure that the categories you describe are carefully defined, and that socioeconomic, cultural and lifestyle variables that may underlie perceived racial disparities are appropriately controlled for. Please also clearly specify whether race or ethnicity is classified as reported by the patient (self-identifying) or as perceived by the investigators. Please note that is not appropriate to use self-reported or investigator-assigned racial or ethnic categories for genetic studies.

Continuing Professional Development (CPD)

SAMJ is an HPCSA-accredited service provider of CPD materials. Principal authors can earn up to 15 CPD continuing education units (CEUs) for publishing an article; co-authors are eligible to earn up to 5 CEUs; and reviewers of articles can earn 3 CEUs. Each month, *SAMJ* also publishes a CPD-accredited questionnaire relating to the academic content of the journal. Successful completion of the questionnaire with a pass rate of 70% will earn the reader 3 CEUs. Administration of our CPD programme is managed by Medical Practice Consulting. To complete questionnaires and obtain certificates, please visit MRP Consulting

Manuscript preparation

Preparing an article for anonymous review

To ensure a fair and unbiased review process, all submissions are to include an anonymised version of the manuscript. The exceptions to this are Correspondence, Book reviews and Obituary submissions.

Submitting a manuscript that needs additional blinding can slow down your review process, so please be sure to follow these simple guidelines as much as possible:

An anonymous version should not contain any author, affiliation or particular institutional details that will enable identification.

Please remove title page, acknowledgements, contact details, funding grants to a named person, and any running headers of author names.

Mask self-citations by referring to your own work in third person.

General article format/layout

Accepted manuscripts that are not in the correct format specified in these guidelines will be returned to the author(s) for correction, which will delay publication.

General:

Manuscripts must be written in UK English.

The manuscript must be in Microsoft Word format. Text must be single-spaced, in 12-point Times New Roman font, and contain no unnecessary formatting (such as text in boxes).

Please make your article concise, even if it is below the word limit.

Qualifications, **full** affiliation (department, school/faculty, institution, city, country) and contact details of ALL authors must be provided in the manuscript and in the online submission process.

Abbreviations should be spelt out when first used and thereafter used consistently, e.g. 'intravenous (IV)' or 'Department of Health (DoH)'.

Include sections on Acknowledgements, Conflict of Interest, Author Contributions and Funding sources. If none is applicable, please state 'none'.

Scientific measurements must be expressed in SI units except: blood pressure (mmHg) and haemoglobin (g/dL).

Litres is denoted with an uppercase L e.g. 'mL' for millilitres).

Units should be preceded by a space (except for % and °C), e.g. '40 kg' and '20 cm' but '50%' and '19°C'.

Please be sure to insert proper symbols e.g. μ not u for micro, α not a for alpha, β not B for beta, etc.

Numbers should be written as grouped per thousand-units, i.e. 4 000, 22 160.

Quotes should be placed in single quotation marks: i.e. The respondent stated: '...'

Round brackets (parentheses) should be used, as opposed to square brackets, which are reserved for denoting concentrations or insertions in direct quotes.

If you wish material to be in a box, simply indicate this in the text. You may use the table format –this is the *only* exception. Please DO NOT use fill, format lines and so on.

SAMJ is a generalist medical journal, therefore for articles covering genetics, it is the responsibility of authors to apply the following:

- Please ensure that all genes are in italics, and proteins/enzymes/hormones are not.
- Ensure that all genes are presented in the correct case e.g. TP53 not Tp53.

****NB:** Copyeditors cannot be expected to pick up and correct errors wrt the above, although they will raise queries where concerned.

- Define all genes, proteins and related shorthand terms at first mention, e.g. '188del11' can be glossed as 'an 11 bp deletion at nucleotide 188.'
- Use the latest approved gene or protein symbol as appropriate:

Human Gene Mapping Workshop (HGMW): genetic notations and symbols

HUGO Gene Nomenclature Committee: approved gene symbols and nomenclature

OMIM: Online Mendelian Inheritance in Man (MIM) nomenclature and instructions

Bennet et al. Standardized human pedigree nomenclature: Update and assessment of the recommendations of the National Society of Genetic Counselors. *J Genet Counsel* 2008;17:424-433: standard human pedigree nomenclature.

Preparation notes by article type

Research

Editorials

CME

In Practice and Case reports

Reviews

Clinical trials

Correspondence

Obituaries

Book reviews

Guidelines

Research

Guideline word limit: 4 000 words

Research articles describe the background, methods, results and conclusions of an original research study. The article should contain the following sections: introduction, methods, results, discussion and conclusion, and should include a structured abstract (see below). The introduction should be concise – no more than three paragraphs – on the background to the research question, and must include references to other relevant published studies that clearly lay out the rationale for conducting the study. Some common reasons for conducting a study are: to fill a gap in the literature, a logical extension of previous work, or to answer an important clinical question. If other papers related to the same study have been published previously, please make sure to refer to them specifically.

Describe the study methods in as much detail as possible so that others would be able to replicate the study should they need to.

Results should describe the study sample as well as the findings from the study itself, but all interpretation of findings must be kept in the discussion section, which should consider primary outcomes first before any secondary or tertiary findings or post-hoc analyses. The conclusion should briefly summarise the main message of the paper and provide recommendations for further study.

Select figures and tables for your paper carefully and sparingly. Use only those figures that provided added value to the paper, over and above what is written in the text.

Do not replicate data in tables and in text .

Structured abstract

This should be 250-400 words, with the following recommended headings:

- o **Background:** why the study is being done and how it relates to other published work.
- o **Objectives:** what the study intends to find out
- o **Methods:** must include study design, number of participants, description of the intervention, primary and secondary outcomes, any specific analyses that were done on the data.
- o **Results:** first sentence must be brief population and sample description; outline the results according to the methods described. Primary outcomes must be described first, even if they are not the most significant findings of the study.
- o **Conclusion:** must be supported by the data, include recommendations for further study/actions.

Please ensure that the structured abstract is complete, accurate and clear and has been approved by all authors.

Do not include any references in the abstracts.

Here is an example of a good abstract.

Main article

All articles are to include the following main sections: Introduction/Background, Methods, Results, Discussion, Conclusions.

The following are additional heading or section options that may appear within these:

Objectives (within Introduction/Background): a clear statement of the main aim of the study and the major hypothesis tested or research question posed

Design (within Methods): including factors such as prospective, randomisation, blinding, placebo control, case control, crossover, criterion standards for diagnostic tests, etc.

Setting (within Methods): level of care, e.g. primary, secondary, number of participating centres.

Participants (instead of patients or subjects; within Methods): numbers entering and completing the study, sex, age and any other biological, behavioural, social or cultural factors (e.g. smoking status, socioeconomic group, educational attainment, co-existing disease indicators, etc) that may have an impact on the study results. Clearly define how participants were enrolled, and describe selection and exclusion criteria.

Interventions (within Methods): what, how, when and for how long. Typically for randomised controlled trials, crossover trials, and before and after studies.

Main outcome measures (within Methods): those as planned in the protocol, and those ultimately measured. Explain differences, if any.

Results

Start with description of the population and sample. Include key characteristics of comparison groups.

Main results with (for quantitative studies) 95% confidence intervals and, where appropriate, the exact level of statistical significance and the number need to treat/harm. Whenever possible, state absolute rather than relative risks.

Do not replicate data in tables and in text.

If presenting mean and standard deviations, specify this clearly. Our house style is to present this as follows:

E.g.: The mean (SD) birth weight was 2 500 (1 210) g. Do not use the $\pm$ symbol for mean (SD).

Leave interpretation to the Discussion section. The Results section should just report the findings as per the Methods section.

Discussion

Please ensure that the discussion is concise and follows this overall structure – sub-headings are not needed:

Statement of principal findings

Strengths and weaknesses of the study

Contribution to the body of knowledge

Strengths and weaknesses in relation to other studies

The meaning of the study – e.g. what this study means to clinicians and policymakers

Unanswered questions and recommendations for future research

Conclusions

This may be the only section readers look at, therefore write it carefully. Include primary conclusions and their implications, suggesting areas for further research if appropriate. Do not go beyond the data in the article.

Illustrations / photos / scans

If illustrations submitted have been published elsewhere, the author(s) should provide consent to republication obtained from the copyright holder.

Figures must be numbered in Arabic numerals and referred to in the text e.g. '(Fig. 1)'.

Each figure must have a caption/legend: Fig. 1. Description (any abbreviations in full).

All images must be of high enough resolution/quality for print.

All illustrations (graphs, diagrams, charts, etc.) must be in PDF or jpeg form.

Ensure all graph axes are labelled appropriately, with a heading/description and units (as necessary) indicated. Do not include decimal places if not necessary e.g. 0; 1.0; 2.0; 3.0; 4.0 etc.

Scans/photos showing a specific feature e.g. *Intermediate magnification micrograph of a low malignant potential (LMP) mucinous ovarian tumour. (H&E stain).* –include an arrow to show the tumour.

Each image must be attached individually as a 'supplementary file' upon submission (not solely embedded in the accompanying manuscript) and named Fig. 1, Fig. 2, etc.

Tables

Tables should be constructed carefully and simply for intelligible data representation. Unnecessarily complicated tables are strongly discouraged.

Large tables will generally not be accepted for publication in their entirety. Please consider shortening and using the text to highlight specific important sections, or offer a large table as an addendum to the publication, but available in full on request from the author

Embed/include each table in the manuscript Word file - do not provide separately as supplementary files.

Number each table in Arabic numerals (Table 1, Table 2, etc.) and refer to consecutively in the text.

Tables must be cell-based (i.e. not constructed with text boxes or tabs) and editable. Ensure each table has a concise title and column headings, and include units where necessary.

Footnotes must be indicated with consecutive use of the following symbols: * † ‡ § ¶ || then ** †† ‡‡ etc.

Do not: Use [Enter] within a row to make 'new rows':

Rather:

Each row of data must have its own proper row:

Do not: use separate columns for *n* and %:

Rather:

Combine into one column, *n* (%):

Do not: have overlapping categories, e.g.:

Rather:

Use <> symbols or numbers that don't overlap:

References

NB: Only complete, correctly formatted reference lists in Vancouver style will be accepted. Reference lists must be generated manually and not with the use of reference manager software. Endnotes must **not** be used.

Authors must verify references from original sources.

Citations should be inserted in the text as superscript numbers between square brackets, e.g. These regulations are endorsed by the World Health Organization,^[2] and others.^[3,4-6]

All references should be listed at the end of the article in numerical order of appearance in the Vancouver style (not alphabetical order).

Approved abbreviations of journal titles must be used; see the List of Journals in Index Medicus.

Names and initials of all authors should be given; if there are more than six authors, the first three names should be given followed by et al.

Volume and issue numbers should be given.

First and last page, in full, should be given e.g.: 1215-1217 **not** 1215-17.

Wherever possible, references must be accompanied by a digital object identifier (DOI) link). Authors are encouraged to use the DOI lookup service offered by CrossRef:

- o On the Crossref homepage, paste the article title into the 'Metadata search' box.
- o Look for the correct, matching article in the list of results.
- o Click Actions > Cite
- o Alongside 'url =' copy the URL between { }.
- o Provide as follows, e.g.: <https://doi.org/10.7196/07294.937.98x>

Some examples:

Journal references: Price NC, Jacobs NN, Roberts DA, et al. Importance of asking about glaucoma. Stat Med 1998;289(1):350-355. <http://dx.doi.org/10.1000/hgjr.182>

Book references: Jeffcoate N. Principles of Gynaecology. 4th ed. London: Butterworth, 1975:96-101.

Chapter/section in a book: Weinstein L, Swartz MN. Pathogenic Properties of Invading Microorganisms. In: Sodeman WA, Sodeman WA, eds. Pathologic Physiology: Mechanisms of Disease. Philadelphia: WB Saunders, 1974:457-472.

Internet references: World Health Organization. The World Health Report 2002 - Reducing Risks, Promoting Healthy Life. Geneva: WHO, 2002. <http://www.who.int/whr/2002> (accessed 16 January 2010).

Legal references

- Government Gazettes:

National Department of Health, South Africa. National Policy for Health Act, 1990 (Act No. 116 of 1990). Free primary health care services. Government Gazette No. 17507:1514. 1996.

In this example, 17507 is the Gazette Number. This is followed by :1514 - this is the notice number in this Gazette.

- Provincial Gazettes:

Gauteng Province, South Africa; Department of Agriculture, Conservation, Environment and Land Affairs. Publication of the Gauteng health care waste management draft regulations. Gauteng Provincial Gazette No. 373:3003, 2003.

- Acts:

South Africa. National Health Act No. 61 of 2003.

- Regulations to an Act:

South Africa. National Health Act of 2003. Regulations: Rendering of clinical forensic medicine services. Government Gazette No. 35099, 2012. (Published under Government Notice R176).

- Bills:

South Africa. Traditional Health Practitioners Bill, No. B66B-2003, 2006.

- Green/white papers:

South Africa. Department of Health Green Paper: National Health Insurance in South Africa. 2011.

- Case law:

Rex v Jopp and Another 1949 (4) SA 11 (N)

Rex v Jopp and Another: Name of the parties concerned

1949: Date of decision (or when the case was heard)

(4): Volume number

SA: SA Law Reports

11: Page or section number

(N): In this case Natal - where the case was heard. Similarly, (C) would indicate Cape, (G) Gauteng, and so on.

NOTE: no . after the v

Other references (e.g. reports) should follow the same format: Author(s). Title.

Publisher place: Publisher name, year; pages.

Cited manuscripts that have been accepted but not yet published can be included as references followed by '(in press)'.

*Unpublished observations and personal communications in the text must **not** appear in the reference list. The full name of the source person must be provided for personal communications e.g. '...(Prof. Michael Jones, personal communication)'.*

Appendix I: Turnitin plagiarism report

Turnitin form a t.docx

ORIGINALITY REPORT

8%

SIMILARITY INDEX

6%

INTERNET SOURCES

4%

PUBLICATIONS

3%

STUDENT PAPERS

PRIMARY SOURCES

wiredspace.wits.ac.za

Internet Source

4%

"15 th International Congress on
Antiphospholipid Antibodies Abstract
Presentations", Lupus, 2016

Publication

1%

ndlethornet.edu.et

Internet Source

1%

Submitted to Robert Kennedy College AG

Student Paper

1%

Submitted to University of the Western Cape

Student Paper

1%

www.ajmc.com

Internet Source

1%

Exclude quotes On

Exclude matches < 1%

Exclude bibliography On
