悪化する日間の咳および過敏性症状

成人慢性咳患者におけるアジア粉じん

由美子、巻崎良弘、本村紀之、藤村雅明、中西紗香、吉崎智之、斎藤清文、早川和知、小林寛久

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Exacerbation of daily cough and allergic symptoms in adult patients with chronic cough by Asian Dust: A hospital-based study in Kanazawa

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Abbreviations
Total suspended particulate (TSP); Particulate matter (PM); Light detection and ranging (LIDAR); Polycyclic aromatic hydrocarbons (PAHs)
Abstract

The health effects associated with Asian dust have attracted attention due to the rapid increase in the number of Asian dust events in East Asia in recent years. The aim of this study was to investigate the associations between Asian dust and daily cough, as well as allergic symptoms, in adult patients who suffer from chronic cough. We enrolled 86 adult patients from Kanazawa University Hospital, Japan, who were diagnosed with asthma, cough variant asthma, atopic cough or a combination of these conditions. From January to June 2011, subjects recorded their symptoms in a diary every day. Asian dust and non-Asian dust periods were defined according to the dust extinction coefficient, measured using the light detection and ranging (LIDAR). The daily levels of total suspended particulates, polycyclic aromatic hydrocarbons (PAHs) and coexisting factors related to allergies, such as the Japanese cedar pollen count, were measured. McNemar’s test showed that there were significantly more cough-positive patients during Asian dust periods than during the non-Asian dust period ($p = 0.022$). In addition, during Asian dust periods when the daily levels of Japanese cedar pollen, Japanese cypress pollen and PAHs were elevated, there were significantly more patients who experienced itchy eyes than during the non-Asian dust period ($p < 0.05$). On the other hand, there were no significant differences in the allergic symptoms, including sneezing or a runny nose and nasal congestion. This is the first report to show that Asian dust triggers cough and allergic symptoms in adult patients with chronic cough.

Key words:

Allergic symptoms; Asian dust; Asthma; Chronic cough; Daily cough symptom
1. Introduction

The health effects of Asian dust have attracted attention due to the rapid increase in the number of Asian dust events in East Asia in recent years (Kurosaki et al., 2011). Asian dust is sand from the Taklamakan Desert, the Gobi Desert and the Loess area of interior China that is blown by westerly winds to countries such as Japan, which are positioned downwind. These sand storms typically occur during the spring and sometimes during the late fall in the Northern Hemisphere (Iwasaka et al., 2003; Minoura et al., 1998). Epidemiological studies have shown that Asian dust increases hospitalizations and emergency visits because it exacerbates asthma (Kanatani et al., 2010; Yang et al., 2005), allergic rhinitis (Chang et al., 2006) conjunctivitis (Yang, 2006) and contact dermatitis (Otani et al., 2012), and it decreases the peak expiratory flow values (Park et al., 2005; Watanabe et al., 2011). These findings are supported by some experiments with laboratory animals: Asian sand dust inhalation enhanced allergen-induced airway inflammation in mice and in guinea pigs (Hiyoshi et al., 2005; Ichinose et al., 2008; Lei et al., 2004) and allergic rhinitis in rats (Ichinose et al., 2009).

Asthma is a chronic airway inflammatory condition characterized by repetitive cough, wheezing, dyspnea, reversible airway narrowing and airway hyperresponsiveness (Ohta et al., 2011). The prevalence of asthma amongst Japanese adults has increased over the past 10 years (Fukutomi et al., 2010), and in recent years, the number of patients who suffer from chronic cough without wheezing or dyspnea has also increased. In Japan, most patients with chronic cough without wheezing or dyspnea are diagnosed with cough variant asthma and atopic cough (Fujimura et al., 2005). Cough variant asthma is considered to be a precursor of typical asthma (Corrao et al., 1979; Irwin et al., 1997) and an atopic cough is defined as a non-asthmatic bronchodilator-resistant chronic cough with an atopic basis (Fujimura et al., 1992). It is known that chronic cough can result from a hypersensitivity reaction to environmental factors such as
chemicals, cold air and smoke (Matsumoto et al., 2012; Ternesten-Hasseus et al., 2011), although there have been no reports about an association between Asian dust and chronic cough symptoms.

We conducted a patient survey to evaluate whether Asian dust exacerbates daily cough and other allergic symptoms in adult patients who suffer from at least one of the following: asthma, cough variant asthma and atopic cough, in Kanazawa City, which is located near the Sea of Japan.

2. Methods

2.1 Patients

We recruited 104 patients who had been diagnosed with and treated for asthma, cough variant asthma or atopic cough (or a combination of these) at the Kanazawa University Hospital, Japan, from January to June 2011. Ethical approval for this study was obtained from Kanazawa University. All patients gave informed consent before participating in the study. Ninety (86.5%) of the 104 patients gave informed consent and completed allergy diaries, and were enrolled in our study. We excluded four of these patients because they were current smokers, and we analyzed the data from the remaining 86 subjects. Asthma was diagnosed on the basis of the Japan Asthma Prevention and Management Guidelines 2011 (Ohta et al., 2011). Cough variant asthma was diagnosed according to the criteria of the Japanese Cough Research Society (Kohno et al., 2006), and atopic cough was diagnosed according to previously reported criteria (Fujimura et al., 1992), which is based on a cough resistant to the use of bronchodilators and cough resolution with the use of histamine H1 antagonists and/or inhaled corticosteroids. The following factors were also taken into consideration: an atopic predisposition, including a history and/or complications of allergic disease, excluding asthma, findings of blood
examinations, chest and sinus radiographs, cough sensitivity to capsaicin and the bronchial responsiveness to methacholine. Some atopic cough patients also had asthma or cough variant asthma. During the study period, the patients continued to take their usual medications, and none of them experienced symptoms evident of chronic obstructive pulmonary diseases or other disorders.

2.2 Patient symptom surveys
At the start of the study, patients were instructed to record their personal daily cough and other respiratory, nasal and ophthalmic allergic symptoms in their allergy diaries every day from January to June 2011, which was the study period. They recorded their symptoms at the end of each day. To ensure that patients remembered to record their symptoms during this period, we requested them to bring their diaries along to all consultations that were scheduled during this study period. Patients had to record asthmatic symptoms, such as the frequency of coughing, the appearance of wheezing, chest tightness, asthma attacks and the presence of sputum, in a diary kept for all “asthmatic” records, while they had to record allergic symptoms such as sneezing, a runny nose, nasal congestion and itchy eyes in a diary kept for “nasal allergy” records (Okubo et al., 2011). We checked the lack of record which was not related with the health symptoms when patients brought their diary along to all consultation during the study period. We also confirmed the all 86 subjects did not dropout during the study period. Missing rate in cough and itchy eyes were 26.7 % and 9.3 %, respectively.

2.3 Definition of the Asian dust and non-Asian dust periods
Asian dust period was estimated by the dust extinction coefficient, measured using a light
detection and ranging (LIDAR) system, with a polarization analyzer that could distinguish between mineral dust, including Asian dust, and other spherical particles (Iwasaka et al., 2004; Shimizu et al., 2004; Sugimoto et al., 2011). We averaged 24 h data, obtained from the atmosphere 1 km above the ground at the Toyama observation station (Imizu City, Toyama Prefecture, Japan) which is approximately 40 km east of Kanazawa City. The standard threshold of the dust extinction coefficient for a Asian dust day was 0.1/km (Sugimoto et al., 2003). The Japan Meteorological Agency identifies a Asian dust day (the occurrence of a Asian dust event) as a day when the visibility is <10 km in a Japanese city. During the study period, five Asian dust days were observed in Kanazawa City: May 2, 3, 4, 13 and June 4. The dust extinction coefficients on these five days were as follows: 0.1/km on three of the days, and 0.09/km and 0.02/km on the other two days, respectively. Therefore, the dust extinction coefficient that could visibly be observed was approximately 0.1/km or more. In this study, the Asian dust periods were defined as four consecutive days when the dust extinction coefficient measured using the LIDAR was > 0.03/km, in order to evaluate the health effects after heavy as well as moderate dust events that could not be visibly observed. During the study period, 15 days with dust extinction coefficients > 0.03/km were observed: April 7, 10, and 12–15 and May 1–4, 6, and 13–16. We identified the consecutive four-day periods of April 12–15, May 1–4 and May 13–16 as Asian dust periods 1, 2 and 3, respectively (Figure 1).

As a control for Asian dust periods, the non-Asian dust periods were defined as four consecutive days with dust extinction coefficient of < 0.01/km. During April and May, the consecutive days with dust extinction coefficient of < 0.01/km was observed only on May 27–31, therefore the period of May 27-30 was selected as the non-Asian dust period (Figure 1).
2.4 Measurement of the total suspended particulates, PAHs and pollen particles

The daily total suspended particulate (TSP) was collected at Kanazawa University (Takaramachi, Kanazawa, Ishikawa Prefecture, Japan) (136.67°E, 36.56°N) using a high-volume air sampler (120SL, KIMOTO, Osaka, Japan) with a glass filter (FIBERFILM T60A20, 8 × 10 in, Pallflex, Putnam, CT, U.S.A.) at a flow rate of 1000 L/min. This method was used throughout the study period. After the TSP was collected for 24 h, the filter was replaced with a new one, and the collected filter was placed in a desiccator for 16 to 48 h. After measuring the TSP weight, the filter was stored in a freezer at -20°C until pretreatment. We measured the total concentration of PAHs, including fluoranthene, pyrene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, and benzo[a]pyrene. PAHs were analyzed using a HPLC as described previously (Yang et al., 2007) with several modifications. Briefly, 5 cm² of each filter was cut into small pieces. The pieces were placed in a 10-mL test tube containing 625 μL of ethanol and 1.88 mL of dichloromethane and treated ultrasonically for 15 min. The extract was passed through membrane filters (polytetrafluoroethylene [pore size, 0.45 μm]; GL Science, Tokyo, Japan) and analyzed using HPLC equipped with a fluorescence detector (RF-20A; SHIMADZU, Kyoto, Japan) and a reversed-phase column (Intersil ODS-P, 4.6 × 250 mm, 5 μm; GL Science).

The levels of Japanese cedar, Japanese cypress and Japanese rice pollen were measured with a Durham sampler (Durham, 1946) at Kanazawa University in a position that allowed free air movement on all sides. Slides covered with glycerin were exposed to the air for 24 h. The total daily pollen count was expressed as the number of pollen particles per square centimeter.

2.5 Statistical analysis

We compared the presence of symptoms (positive or negative) during Asian dust and non-Asian
dust periods for each of our patients. Patients who experienced symptoms for at least one day during each of the four-day periods were considered to be positive for symptoms. The McNemar’s test was used to compare the symptoms within the Asian dust and non-Asian dust periods. Total number of patients evaluated in each symptom was not always equal due to the missing of records.

Data were analyzed using the SPSS software program for MS Windows, version 19.0 (SPSS, Inc., New York, NY, U.S.A.). Significance was set at $p < 0.05$ for all analyses.

3. Results

3.1 Characteristics of the subjects

The ages of the subjects in our study varied between 23 and 84 years. Twenty-eight and 58 of the 86 subjects were male and female, respectively (Table 1). In all, 49, eight and 15 subjects were diagnosed with asthma, cough variant asthma, and atopic cough, respectively. Four of the patients had asthma as well as atopic cough, and 10 of the subjects had cough variant asthma as well as atopic cough. The mean ± standard deviation (SD) of the disease duration was $15.6 \pm 9.4$ years. The mean concentration of TSP was the highest during Asian dust period 2 (Table 2), whereas Asian dust period 1 had the highest levels of PAHs, Japanese cedar pollen and Japanese cypress pollen (Table 2).

3.2 Cough symptoms during Asian dust and non-Asian dust periods

During the study period, the prevalence of cough and sputum symptoms varied between 20% and 50% (Figure 2A). To examine the effects of Asian dust on cough symptoms, the existence of cough symptoms (positive or negative) in each patient was compared between Asian dust and non-Asian dust periods. During Asian dust period 1, a total of 11 patients, who were
cough-negative during the non-Asian dust period, became cough-positive. There were two patients who were cough-positive during the non-Asian dust period but cough-negative during the Asian dust period. There were significantly more cough-positive patients during the Asian dust period than that during the non-Asian dust period ($p = 0.022$). Similarly, during Asian dust periods 2 and 3, a total of 12 and 10 patients were cough-positive, but had been cough-negative during the non-Asian dust period, respectively. During Asian dust period 3, only one patient was cough-positive in the non-Asian dust period, but was cough-negative during the Asian dust period. None of the patients were cough-negative during Asian dust period 2. We found an association between the cough symptoms and Asian dust periods ($p = 0$ and $p = 0.012$ for Asian dust periods 2 and 3, respectively). There was no significant difference in the symptom of sputum production between Asian dust periods 1, 2 and 3 and the non-Asian dust period ($p = 1$, $p = 0.344$ and $p = 0.727$, respectively). As only a few patients experienced wheezing ($n = 3$), chest tightness ($n = 5$) or an asthma attack ($n = 4$) during the study period, we excluded these symptoms from our analyses.

We also evaluated the presence of allergic symptoms in each of the patients in order to estimate the modified effects on allergic symptoms by the interaction of exposures to Asian dust and pollen. During the study period, the prevalence of a runny nose and sneezing varied between 0% and 20%, and the prevalence of nasal congestion and itchy eyes varied between 0% and 60% (Figure 2B). We compared the number of patients who experienced allergic symptoms during Asian dust and non-Asian dust periods (Table 4). There were significantly more patients with itchy eyes during Asian dust period 1 than during the non-Asian dust period ($p = 0.019$). None of the other allergic symptoms, including sneezing, a runny nose or nasal congestion, differed significantly during the Asian dust and non-Asian dust periods.
4. Discussion

To the best of our knowledge, this is the first study to report that Asian dust exacerbated daily cough symptoms in adult patients suffering from chronic cough. We evaluated the presence of cough symptoms in each of the patients during Asian dust periods and during a non-Asian dust period. As a result, we clearly found that there were significantly more cough-positive patients during Asian dust periods than during the non-Asian dust period. According to a telephone survey within three days after an Asian dust event, Watanabe et al. reported that about 14% of asthma patients experienced exacerbation of cough symptoms on the Asian dust event days (Watanabe et al., 2011), although there were no statistical tests performed to determine the significance of this finding. We showed that about 12% of asthma patients experienced exacerbation of cough symptoms based on a survey with personal daily diaries. In the present study, the patients recorded their cough and other allergic symptoms in their allergy diaries every day irrespective of Asian dust events. The benefit of this method is to prevent the patients from being influenced by the information about the Asian dust event. In the beginning of the study, many patients might feel that it was difficult to record their symptoms every day, however, they soon became accustomed to keeping the diary and made it a habit. In addition, there was a clinical benefit for sharing the information, because it allowed physicians to better understand the patients’ conditions. Accordingly, 86.5% of the patients completed allergy diaries in this study.

Although Asian dust events (Asian dust days) in Japan are determined by visibility-based observations made by the Japan Meteorological Agency, the data measured with the LIDAR system was used in our assessment. The LIDAR system distinguishes between mineral dust particles, including Asian dust, and other spherical particles by identifying differences in the shapes of the particles (Sugimoto et al., 2003). Recently, some
epidemiological studies have used LIDAR data to evaluate the health effects of Asian dust. One study showed that a dust extinction coefficient of > 0.1/km resulted in an increase in asthmatic hospitalization (Kanatani et al., 2010), and another study found that emergency ambulance dispatches, in response to illnesses such as cardiovascular stress, increased on days with a dust extinction coefficient of > 0.066/km (Ueda et al., 2012). During this study period, the dust extinction coefficients of the five Asian dust days determined by visibility-based observation were approximately 0.1/km or more. In the present study, to investigate the health effects of heavy, as well as moderate, dust events that could not be observed visibly, we defined a Asian dust period as four consecutive days with a dust extinction coefficient of > 0.03/km. Consequently, we found that even though none of the patients needed emergency treatment during these Asian dust periods, a significant number of patients experienced a worsening of cough symptoms during moderate dust events.

We also estimated the modified effects of allergic symptoms by the interaction of exposure to Asian dust and the coexisting factors that were related to allergies, including Japanese cedar pollen, Japanese cypress pollen and Japanese rice pollen. Japanese cedar pollinosis is the most common pollinosis in the spring season (Nakamura et al., 2003). In recent years, this condition has become a public concern because the prevalence of common pollinosis has increased (Kaneko et al., 2005). We found that there were significantly more patients who experienced itchy eyes during the Asian dust period that coincided with the presence of pollen than during the non-Asian dust period. On the other hand, there were no significant differences in the other allergic symptoms, including sneezing or a runny nose and nasal congestion. A possible reason for this finding was that the prevalence of nasal congestion was high throughout the study period (Figure 2B), therefore, other allergic symptoms were hardly affected. However, it is not yet clear whether a worsening of itchy eyes depends on the quantity of pollen or Asian
dust, because the pollen count and Asian dust increased simultaneously during the Asian dust periods.

From October to April, PAHs from coal emissions are transported over long distances from the Asian continent to Japan. However, when ambient temperatures in China start to rise, around April each year, the coal emissions decline (Yang et al., 2007). Consistent with this, the atmospheric concentration of PAHs was the highest during Asian dust period 1, which included April 12–15. As diesel exhaust particles containing PAHs are known to adversely affect asthma patients (McCreanor et al., 2007), the asthma-related symptoms may be exacerbated due to the simultaneous inhalation of PAHs and Asian dust.

In this study, the control period was located only at the end of May. Because Figure 2A had indicated the decreasing trend in the prevalence of cough in May, there is a possibility that the association between Asian dust and cough symptoms could be influenced by the seasonal weather variables. We analyzed the effects of Asian dust in the 86 patients enrolled in our study. Further studies with a larger number of patients are needed to reveal the environmental factors, including pollens, chemicals, weather conditions and bioaerosols, which could adversely affect these respiratory diseases (Griffin, 2007; Maki et al., 2010; Tobo et al., 2010), and to demonstrate whether the symptoms could vary in terms of asthma, cough variant asthma and atopic cough.

5. Conclusions
This is the first report to show that Asian dust triggers cough and allergic symptoms in adult patients suffering from chronic cough. We found that there were significantly more cough-positive patients during Asian dust periods than that during the non-Asian dust period. In addition, when daily levels of coexisting factors related to allergies, such as the Japanese cedar
pollen count, were elevated, a significantly higher number of patients experienced itchy eyes during Asian dust periods. Therefore, the present study suggests that Asian dust enhances allergic reactions to Japanese cedar pollen.

Acknowledgments

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Sugimoto, N., Hara, Y., Shimizu, A., Yumimoto, K., Uno, I., Nishikawa, M., 2011. Comparison of
Figure legends

Figure 1. The daily concentrations of airborne particulate matter during the study period. AD1: Asian dust period 1, AD2: Asian dust period 2, AD3: Asian dust period 3, non-AD: non-Asian dust period.

Figure 2. The daily prevalence of cough and sputum production (A), sneezing, nasal congestion, runny nose and itchy eyes (B) during the study period. AD1: Asian dust period 1, AD2: Asian dust period 2, AD3: Asian dust period 3, non-AD: non-Asian dust period.
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### Table 1. Characteristics of patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No (%) or Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>86</td>
</tr>
<tr>
<td>Age (years)</td>
<td>64.0 ± 12.7</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>28 (32.6)</td>
</tr>
<tr>
<td>Female</td>
<td>58 (67.4)</td>
</tr>
<tr>
<td>Disease</td>
<td></td>
</tr>
<tr>
<td>Asthma</td>
<td>49 (57.0)</td>
</tr>
<tr>
<td>CVA</td>
<td>8 (9.3)</td>
</tr>
<tr>
<td>AC</td>
<td>15 (17.4)</td>
</tr>
<tr>
<td>Asthma and AC</td>
<td>4 (4.7)</td>
</tr>
<tr>
<td>CVA and AC</td>
<td>10 (11.6)</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>15.6 ± 9.4</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
</tr>
<tr>
<td>non-smoker</td>
<td>60 (69.8)</td>
</tr>
<tr>
<td>ex-smoker</td>
<td>26 (30.2)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.8 ± 3.3</td>
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</tbody>
</table>

* CVA, cough variant asthma; AC, atopic cough
Table 2. Average airborne particulate matter during the Asian dust and non-Asian dust periods

<table>
<thead>
<tr>
<th></th>
<th>non-Asian dust period (27-30 May)</th>
<th>Asian dust period 1 (12-15 April)</th>
<th>Asian dust period 2 (1-4 May)</th>
<th>Asian dust period 3 (13-16 May)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dust extinction coefficient (km)</td>
<td>0.003 ± 0.003</td>
<td>0.045 ± 0.016</td>
<td>0.166 ± 0.121</td>
<td>0.091 ± 0.061</td>
</tr>
<tr>
<td>TSP (µg/m³)</td>
<td>17.5 ± 6.63</td>
<td>68.4 ± 12.8</td>
<td>125.1 ± 69.7</td>
<td>90.3 ± 24.5</td>
</tr>
<tr>
<td>PAHs (ng/m³)</td>
<td>0.51 ± 0.35</td>
<td>3.28 ± 1.11</td>
<td>0.86 ± 0.42</td>
<td>1.81 ± 0.68</td>
</tr>
<tr>
<td>Japanese cedar pollen (/cm²)</td>
<td>0</td>
<td>49.3 ± 33.9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Japanese cypress pollen (/cm²)</td>
<td>0</td>
<td>31.5 ± 19.7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Japanese rice pollen (/cm²)</td>
<td>1.75 ± 1.71</td>
<td>0</td>
<td>8.5 ± 10.0</td>
<td>6.0 ± 6.0</td>
</tr>
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Table 3. The number of patients who experienced symptoms during the Asian dust and non-Asian dust periods

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>non-Asian dust period (27-30 May)</th>
<th>Asian dust period 1 (12-15 April)</th>
<th>Asian dust period 2 (1-4 May)</th>
<th>Asian dust period 3 (13-16 May)</th>
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<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td>p-value</td>
<td>Negative</td>
</tr>
<tr>
<td>Cough</td>
<td>31</td>
<td>11</td>
<td>0.022</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>19</td>
<td>0.012</td>
<td>0</td>
</tr>
<tr>
<td>Sputum production</td>
<td>40</td>
<td>5</td>
<td></td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>23</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

Note: p-values indicate the significance of the difference between the Asian dust and non-Asian dust periods.
<table>
<thead>
<tr>
<th>Symptoms</th>
<th>non-Asian dust period (27-30 May)</th>
<th>Asian dust period 1 (12-15 April)</th>
<th>Asian dust period 2 (1-4 May)</th>
<th>Asian dust period 3 (13-16 May)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td>p-value</td>
<td>Negative</td>
</tr>
<tr>
<td>Sneezing</td>
<td>Negative</td>
<td>18</td>
<td>13</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>8</td>
<td>22</td>
<td>7</td>
</tr>
<tr>
<td>Runny nose</td>
<td>Negative</td>
<td>17</td>
<td>11</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>5</td>
<td>28</td>
<td>4</td>
</tr>
<tr>
<td>Nasal congestion</td>
<td>Negative</td>
<td>43</td>
<td>7</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>5</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Itchy eyes</td>
<td>Negative</td>
<td>55</td>
<td>15</td>
<td>61</td>
</tr>
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<td></td>
<td>Positive</td>
<td>4</td>
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</table>