

Peer Review File

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Reviewer A

The author should be congratulated on this extensive and timely review. The review is written from the point of MU, but it goes much further than that. I have the following suggestions:

1. Author should follow the current accepted/recommended conventions for VWF, VWF assays & VWD: VWD = von Willebrand disease (not von Willebrand's disease); the v in von should be lower case (name), but the v in VWF and VWD should always be uppercase. The correct abbreviations for the VWF assays are: VWF:Ag, VWF:RCo, VWF:CB (not RiCof, CBA or other used abbreviations). Please correct throughout. There were studies also published on other VWF assays related to COVID-19 (for example as indicated in the referenced review - ref 23). These could also be mentioned briefly (using the correct abbreviation as relevant).

Thank you for pointing out the inconsistency in nomenclature throughout the manuscript. I have reviewed the document and made the following corrections:

Von Willebrand Disease on lines 48, the remaining 81 references to VWD (including references) comply with the appropriate nomenclature

Lower case “v” has been placed in all cases of von, with the exception of where the V is at the beginning of a sentence or paragraph. Changes are tracked and are present on lines 48, 682, 866. The remaining 83 references to von (including references) comply with the appropriate nomenclature

The document has been checked and 6 instances of VWD were found. Now all references to VWD comply with the appropriate nomenclature

The document was checked and 152 instances of VWF were found. Changes were made on lines 65, 70, 372, 373, 1059, 1069

Review of the manuscript for assay nomenclature was performed. Changes were made on lines 124, 125, 269, 323, 514. The 5 examples found in the references where VWF Antigen is used was left as is, as this is the format used by the original author in the reference. I hope this is acceptable

On line 375 collagen binding assay was retained as it references the method used for ADAMTS13 activity quantification

The additional VWF assays referenced in ref 23 have been added into lines 212-213

2. The main concern is with Table 1, which is very large. I wonder if this would be better as a Supplementary Table, and an abbreviation used for the main text.

I agree completely I have reviewed selected columns as a new Table 1 for insertion into the body of the manuscript, whilst retaining the detailed descriptions in the supplementary table 1

Much of the text in the table seems to have been copied directly from the source files, and thus there are many unexplained abbreviations, and many non standard abbreviations. Using the abbreviations listed in point 1 above and explained in the Table legend would cut out a significant amount of text. All abbreviations used in the table should be explained in the table footer, and some commonly used text could be abbreviated and explained in the table footer.

In the supplementary table I have reviewed all abbreviated text and defined in the table footer. Additionally, I have reviewed the text contained within the table and have condensed where possible to improve readability

The order of entries in the table was unclear to me, but then I saw the few entries seemed to be in order of 'Clinical Scenario', but this sequence seemed to break down later. I suggest the sequence be in Body Classification (col 4) order, and then perhaps col 3. That would better align to Figure 2.

Thanks for pointing this out, the order was as you correctly stated based on clinical scenario. I am unsure why it was lost later in the table. I have reviewed the table and have applied the sorting on columns 4 first and 3 second as requested to help with interpretation

Several minor errors in the table could also be corrected. Sometimes abbreviations are not defined or explained.

I have reviewed the supplementary table and corrected abbreviations according to the guidance above and have defined additional abbreviations in the footer.

Minor:

1. try to not use words like 'it' as these have unclear meaning. Example, replace 'it' on line 39 with 'VWF'.

I have corrected line 39 (now moved to line 40)

I checked the use of "it" throughout the document and made the following changes:

Line 28 – this review

Line 372-373 – sentence truncated to remove section containing "it"

Line 409 – its replaced with ADAMTS13

Line 461 it is replaced with VWF-ADAMTS13 ratio

Line 465 – sentence rephrased to remove "it"

Line 482 – it's replaced with bias

Line 583 sentence rephrased to remove "it"

Line 602 sentence rephrased to remove "it"

- lines 45,46: Text "Lack of a true catalytic activity focuses measurement on antigenic measures in plasma" for VWF has unclear meaning; please revise/clarify. VWF has several activities that could be assessed in these studies, especially those potentially reflective of HMW VWF, such as VWF:RCo & VWF:CB.

I have removed the reference to a non-catalytic activity as I accept this was confusing and in reality not required for the point being made

- Lines 102-104: definition of MU attributed to Ref 15; I think it should be 14? Thank you for pointing out the error in referencing that is indeed correct it should reference the VIM document as reference 14. This has been changed

- Line 185: "range (49.77%)" can probably be limited to one dec point 49.8; I doubt it was that accurate as to require 2 dec points, even if so stated on the original paper.

I have changed the result as 49.8%

- The text uses mixture of abbreviations and full text after an abbreviation is defined; suggest define abbreviation on first appearance and then use abbreviation thereafter.

Thank you for pointing out the error in abbreviation compliance I have made the following changes:

Line	Original	Corrected to
45	Von Willebrand Factor	VWF
357	Von Willebrand Disease	VWD
305, 306, 484, 554, 595	Measurement uncertainty	MU
69	T h r o m b o t i c Thrombocytopaenic Purpura	TTP

- Line 199: "reported ratios as" - be clear, what ratios? "reported VWF/ADAMTS13 ratios as"

This has been changed as suggested

- Lines 235-236: "ratio (4.94 (3.13-7.21) compared to survivors (3.18 (2.59-4.88))" seems to be an ')' missing in each case.

Thank you this was indeed an error. I have removed the leading bracket as I considered this to be unnecessary

- Lines 265 and 266: "Hepatology and inflammatory conditions showed the largest ratio increases. Hepatology showed significant VWF increase" I guess to be pedantic it is the field of Hepatology and related diseases?

I have updated the text to reflect inclusion of diseases related to Hepatology as well

- Line 270: "[42]"

Thank you, correct bracket usage has been updated in the document

10. Line 315, 316: Sample types were largely from standard sodium citrate although EDTA was explicitly reported in at least one study" for what test and why? Sometimes used for multimer analysis samples, but not generally suitable for VWF assays.

I have updated the manuscript to reflect the original paper (Farkas, 2017) that quotes an in house ELISA for VWF:Ag based on the original work of Cejka 1982.

11. Line 356: misspelling "multimers"

Corrected

12. Lines 476 - 478: "The type of collagen used will result in differential sensitivity for ULVW multimers with type III collagen being more sensitive [35]." true but is ref 35 the correct ref for this statement?

Thank you for pointing that out, the correct reference is reference 3, I assume this was a typo. It has been changed now

13. Figure 1: should 2022 be omitted as only up to March data; otherwise looks like a sharp drop in 2022; will likely be reduced reports, but let's wait till end of 2022. Author could make a comment about the two larger humps for Hepatology & Cardiology; seems quite a few papers in some years and the idea didn't take root in those specialties?

I agree and thank you for the suggestion. I have reworked the figures to exclude the data from 2022 as this gives a misleading representation of the publications to date for a partial year.

Reviewer B

Interesting and original paper.

- The paper contains a lot of useful information that is not currently summarized in the abstract --> the author should improve the abstract.

Thank you for the comment. The abstract in its initial form indeed did not completely reflect the body of the manuscript and as such I have improved it to hopefully reflect the content more closely.

- Sometimes information about ratio or imbalance between VWF and ADAMTS13 are available but not calculated. This should be mentioned to alert about the usefulness to calculate the ratios in some clinical settings for example: PMID: 33200086

I have updated the abstract to include the importance of recognizing the need to derive the calculated parameter as it gives potential additional disease specific information otherwise not available if the individual parameters are used in isolation

- Keywords: suggest to add: COVID-19, biological variation, measurement uncertainty.

Measurement Uncertainty and biological variation have been added. SARS CoV-2 was in the keywords so I hoped COVID-19 was not additionally required

- Section 8: please add references.

Sorry I don't understand. Section 8 is the reference section. All references used within the manuscript are quoted below the section 8 header

- the author should add some info about how to calculate biological variation and measurement uncertainty in a haemostasis laboratory (and summarize the literature about the (few) data available in hemostasis).

This was originally considered for the original review however, the extent to which this was written meant the manuscript reached an unmanageable, and unpublishable size according to the regulations available. I am considering a follow up article regarding the specifics of how to do so, as requested. I hope this is satisfactory. The intention was to introduce a thematic discussion of the aspects of MU assessment that are rarely considered when investigating new, or combinations of, haemostasis measurands. The intention was not, in the end, to review MU in haemostasis as a whole.

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Conclusion: the author should add some guidance for the next future.

Minor corrections.

L125 ADAMST13 --> ADAMTS13

Corrected

L185 49.77% --> 49.8%

Corrected, thank you

L200 staph aureus to be reworded

Thank you, the full name has been placed in the manuscript

L418 multimers --> multimers

Corrected, thank you

Reviewer C

Congratulations on an in depth and important analysis.

- Could the author expand on the importance of high molecular weight VWF measurements in the overall assessment of ratio utility.

I have added a section (see below) commenting on the limited number of studies using multimers and the potential barriers to use in a routine clinical setting

VWF multimer analysis is used rarely, and as it is a laborious and highly specialised method, the utility in routine clinical practice is questionable despite the additional information obtained by the relative distribution of VWF multimers.

- Could the author comment on the utility of functional assays of coagulation (e.g. TEG) to be used to help define the utility of the ratio and its meaning.

I have added a short section at the end of the conclusion (see below) to address this point

Not discussed in this review is the utility of functional assays of haemostasis including viscoelastometry for use in these patient cohorts. This was not investigated in any of the publications reviewed. Taken separately, viscoelastic testing does not add additional benefit to those assays routinely used for assessment of ADAMTS13 or VWF so it is reasonable to expect that no additional benefit would be seen with viscoelastic testing although we await data to assess this.

- Currently, ADAMT13 therapy is being studied in sickle cell disease based on the VWF/ADAMTS13 ratios. Could the author add a section on genetic anemias that predispose to coagulopathy.

Thank you for the comment. I acknowledge the studies using rhADAMTS to treat sickle cell disease. The studies, from what I can find are not using the VWF/ADAMTS13 ratio as an endpoint as they are assessing each parameter individual according to the study design. As such a discussion of the role of VWF and ADAMTS13 balance in SCD and other genetic anaemias was restricted to discussing those publications where the ratio was explicitly calculated or at least referred to as a marker of disease progression or severity.

Reviewer D

1. Methods: variability is expected because of heterogeneity in measurands, with some studies using VWF antigen and others VWF activity when reporting ratios.
Line 149: concern about calculating ratios when not done by the article authors- perhaps the authors had a reason for not reporting the ratios. If you omit your calculated ratios, are the imbalances shown in Fig 2 retained?

The point about the authors having a reason to exclude calculations is an excellent one, I thank the reviewer for bringing this to my attention. Having reviewed the data there appears to be no effect of data that was constructed by the review author being added to that which had been published as a ratio.

2. (Heading RESULTS should be inserted). Suggest moving Table 1, which displays details of the individual studies, to the Supplementary Material section.

Line 267: Perhaps because hepatic stellate cells are principal site of ADAMTS13 synthesis

Thank you, this was perhaps not clear in the original text, this has been emphasised on lines 269-270 to make it clearer

Line 339: For example, do laboratories participate in standardization exercises? Even when all labs assay the same sample using the same assay method, variability in results occurs and might be due to differences in reagents, equipment, and technical errors.

Thank you for this important point. I have added a short section stating the lack of reporting of such participation and the difficulty that may introduce into interpreting studies

Line 348: ?specialism (specialty).

Changed – now on line 356

Line 349: reword: "same studies that they assay were..."

Thanks for this observation. This was a typo and has been corrected