Nasal Secretion Concentrations of IL-5, IL-6, and IL-10 in Children With and Without Upper Respiratory Tract Viruses

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Objective: To determine if levels of interleukin (IL) 5, IL-6, and IL-10 or their ratios in nasal secretion are diagnostic of viral upper respiratory tract infections (vURTIs) and coldlike illnesses (CLIs) in children.

Design: Longitudinal study of children for vURTIs, CLIs, and concentrations and ratios of nasal cytokines.

Setting: Outpatient assessments of children.

Participants: A total of 224 children, aged 1 to 9 years.

Main Outcome Measures: Concentrations of IL-5, IL-6, and IL-10 in nasal secretions, vURTIs diagnosed by polymerase chain reaction (PCR) detection of upper respiratory tract viruses, and concurrent CLIs diagnosed by parents.

Results: Of 1269 secretion samples, 552 (43.5%) were collected during a vURTI (PCR findings positive for an assayed virus [PCR+/H11001]). A concurrent CLI was diagnosed for 34% of the PCR+/H11001 samples and for 18% of the samples found to be negative by PCR analysis (PCR−). Cytokine concentrations and ratios were highly variable and skewed to the lower values. The significance of the cytokine concentrations and ratios as discriminators of groups defined by the presence or absence of virus and of subgroups defined by the presence or absence of a CLI was evaluated using receiver operating characteristic curves. All measures were significant discriminators of the PCR+ vs PCR− groups, and most were significant discriminators of the paired CLI subgroups. The concentration of IL-6 and the IL-5/IL-6 ratio were the best discriminators across all groups and subgroups. However, the sensitivities and specificities of those discriminators at the best cutoff values were on the order of 0.7 for the most extreme pairwise comparison (PCR+/CLI+ vs PCR−CLI−) and lower for the other comparison groups.

Conclusion: The low sensitivities and specificities for cytokine-based assignment of specimens to the paired groups and subgroups limit their usefulness for diagnosis of infection or illness.

periods\textsuperscript{11-13}, (2) in vURTIs accompanied by CLIs vs vURTIs without CLIs\textsuperscript{14}; and (3) in vURTIs with airway complications.\textsuperscript{15-17} Thus, local cytokine levels may serve as markers for the presence of a vURT and for the degree of illness during a vURT.\textsuperscript{18}

Herein, we focus on the nasopharyngeal concentrations of 3 cytokines: interleukin (IL) 6, a proinflammatory cytokine the nasopharyngeal levels of which are known to track sign and symptom expression during the course of a vURT; IL-10, an anti-inflammatory cytokine known to be produced during recovery from a vURT; and, as a control, IL-5, which is not produced during a vURT.\textsuperscript{10,19,20} Concentrations and ratios of these cytokines were assayed in nasopharyngeal secretions and wash fluids collected periodically from children over the course of the typical “cold season.” The samples were grouped by the presence or absence of one or more common upper respiratory viruses, as detected by polymerase chain reaction (PCR) and subgrouped by the presence or absence of a concurrent CLI on the day of sample collection for both virus-positive (PCR\textsuperscript{+}) and virus-negative (PCR\textsuperscript{−}) specimens. The hypothesis tested is that nasal secretion concentrations of IL-6 and/or IL-10 and/or ratios of cytokine concentrations are diagnostic of a vURT and of a CLI in children.

### METHODS

Nasopharyngeal secretion samples were collected during the course of a 2-center longitudinal study of the relationship between otitis media and vURTIs. Families with at least 2 children aged between 1 and 5 years were enrolled. The 2 index children and any older sibling younger than 10 years who attended to participation were included as subjects. Approximately 30 families in each of 4 consecutive years were enrolled during the month of October and observed through April of that year. Families were reimbursed $100/mo for their participation, and the study was approved by the institutional review boards at the 2 study sites.

The general protocol included initially collecting demographic and related information on the enrolled children, followed by the following regular activities: (1) daily parental assignment of their children’s status with respect to the presence or absence of a CLI and of 7 illness signs (i.e., runny nose, nasal congestion, sore throat, cough, fever, irritability, and earache); and (2) weekly assessments of the presence or absence of otitis media by pneumatic otoscopy and periodic collection of nasal secretion samples during parent-identified CLI episodes in the child or in an enrolled sibling, at the onset of a new otitis media episode in the child or in a sibling, and at random times during illness-free periods. The data analyzed herein are the results of nasal secretion sample assays for the presence or absence of common upper respiratory viruses; IL-5, IL-6, and IL-10 protein concentrations and the ratios of these concentrations; and the presence or absence of a CLI on the day of secretion collection.

Nasal secretions were collected by aspiration of free secretions or, in their absence, by introducing sterile saline solution fluids collected periodically from children over the course of the typical “cold season.” The samples were grouped by the presence or absence of one or more common upper respiratory viruses, as detected by polymerase chain reaction (PCR) and subgrouped by the presence or absence of a concurrent CLI on the day of sample collection for both virus-positive (PCR\textsuperscript{+}) and virus-negative (PCR\textsuperscript{−}) specimens. The hypothesis tested is that nasal secretion concentrations of IL-6 and/or IL-10 and/or ratios of cytokine concentrations are diagnostic of a vURT and of a CLI in children.

### RESULTS

Samples were collected from 224 children (118 boys, 190 white, 26 black, and 8 mixed race or other) aged 1 to 8.6 years (mean [SD] age, 3.6 [1.6] years). A total of 1269 samples were assayed for virus and the 3 cytokines; 532
absence (−) of virus.

...in 15 (2.7%), and multiple viruses in 55 (10.0%). Assign-
...eted virus was rhinovirus in 367 samples (66.5%), RSV
...tection (PCR) for at least 1 of the assayed viruses.

...of these were PCR+ for at least 1 of the assayed
...ected virus was rhinovirus in 367 samples (66.5%), RSV
...in 37 (6.7%), coronavirus in 36 (6.5%), influenza virus in
...IL-5/IL-10 concentrations in secretion samples and their ratios
...data for the IL-5, IL-6, and IL-10 concentrations in secretion
...anxiety or absence of illness (CLI+ vs CLI−) on the day of

(43.5%) of these were PCR+ for at least 1 of the assayed
virus. The detected virus was rhinovirus in 367 samples
(66.5%), RSV in 37 (6.7%), coronavirus in 36 (6.5%), influenza
in 27 (4.9%), parainfluenza virus in 15 (2.7%), adenovirus
in 15 (2.7%), and multiple viruses in 55 (10.0%). Assign-
mments of CLI for the day of sample collection could be made
for 502 of the PCR+ samples (90%) and 645 of the PCR−
samples (90%) (missing assignments resulted from incom-
plete parental diary data). A concurrent CLI was present
for 171 of the 502 PCR+ specimens (34%) and 118 of the
645 PCR− samples (18%) (P < .001 for the χ2 test).

**Table 1** summarizes data for the IL-5, IL-6, and IL-10
concentrations in secretion samples and their ratios for
groups defined by the presence or absence of virus de-
tection (PCR+ vs PCR−) and, for both groups, by the pre-

Table 1. Cytokine Concentrations and Ratios in the Study Groups and Subgroups

<table>
<thead>
<tr>
<th>Cytokine Characteristic</th>
<th>Sample Findinga</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>PCR+ (n = 552)</td>
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<tr>
<td>Concentration, pg/mL</td>
<td></td>
</tr>
<tr>
<td>IL-5</td>
<td>6.98 (2.00-1794.32)</td>
</tr>
<tr>
<td>IL-6</td>
<td>1.98 (0.05-204.00)</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.10 (0.10-120.24)</td>
</tr>
<tr>
<td>Ratio IL-5/IL-6</td>
<td>4.34 (0.05-4321.80)</td>
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<tr>
<td>Ratio IL-5/IL-10</td>
<td>20.35 (0.02-17454.50)</td>
</tr>
<tr>
<td>Ratio IL-6/IL-10</td>
<td>5.65 (0.00-2040.00)</td>
</tr>
</tbody>
</table>

Abbreviations: CLI, coldlike illness, present (+) or absent (−); IL, interleukin; PCR, polymerase chain reaction assay, the test used to determine the presence (+) or absence (−) of virus; SE, standard error.

**Table 2** summarizes the statistical data for the ROC curves (AUC [standard error (SE)], Z value, and 2-tailed, P value) constructed for each of the 3 cytokine concentrations and their ratios with respect to assigning samples to the appropriate paired group and subgroup. For the PCR+ vs PCR− comparison, all 3 cytokines and their ratios were significant discriminators. However, while the IL-6 and IL-10 concentrations and the IL-6/IL-10 ratio assigned the samples to the appropriate group, the IL-5

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concentration and the IL-5/IL-10 and IL-5/IL-6 ratios assigned the samples to the opposite group (eg, PCR+ samples assigned to PCR− group). For the PCR+ CLI+ vs the PCR− CLI− groups based on interleukin (IL) 6 concentration (A) and on the IL-5/IL-6 ratio (B). The values increase from left to right. Note that the IL-6 concentrations are higher for the PCR− CLI+ group while the IL-5/IL-6 ratios are higher for the PCR− CLI− group. Best discrimination is achieved at an IL-6 concentration and IL-5/IL-6 ratio that maximizes the vertical difference between the ROC curve and the line of identity. PCR indicates polymerase chain reaction, the test used to determine the presence (+) or absence (−) of virus; CLI, coldlike illness present (+) or absent (−).

Figure. Receiver operator characteristic (ROC) curves for sample assignment to the PCR− CLI− vs the PCR+ CLI+ groups based on interleukin (IL) 6 concentration (A) and on the IL-5/IL-6 ratio (B). The values increase from left to right. Note that the IL-6 concentrations are higher for the PCR− CLI+ group while the IL-5/IL-6 ratios are higher for the PCR− CLI− group. Best discrimination is achieved at an IL-6 concentration and IL-5/IL-6 ratio that maximizes the vertical difference between the ROC curve and the line of identity. PCR indicates polymerase chain reaction, the test used to determine the presence (+) or absence (−) of virus; CLI, coldlike illness present (+) or absent (−).

A large number of cytokines could be assayed in this type of study,10 and so it is important that judicious choices be made regarding those selected for assay. Studies that have captured the temporal dynamics for a variety of cytokines elaborated during the course of experimental vURTIs provide some guidance.10,19,20,22 Across studies and viruses, postinfection changes in the concentrations of IL-6, IL-8, IL-10, interferon alfa, and tumor necrosis factor in nasal secretions have been reported, but only IL-6 and IL-8 were consistently detected. In the present study, IL-6 was chosen for assay because of (1) its low basal nasal secretion concentration (as opposed to IL-8, which, like IL-5, is constitutively produced at relatively high levels); (2) its significant-fold increases postinfection; and (3) its direct relationship to CLI signs and symptoms. Interleukin 10 was chosen because it is an anti-inflammatory cytokine produced during the late period of a vURTI and has been related to the development of complications. Interleukin 5 was chosen as a control cytokine because its concentration is not increased during vURTIs.10

Researchers disagree on how to measure and report cytokine concentrations in nasal secretions. Some investigators favor the use of raw cytokine levels,19,23 while others recommend that the concentrations be corrected for saline dilution by dividing by the urea concentration in the sample.24 In the analyses presented herein, the raw cytokine concentrations were used, but in separate analyses, the concentrations were corrected for urea, as previously described.23 The results of the ROC analyses for the 2 data sets were similar, but the raw cytokine concentrations yielded higher AUCs for IL-6 and IL-10 compared with the adjusted concentrations (data not shown).

The findings of our ROC analyses supported the expectation that nasal secretion concentrations of IL-6 and IL-10, but not IL-5, are higher during times of virus detection and when symptoms and/or signs are expressed on the day of secretion collection. Specifically, the results show that the IL-6 concentration was significantly greater in PCR+ nasal secretions than in those without evidence of virus. For PCR+ and PCR− samples, IL-6 concentrations were significantly greater in samples collected on an illness day than those collected on a day without illness. These results suggest that IL-6 concentration...
is a marker of virus infection during a vURT I and of local inflammation reflected as nasal symptoms and signs, regardless of an identified vURT I. While IL-10 concentration was also significantly greater for those groups and subgroups, that protein was not detected in most samples assayed, which limits its usefulness as a discriminator.

In contrast, IL-5 concentration was measurable in a large percentage of samples, which suggests that it is constitutively produced, but its concentration was lower in the PCR− samples than in the PCR+ samples; its concentration did not differ among subgroups defined by the presence or absence of concurrent illness.

While the measured concentrations of the assayed cytokines and their ratios were capable in most cases of discriminating groups defined by the presence or absence of viral infection and the presence or absence of a CLI, the sensitivities and specificities of the discriminations were low. Indeed, while IL-6 concentration and the IL-5/IL-6 ratio were the best overall between-group discriminators based on their AUCs, the specificities and sensitivities of the discrimination for the 2 most extreme comparisons, PCR+CLI+ vs PCR−CLI−, were only about 0.7. The specificities and sensitivities of the other tested comparisons based on the cytokines and cytokine ratios were much lower.

The moderate to low sensitivities and specificities of the various cytokine discriminations for group and subgroup assignments reflect the observed high variability in the concentrations and ratios of these cytokines for the different groups and subgroups. This large variability was not unexpected. Reports of experimental vURTIs caused by different viruses have described the temporal kinetics for cytokine production during the course of infection.10,20,22 Those results document well-defined virus- and cytokine-specific postexposure changes in cytokine levels (curves) characterized by successive temporal periods of increase, plateau, and decrease that are phase locked to the time of virus exposure.10 In natural vURTIs, the time of exposure and onset of infection are unknown; therefore, sampling covers all times represented by these curves and all cytokine concentrations associated with those times.

In addition, we did not assay all potential viruses (eg, metapneumovirus, bocaviruses), and this may have contributed to the observed variability in the main groups and biased our assignment of samples to the PCR+ group. However, the low natural incidence of these excluded viruses implies that such biases would exert a minimal effect on the analyses. Also, parent-assigned signs and symptoms used to determine the presence or absence of a CLI might have varied between episodes and among subjects, thus introducing errors in the CLI assignments. Finally, other natural illnesses not associated with a vURT I (eg, allergy, rhinitis, asthma) can affect the nasal cytokine concentrations, and the presence or absence of these illnesses was not systematically quantified in this study.

In summary, none of the cytokine markers examined in this study are expected to be very useful for assigning nasal secretion samples to groups defined by the presence or absence of virus infection or to subgroups defined by the presence or absence of the signs and symptoms of a CLI. Because we chose cytokines that are arguably the best makers of infection and inflammation, it is unlikely that a single cytokine or panel of cytokines can be used with a high specificity and sensitivity to prospectively identify patients with a vURT I who are “at risk” for complications.

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Author Contributions: Dr Doyle had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Alper, Li-Korotky, Winther, and Doyle. Acquisition of data: Alper, Lo, Cullen Doyle, and Winther. Analysis and interpretation of data: Alper, Li-Korotky, Lo, and Doyle. Drafting of the manuscript: Alper, Cullen Doyle, and Doyle. Critical revision of the manuscript for important intellectual content: Alper, Li-Korotky, Lo, Cullen Doyle, Winther, and Doyle. Statistical analysis: Doyle. Obtained funding: Alper and Doyle. Administrative, technical, and material support: Alper, Lo, and Cullen Doyle. Study supervision: Alper, Li-Korotky, Lo, and Winther.

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REFERENCES


