Impact of Respiratory Viruses on Pediatric Asthma Exacerbations

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Abstract

Objective: Asthma exacerbations (AEs) have a great influence on asthma related morbidity. Respiratory viruses were known to trigger these exacerbations. We aimed to determine the impact of respiratory viruses on pediatric AE and to compare them with those in non-asthmatic children with acute respiratory tract infections.

Material and Methods: A total of 125 asthmatic children with AE between December 2013 and April 2014 were recruited. Age-matched 125 non-asthmatic children with respiratory tract infections over the same time period were enrolled to compare the triggering respiratory viruses. Demographic and clinical features were recorded. A multiplex PCR assay was used to detect 21 different respiratory pathogens and *Mycoplasma pneumoniae*.

Results: Respiratory pathogens were detected in 84.8% of patients with AE. Rhinovirus was the most common virus detected (30.4%). Non-asthmatic children with ARTI were 92% positive for a respiratory pathogen and were mostly infected with influenza A (29.6%). Patients with virus positive AE were younger in age than those with virus negative AE (p=0.001). A higher hospitalization rate (p=0.023) and longer duration of symptoms (p=0.031) were observed in virus positive AEs. The type of maintenance treatment for asthma did not have a significant effect on virus positivity and severity of exacerbation (p>0.05).

Conclusion: A high prevalence of respiratory viruses with the predominance of rhinovirus in pediatric AEs was confirmed in this study. Furthermore, the viral epidemiology of AEs was found to show some differences from that of respiratory tract infections at the same time period. This study also revealed an increase in the severity of AEs with the presence of respiratory viruses, highlighting their importance in treatment and prevention strategies of AEs. *(J Pediatr Inf 2016; 10: 14-21)*

Keywords: Asthma exacerbation, children, influenza viruses, respiratory viruses, rhinovirus

Received: 28.01.2016

Accepted: 16.03.2016

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This study was presented at the EAACI (The European Academy of Allergy and Clinical Immunology) Congress 6-10 2015, Barcelona, Spain.

DOI: 10.5152/ced.2016.2286

Introduction

Asthma is one of the most common chronic childhood disease, causing intermittent and reversible bronchial obstruction. Viral respiratory infections can trigger asthma exacerbations (AEs) in children. These exacerbations have a great influence on total asthma related morbidity, mortality, and cost. Approximately 80% of AEs has been associated with respiratory viral infections (1, 2). Rhinoviruses (RVs), respiratory syncytial virus (RSV), coronaviruses (CVs), influenza A and B, parainfluenza viruses (PIVs), adenovirus (AV), human metapneumovirus (HMPV), human bocavirus (HBOV), and enteroviruses (EVs) have been related to AEs (3). The viral detection rate and type of respiratory virus in AEs depend on age, seasonality, and locality (3). RV is the predominant viral pathogen in older children with AE accounting approximately for 60% (2). On the other hand, RSV predominates in asthma exacerbations of younger children (4).

Surprisingly, *Mycoplasma pneumonia* was not found to be a frequent trigger of acute asthma in children despite the frequent use of macrolides in AEs (5).

Because the prevalence and spectrum of viral infections may greatly vary depending on seasonality and locality, epidemiological data regarding the association of viral respiratory infections and AEs in our region would help in the accumulation of data on this issue. Furthermore, information regarding clinical differences of viral triggered AEs may give new perspectives on the prevention and treatment of these AEs. The difference of viral epidemiology of AEs and that of respiratory tract infections in non-asthmatic children is not extensively studied, which may confirm the specific role of some viruses in triggering AEs. Hence, this study was planned to determine the role of different viruses in precipitating AEs in Istanbul, to define the clinical differences of viral triggered AEs, and to compare viral etiology of AEs with that of respiratory tract infections in children without asthma over the same time period.

Material and Methods

Study population and design: This is a prospective descriptive study conducted in the pediatric emergency room of a tertiary university hospital. Study population consisted of 125 children with physician diagnosed asthma aged 2–18 years, who were admitted to the emergency room with AE between December 2013 and April 2014. Children <2 years of age were not included even if they had a history of three or more episodes of wheezing attacks relieved by bronchodilators to rule out the confounding factors due to differential diagnosis. Age-matched 125 peers without known asthma or current wheezing who were admitted to the emergency room with sign and symptoms of acute respiratory tract infection over the same time period were also recruited in the study to compare viral etiologies of AEs and acute respiratory tract infections in non-asthmatic children. Asthma was excluded in these children by questioning their medical history regarding clinical features, which defines asthma. Sex and age-matched children without asthma were recruited within 3 days of a child with asthma entering the study.

Demographic features, acute symptoms, and physical examination on admission were recorded on a standardized data collection sheet. Detailed medical history regarding asthma were taken in asthmatic children. Results of the skin prick allergy tests were obtained from medical records. The severity of AEs was classified according to the Global Initiative for Asthma guidelines (6, 7). Hemogram with differentials, C reactive protein (CRP), and chest X-ray were obtained if ordered by the physician dealing with the patient in the emergency room. Chest X-rays were evaluated by a radiologist blinded to the group of the patient and result of the viral detection. Need for hospital admission (to pediatric wards or to pediatric intensive care unit) was recorded. All patients were followed up via phone calls after discharge until they were free of symptoms regarding the current exacerbation and/or respiratory infection.

This study was approved by the Ethics Committee of İstanbul School of Medicine İstanbul University with the number 2013/1845. Informed consents were obtained from the parents/patients before participation.

Definitions: Asthma was defined as a history of at least three episodes of wheeze and/or dyspnea, which clinically improved with the use of β_2 agonists.

Asthma exacerbation was defined as signs and symptoms like cough, dyspnea, chest tightness, or wheezing, which required admission to the emergency department and nebulized bronchodilators to relieve previously diagnosed asthmatic children (8).

The asthma control level of asthmatic patients was determined by questioning daytime symptoms, limitation of activities, nocturnal symptoms, and need for reliever/rescue treatment in the past 4 weeks according to the criteria previously defined in the Global Initiative for Asthma (GINA) guidelines (6, 7).

Non-asthmatic children with respiratory tract infections were diagnosed based on clinical and radiological findings (9). Bronchiolitis was defined as the presence of tachypnea, dyspnea, and wheezing in younger children, frequently seen with air trapping on chest X-ray. Acute bronchitis was characterized by productive cough and variable rhonchi on auscultation without a consolidation on chest X-ray. Pneumonia was confirmed when patient showed symptoms of lower respiratory tract infections together with rales on auscultation and consolidation on chest X-ray.

Nasopharyngeal sample collection and virus analysis: On the admission day to the emergency room, nasopharyngeal samples were collected by inserting the cotton swab in both nostrils till the nasopharynx and turning it around 360°. All specimens were obtained without administering any solution into the nostrils. The cotton swabs were maintained in the transport medium containing tubes (Virocult, Medical Wire & Equipment, UK). Samples were transported to the virology laboratory immediately during the working hours and analysis was performed on the same day. If the sample was not taken during working hours, it was kept at room temperature for a maximum of 24 h before transporting to the laboratory. In the virology laboratory, EZ1 Virus mini kit V2.0 (Catalog number: 955134, Qiagen, Germany) was used for total nucleic acid extraction in accordance with the manufacturer's instructions. Real-time polymerase chain

reaction (RT-PCR)-based multiplex FTD® Respiratory Pathogens 21 kit (Fast-track diagnostics Ltd. Malta) was used for the detection of respiratory pathogens on RotorGene Q platform (Qiagen, Germany). The kit is able to detect the following 21 respiratory pathogens: influenza A and B; H1N1; RV; CV, NL63, 229E, OC43, HKU1; parainfluenza 1, 2, 3, 4; HMPV A/B; HBOV; *M. pneumoniae*; RSV A/B; AV; EV; and parechovirus and has an internal control. For the detection of influenza H3 subtype, influenza B Yamagata and Victoria lineages, real-time RT-PCR was performed using an ABI 7500 platform with CDC primers and probes according to the CDC protocol (10).

Statistical analysis

Normality was assessed using the Shapiro Wilk test, Kolmogorov–Smirnov test, and histogram graphics. Data were presented as median, minimum, maximum, frequency, and percentage. T test was used for the comparison of normally distributed continuous data between independent groups. Mann–Whitney U test was chosen for data that were not normally distributed. Categorical variables were evaluated using the chi-square test, chi-square with Yates' correction and Fisher's exact test. All p values are based on two tailed statistical analyses, and p values <0.05 were considered statistically significant. All statistical analyses were performed with SPSS (SPSS Statistics for Windows, Version 21.0.; Armonk, NY, IBM Corp).

Results

Patient characteristics

A total of 250 children admitted to the pediatric emergency room, 125 with AE and 125 with respiratory tract infections were recruited to the study between December 2013 and April 2014. The most common clinical diagnosis of non-asthmatic children with respiratory tract infections were upper respiratory tract infection (69.6%), followed by acute bronchitis (12.8%), bronchopneumonia (10.4%), pneumonia (4%), and acute bronchiolitis (3.2%).

Comparison of viral etiology in asthma exacerbations and other respiratory tract infections:

Respiratory pathogens were detected in 84.8% of patients with AE and in 92% of age-matched non-asthmatic children with a respiratory tract infection (Table 1). RV was the most common virus detected (30.4%) in patients with AE. In contrast, non-asthmatic children were mostly infected with influenza A (29.6%), followed by influenza B (15.2%). The distribution of viral pathogens is summarized in Figure 1.

Detection of ≥2 respiratory pathogens was more frequent in AEs (12.8%, p=0.006) and all patients coinfected with RV; 5 with AV; 3 with RSV; 3 with HBOV; 2 with CV; and 1 each with PIV, influenza B, and HMPV. In contrast, coinfection was detected in only four non-asthmatic children with respiratory tract infections: three with HBOV+RSV and one with RV+EV.

Hospitalization rate and duration of symptoms were similar in children with AE and in non-asthmatic children with respiratory tract infections.

Comparison of virus positive and negative asthma exacerbations.

Table 1. Comparison of patients with asthma exacerbations and other respiratory tract infections

	Children with asthma exacerbation (n=125)	Non-asthmatic children with respiratory tract infections (n=125)	p
Age, years, median (range)	5.5 (2.5-15)	5 (2.5-16)	0.386
Age 2–5 years / >5 years, n	63/62	53/72	0.205
Sex, M, n (%)	89 (71.2)	73 (58.4)	0.034
Presence of any respiratory pathogen, n (%)	106 (84.8)	115 (92)	0.115
Admission time; n (%)			
December	32 (25.6)	28 (22.4)	0.864
January	36 (28.8)	44 (35.2)	
February	24 (19.2)	21 (16.8)	
March	24 (19.2)	23 (18.4)	
April	9 (7.2)	9 (7.2)	
Hospitalization, n (%)	33 (26.4)	31 (24.8)	0.772
PICU admission, n (%)	8 (6.6)	3 (2.4)	0.123
Duration of symptoms, days, median (range)	7 (2–20)	6 (4–21)	0.106

PICU: Pediatric Intensive Care Unit

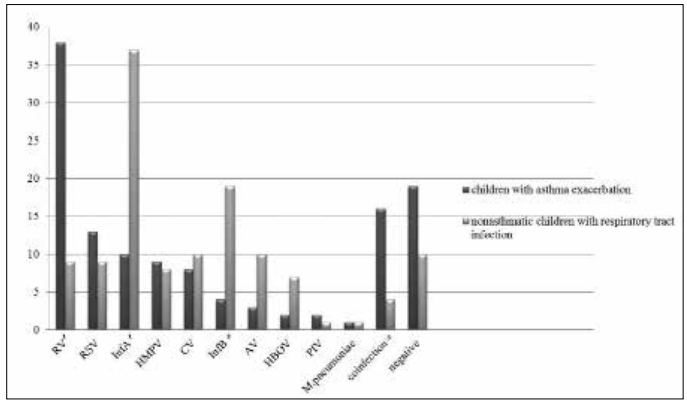

Figure 1. Distribution of respiratory viruses detected in children with asthma exacerbation (n=125) and in non-asthmatic children with respiratory tract infection (n=125)

RV: rhinovirus; RSV: respiratory syncytial virüs; InfA: influenza A; HMPV: human metapneumovirus; CV: coronavirus; InfB: influenza B; AV: adenovirus; HBOV: human bocavirus; PIV: parainfluenza virus

Table 2. Clinical and laboratory features of asthma exacerbations in relation to viral infection

	Virus positive asthma n=106 (84.8%)	Virus negative asthma exacerbations n=19 (15.2%)	p
Age, years, median (range)	5.5 (2.5-15)	8 (3.5-14)	0.001
Sex M; n (%)	76 (71.7)	13 (68.4)	0.771
Domestic tobacco exposure, n (%)	45 (42.5)	12 (63.2)	0.156
Presence of atopic sensitization, n (%)	57 (53.8)	17 (89.5)	0.008
Severity of AE, n (%)			
Mild	48 (45.3)	15 (78.9)	0.006
Moderate	22 (20.8)	4 (21.1)	
Severe	36 (34)	0	
Hospitalization, n (%)	32 (30.2)	1 (5.3)	0.023
PICU admission, n (%)	8 (7.7)	0	0.214
Duration of symptoms, days	7 (4-20)	4 (2-5)	<0.001
Positive CXR finding, n (%)	51 (48.1)	2 (10.5)	0.002
Laboratory parameters, median (range)			
Leucocyte count*	10700 (3870-29300)	9380 (4680-13200)	0.172
Neutrophil count*	7000 (200-24900)	6500 (2930-10200)	0.273
Lymphocyte count*	2500 (500-7300)	2490 (500-4650)	0.891
Eosinophil count*	110 (0-1060)	400 (0-900)	0.011
CRP, mg/dL	9.18 (0.01-100)	1.54 (0.18-10.85)	<0.001

AE: asthma exacerbation; CXR: Chest X ray; PICU: Pediatric Intensive Care Unit

Demographic, clinical, and laboratory characteristics of patients who had an AE with and without a detectable respiratory pathogen were compared (Table 2). Patients with AEs triggered by respiratory pathogens were younger in age (p=0.001). Male gender was predominant in both subgroups. Atopic sensitization defined by positive skin prick tests was more common in children with a virus negative AE (p=0.008). The presence of ≥2 symptoms related to respiratory tract infections, including fever, rhinorrhea, nasal congestion, hoarseness, sore throat, myalgia, and fatigue, was indicative of viral positivity (p<0.001). Fever, pharyngitis, and hyperemia of nasal mucosa were associated with a detectable virus in AEs (p < 0.001 for all). On the other hand, the paleness of nasal mucosa was detected in 68.4% of virus negative asthma exacerbations (p<0.001).

Table 3. Clinical features of asthma exacerbations in patients aged between 2–5 years and >5 years

Patients with asthma exacerbation			
	2–5 years (n=53)	6–17 years (n=72)	p
Organism detected in nasal sample, n (%)			
Negative	2 (3.8)	17 (23.6)	0.005
Rhinovirus	18 (34)	20 (27.8)	0.585
RSV	10 (18.9)	3 (4.2)	0.018
Influenza A	5 (9.4)	5 (6.9)	0.742
HMPV	3 (5.7)	6 (8.3)	0.732
Coronaviruses	2 (3.77)	5 (6.9)	0.698
Influenza B	0	4 (5.6)	0.136
Adenovirus	1 (1.9)	2 (2.8)	1.00
HBOV	2 (3.8)	0	0.178
Parainfluenza viruses	1 (1.9)	1 (1.4)	1.00
Mycoplasma pneumoniae	0	1 (1.4)	1.00
Coinfection	9 (17.6)	8 (14.5)	0.495
Presence of atopic sensitization, n (%)	16 (30.2)	58 (80.6)	<0.001
Severity of AE, n (%)			
Mild	19 (35.8)	44 (61.1)	0.005
Moderate	11 (20.8)	15 (20.8)	
Severe	23 (43.4)	13 (18.1)	
Hospitalization, n (%)	21 (39.6)	12 (16.7)	0.008
PICU admission, n (%)	8 (15.1)	0	0.001
Duration of symptoms, days, n (%)	7 (4–20)	6 (2–21)	0.037

AE: asthma exacerbation; HBOV: human bocavirus; HMPV: human metapneumovirus; PICU: Pediatric Intensive Care Unit; RSV: respiratory syncytial virus

Out of the 125 children with an AE, 51.2% were not using inhaled corticosteroids (ICS) either alone or in combination with leukotriene receptor antagonists (LCRA). The use of short acting β_2 agonists as needed was the most common asthma treatment in either group (37.1% and 47.4% in the virus positive and negative groups, respectively). The type of asthma treatment, i.e., only LCRA, only ICS, combination of ICS and LCRA, or only short acting β2 agonists as needed, did not have a significant effect on virus positivity and severity of AE (p>0.05 for all). Most of the children had partly controlled asthma (70.4%). Children with controlled and uncontrolled asthma had similar frequencies (16.8% and 12%, respectively). Asthma control level affected neither virus detection nor the need of hospitalization (p>0.005).

Presence of a respiratory virus was associated with the severity of AE (Table 2, p=0.006). Hospitalization rate were also significantly higher in virus induced AEs (p=0.024). A respiratory pathogen was detected in all severe AEs (n=36 out of 125), RV was the most prevalent among them (n=15). Duration of the symptoms was longer in children with a virus positive AE (p=0.031). A chest X-ray was ordered in 64.8% of children with AE: peribronchial infiltration was present in 32.5%, consolidation in 7.9%, and atelectasis in 1.6%. A positive radiological finding was significantly associated with a virus positive AE (Table 2, p=0.002). There were no difference in leucocyte, neutrophil, and lymphocyte counts. However, eosinophil counts were found to be significantly higher in children with a virus negative AE (p=0.011). CRP was higher in virus positive AEs (p<0.001).

Comparison of asthma exacerbations in regard to age groups (2–5 years vs 6–17 years).

Detection of a respiratory virus in AEs was associated with younger age (p=0.002). With regard to the detected virus types, only RSV showed a difference in detection rate, with higher detection rate in 2–5 years old asthmatic children (Table 3). RV was the most common respiratory pathogen in both groups. Atopic sensitization was more frequent in patients >5 years old (p<0.001). Severity of AEs increased with younger age (p=0.005). Accordingly, need for hospitalization and pediatric intensive care unit admission was more frequent, and the duration of symptoms were longer in the younger age group (Table 3).

Discussion

This study evaluated the role of respiratory viruses in precipitating AEs of children, with a specific focus on determining the viral epidemiology of AEs and concurrent respiratory tract infections of the non-asthmatic children in the community. It also described the clinical features of virus associated AEs. A respiratory virus was detected in a high proportion of children with AEs (84.8%). Recent studies report a less pronounced rate of virus positivity in AEs (34.1%–63.1%), which may be due to regional and seasonal differences (11-15). It is proposed that altered innate immunity in asthmatics may contribute to viral induced exacerbations, owing to epithelial damage, mucus metaplasia, and immune system polarization towards a T helper 2 phenotype, thus, decreasing the interferon-γ response, which is important in antiviral immunity (16). RV with a rate of 43.2%, alone or as a co-infection, was the predominant virus detected in respiratory secretions of children with AEs. Several studies confirm the association between RV and AEs (2, 5, 11-15). Association between RV and asthma has been thoroughly studied. It was suggested that RV infection induces the release of chemokines from airway epithelial cells, which potentiates preexisting allergic inflammation (16).

In this study, a significantly higher prevalence of RV was detected in children with AEs. On the contrary, influenza viruses were the most common respiratory viruses in the non-asthmatic children with respiratory tract infection, and RV was the fifth most common. The frequency of coinfections also significantly differed between the two groups, with higher incidence seen in children with AEs, and strikingly all coinfections included RV. In brief, data presented here showed that viral epidemiology of asthma does not reflect viral epidemiology in the community for the study period. RV still predominates in AEs, even if the circulating virus in the community is different at that time. Studies evaluating this issue by the inclusion of age-matched asthmatic and non-asthmatic respiratory illnesses at the same time period are limited in number. Rawlinson et al. have found a higher association of HMPV and EVs with respiratory infections in children without asthma than in children with asthma, in whom RV was the most prevalent (17).

Respiratory syncytial virus has been reported as the most common underlying viral cause of AEs in preschool ages (2, 4, 18). Although RSV showed a significantly higher incidence in preschool aged children than in school aged children, RV was still the leading viral cause of AEs in our preschool aged children. The overall low prevalence of RSV infection may be due to the exclusion of wheezy children below 2 years of age in this study.

Most pediatric studies report an overall low prevalence of influenza from 0 up to 7% (14, 19). In this study, influenza A and B were present in 11.2% of children with AEs compared with 44.8% in non-asthmatic children with respiratory infections. The time period of the study should be taken into account in the interpretation of influenza prevalence in AEs because it produces yearly epidemics during the winter months. In 2013–2014 cold season, a high level of influenza activity was observed in İstanbul. It peaked in January and February, 2014, a time period in which 48% of children with AEs were enrolled in the present study. This may explain the relatively high incidence of Influenza in our asthmatic patients. Some studies reported that AEs triggered by Influenza may be severe and lead to hospitalizations (20). However, only 2 out of 14 children with Influenza related AEs were hospitalized in this study.

Children with virus negative AEs were older (2–5 years vs >5 years, p=0.001); a similar finding was reported by Chang et al. (8). Similarly, atopic sensitization was detected more frequently in older children (p<0.001). Furthermore, the presence of atopy was more common and blood eosinophil counts were higher in children with virus negative AEs (p=0.008 and p=0.011 respectively). All of these data, which support one another, imply that older the age, the higher the probability that AEs were

related to allergen exposure and not to a respiratory virus. Previously reported data showed the synergistic interaction between allergen exposure and viral infection in an allergen sensitized host, which increased the risk and severity of asthma exacerbation (21, 22). Findings of the present study did not indicate a synergistic effect of atopic sensitization and presence of a respiratory virus on the severity of asthma exacerbation.

Influence of respiratory virus detection on the severity of AEs and duration of symptoms is not a frequently studied. Studies that included both in and outpatients, like the present study, could make a better and objective evaluation of hospitalization and disease severity. Two trials with such a design, in which almost half of the AEs were virus positive, did not associate respiratory virus positivity and AE severity (5, 13). In contrast, our data revealed positive correlation between respiratory virus detection and severity of AEs, duration of symptoms, need for hospitalization, and pediatric intensive care unit admission ($p<0.05$ in all). This finding indicates that respiratory viruses not only trigger AEs but also increase their severity.

Half of the asthmatic patients were using regular ICS. The influence of the presence and type of controller treatment on virus associated AEs has been seldomly evaluated. In the present study, it did not the affect attack severity and frequency of respiratory virus detection in AEs. In an adult study, it was found that patients with RV infection were significantly less likely to be ICS users (23). Another study reported similar findings to the current study. Authors have found no relation of ICS use with the frequency of virus detection, hospitalization, and attack severity (5). The efficacy of ICS in preventing intermittent viral induced asthma is not well established (24). Based on these findings, it may be proposed that other therapeutic options than the current ones are needed to prevent or decrease the severity of virus associated AEs.

This study has some limitations that should be mentioned. We started patient enrollment in December because the respiratory virus analysis using RT-PCR could be available at that time in our institution. Because of a limited financial source, we could not cover the whole year, which would provide us with a better epidemiologic data. Molecular testing is increasingly used in clinical virology laboratories for diagnosis of viral respiratory tract infections. The sensitivity of PCR testing is higher when compared to traditional tests such as culture and antigen detection (25). Acute viral respiratory infections are caused by the multitude of viruses, which have no specific signs and symptoms. PCR testing for every individual virus is laborious and time-consuming. Multiplex-PCR methods enable us to search for many viruses in a single analysis. The sensitivity of the kit used in our study (multiplex FTD® Respiratory Pathogens 21 kit; Fast-track Diagnostics Ltd. Malta) was compared with the in-house singleplex real-time RT-PCR assays and comparable results were obtained (26, 27). On the other hand, there are concerns about specificity, false positivity, of these methods due to carriage, persistence, and contamination. Determination of the presence of viral nucleic acid in the sample does not necessarily mean that it is viable and infective (28). A comparison of traditional viral testing with multiplex PCR revealed that PCR detected a higher rate of viral presence in healthy controls than traditional methods (29). Despite these limitations, we believe that this study presents important data regarding the association of respiratory viruses and AEs.

Conclusion

This study reveals a high prevalence of respiratory viruses in AEs, with the predominance of RV, in accordance with the vast majority of the literature. Furthermore, it delineates the difference in the viral epidemiology of AEs and other respiratory tract infections of non-asthmatic children. Data presented here show an increase in the severity of AEs with the presence of respiratory viruses, highlighting their importance in treatment and prevention strategies of AEs. Controller treatment with ICS and/or LTRA does not appear to have an influence on virus provoked AEs. Accordingly, future researches should evaluate specific antiviral medications and agents that will augment viral immunity.

Ethics Committee Approval: Ethics committee approval was received for this study from İstanbul University İstanbul School of Medicine with the number 2013/1845.

Informed Consent: Informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - H.A., N.S.; Design - H.A., N.S., Z.T., N.G., A.S.; Supervision - N.S., A.S., Z.T., N.G.; Materials - H.A., M.S., O.B.E., E.Y., M.U., M.C.; Data Collection and/or Processing - H.A., M.S., O.B.E., E.Y., M.U., S.H.T.; Analysis and/or Interpretation - H.A., M.S., N.S.; Literature Review - H.A., N.S.; Writing - H.A.; Critical Review - N.S., A.S., Z.T., N.G.; Other - M.C., M.U., O.B.E, E.Y., S.H.T.

Acknowledgements: We are grateful to Hayriye Vehid, PhD, for her contribution in statistical evaluation, to Deniz Özceker, MD, for her help in caring the patients.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This study was funded by İstanbul University Division of Scientific Research Projects (project number: 40127).

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