Received: 22 January 2021 | Revised: 12 March 2021

DOI: 10.1111/ijlh.13532

LETTER TO THE EDITOR

ISLH International Journal of Laboratory Hematology WILEY

Diagnostic value of plasma viscosity testing for patients with COVID-19

Dear Editors,

An unusual cluster of patients suffering from a pneumonia-inducing condition was first reported within Wuhan, Hubei Province, China, in late December 2019. It was determined that the causative pathogen was a novel coronavirus termed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] The disease that is consequently attributed to this is now commonly known as COVID-19. The World Health Organization (WHO) has declared this a public health emergency of international concern due to the global pandemic of unprecedented proportion that has been implicated in over 2 million deaths worldwide.[2] The symptoms are variable ranging from a cough, pyrexia, and loss of taste and/or smell to more severe respiratory disorders, cytokine storms, microclot formation and multi-organ failure, up to fourteen days after exposure to the virus. It has been reported that approximately 20% of COVID-19–positive patients are asymptomatic, 14% develop severe symptoms, which require medical intervention, and 5% require critical care.[3,4] A large diversity of symptoms and outcomes has been seen, but there is at present no specific blood test for early detection or indication of progression severity.

Here, we describe the first prospective evaluation of the diagnostic potential of using plasma viscosity testing for patients with suspected COVID-19. Plasma viscosity is often regarded as an alternative to the erythrocyte sedimentation rate (ESR) or C-reactive protein. It has shown to be significantly increased compared with those testing negative for COVID-19 and above that expected with viral infections. Whilst a physiological change in the concentration of acute-phase proteins with a consequent increase in plasma viscosity would be expected in this condition, this study demonstrates that this is statistically significant. Indeed, a plasma viscosity result above the identified cut-off is shown to have good correlation with disease progression. The measurement of plasma viscosity is a cheap and reliable test already available in many haematology laboratories. It is frequently performed alongside a routine full blood count (FBC) without the need for taking an additional blood sample.

Patients were admitted at Addenbrooke's Hospital (Cambridge University Hospitals NHS Foundation Trust) with symptoms suggestive of COVID-19 and were subsequently confirmed for the presence or absence of SARS-CoV-2 RNA using reverse transcription (RT)-PCR of upper respiratory swabs. Whole blood specimens anticoagulated in EDTA were also taken at the same time as part of a standard laboratory assessment panel.

An FBC was performed using an ADVIA 2120i (Siemens Healthcare Ltd) analyser. Specimens were then centrifuged at 4000 rpm for six minutes, and plasma viscosity analysis was performed at room temperature using the BV200 Clinical Viscometer (Benson Viscometers Ltd). All calibration and quality control procedures were performed in accordance with the manufacturer's information for use and local standard operating procedures.

All statistical analyses were performed using Analyse-it for Microsoft Excel version 3.80 (Analyse-it Software, Ltd. 2012; http://analyse-it.com).[5] Statistical analysis comprised calculation of mean, standard deviation (SD) and 95% confidence intervals (CI) for the plasma viscosity (PV), white blood cell (WBC), platelet, neutrophil, lymphocyte and large unstained cell (LUC) counts. The Shapiro-Wilk test was used to ascertain the normality of data and provided a $P < .0001$ for each of these results. The Mann-Whitney U test was selected based on this. A p-value of <0.05 was considered significant. In addition, receiver operator characteristic (ROC) curve analysis was used to determine the area under curve (AUC), sensitivity, specificity, 95% confidence intervals, likelihood ratios, Youden's index, positive predictive value (PPV) and negative predictive value (NPV). The maximum weighted Youden's index was used to elucidate the optimum cut-off value for the plasma viscosity as a positive indicator of COVID-19 infection.

There were a total of 395 samples analysed between April and May 2020. Of these, 224 (56.7%) and 171 (43.3%) were from patients who were PCR-negative and PCR-positive for SARS-CoV-2, respectively. There were 147 (37.2%) female and 248 (62.8%) male patients, with a mean age of 54 (SD = 20, 95% CI: 51.1-57.5) and 56 (SD = 17.7, 95% CI: 53.6-58), respectively.

The mean plasma viscosity was 1.62mPas (SD = 0.13, 95% CI: 1.6-1.64) in those testing negative for COVID-19 and 2.00mPas (SD = 0.36, 95% CI: 1.95-2.06) in those testing positive. There was a significant difference between the plasma viscosity of patients with negative and positive COVID-19 infections confirmed by RT-PCR, $U = 3304$, $z = -14.14$, $P < .0001$, when tested for the hypothesis that plasma viscosity levels would be significantly elevated in patients testing positive as opposed to negative for COVID-19.

ROC curve analysis demonstrated an area under curve (AUC) of 0.914 (95% CI: 0.89-0.94) (Figure 1). A cut-off plasma viscosity of 1.83mPas was selected using the maximum weighted Youden's index of 0.683 as the discriminator. A PV of 1.83mPas has a sensitivity of 69.6% (95% CI: 62.1%-76.4%) and a specificity of 98.7% (95% CI: 96.1%-99.7%) for COVID-19. The PPV is 97.5% (95%

CI: 92.8%-99.2%) and NPV is 80.6% (95% CI: 77.2%-84.2%) for COVID-19 at this plasma viscosity cut-off. A range of plasma viscosity levels and their associated sensitivity, specificity, confidence intervals, likelihood ratios and Youden's index are detailed in Table 1.

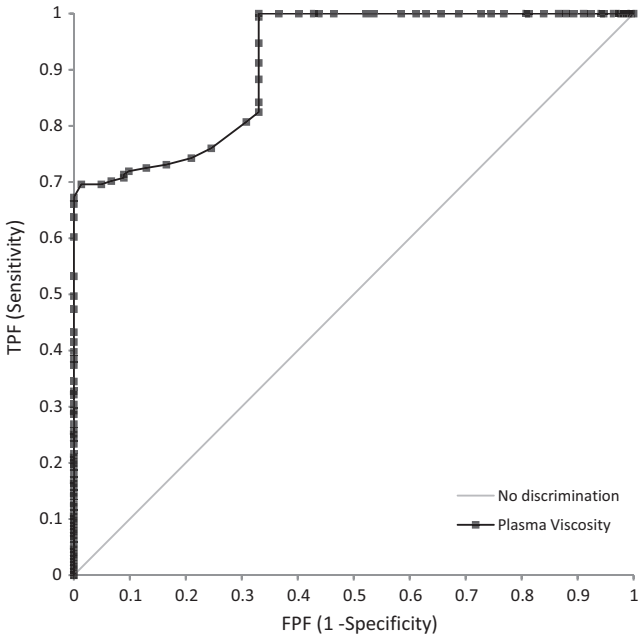

FIGURE 1 Receiver operating characteristic (ROC) curve analysis provided an area under curve (AUC) of 0.914 (95% CI: 0.89-0.94)

In comparison with non–COVID-19 patients, COVID-19–positive patients had reduced lymphocyte counts (1.37 ± 0.67 vs 1.07 ± 0.48 × 10^9/L, U = 7991, z = −2.08, *P* < *.037*. There was no significant difference between the WBC counts (9.44 ± 5.38 vs 8.73 ± 4.58 × 10^9/L, U = 9138, z = −0.36, *P* < *.726*), platelet counts (252 ± 139.3 vs 250 ± 165 × 10^9/L, U = 8728, z = −0.97, *P* < *.332*), neutrophil counts (7.51 ± 5.30 vs 7.21 ± 4.61 × 10^9/L, U = 9083, z = −0.44, *P* < *.659*) or the LUC count (0.19 ± 0.23 vs 0.22 ± 0.29 × 10^9/L, U = 8871, z = 0.758, *P* < *.447*) between these patient cohorts.

We report here a sensitive and original use of plasma viscosity in the risk stratification of patients with COVID-19 infection. It has been shown that systemic infection with COVID-19 causes an increase in serum immunoglobulins as well as fibrinogen and clotting factors. Our findings demonstrate additional evidence of the increase in serum proteins that contribute to the combined hyperinflammatory and prothrombotic states that have been well reported. Our results suggest that the level at which inpatient admission is required can be directly correlated with the degree of increase in plasma viscosity. We showed that plasma viscosity is highly sensitive at discriminating between inpatients with symptomatic COVID-19 and without.

Previous studies have documented significantly raised plasma viscosity levels in COVID-19, 1.9-4.2mPas and 2.6-4.2mPas.[6,7] Importantly, however, these results were based on two smaller studies and only included patients who were critically ill with COVID-19.

TABLE 1 Plasma viscosity results between the greatest sensitivity and specificity for COVID-19. This includes confidence intervals (CI), likelihood ratios and supplementary Youden's index used as the discriminator

Plasma viscosity (mPas)	Sensitivity (95% CI)	Specificity (95% CI)	Likelihood ratio (+)	Likelihood ratio (−)	Youden's index
1.66	100% (97.9-100)	66.9% (60.4-73.1)	171	150	0.670
1.67	99.4% (96.8-99.9)	66.9% (60.4-73.1)	170	150	0.664
1.68	94.7% (90.2-97.6)	66.9% (60.4-73.1)	162	150	0.617
1.69	91.2% (85.9-95.0)	66.9% (60.4-73.1)	156	150	0.582
1.70	88.3% (82.5-92.7)	66.9% (60.4-73.1)	151	150	0.553
1.71	84.2% (77.9-89.3)	66.9% (60.4-73.1)	144	150	0.512
1.72	82.5% (75.9-87.8)	66.9% (60.4-73.1)	141	150	0.494
1.73	80.7% (73.9-86.3)	69.2% (62.7-75.2)	138	155	0.499
1.74	76.0% (68.9-82.2	75.5% (69.3-80.9)	130	169	0.515
1.75	74.3% (67.0-80.6)	79.0% (73.1-84.2)	127	177	0.533
1.76	73.1% (65.8-79.6)	83.5% (77.9-88.1)	125	187	0.566
1.77	72.5% (65.2-79.1)	87.1% (81.9-91.2)	124	195	0.596
1.78	71.9% (64.6-78.5)	90.2% (85.5-93.7)	123	202	0.621
1.79	71.4% (63.9-77.9)	91.1% (86.6-94.5)	122	204	0.624
1.80	70.8% (63.3-77.5)	91.1% (86.6-94.5)	121	204	0.618
1.81	70.2% (62.7-76.9)	93.3% (89.2-96.2)	120	209	0.635
1.82	69.6% (62.1-76.4)	95.1% (91.4-97.5)	119	213	0.647
1.83	**69.6% (62.1-76.4)**	**98.7% (96.1-99.7)**	**119**	**221**	**0.683**
1.84	67.6% (59.7-74.2)	100% (98.4-100)	115	224	0.673

Note: The optimum cut-off PV of 1.83mPas is highlighted in bold.

Our study provides a broader evaluation of plasma viscosity levels in a range of symptomatic hospital inpatients.

This cheap and simple test may provide more useful information in risk-stratifying patients who present with symptoms of COVID-19 as to date; there have been no other reported laboratory tests, which have been able to quantify the degree of individual disease burden. Based on these data, we conclude that plasma viscometry analysis can be implemented into assessment algorithms to help quickly categorize patients with suspected symptoms of COVID-19 into high or low disease complication risk groups.

Our study, however, does have some limitations: firstly, it is single centre and uses only one specific analyser. The Benson Viscometer is available in other centres and should be able to allow these units to confirm or refute our results. Secondly, these data are prospective and although show an excellent ability to discern between two inpatient groups, this may be due to confirmation bias; patients may be asymptomatic but still be prone to a hyperinflammatory state.

The simplicity of the plasma viscosity test is that this is rapidly available, requires only limited operator training and may help facilitate quick decision-making when combined with additional clinical data such as degree of hypoxia or measurement of other inflammatory proteins.

To validate our work, we are now planning on collecting the data on all patients admitted to our centre who have had an FBC and plasma viscosity performed in order to see whether this can be used to create a simple triage tool.

KEYWORDS

COVID-19, hyperinflammatory, plasma viscosity

CONFLICT OF INTEREST

The authors have no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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