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Verification of a semi-automated Von Willebrand factor multimer assay and its clinical utility in hiv seropositive patients

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VERIFICATION OF A SEMI-AUTOMATED VON WILLEBRAND FACTOR MULTIMER ASSAY AND ITS CLINICAL UTILITY IN HIV SEROPOSITIVE PATIENTS

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A research report submitted as a “submissible article” to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfillment of requirements for the degree of Master of Medicine (Haematology)

Johannesburg, 2021

DECLARATION

I, Marcel Engelbrecht, declare that this Research Report is my own, unaided work. It is being submitted for the degree of Master of Medicine (Haematology) at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other university.

_____(Signature)

____ day of _____ 2021 in Johannesburg, South Africa.

To my mother Ronell and sister Celeste

PRESENTATIONS FROM THIS STUDY

- **Article 1**: Poster presentation: Annual conference of the South African Society of Thrombosis and Haemostasis (2019).

PUBLICATIONS FROM THIS STUDY

- **Article 1**: This article has been submitted to the International Journal of Laboratory Haematology for peer review and publication (original article, outcome pending).
- **Article 2**: This article has been submitted to the journal Clinical Laboratory for peer review and publication (short communication, outcome pending).

ABSTRACT (ARTICLE 1): VERIFICATION OF A NEW VON WILLEBRAND FACTOR MULTIMER ASSAY

Please note: submissible format with word limit of 250 words as guided by the author guidelines of the International Journal of Laboratory Hematology.

Introduction

Accurate diagnosis and subtyping of Von Willebrand disease (VWD) is critical to informing appropriate therapy and relies on several laboratory assays, including von Willebrand factor (VWF) multimer analysis. Traditional electrophoresis methods are labour-intensive, poorly standardized, and time-consuming. A newly-introduced commercial assay, the Hydragel 5 VWF multimers kit® (Sebia, Lisses, France), aims to overcome these challenges by improving standardisation and result turnaround time. This study verified the laboratory performance of this kit.

Methods

Twenty-five normal samples; fifteen abnormal samples from a specialist VWD management center; and nine external quality assurance (EQA) samples were analysed with the Hydragel 5 VWF multimers kit®. The agreement of results with the conventional in-house reference method (accuracy), inter- and intra-gel variability (reproducibility), and the limit of detection were assessed.

Results

Qualitatively, there was complete concordance of the results on the Hydragel 5 VWF multimers kit® with those of manual electrophoresis on both normal and abnormal samples. On all EQA surveys the multimer results obtained with the Hydragel 5 VWF multimers kit® agreed with EQA designated results. In addition, using densitometric scanning quantitative data were produced for each sample tested. Using this data, the intra- and inter-run reproducibility were demonstrated to be acceptable. The VWF antigen concentration at which multimers were still detectable was established to be between 5% and 10%.

Conclusion

The Hydragel 5 VWF multimers kit®, producing both qualitative and quantitative data, is an easy-to-use, standardised assay with acceptable analytical performance which can assist in accurate subtyping of patients with VWD.

ABSTRACT (ARTICLE 2): VON WILLEBRAND FACTOR MULTIMER PATTERNS IN PEOPLE LIVING WITH HUMAN IMMUNODEFICIENCY VIRUS INFECTION

Introduction

Endothelial dysfunction contributes to hypercoagulability in people with HIV (PWH). A surrogate marker of this is elevated von Willebrand factor (VWF) which is documented in PWH. This study compared the VWF multimer patterns in PWH who were anti-retroviral therapy (ART) naïve and immune reconstituted on ART.

Methods

VWF multimer analysis was performed with a semi-quantitative electrophoresis assay on plasma samples from 79 PWH (39 ART-naïve and 40 virally suppressed on ART) and 25 normal control samples.

Results

The total optical density mean VWF level was significantly increased in PWH, and higher in the ART-naïve versus the ART-exposed, virally suppressed group ($p<0.0001$). No quantitative difference was, however, demonstrated in the multimer distribution between the groups.

Conclusion

Our findings suggest that there is no difference in multimer distribution between ART-naïve and virally suppressed PWH. Due to a small sample size, confirmation in a larger study is required.

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TABLE OF CONTENTS

DECLARATION....ii
PRESENTATIONS FROM THIS STUDY....iv
PUBLICATIONS FROM THIS STUDY....iv
ABSTRACT: ARTICLE 1....v
ABSTRACT: ARTICLE 2....vi
ACKNOWLEDGEMENTS....vii
LIST OF FIGURES....ix
LIST OF TABLES....x
LIST OF ABBREVIATIONS....xi
1. ARTICLE 1: VERIFICATION OF A NEW VON WILLEBRAND FACTOR MULTIMER ASSAY
1.1 INTRODUCTION....1
1.2 MATERIALS AND METHODS....3
1.3 RESULTS....6
1.4 DISCUSSION....11
1.5 CONCLUSION....13
1.6 REFERENCES....14
2. ARTICLE 2: VON WILLEBRAND FACTOR MULTIMER PATTERNS IN PEOPLE LIVING WITH HUMAN IMMUNODEFICIENCY VIRUS INFECTION
2.1 INTRODUCTION....17
2.2 MATERIALS AND METHODS....19
2.3 RESULTS....21
2.4 DISCUSSION....24
2.5 CONCLUSION....25
2.6 REFERENCES....26
3. APPENDICES
3.1 APPENDIX A: Ethics clearance certificate....28
3.2 APPENDIX B: Turn-it-in report....30
3.3 APPENDIX C: Approved research protocol....35

LIST OF FIGURES: ARTICLE 1

Figure 1: Study sample composition……..……3
Figure 2a: A typical Hydragel 5 gel following completion of the assay……..8
Figure 2b: A densitometric tracing of lane 2, revealing a normal multimer distribution.....…....8
Figure 2c: Type 1 VWD with a relatively normal multimer distribution…….....…….8
Figure 2d: Type 2A VWD. Loss of intermediate and high molecular weight multimers……8
Figure 2e: Type 2B VWD. Loss of high molecular weight multimers……8
Figure 2f: Type 2M VWD. High molecular weight multimers are present……8
Figure 2g: Type 3 VWD. Note complete loss of all VWF multimers……..8
Figure 3: Photographs of each RCPA EQA sample (gel) analysed with the Hydragel 5 VWF multimers kit®……..9

LIST OF FIGURES: ARTICLE 2

Figure 1a: An example of a typical electrophoresis gel after testing……..20
Figure 1b: A typical example of a normal densitometrogram, highlighting the different multimer subsets……..……20

LIST OF TABLES: ARTICLE 1

Table 1: Results of the comparison between the in-house method and the new Hydragel 5 VWF multimers kit® using densitometric scanning……7
Table 2: Comparison of in-house manual method and Hydragel 5 VWF multimers kit®…….…....10

LIST OF TABLES: ARTICLE 2

Table 1: Patient characteristics and results……..……..21
Table 2: ART+ subgroup analysis (non-PI-containing versus PI-containing regimen)……22

LIST OF ABBREVIATIONS

ADAMTS13	A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13
ART	Antiretroviral therapy
AUC	Area under the curve
CV	Coefficient of variation
ECAT	External Quality Control of diagnostic Assays and Tests
EQA	External quality assurance
FVIII	Factor 8
GLM	General linear model
GP1b	Glycoprotein receptor 1b
HIV	Human immunodeficiency virus
HIV+ART+	HIV-seropositive, ART-initiated
HIV+ART-	HIV-seropositive, ART-naïve
HMWM	High molecular weight multimer
HREC	Human research ethics committee
IMWM	Intermediate weight multimer
kDa	Kilodalton
LMWM	Low molecular weight multimer
NHLS	National Health Laboratory Service
OD	Optical density
PI	Protease inhibitor
PPP	Platelet poor plasma
PWH	People with human immunodeficiency virus infection
RCPA	Royal College of Pathologists of Australasia
SD	Standard deviation
UL-VWF	Ultra-large von Willebrand factor
VWD	Von Willebrand disease
VWF	Von Willebrand factor
VWF:Ag	Von Willebrand factor antigen