Association of Viral Load With Disease Severity in Outpatient Children With Respiratory Syncytial Virus Infection

Erika Uusitupa[1], Matti Waris[2], Terho Heikkinen[1,3]

[1] Department of Pediatrics, University of Turku, FI-20520 Turku, Finland

[2] Department of Clinical Microbiology, Turku University Hospital, and Institute of Biomedicine, University of Turku, FI-20520 Turku, Finland

[3] Department of Pediatrics, Turku University Hospital, FI-20520 Turku, Finland

Corresponding author: Terho Heikkinen, MD, PhD, Professor of Pediatrics, Department of Pediatrics, University of Turku, FI-20520 Turku, Finland. E-mail: terho.heikkinen@utu.fi Tel: +358-50-5359095.

Alternate corresponding author: Erika Uusitupa, MD, Department of Pediatrics, University of Turku, FI-20520 Turku, Finland. E-mail: erika.m.uusitupa@utu.fi. Tel: +358-40-8311072.

Summary: In a prospective study of respiratory infections, children with higher respiratory syncytial virus load had significantly longer durations of rhinitis, cough, fever, and any symptoms than those with lower viral load.

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Correspondence and reprint requests

Terho Heikkinen, Department of Pediatrics, University of Turku, FI-20520 Turku, Finland.

E-mail: terho.heikkinen@utu.fi

ABSTRACT

Background. There are scarce data on whether viral load affects the severity of respiratory syncytial virus (RSV) disease in outpatient children.

Methods. We analyzed the association between viral load and disease severity among children who participated in a prospective cohort study of respiratory infections. The children were examined and nasal swabs for the detection of RSV were obtained during each respiratory illness. Quantification of RSV load was based on the cycle threshold (Ct) value. For the primary analysis, the children were divided into 2 groups: higher (Ct <27) and lower viral load (Ct ≥27).

Results. Among 201 episodes of RSV infection, children with higher viral load had significantly longer median durations of rhinitis (8 vs 6 days; *P* = .0008), cough (8 vs 6 days; *P* = .034), fever (2 vs 1 days; *P* = .018), and any symptom (10 vs 8 days; *P* = .024) than those with lower viral load. There were statistically significant negative correlations between the Ct values and the durations of all measured symptoms.

Conclusions. Our findings support the concept that viral load drives the severity of RSV disease in children. Reducing the viral load by RSV antivirals might provide substantial benefits to outpatient children.

Keywords:

Respiratory syncytial virus; children; viral load, disease severity; antiviral agents.

BACKGROUND

Respiratory syncytial virus (RSV) is a major cause of acute respiratory tract infection in children worldwide [1-5]. More than 3 million children <5 years of age are hospitalized with RSV infection every year, and the annual RSV-associated mortality in this age group has been estimated at 118 000 [5]. Although young infants are frequently hospitalized with RSV-associated bronchiolitis, the burden of RSV is substantial also among children treated as outpatients [1, 6, 7]. In the absence of vaccines and antiviral drugs against RSV, the treatment of RSV infections has remained largely supportive [8]. In recent years, however, several candidate RSV vaccines, antivirals, and monoclonal antibodies have been developed and are currently being tested [8, 9].

One of the main reasons hindering the development of RSV antivirals has been the lack of evidence that higher RSV loads are associated with more severe manifestations of the illness. This question is important because if viral load is a leading factor affecting the clinical RSV illness, reduction of the viral load by use of antiviral agents could be expected to ameliorate the illness. Previous studies assessing the impact of viral load on RSV disease severity have been carried out mainly among hospitalized children, using highly variable study designs and outcomes to measure disease severity. Although several studies have demonstrated a positive correlation between RSV load and the severity of the illness [10-17], a number of studies have failed to show a similar association [18-24].

The largest numbers of RSV-infected children are treated in the outpatient setting, where the availability of effective RSV antivirals could provide substantial benefits [1, 6]. However, there is

little information about the association between RSV load and any measure of disease severity among outpatient children [13]. We sought to determine whether RSV load in naturally infected outpatient children is associated with the duration of symptoms and the rates of complications.

METHODS

Subjects

This analysis was based on data from a prospective cohort study of respiratory infections among outpatient children ≤13 years of age that was performed during 2 consecutive winter seasons (October-May 2000-2001 and 2001-2002) in Turku, Finland [6, 25]. The participants were recruited through day care centers, family day care, and schools, and there were no exclusion criteria for enrollment. Overall, the study comprised 2231 child-seasons of follow-up. The study protocol was approved by the Ethics Committee of the Hospital District of Southwest Finland, and the study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from the parents or guardians of all participating children before the commencement of the study.

Study Conduct

During each season, the parents were instructed to bring their child to the study clinic every time the child had fever or signs or symptoms of a respiratory infection. All visits were free of charge, and there was no limit for the number of visits made. The study clinic was open every day. At each visit, a study physician examined the child and filled out a structured medical record that contained the history, signs and symptoms, clinical findings, diagnosis, and treatment. Children

without any complications at the first visit were routinely reexamined after 5-7 days and additionally whenever the parents deemed it necessary.

Symptom Diaries

Throughout the follow-up period, the parents filled out daily symptom diaries that consisted of charts inquiring about the signs and symptoms of the child, and especially about the objective signs of fever, rhinitis, and cough. The durations of symptoms for this analysis were retrieved from these daily symptom diaries. When calculating the overall duration of illness, all consecutive days on which the child had fever, rhinitis, or cough were included.

Virological Assays

During each respiratory infection, regardless of the severity of symptoms or the presence or absence of fever, a nasal swab was obtained for determination of the viral etiology of the illness. All virological analyses were performed at the Department of Virology, University of Turku. The detection of RSV in the specimens was based on both viral culture and reverse-transcription polymerase chain reaction (RT-PCR). Nucleic acids were extracted by using the High Pure Viral Nucleic Acid Kit or the MagNA Pure LC extractor (Roche Diagnostics, Espoo, Finland) according to the manufacturer's protocols. The extracts were stored at −70°C and later analyzed for RSV N gene RNA by RT-PCR [26]. Quantification of the viral load in specimens positive for RSV by RT-PCR was based on determination of the cycle threshold (Ct) value. The Ct value is defined as the calculated cycle number at which the PCR product crosses a threshold of detection, and it provides a semiquantitative measure of viral load. Ct values are inversely proportional to the amount of

target nucleic acid in the sample: the lower the Ct value, the greater the amount of target nucleic acid in the specimen.

RSV Illnesses Analyzed

Among a total of 302 RSV infections diagnosed in the children, Ct values and full clinical and diary data were available for 268 episodes. To allow for focusing on new-onset RSV illnesses in young children, we limited this analysis to children <10 years of age in whom the maximum duration of respiratory symptoms before sampling at the study clinic was 6 days. During the 2-year study period, 9 children each included in this analysis had 2 separate episodes of RSV illness. For the purposes of this study, these children were considered separate children in the analyses, and they were analyzed in the Ct value and age group that they belonged to at the time of the illness. The final analysis included 201 RSV illnesses in 192 children. The distribution of the Ct values during these episodes is presented in Figure 1; the mean Ct value was 27.7.

Statistical Analysis

In the primary analysis to explore the association between viral load and disease severity, we first divided the children into 2 groups on basis of the mean Ct value: higher viral load (Ct value <27) and lower viral load (Ct value ≥27) (Fig. 1). In a secondary analysis to assess any trends, the 201 children were further divided into 3 equal-sized groups according to their Ct value (high, intermediate, and low viral load; n = 67 in each group). Finally, we determined the correlations between the Ct values and the durations of rhinitis, cough, fever, and any symptom among all 201 children.

The unpaired *t* test was used for comparing differences in means, and the Mann–Whitney *U* test for comparing differences in medians between 2 groups. Comparison of differences in means between 3 groups was performed by one-way analysis of variance, and comparison of medians between 3 groups by the Kruskal-Wallis test. Proportions between the groups were compared by the χ^2 test. Spearman´s rank correlation was used to analyze correlations between Ct values and durations of symptoms. Two-sided *P* values of < .05 were considered to indicate statistical significance. All statistical analyses were performed with SPSS Statistics, version 25 (IBM SPSS Statistics).

RESULTS

Clinical Characteristics

Of the 201 children with a new-onset RSV illness, 106 (52.7%) children had a Ct value <27 (higher viral load) and 95 (47.3%) had a Ct value ≥27 (lower viral load). The baseline characteristics of children in these groups are shown in Table 1. The median age of the children was 2.9 years in the higher viral load group and 3.2 years in the lower viral load group (difference of 4 months; *P* = .02). There were no statistically significant differences between the groups in the duration of rhinitis (*P* = .56), cough (*P* = .78), or fever (*P* = .98) prior to viral sampling at the study clinic. Viral culture was positive in 64 (60.4%) children with a Ct value <27, compared with 12 (12.6%) children with a Ct value ≥27 (*P* <.0001).

Duration of Symptoms

In the primary analysis between the 2 groups, children with higher viral load had significantly longer median durations of rhinitis (8 vs 6 days; *P* = .0008), cough (8 vs 6 days; *P* = .034), fever (2 vs 1 days; *P* = .018), and any symptom (10 vs 8 days; *P* = .024) than those with lower viral load (Table 2).

In the secondary analysis to assess any trends among children divided into 3 equal-sized groups on basis of their Ct value (high, intermediate, and low viral load), children with high viral load had consistently longest durations of rhinitis, cough, fever, and any symptom, whereas the durations of these symptoms were shortest in children with low viral load (Figure 2). The differences in the mean durations between the 3 groups did not reach statistical significance. However, the differences in the median durations of rhinitis between the 3 groups were statistically significant (*P* = .008).

The correlations between the Ct value and the duration of various symptoms among all 201 children are presented in Figure 3. For all measured outcomes, there was a statistically significant negative correlation between the Ct value and the duration of the symptom, indicating that higher viral load was associated with longer duration of symptoms.

Because children with higher viral load were younger than those with lower viral load, we further compared the durations of various symptoms between high and low RSV load in different age groups of children (Figure 4). Except for children <2 years of age, there were trends towards longer durations of symptoms among children with higher viral load when adjusted for age group.

The differences in the median durations of rhinitis (9 vs 5 days; *P* = .0003) and any symptoms (10 vs 8 days; *P* = .012) in the group of children 2-3 years of age were statistically significant.

Complications and Antibiotic Treatment

In the primary analysis between the 2 groups, acute otitis media was diagnosed in 56 of 106 (52.8%) children in the higher viral load group, compared with 44 of 95 (46.3%) children with lower viral load (*P* = .36). Sixty-three (59.4%) children with higher viral load and 47 (49.5%) children with lower viral load were treated with antibiotics (*P* = .16).

DISCUSSION

Our study performed in a real-life setting among outpatient children demonstrates that higher RSV load is associated with a longer duration of illness. The main strengths of the study include the prospective follow-up of children who were clinically examined during each episode of respiratory illness, nasal sampling for viruses during each illness regardless of the severity of symptoms, and real-time recording of daily symptoms by the parents. Furthermore, the durations of respiratory symptoms before viral sampling were similar in children with higher and lower viral loads, and the association between the viral load and duration of illness was analyzed and confirmed by several methods.

To our knowledge, the present analysis consisting of 201 children with RSV infection is the largest one to assess the relationship between RSV load and clinical illness among outpatient children. In a previous study including 30 infants with RSV infection in the community, Houben et al. reported

a positive correlation between RSV load and a disease severity score but found no association between viral load and the duration of illness [13]. Their finding of no association may be due to the relatively small sample size, but it is also probable that the method of determining the duration of illness was different from that used in our study because the median duration of RSV illness in their study was only 3 days. In our analysis, the durations of various symptoms were retrieved directly from the symptom diaries that the parents filled out daily during their child´s illness, and it is highly likely that those data provided the most accurate information about the duration of symptoms in the children.

Most previous studies on RSV load and disease severity have been carried out among hospitalized children by using various outcomes to measure disease severity. Although several studies, especially the largest ones, have demonstrated a direct association between viral load and disease severity [10-17], not all of them have found such an association [18-24]. However, there are plenty of potential reasons, ranging from small sample sizes to low sensitivity of the outcomes used for determining severity, for the lack of finding an association between RSV load and disease severity. Therefore, studies failing to show a significant association should not be automatically interpreted to prove the nonexistence of an association.

Because children treated as outpatients have generally milder illnesses than hospitalized children, the outcomes used for determining disease severity among hospitalized children are not usually applicable to the outpatient setting. As only a small proportion of outpatient children with RSV are eventually hospitalized [6], the duration of illness and the development of complications that are managed in the outpatient setting are relevant indicators of disease severity that also have a

direct economic impact on the families in terms of parental work absenteeism and costs of treatment.

Our study serves as a proof of concept that RSV load is associated with clinical illness also among outpatient children. It is possible that also other factors, for instance host-related ones, play a role in the clinical presentation of RSV illness. Age is a particularly important factor in this context because, as observed also in the present study, young children have higher viral loads than older children or adults [27]. Furthermore, the duration of respiratory illness is generally longer among younger than older children [28]. All in all, it appears that child´s age, viral load, and duration of symptoms are all associated with each other. Although it is clear that association does not prove cause and effect, the observed association between viral load and duration of symptoms suggests a mechanism that could be subject to intervention to reduce the severity of RSV illness. It is theoretically possible that effective RSV antiviral agents, especially when started early in the course of the illness, might substantially shorten the duration of the illness and reduce the incidence of complications, analogously to influenza antivirals in the treatment of influenza [29].

Our study has also some limitations. Although it was so far the largest outpatient study on this topic, the sample size was still modest. This reduced the statistical power to demonstrate differences between smaller subgroups of children and for categorical outcomes such as acute otitis media and antibiotic treatment. The nasal swabs were obtained as part of a clinical follow-up study, and the procedure for the collection of the specimens was not strictly standardized. However, any major variation in the quality of the samples was minimized by two factors: the specimens were collected by few members of the study personnel, and they were all specifically

trained to do that prior to the commencement of the study. Moreover, variation in the quality of the specimens would have increased the variability in the Ct values, which would have biased the results in the direction of making it more difficult to demonstrate associations between the viral load and various outcomes.

In conclusion, our follow-up study among outpatient children provides support for the concept that viral load drives the severity of RSV disease in children. Because it is plausible that reduction of the RSV load by effective antiviral agents could decrease the severity of the illness in children [30, 31], development of such agents can be regarded as a high priority.

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Table 1. Baseline characteristics of the 201 children with RSV illness.

Variable	**Ct <27**	**Ct ≥27**
No. of children	106	95
Age group, n (%)		
<2 years	22 (20.8)	14 (14.7)
2-3 years	63 (59.4)	51 (53.7)
4-9 years	21 (19.8)	30 (31.6)
Gender, n (%)		
Girls	53 (50.0)	42 (44.2)
Boys	53 (50.0)	53 (55.8)
Duration of symptoms before viral sampling, days, mean (SD)		
Rhinitis	2.5 (1.4)	2.6 (1.8)
Cough	2.7 (1.3)	2.7 (1.6)
Fever	0.9 (0.9)	0.9 (1.1)
Viral culture, n (%)		
Positive	64 (60.4)	12 (12.6)
Negative	42 (39.6)	83 (87.4)

SD, standard deviation

Table 2. Duration of symptoms in children with higher (Ct <27) and lower (Ct ≥27) viral load.

Symptom	**Ct <27 (n = 106)**	**Ct ≥27 (n = 95)**	***P***
Rhinitis (days)			
Mean (SD)	9.0 (5.2)	6.9 (6.4)	0.012
Median (IQR)	8.0 (6.0-11.0)	6.0 (3.0-10.0)	0.0008
Cough (days)			
Mean (SD)	8.4 (4.7)	7.2 (5.1)	0.079
Median (IQR)	8.0 (6.0-11.0)	6.0 (4.0-10.0)	0.034
Fever (days)			
Mean (SD)	2.3 (2.1)	1.7 (1.9)	0.029
Median (IQR)	2.0 (0.0-4.0)	1.0 (0.0-3.0)	0.018
Any symptom (days)			
Mean (SD)	10.9 (5.7)	9.6 (6.4)	0.12
Median (IQR)	10.0 (8.0-13.0)	8.0 (6.0-12.0)	0.024

SD, standard deviation; IQR, interquartile range

FIGURE LEGENDS

Figure 1.

Distribution of Ct values among the 201 children with RSV illness. Black bars, children with higher viral load (Ct <27, n = 106); grey bars, children with lower viral load (Ct ≥27, n = 95).

Figure 2.

Mean durations of symptoms in children divided into 3 equal-sized groups based on their Ct value (n = 67 in each group). Black bars, children with high viral load (Ct <24.35); grey bars, children with intermediate viral load (Ct 24.35-29.30); dotted bars, children with low viral load (Ct >29.30). *P* values between the 3 groups were calculated by one-way analysis of variance.

Figure 3.

Correlations between the Ct value and the duration of rhinitis (A), cough (B), fever (C), and any symptom (D) among 201 children with RSV illness (Spearman´s rank correlation).

Figure 4.

Median durations of symptoms in children adjusted by age: <2 years (n = 36), 2-3 years (n = 114), and 4-9 years (n = 51). Black bars, children with higher viral load (Ct <27); grey bars, children with lower viral load (Ct ≥27). Asterisks indicate statistically significant differences (Mann-Whitney *U* test).

Figure 1.

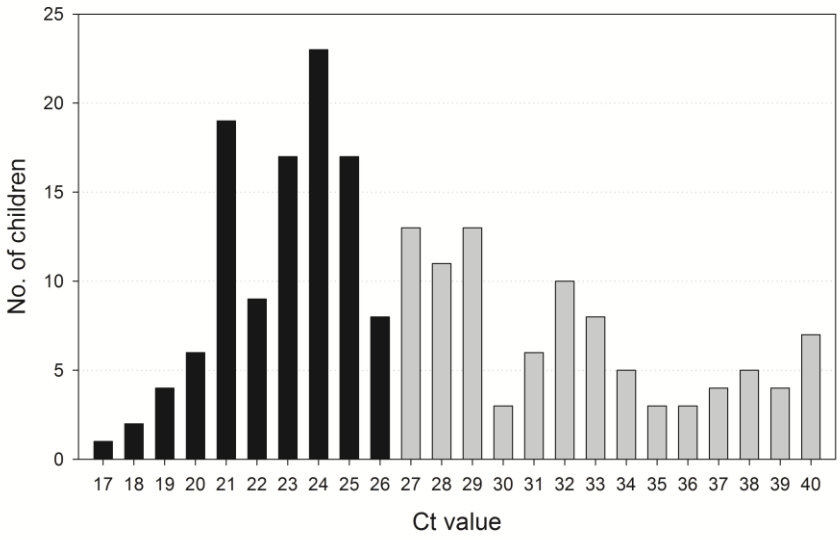
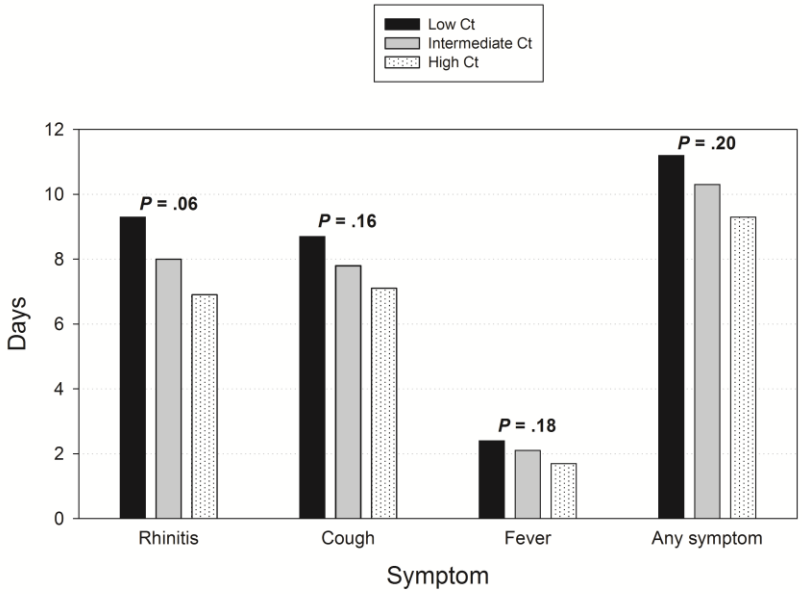


Figure 2.



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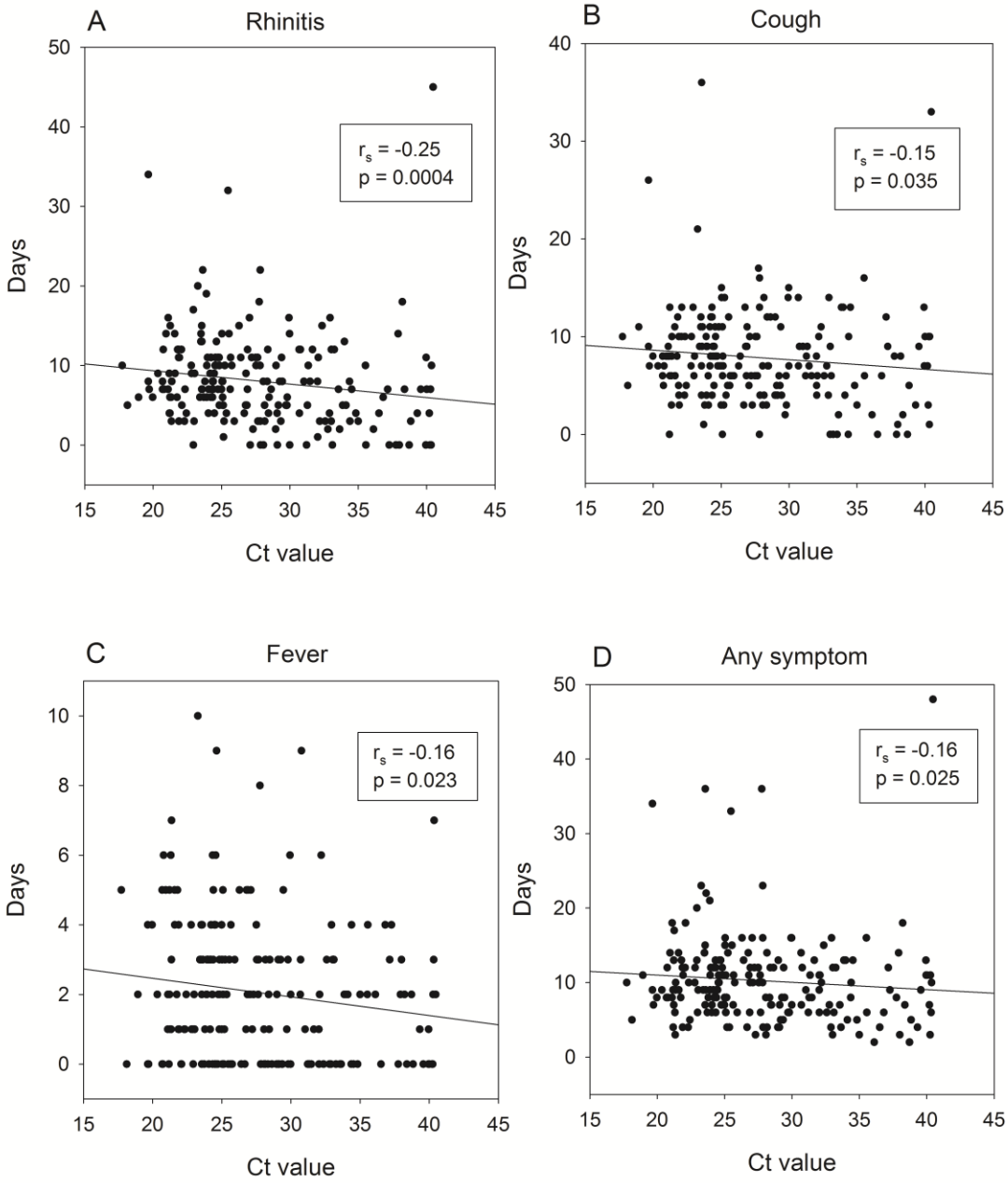

Figure 4.