Abstract

Objectives: Upper respiratory virus infection is associated with the expression of symptoms and signs of illness, and with the development of complications in anatomically contiguous structures. In most epidemiological studies, the frequency of the various complications is expressed as a fraction of the total population judged to be ill by report, signs, or symptoms. Because not all infected subjects become ill and because infected non-ill subjects may develop complications, such risk estimates could be inaccurate. The objective of this study was to estimate the magnitude of the presentation bias during controlled, experimental infections.

Study Design: This was a prospective, experimental study of the relationship between illness and otological complications during experimental upper respiratory virus infection in 316 adult volunteers.

Methods: The data for illness and for abnormal middle ear underpressure in adult (18-54 y) volunteers experimentally infected with one of three viruses (rhinovirus type 39, rhinovirus strain hanks, influenza A virus) were analyzed and expressed as the relative frequencies of infected subjects reporting illness, developing abnormal middle ear pressure, and developing abnormal middle ear pressure in the absence of illness.

Results: For all three viruses, illness was documented in approximately 50% of the infected subjects. While the frequency of persons developing abnormal middle ear underpressure was greater in the infected-ill subjects, approximately one third of all infected subjects developing that complication did not report illness.

Conclusions: These results support a large presentation bias in epidemiological surveys of viral upper respiratory infections, and infer that those surveys underestimate the true frequency of complications resulting from such infections.

INTRODUCTION

Upper respiratory tract infection (URI) is the most common disease in both the pediatric and adult populations.1,2 The majority of URIs are caused by viruses, with the rhinoviruses contributing to more than 30% of these infections. URIs are associated with nasal, throat, and systemic signs and symptoms and contribute greatly to morbidity in the human population. Also, URIs precede and predispose to complications such as sinusitis, otitis media, pneumonia, and exacerbations of asthma, that further contribute to morbidity and economic costs.3

Otitis media is characterized by the presence of inflammation within the middle ear and is second in frequency for diseases affecting infants and children only to URIs.4 Persistent otitis media with effusion is refractory to current methods of medical treatment and can persist for weeks, months, or even years. Epidemiological studies show that more than 50% of the new episodes of otitis media are temporally associated with a viral URI and experimental studies document a causal relationship between the two diseases.5-9 Thus a significant impact on the incidence and prevalence of otitis media could be realized by rational interventions in URI pathogenesis that prevent otitis media as a complication.
Experimental respiratory virus infection of adults has been developed as a model to study the pathogenesis of the infection and its complications. In studies with different viruses, experimental infection caused sequential otological complications that were consistent with a causal pathway leading from virus infection to otitis media via the intermediate development of eustachian tube dysfunction and middle ear underpressure. Using that model, a number of medical interventions were evaluated with respect to their ability to reduce symptom load and decrease the frequency of complications. Interestingly, studies with anti-inflammatory, antimi auditory, and antiviral drugs documented different response domains for the various symptoms, signs, and complications. For example, treatment of subjects infected with influenza A virus using the antiviral rimantadine caused a rapid and significant reduction in total symptom load, but had no effect on the frequencies of persons with earache, abnormal eustachian tube function, middle ear underpressure, or otitis media. These observations suggest that in disease course, the pathogenic pathway or pathways for the development of symptoms are disassociated from that for these otological complications.

In the present study, this hypothesis is tested using the data available for adults experimentally exposed to one of three viruses, rhinovirus type 39 (RV39), rhinovirus strain Hanks (RVH), and influenza A virus (INF). The data for infection, illness, and one measure of an otological complication (abnormal middle ear underpressure) were used to define the relative frequencies of infected subjects reporting illness, developing complications, and developing complications in the absence of illness. Results were examined with respect to the predictive accuracy of illness presentation for identifying persons with otological complications of a viral URI.

MATERIALS AND METHODS

Subjects included in this report were recruited by newspaper advertisement from the population and surrounding community of the University of Pittsburgh and were studied in conformity with the requirements of two different protocols approved and funded by the National Institutes of Health. Potential subjects had a general physical examination, a urological history, and a history taken for assay of markers of hepatic and renal function and for assay of serum antibodies to HIV using standard methods. Subjects were excluded if presenting with findings or a history suggestive of systemic illness or recent URI, if they had marked elevations in the assay parameters indicative of hepatic or renal impairment, if they required prescription medication for any condition other than birth control, or if they had antibodies to HIV. The rhinovirus study protocol was approved by the Human Rights Committee at the University of Pittsburgh and the influenza A study protocol was approved by the Committee at the Children's Hospital of Pittsburgh. All subjects provided written informed consents for HIV screening and study participation.

The population included in this report consisted of 316 healthy, adult volunteers (age range, 18-54 y) who had bilaterally normal middle ear pressure on baseline testing. The subjects were experimentally exposed to either rhinovirus (RV39, n = 141; RVH, n = 123; all untreated) or INF (n = 52; placebo-treated group) as previously described. Details of the hypotheses, methodologies, and specific results for each of the two parent studies were published previously. Middle ear testing protocols, procedures for monitoring illness, cloister site, and study personnel were identical for the three groups. All subjects and investigators were blinded to the presence or absence of infection and, for the influenza A study protocol, to the treatment assignment (rimantadine vs. placebo) of the subjects.

Subjects in all groups were cloistered in separate rooms of a local hotel for a 6-day (rhinovirus) or 8-day (influenza A virus) period. On each day of cloister, symptoms were scored by the subjects, signs were evaluated by a physician, and objective measurements of nasal mucociliary transport rate, airway patency, secretion production, and middle ear pressure were made. Also, a nasal lavage was performed and samples were submitted for virus culture. Twenty-four hours after admission to cloister (end study day 0), the subjects were intranasally inoculated with a safety-tested strain of rhinovirus or influenza A virus. Pre-exposure and postexposure (approximately 3-4 w) bloods were drawn for assay of virus-specific antibody titers in accordance with standard methods.

The data for this report consisted of measures of infection, illness, and middle ear pressure. Infection was defined for all groups as recovery of the challenge virus from the nasal lavage fluid on at least one post-challenge day of cloister and/or a fourfold increase in virus-specific serum antibody titer between the prechallenge and convalescent blood serum samples. For rhinovirus exposures, eight symptoms including sneezing, headache, malaise, chilliness, nasal discharge, nasal congestion, cough, and sore throat were scored by the subject on a five-point scale (none, mild, moderate, moderate-severe, and severe) corresponding to none, mild, moderate, moderate-severe, and severe degrees. Additionally, the subjects were questioned as to whether they believed that they had a cold on that day. Subjects were defined as ill if they reported on any day during the period of cloister that they had a cold. For influenza A challenges, 15 specific symptoms including sneezing, nasal discharge, nasal congestion, earache, sinus pain, sore throat, cough, chest congestion, malaise, headache, chilliness, muscle ache, joint pain, sweats, and fever were rated by the subjects on a four-point scale. Subjects were defined as ill if they had a total symptom score of at least 4 on 2 consecutive days of cloister. Symptom load for all groups was defined as the baseline adjusted total symptom score for the post-exposure period of cloister. For all groups, middle ear status was assessed bilaterally by tympanometry using a commercially available clinical instrument (Teledyne Impedance Screener, Teledyne, Charlottesville, VA). Abnormal middle ear underpressure was defined as a tympanometric value of -100 mm H2O.
In the analysis, the frequency of days with abnormal middle ear underpressure (either unilateral or bilateral) was compared among three subgroups of subjects; i.e., uninfected, infected non-ill, and infected ill. Pairwise comparisons for the number of days with abnormal middle ear underpressure were made among these subgroups for each of the viruses. Statistical significance was assigned using a Mann-Whitney U test evaluated at [alpha] 0.05 (two-tailed). In the data presentation, the convention mean ± standard deviation is used throughout.

RESULTS

The results for the distribution of subjects, total symptom load, frequency presenting with abnormal middle ear underpressure, and the average number of days with abnormal middle ear underpressure for each of the three subgroups are summarized in Table I. The daily frequencies of persons with abnormal middle ear underpressure in each of these subgroups are depicted in Figure 1.

<table>
<thead>
<tr>
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<th>Not Infected</th>
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<th>Infected-Ill</th>
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</thead>
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<tr>
<td>Number of subjects</td>
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<td></td>
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<tr>
<td>Influenza A</td>
<td>4 (8%)</td>
<td>25 (48%)</td>
<td>23 (44%)</td>
</tr>
<tr>
<td>Rhinovirus type 39</td>
<td>13 (9%)</td>
<td>69 (49%)</td>
<td>59 (42%)</td>
</tr>
<tr>
<td>Rhinovirus strain Hanks</td>
<td>27 (22%)</td>
<td>52 (42%)</td>
<td>44 (36%)</td>
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<td>Symptom load</td>
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<td>Influenza A</td>
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<td>32.8 ± 14.2, 26.8–38.9</td>
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<td>5.9 ± 6.9, 4.2–7.5</td>
<td>23.2 ± 12.8, 19.9–26.5</td>
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<td>Rhinovirus strain Hanks</td>
<td>3.6 ± 4.3, 1.9–5.3</td>
<td>7.6 ± 7.9, 5.5–9.7</td>
<td>24.7 ± 12.5, 21.0–26.5</td>
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<td>Number with Abn MEP</td>
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<td></td>
<td></td>
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<tr>
<td>Influenza A</td>
<td>0 (0%)</td>
<td>14 (56%)</td>
<td>19 (83%)</td>
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<tr>
<td>Rhinovirus type 39</td>
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<td>26 (38%)</td>
<td>38 (64%)</td>
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<td>Rhinovirus strain Hanks</td>
<td>10 (37%)</td>
<td>23 (44%)</td>
<td>30 (68%)</td>
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<tr>
<td>Number days with Abn MEP</td>
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<td>Influenza A</td>
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<tr>
<td>Rhinovirus type 39</td>
<td>0.2 ± 0.8, 0.3–0.7</td>
<td>0.8 ± 1.3, 0.5–1.1</td>
<td>1.8 ± 1.8, 1.4–2.3</td>
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<tr>
<td>Rhinovirus strain Hanks</td>
<td>0.7 ± 1.2, 0.2–1.2</td>
<td>1.3 ± 1.8, 0.8–1.8</td>
<td>1.8 ± 1.8, 1.3–2.3</td>
</tr>
</tbody>
</table>

**TABLE I.** Number of Subjects (%), Average Total Symptom Load (±STD; 95% CI), Number Developing Abnormal Middle Ear Underpressures (%), and Average Number of Days (±STD; 95% CI) With Abnormal Middle Ear Underpressures (Abn MEps) for the Three Subgroups of the Influenza- and Rhinovirus-Exposed Subjects.
Influenza Virus

Of the 52 subjects exposed to INF virus and treated with a placebo medication, four (8%) were not infected and none of these developed abnormal middle ear underpressure or qualified as having an illness. Twenty-five (52%) of the infected subjects were not ill. The average total symptom load for the infected non-ill and the infected ill subgroups was 6.5 ± 6.3 and 32.8 ± 14.5, respectively, ($P < .05$). Fourteen (56%) of the subjects in the infected non-ill group and 19 (83%) in the infected ill group had at least 1 day of abnormal pressure. The average number of days with abnormal middle ear underpressure was not different between the infected non-ill (2.16 ± 2.50) and the infected ill (2.78 ± 2.30) subjects, but that measure for both infected subgroups was greater than that of the uninfected subjects ($P < .05$). For both the infected ill and the infected non-ill subgroups, the frequency of persons with abnormal middle ear underpressures increased after INF exposure and remained elevated throughout the period of study (Fig. 1).
Rhinovirus

Thirteen of the 141 subjects (9%) exposed to RV39 and 27 of the 123 subjects (22%) exposed to RVH were not infected. One uninfected subject (4%) in the RVH group and two (15%) in the RV39 group reported having a cold. Of the infected subjects, 59 (46%) exposed to RV39 and 44 (46%) exposed to RVH reported having a cold. The average total symptom load for the uninfected, infected non-ill, and infected ill groups was 3.6 ± 4.4, 5.9 ± 6.9, and 23.2 ± 12.8 for the RV39 subjects and 3.6 ± 4.3, 7.6 ± 7.9, and 24.7 ± 12.5 for the RVH subjects. Pairwise comparisons were significant between those values for the infected ill and those for both of the other two subgroups.

For RV39, one (8%), 26 (56%), and 38 (64%) of the subjects in the uninfected, infected non-ill, and infected ill subgroups had abnormal middle ear underpressure on at least 1 day. Corresponding values for those subgroups of subjects exposed to RVH were 10 (37%), 23 (44%), and 30 (68%), respectively. For RV39, the average number of days with abnormal middle ear underpressure was 0.23 ± 0.83, 0.80 ± 1.31, and 1.83 ± 1.79 for the uninfected, infected non-ill, and infected ill subgroups, respectively. All pairwise comparisons between subgroups were statistically significant. Corresponding values for those subgroups of the RVH-exposed population were 0.70 ± 1.23, 1.31 ± 1.81, and 1.80 ± 1.64, respectively. That summary value was significantly different between the infected ill subgroup and the other two subgroups, but not between the uninfected and infected non ill subgroups exposed to RVH. The patterned relationship among subgroups for the frequency of persons with abnormal middle ear underpressures was similar for the two rhinoviruses. Specifically, on all postexposure days, that frequency was greatest for the infected ill subgroup, intermediate for the infected non-ill subgroup, and least for the uninfected subgroup.

DISCUSSION

The mechanism by which viral URIs cause otitis media is not well understood, but considerable evidence supports mediation by the intermediate development of eustachian tube dysfunction and middle ear underpressure. Indeed, tubal dysfunction and middle ear underpressure were documented in adults and children with natural URIs, and in adults experimentally infected with different viruses.6-13,15 For example, Sanyal and colleagues reported that 75% of their subjects had abnormal tympanograms during a natural URI episode. The majority (81%) of these abnormalities occurred by day 1 or 2 after symptom presentation, and middle ear underpressure was documented prior to the development of otitis media in five of the seven new episodes observed.15 Similarly, adults with experimental viral URIs develop sequential otological manifestations including eustachian tube dysfunction, middle ear underpressure, and otitis media.8-10 In contrast, cross-sectional screening studies and longitudinal repeated measure studies show that in the absence of eustachian tube dysfunction or conditions that predispose to such dysfunction, middle ear underpressure is relatively stable, ranging from -50 to +50 mm H2O for adults.6-13,15,16 Because abnormal middle ear underpressure heralds the development of otitis media in children and adults with URIs, that parameter was used in the current study to estimate the frequency of otological complications after experimental rhinovirus and influenza virus exposures.

In this study, all subjects were cloistered under the same conditions and at the same site. The frequency of illness (7%) and the total symptom load were low in the combined group of uninfected subjects. Approximately 50% of the subjects with documented infection were judged to be ill, and for all groups the symptom load for the infected ill subgroup was much greater than that of the infected non-ill subgroup. Of importance, the frequency of abnormal middle ear underpressure in the infected non-ill subjects was intermediate between that for the infected ill and the uninfected subjects. Indeed, for all three viruses approximately one third of the infected subjects who developed this complication were not ill.

These results are consistent with those of previous studies showing different response domains for the symptoms, signs, and complications of viral URIs. In those studies, intervention with specific antihistamines had consistent and predictable effects on those symptoms associated with the targeted mediator, but not others; and antiviral treatment decreased virus load and local proinflammatory cytokine concentrations and total symptoms, but not the frequency of otological complications.11-13 The existence of mediator-specific domains for different symptoms, signs, and pathophysiologies implies a complex pathogenic process for disease presentation during viral URIs, with divergent pathways being activated soon after infection.

These observations show that a significant proportion of individuals infected with upper respiratory viruses is unaware of the infection, though this subpopulation remains at risk to develop certain otological complications. Thus illness presentation is not a sufficient signal to circumscribe the population experiencing complications of the underlying virus infection. While it is possible that the observed co-incidences among infection, illness, and complications are unique to the experimental model, published data for the model agree with those for natural exposures.16 Thus epidemiological studies that rely on illness to define infection are limited by a significant presentation bias and underestimate the true frequency of infected individuals in the population. Also such studies underestimate the risk of complications associated with a viral URI and misassign with respect to etiology a high percentage of those episodes.
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Key Words: Viral upper respiratory infection; otitis media; influenza virus; rhinovirus

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**Table I**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>Influenza</td>
<td>12.3 ± 0.6</td>
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<td>Rhinovirus 39</td>
<td>12.2 ± 0.6</td>
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<td>Rhinovirus H1N1</td>
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</tr>
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<td>Rhinovirus H3N2</td>
<td>12.2 ± 0.6</td>
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**Fig. 1**