Carving out a niche for SARS-CoV-2 plasma RNA testing

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Diagnostic methods for coronavirus disease 2019 (COVID-19) provide the means to confirm infection with its etiological agent, severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2). However, tools for COVID-19 clinical risk stratification are currently lacking. Severely ill individuals represent approximately 10% of those with confirmed SARS-CoV-2 infection, and disproportionately account for resource utilization, including need for intensive care unit (ICU) admission and invasive mechanical ventilation [1]. Furthermore, COVID-19 surge settings can massively reduce availability of critical resources, requiring the implementation of rapid and effective patient triaging. Several manuscripts have explored the potential prognostic utility of laboratory markers including absolute lymphocyte count, C-reactive protein, ferritin, D-dimer, interleukin-6, procalcitonin and cardiac troponin [1-3]. However, test performance of these biomarkers, used singly or in combination, has largely proven insufficient to be employed for routine clinical decision-making. Similarly, clinical scoring systems have been proposed but require further validation and study to ensure robust generalizability [4]. Recently, it has been shown that reverse transcription polymerase chain reaction (RT-PCR), as well as droplet-based digital PCR, could be used to detect SARS-CoV-2 RNA in the bloodstream [5, 6]. RNAemia was more prevalent than previously documented, and was associated with clinical severity. Previous literature had shown low RNAemia prevalence in COVID-19 cohorts, with the largest study to date detecting viral RNA in plasma in only 1% of those with confirmed infection [7].

In this issue, Prebensen and colleagues present results from a prospective cohort study of individuals admitted with COVID-19 at an academic hospital in Norway. They assessed the association between detection of SARS-CoV-2 RNA in plasma and a composite clinical primary outcome of ICU admission for COVID-19 and/or in-hospital mortality. The cohort included 135 individuals, of whom 31 were admitted to the ICU and four died. Of the 123

individuals who underwent plasma testing, RNAemia was common with detection in 48 (39%) at baseline, and in at least one sample from either of three timepoints in 58 (47%). RNAemia was more frequent in patients who required ICU admission or died from COVID-19-related complications. In addition, plasma viral loads were statistically higher in individuals who developed the primary outcome, though it is unclear if this difference is clinically meaningful. In contrast, upper respiratory cycle threshold (Ct) values were not associated with clinical severity, or with detection of RNAemia, and serological responses were similar in chronology and intensity in both groups. Overall, this is a well-performed study with a clear research question and design that was well suited to prospectively assess clinical severity outcomes. Despite concern for selection bias in their cohort, that an association was detected in individuals with more severe disease supports the importance of RNAemia. Another limitation is the observed attrition in the proportion of samples collected over time. However, the association between RNAemia and clinical severity was maintained over time and after adjustment for potential confounders. In summary, these data demonstrate that RNAemia is this population was similar to what was found in a retrospective study in Northern California and reinforced the potential prognostic utility of plasma as an adjunctive sample type.

How do we reconcile these observations with previous studies, and best move forward? As hypothesized by the authors, patient selection may explain part of the observed high frequency of RNAemia. Similarly, pre-analytical considerations such as time between sample collection and testing, storage conditions, and number of freeze-thaw cycles may have played a role in explaining lower frequency in some previous studies. Specimen volume and differences in viral assay targets may have further contributed. Additional prospective work with optimized testing on fresh samples and standardized methods will be required to fully

assess the importance of RNAemia. The high prevalence of RNAemia in symptomatic individuals also raises theoretical concern for occupational bloodborne exposure. However, plasma appears to be a low-risk sample type compared to respiratory samples based on viral load data [5, 7]. To our knowledge, SARS-CoV-2 has not yet been successfully cultured from plasma and no case of blood-borne transmission has been reported. This area will require further study and additional surveillance.

So what is the role of testing plasma for detection of SARS-CoV-2 at a time when availability of nucleic acid amplification testing reagents and supplies is unstable and laboratories constantly struggle to stay atop demand? Some will certainly point out that the current laboratory testing capacity in many settings falls short for respiratory samples alone, and that this should be prioritized. This is a compelling argument, and we agree that sound prioritization and application of diagnostic stewardship principles should prevail. However, we would argue that these recent data support added value for plasma RNA testing in specific scenarios. First, patient selection is key and testing should only be performed in specific populations where it may help guide clinical management. This potential prognostic tool would be most useful in individuals who present illness that warrants hospital admission, but in whom the risk for progression to severe disease is unclear. Timing on this front may be an issue, as individuals with COVID-19 often present to medical attention one week or more after symptom onset, resulting in a short interval between hospital admission and ICU transfer as seen in this study. Second, plasma RNA testing should be performed only after a confirmed diagnosis, unless strong clinical suspicion persists despite a negative upper respiratory tract test. In those cases, plasma RNA testing may also provide a useful diagnostic alternative in addition to its prognostic utility. This may allow more rapid testing in cases that would otherwise require an invasive aerosol-generating procedure, such as bronchoscopy, to

confirm the diagnosis [8]. This would also provide a benefit in cases where SARS-CoV-2 confirmation is a prerequisite to access therapy such as remdesivir. What are the downsides? Such testing will require separate validation of plasma as a sample type for SARS-CoV-2 nucleic acid amplification testing in clinical laboratories, or consideration for send-out to reference laboratories with the associated additional turnaround time and expense. Access to plasma samples from individuals with confirmed SARS-CoV-2 infection is required for this validation, and may involve biobanking efforts. Nonetheless, this is not out of reach of institutions who regularly perform molecular infectious diseases testing, and for which limited adaptation is required for testing plasma.

In summary, RNAemia-based stratification has the potential to guide clinical decision-making, but we need to better understand under what circumstances and in what patient populations this is most likely to translate to real-world utility. We look forward to future prospective studies that incorporate plasma RNA testing in parallel with comprehensive testing strategies and clinical parameter assessment to better understand and incorporate the added value of RNAemia as a marker for COVID-19 severity. In the meantime, let us mitigate the important challenge of ensuring sustainable clinical molecular testing, after which we can extend its scope to fully leverage the potential of RNAemia as a prognostic and diagnostic tool.

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