





The need for accurate D-dimer reporting in COVID-19: Communication from the ISTH SSC on fibrinolysis

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1 | INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic continues to claim many lives across the world. In the attempts to identify a reliable prognostic indicator, marked elevation of D-dimer has been a strong contender.^{1,2} In many studies, D-dimers have consistently been shown to be the most significant marker for illness severity and death risk prediction.^{3,4} Despite the usefulness of this fibrinolytic marker, along with a recent letter by Gris et al,⁵ we note several problems across the medical literature with D-dimer reporting creating confusion and potentially misleading data interpretation.

Since the arrival of the first monoclonal antibody-based assays in the 1980s, D-dimer measurements have consistently proven to be a reliable diagnostic tool.⁶ Recent heightened awareness among the health care professionals and public about the risk of venous thromboembolism and disseminated intravascular coagulation, coupled with ease of use and accessibility of the D-dimer tests led to its increased popularity. The increased demand has led to proliferation of commercially available assays, but the manufacturers, and

consequently workers, publishing their work have not been consistent with the reporting mechanisms of laboratory data, thus leading to confusion and causing outright errors. These issues have been recurring themes in the D-dimer saga. In over two dozen COVID-19 related papers published involving D-dimer levels this year, we noted the following problems:

- Most failed to identify the manufacturer or type of D-dimer assay used
- Most did not clearly report the analytical performance of the assay (ie, variations in sensitivity, specificity, and linearity of the quantitation methods)
- There was limited information on whether D-dimer units or fibrinogen equivalent units were used
- There were inconsistencies in the magnitude of units chosen (eg, mg/L, µg/mL, ng/mL)
- A normal or disease specific cutoff value was not reported in some and most did not include age-related cutoffs
- Distinction was typically not made between thromboembolism and DIC
- The statistical analysis used for comparing data was often vague

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2 | THE PROBLEM WITH DIFFERENT ASSAYS

Plasmin degradation of cross-linked fibrin during fibrinolysis creates complex fragments, which contain D and E fragments.^{7,8} A D-dimer refers to the covalently bound D-domains of adjacent fibrin monomers along with an E-domain of the opposite and staggered strand. Monoclonal antibodies to D-dimer were created in the 1980s, which were specific for epitopes on D-D fragments and absent on fibrinogen and non-cross-linked fibrin fragments.^{9,10}

However, fragments released from proteolysis of fibrinogen and fibrin by elastase and other enzymes in the circulation could interfere with this test, especially in the setting of sepsis or inflammation (as commonplace in the COVID-19 scenario).¹¹ Because the different D-dimer assays use monoclonal antibodies to fibrin fragments, variability in test results between kits is very much possible. The Fibrin Assay Comparison Trial study distributed a set of 86 samples from patients with different clinical conditions to 12 D-dimer manufacturers.¹² There were considerable variations in specificity for crosslinked fibrin in this study, possibly because of fibrin complexes, or fibrin degradation products (FDPs).¹² There are frequent reports of very high fibrinogen levels in COVID-19 patients and it is not known what effect this may have on D-dimer test performance.^{1,13,14} High circulating fibrinogen, fibrinogen degradation products, or changes in fibrin structure may all potentially affect test specificity and sensitivity.

There are also issues with the specificity and sensitivity of the D-dimer assay based on the diagnostic purpose. A meta-analysis including 97 studies of patients with suspected deep vein thrombosis (DVT) reported an overall estimated sensitivity and specificity of D-dimer of 90.5% and 54.7%, respectively, but both estimates were subject to significant heterogeneity.¹⁵ Sensitivity and specificity also varied across the different types of tests and the clinical probability for DVT.¹⁵ For the diagnosis of DIC, D-dimer quantification is more crucial, where the variability can pose significant issues.¹⁶ FDPs can also affect D-dimer causing over- or underestimation of D-dimer.¹⁷ The poor specificity of D-dimer tests leads to high rates of false positive results but reliable negative test results, and explains why D-dimer screening is commonly used to exclude DVT.

3 | INCONSISTENCIES OF UNITS

D-dimer can be reported as fibrinogen equivalent units (FEU) or D-dimer units (DDU).¹⁸ One FEU compares the mass of the D-dimer to that of fibrinogen with a calibrator prepared from plasmin degradation of purified fibrinogen.^{19,20} DDU is an estimated mass of the D-dimer unit with purified D-dimer used as the calibrator.^{18,20} FEU is approximately two-fold higher than that of DDU. If laboratory personnel or clinicians are not aware of this distinction, results interpretation can be inaccurate. Olson et al performed a survey among several US laboratories and noted that almost one-third of the laboratories changed the units from that recommended by the manufacturer.¹⁹ Another web survey revealed that 28 different combinations

of measure units are currently used for reporting D-dimer test results.²¹ Second, there is tremendous variability in the magnitude of units reported. Different publications use ng/mL, µg/mL, mg/L, and µg/L to report D-dimer results, which can cause considerable confusion among non-laboratory health care personnel. This heterogeneity in the reporting units was identified in the web survey, in which one-third each was using "ng/mL" and "mg/L," whereas 18% used "µg/L".²¹

4 | THE PROBLEMS WITH CUTOFFS

D-dimer is commonly used to exclude venous thromboembolism in patients with low clinical probability. A threshold or cutoff value is important for application of this exclusion. Once again, the D-dimer cutoff level is dependent on the different assay methods and calibrators.¹⁸ D-dimer users should be aware that cutoff values are not transferable between methods and even between institutions. The assay should ideally be validated in prospective studies or at least compared with already validated assays.¹⁹ The British Committee for Standards in Haematology guidelines state that testing a minimum of 200 subjects should be done before local approval of a D-dimer assay,²² although this may be difficult to achieve in all laboratories.

5 | THE NEED FOR HARMONISATION THAN STANDARDIZATION

Prothrombin time used in patients receiving vitamin K antagonists was in similar turmoil as D-dimer. Collaborative efforts in this area led to development and widespread use of the International Normalized Ratio. Similarly, our group has been investigating potential ways to generate standardization of D-dimer to help address current issues. The presence of different D-dimer fragments and patient characteristics makes preparation of a "universal standard" not easy and straightforward.^{16,17,23} Harmonization rather than standardization of test results may be a possible solution to this conundrum.^{17,24} Harmonization would involve conversion of D-dimer values from different assays to a common scale, by applying a validated conversion factor.²⁵ Major commitment of manufacturers is required to investigate kit performance in COVID-19 patients and develop new or updated methods if appropriate.

6 | POTENTIAL UTILITY IN MONITORING COVID-19 PATIENTS

Consistent elevation in D-dimer in all hospitalized patients with COVID-19 has led some clinicians to use this marker to decide on low-intensity or high-intensity anticoagulation based on fold increase in D-dimers.²⁶ This approach is probably based on the presumption that all the D-dimers are coming from clot breakdown, which may not be the case in all patients. For the purpose

of this communication, it is premature to confirm this is a safe strategy without evidence from randomized trials. Another clinical strategy is intensification of anticoagulation in patients who have increase in D-dimers despite prophylactic anticoagulation, which again is presumptive and not yet proved to be a safe approach.²⁷ Last, some studies have already shown that decrease in D-dimers may signify the patient is improving and could mean downgrading the anticoagulation intensity.²⁸ This is certainly a novel use for D-dimer measurements but requires serial monitoring with an accurate method. Currently, there is no evidence to prove that findings with one D-dimer kit will necessarily translate to other D-dimer kits, and harmonization will expectedly improve comparability.

7 | RECOMMENDATIONS

We can hence summarize here a set of indications that we recommend be used when reporting data on D-dimer testing, with special focus on studies in COVID-19, where D-dimer may evenly influence the clinical decision making.

- The type of the of the D-dimer assay (name and manufacturer) must always be clearly reported
- The minimal analytical performance of the assay (including at least the functional sensitivity, total imprecision, linearity, and potential interference from FDPs) should be described
- A standardized measuring unit should be used for reporting data (FEU, as either "µg/L" or "mg/L")
- The cutoff value used in the study should be clearly indicated
- The statistical analysis should be appropriately selected according to sample size and value distribution (normal or not)

CONFLICT OF INTEREST

All authors have no conflict of interest to report.

AUTHOR CONTRIBUTIONS

Dr. Thachil wrote the manuscript and Drs. Lonstaff, Favaloro, Lippi, Urano, and Kim participated in discussion and critical editing of the manuscript.

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