Associations Between Relative Viral Load at Diagnosis and Influenza A Symptoms and Recovery

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Background. Rapid point-of-care polymerase chain reaction (PCR) diagnostic tests generally provide a qualitative result of positive or negative only. Additional information about the relative viral load could be calculated. Such quantitative information might be useful for making treatment decisions.

Methods. We enrolled students at a university health center who presented with cough and 1 additional flu-like symptom from December 2016 to February 2017. Data were collected before, during, and 5 days after the clinic visit. All those enrolled in the study received a point-of-care PCR test (cobas Liat). For those patients that tested positive for influenza A, we investigated correlations between the relative viral load and measures of disease severity and recovery.

Results. One hundred thirty-five students tested positive for influenza A. We found a positive correlation between viral load and body temperature. Time since symptom onset seemed to have a negative correlation but was not statistically significant. We did not find any correlations between viral load and overall symptom severity or outcomes related to recovery.

Conclusions. Although we found a correlation between relative viral load and body temperature, for our study population of young, overall healthy adults, we did not find that relative viral load provided additional information that could help in determining treatment and disease outcomes. It could be that viral load does provide useful additional information for other groups of patients, such as young children or older adults. Further studies on those populations are warranted.

Keywords. infection outcomes; influenza; point-of-care testing; viral load.

Diagnostic polymerase chain reaction (PCR) tests are a sensitive and specific method for determining the presence of many pathogens. Until recently, PCR methods were expensive, time-consuming, and required specialized equipment and staff. As a result, the application of PCR tests for diagnostic purposes is limited. There are 2 Clinical Laboratory Improvement Amendments (CLIA)-waived point-of-care (POC) PCR systems, Xpert Xpress by Cepheid and cobas Liat by Roche [1, 2], available to physicians. These systems can provide highly accurate results in 20–30 minutes without the need for a laboratory or highly trained staff. As the price decreases and the number of pathogens that can be detected increases, these systems will likely have a positive impact on the care of patients.

Currently, the cobas Liat system is only used to produce a qualitative result based on the internal threshold of optical brightness. The system provides the result as either positive (present) or negative (absent) for the pathogen. Although these systems are not currently used to estimate the viral load in the sample, it is possible to estimate the viral load using the number of cycles required to generate a positive test, with more cycles associated with a lower viral load [3–5]. This quantitative measurement could potentially give a physician additional information that could help determine the appropriate treatment and advice regarding the prognosis for patients. For both influenza and other pathogens, the pathogen load often correlates with factors such as disease severity, treatment success, and risk of transmission [6–18].

Several previous studies have looked at the relationship of viral load at diagnosis and the characteristics of the disease in patients with seasonal influenza [4, 19–23]. The results of these studies have been mixed with some reporting associations [19–21, 23–25] and others reporting no associations [4, 22] with clinical characteristics of disease. The time since onset of symptoms and the viral load has been explored in 5 studies [4, 19–21, 23], and all but 1 found a relationship [23, 25]. Two studies have looked at disease outcomes of hospitalized patients with influenza [23, 25]. Analyses from other seasonal influenza infection studies based on repeated measurement of viral load show a reduction of viral load correlates with a decrease in symptoms as well as other clinical outcomes [24, 26–30]. All previous studies

relied on standard quantitative PCR methods that require significant resources to implement. The potential usefulness of viral load data obtained from a rapid POC test has not previously been explored for influenza.

We set out to study outpatient-based PCR results from the cobas Liat POC test to determine whether viral load measurement provided useful additional information about a patient's disease progression or recovery. Our study is unique in that our study population was from a primary care setting, a POC PCR test was used, and we had some data regarding outcomes for disease resolution 5 days after the patients visit. The goal of our analysis was to determine whether the relative viral load (RVL) at diagnosis based on POC PCR has potential relevance to physicians for treatment or prediction of recovery speed.

METHODS

Data Collection

The study used a prospective, nonrandomized, sequential-patient design. Participants were recruited from patients who scheduled a clinical appointment due to an upper respiratory complaint at the student health center at the University of Georgia during the 2016–2017 flu season from December 2016 to February 2017. Patients eligible for the study had an upper respiratory chief complaint before their clinic visit, exhibited cough and 1 other symptom of influenza-like illness, and were seen at the clinic within 1 week of symptom onset. If all criteria were met and patients gave informed consent, they were enrolled in the study at the start of their clinic visit. The enrolled patients received a POC PCR (Roche cobas Liat) diagnostic test for influenza. Study inclusion and exclusion criteria have been previously published [31]. All eligible patients were enrolled in the study until 300 study participants were enrolled. One hundred forty-eight patients tested positive for influenza, and 13 of those were positive for influenza B. We considered the influenza B sample too small to analyze and thus did not further consider them. The study population for our analysis consisted of 135 patients who had a test that was positive for influenza A.

We obtained data from patients at the time they scheduled an appointment, during their visit, and 5 days after their visit. Patients with an upper respiratory chief complaint who tried to make an appointment with the health center were required to fill out a survey before a clinic visit. Responses were required for all the survey questions, and, once submitted, the answers were captured in the patient's electronic health record (EHR). During the clinical visit, a healthcare provider recorded signs and symptoms, laboratory results, diagnosis, and prescribed treatments in the patient's EHR. Finally, 5 days after the clinic visit, each patient was sent a link to a follow-up survey (the link closed 24 hours after the email was sent). All PCR results were joined to the EHR, and follow-up survey data were joined to the EHR using an anonymized identifier, which was unique to every clinical visit. Copies of the redacted data collection forms are available in the Supplementary Material.

Patient Consent Statement

The patient's written informed consent was obtained. The institutional review board approved the study protocol.

Relative Viral Load Computation

The POC PCR machine reports a cycle threshold (CT) for each patient sample, which is the number of amplification cycles the machine runs before a sample was judged to be positive, and can be used to estimate the viral load from the sample [3, 5]. The CT values are inversely proportional to the amount of ribonucleic acid target present in the sample. The Roche cobas Liat machine performs a set number of amplification cycles; therefore, each patient's RVL was calculated using the equation $RVL = 2^{(M-CT)}$ (M is the maximum number of cycles up to which a sample can be considered positive). (This value is considered proprietary by Roche but was given to us for the purpose of our analysis.) All analyses were made using the base-10 logarithm of the RVL, because it spans multiple orders of magnitude.

Constructing Symptom Scores

As a measure of disease severity, we constructed a total symptom score [14]. Two versions of the total symptom score were created. One was based on the symptoms reported by the physician at the time the diagnostic test was given, and the other was based on the patients' self-reported symptoms (1–24 hours) before the diagnostic test was given.

A single point was added for each symptom that was recorded as present. For the patient-based score, 27 symptoms were considered, and for the physician based score, 29 symptoms were considered resulting in maximum scores of 27 and 29, respectively. Physicians were required to provide an answer for some but not all symptoms. As a result, we classified symptoms as reported or not reported. We calculated 2 total symptom scores: one based on the number of symptoms reported by the patient, and the other based on the symptoms reported by the physician for each patient. The 2 versions of the score showed an intermediate level of correlation (see Supplementary Material Figure 1).

To account for the potential of strong correlations between symptoms, we also performed a sensitivity analysis for which we computed the total symptom scores after removing highly correlated variables. Details are provided in the Supplementary Material.

Statistical Analysis

To determine the relationship between numeric outcome variables and the RVL, we used linear regression and Spearman's rank correlations [32, 33]. For categorical outcome variables, relationship with RVL was assessed with analysis of variance
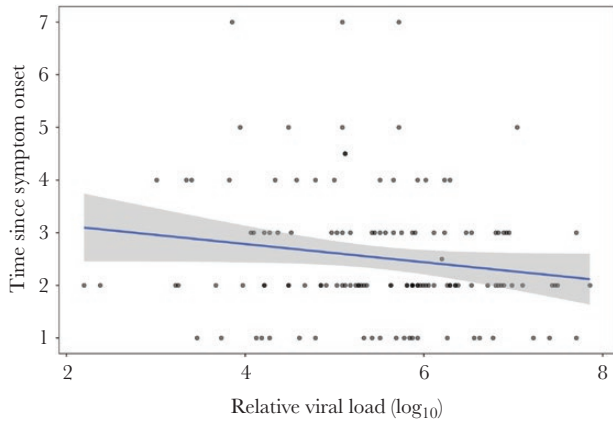
Figure 1. Correlation between the relative viral load ($\log_{10}$) at diagnosis and time since symptom onset. The solid line is the linear regression fit. The shaded area is the 95% confidence interval for the linear regression.

(ANOVA) and Spearman's rank correlation. Because we consider RVL the predictor variable in our analyses, we plot it on the x-axis, with the different outcomes under investigation shown on the y-axis. All analyses were completed in R version 4.0.2 [34]. The code and data required to generate all results are provided in the Supplementary Material.

RESULTS

Study Population
All participants enrolled in the study were college students, age 18 to 25 years, at a major public university. Data were collected at 3 different times. First, patients completed a previsit electronic survey, then data from the visit was recorded in the EHR, and, finally, a postvisit survey was sent 5 days after the visit. For our analysis, only patients with a positive test result for influenza A were included, resulting in 135 observations. Of the 135 records we included in our analysis, 122 had complete data for the previsit survey. Thirteen patients enrolled in the study did not fill out the previsit survey when they made their appointment. The enrollment of these 13 patients is likely the result of including patients with 2 influenza-like symptoms instead of cough plus 1 additional influenza-like symptom. Second, patients may have reported cough verbally to the enrollment staff but not to the physicians. Data recorded during the visit were available for all 135 patients. Finally, 114 of the 135 84.4% completed the postvisit survey. Complete tables for each point of data collection are provided (see Supplementary Material Tables 1–3).

Correlation of Relative Viral Load With Time Since Symptom Onset
Previous studies have shown a reduction in average viral load as days since symptom onset increases [12, 14, 28]. We see a similar pattern in our data based on visual inspection (Figure 1). However, interpatient variation in RVL is so strong that it

dominates any potential time-dependent signal. The linear model did not indicate a statistically significant negative trend ($\beta = -0.17$; 95% confidence interval [CI], -0.36 to 0.01; $P = .07$), neither did Spearman's rank correlation ($r = -0.15$; 95% CI, -0.31 to 0.02; $P = .09$).

Correlation of Relative Viral Load With Total Symptom Scores
We investigated correlations between the total symptom scores (see Methods section) and viral load. We did not find any correlations for either the patient-reported scores (linear regression: $\beta = 0.08$, 95% CI = -0.48 to 0.64, $P = .78$; Spearman's correlation coefficient: $r = -0.00$, 95% CI = -0.18 to 0.18, $P = .98$) or the physician-reported scores (linear regression: $\beta = 0.21$, 95% CI = -0.25 to 0.68, $P = .36$; Spearman's correlation coefficient: $r = 0.04$, 95% CI = -0.13 to 0.21, $P = .65$) (Figure 2).

A sensitivity analysis of the symptom scores, for which we excluded strongly correlated symptoms, showed the same results (see Supplementary Material Figure 2). We also explored a model that included both RVL and time since symptom onset as predictor variables. In that analysis, neither variable showed a significant correlation with symptom scores (see
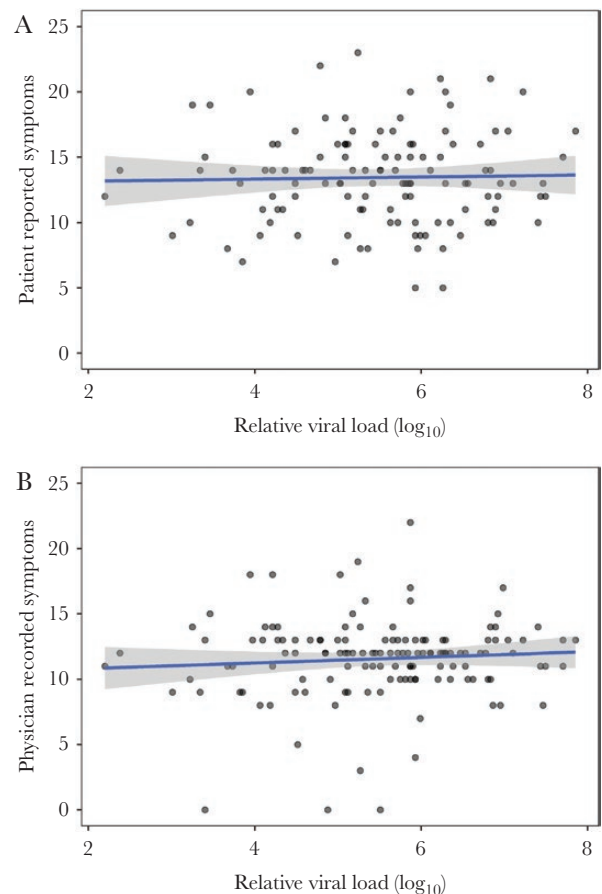

Figure 2. Correlation between the relative viral load ($\log_{10}$) at diagnosis of the patients and the calculated total symptom scores, using (A) symptoms reported by the patient and (B) symptoms reported by the physician.
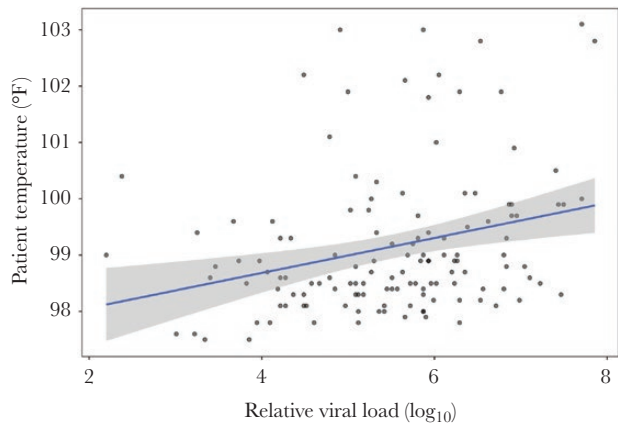
Figure 3. Correlation between relative viral load ($\log_{10}$) and patient temperature at the clinic visit.

Supplementary Material). Another ancillary analysis of self-reported patient activity level, which could be considered as a proxy of disease severity, also did not show a correlation with RVL (see Supplementary Material Figure 3).

Correlation of Relative Viral Load With Fever

Two previous studies showed a relationship between viral load and fever [21, 28]. We wanted to determine whether a similar result holds for our POC rapid test data. To maximize statistical power, we treated body temperature as a continuous variable. We found a positive relationship between body temperature during the clinic visit and RVL (Figure 3). The linear model indicated a statistically significant trend ($\beta = 0.31$; 95% CI, 0.13–0.50; $P = .001$) as did Spearman's rank correlation ($r = 0.29$; 95% CI, 0.13–0.44; $P = 7.2\text{e-}04$).

Correlation of Relative Viral Load With Recovery

Arguably, RVL could be most useful if it was predictive of disease progression and outcomes and could provide the physician with additional useful prognostic information. To investigate this, we explored whether the RVL was predictive of recovery speed, using the data from the postvisit survey.

There was no correlation between RVL and the number of days of work or class that a patient missed (Figure 4A) (linear regression: $\beta = -0.01$, 95% CI $= -0.23$ to 0.20, $P = .91$; Spearman's correlation: $r = -0.00$, 95% CI $= -0.19$ to 0.18, $P = .98$).

Likewise, there was no relationship between a patient's RVL and the number of days the patient reported a subjective fever on the follow-up questionnaire (Figure 4B) (linear regression: $\beta = 0.02$, 95% CI $= -0.19$ to 0.22, $P = .88$; Spearman's correlation: $r = 0.00$, 95% CI $= -0.18$ to 0.19, $P = 0.97$). Finally, there was no relationship between patient-reported cough recovery and RVL at diagnosis (Figure 4C) (ANOVA F-test: $F = 0.7715$, $P = .38$).

DISCUSSION

Studies that track influenza infection time-course in patients have found relationships between viral load and disease [12, 26–29]. However, the usefulness of a single viral load measurement at diagnosis for clinical decision making is unclear. Our study is the first we are aware of to use data obtained from a CLIA-waived POC PCR assay given to patients seeking care in a primary care setting. We were able to assess correlations between RVL and symptoms. We were also able to investigate the potential predictiveness of RVL on prospective outcomes,
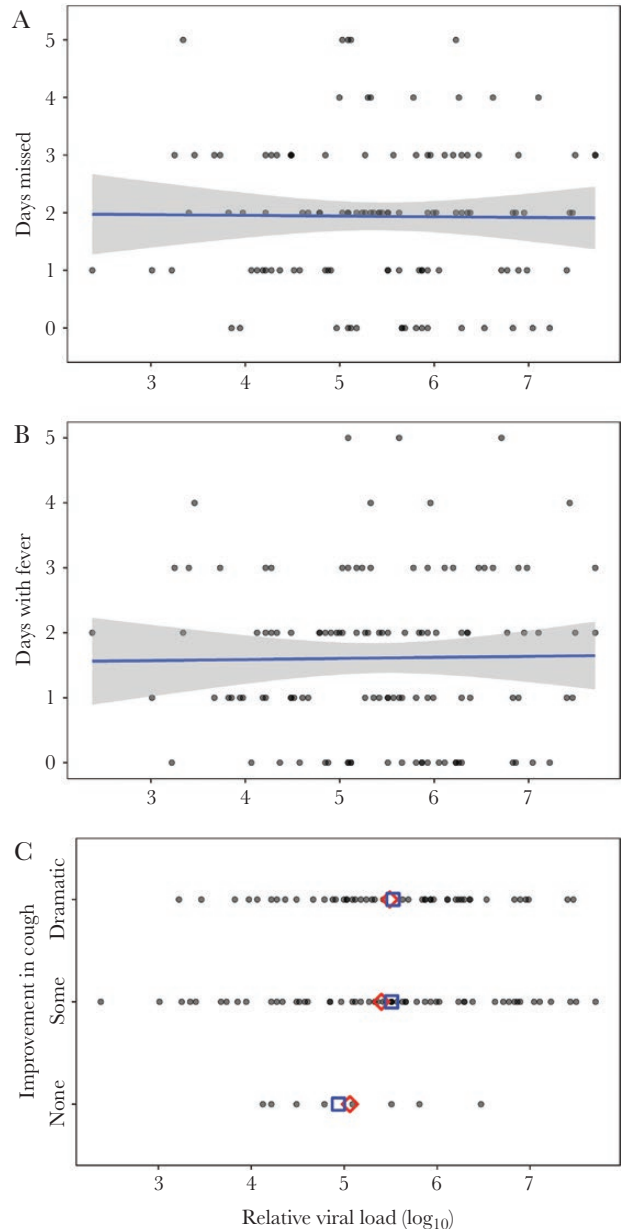

Figure 4. Correlation between relative viral load ($\log_{10}$) and (A) days of work or class missed, (B) reported days fever was present 5 days after the clinic visit, (C) reported recovery from cough 5 days after the clinic visit (diamond = mean, square = median). Sample includes those who completed the postsurvey n = 114.

namely, days of missed work or class, days of fever after diagnosis, and cough improvement.

We found a positive correlation between RVL and body temperature, similar to previous reports based on laboratory PCR assays [21, 28]. We did not find any noticeable correlation between RVL and any of the other outcomes we investigated, including those related to recovery.

There are limitations to our current study. The study population only included generally healthy college students. We also do not know the subtype of the viruses because the current POC test cannot distinguish between subtypes. During the 2016/2017 influenza season, the Centers for Disease Control and Prevention surveillance found that 97.2% of the samples subtyped were H3N2 [35]. In the state of Georgia, surveillance up to week 8 of the influenza season showed that among all the samples positive for influenza A, 97.8% were H3N2 [36]. Thus, we can be reasonably confident that the majority of our patients were infected with H3N2 influenza. We were also not able to assess sample quality, which can impact the number of cycles required to reach a positive threshold [4]. Samples of inferior quality may result in an artificially reduced estimate of viral load. Finally, this study is a secondary analysis of data that were collected to address a different question [31]. As such, the postvisit questions were not as detailed and focused as they could have been if the data were collected primarily for the analysis of clinical outcomes.

CONCLUSIONS

In summary, we found that for the data analyzed here, having information on viral load in addition to a dichotomous (positive/negative) influenza diagnosis was only correlated with body temperature and did not provide any information that could be considered useful for informing clinical practice. Because the data we analyzed were obtained in a clinical setting, we only have viral load at a single point during the infections, and each patient is at a different point in their infection time-course. This is compounded by interpatient variability in viral load. Although more detailed and controlled data would allow potential detection of further correlations, our data is the kind of information that a clinician would obtain, thus it mimics a real-world setting. It is also important to note that our findings may not generalize to other populations. It could be possible that viral load contains important independent information for specific groups of patients such as young children or older adults. Further studies on those populations are warranted.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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