The Role of Microbes in the Pathogenesis of Acute Rhinosinusitis in Young Adults

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Objectives/Hypothesis: To provide information on the course of acute rhinosinusitis (ARS) with sequential nasal and paranasal microbiological data and their correlation with clinical outcomes.

Study Design: We conducted a prospective cohort study among 50 Finnish military recruits with clinically diagnosed ARS in spring 2012.

Methods: We collected symptom, nasal endoscopy, and cone-beam CT (CBCT) scores during the early (2–3 days from onset) and later phases (9–10 days). We took viral samples from the nasopharynx (multiplex respiratory virus polymerase chain reaction [PCR]), bacterial culture from the middle meatus during both phases, and both viral and bacterial samples from the maxillary sinus aspirate (respiratory virus PCR, bacterial culture, broad-range bacterial PCR) during the later phase. Cilia destruction and microbial biofilms were sought from a nasal mucosal biopsy sample.

Results: We found that 42 (84%) of the subjects had viral nucleic acid in the nasopharynx during ARS. During the early phase, 28 (56%) of the subjects had nontypeable *H. influenzae* (NTHi) in the middle meatus, which was associated with wider paranasal mucosal changes in CBCT scans and increased symptoms during the study period. After 9 to 10 days from the onset, NTHi was found in the maxillary sinus in eight subjects (40%, 8/20) and led to prolonged symptoms. Bacterial biofilm was ruled out in 39 (78%) cases, and cilia destruction did not correlate with microbiological or clinical outcomes.

Conclusion: Nasal and paranasal *H. influenzae* coinfection during viral infection may modify the symptoms and the extent of sinonasal mucosal disease observed in CBCT scans already from the beginning of the ARS episode.

Key Words: Acute rhinosinusitis, virus, pathogenic bacteria, sinus, pathophysiology.

Level of Evidence: N/A.

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INTRODUCTION

Acute rhinosinusitis (ARS) is among the most common infections in primary care.[1–3] Recent guidelines summarize the major symptoms and signs of ARS.[4,5] Similarly, reports have shown how in most cases ARS involves not just the nasal passages but also the paranasal sinuses.[6–8] Both viruses and pathogenic bacteria have been shown to be involved in the pathogenesis of ARS.[9–11] It is theorized that most cases of ARS are first caused by a virus and then in some cases are complicated by bacterial coinfection or secondary infection.[12,13]

We still need additional information on the role of viruses and bacteria in the development of ARS.

We followed a group of military recruits during a single episode of ARS and took microbiological samples sequentially from the nasopharynx (multiplex-respiratory virus polymerase chain reaction [PCR]), nasal middle meatus (bacterial culture), and maxillary sinus (multiplex-respiratory virus PCR, bacterial culture, broad-range bacterial 16S ribosomal DNA PCR). We also recorded symptoms, clinical signs, and imaging findings (cone-beam CT [CBCT]). Our three aims were: 1) to describe viral and bacterial findings in samples taken sequentially from one person during the various stages of an ARS episode; 2) to look at the relationship between various viral and bacterial findings; and 3) to determine the clinical relevance of these findings.

MATERIALS AND METHODS

Design and Setting

We conducted a prospective cohort study among military recruits in the Kajaani Garrison, Kainuu Brigade, in Finland. The patients were recruited and examined at the garrison health center (primary care). All the patients provided written informed consent, and the study protocol was approved by the Oulu University Hospital Ethics Committee. The study protocol was registered in the ClinicalTrials.gov database (NCT01580137).

Study Population

The participants were enrolled from consecutive recruits who sought medical care because of acute respiratory symptoms between February 1, 2012, and April 15, 2012. The participants had to have ARS based on the following inclusion criteria: acute onset within the preceding 4 days, the presence of nasal symptoms (blockage or discharge),[5] and abnormal nasal findings (mucosal edema or secretion). Exclusion criteria were concomitant infection requiring antimicrobial treatment, respiratory infection or antimicrobial treatment within 3 weeks preceding the screening visit, nasal allergy or asthma necessitating medication, chronic nasal symptoms or polyps, and prior (para)nasal surgery.

Study Protocol

The protocol included two visits (Fig. 1). The first represented the early phase of ARS, and the second represented the later phase. On the first visit, we collected information from the participants, gave them symptom diaries, examined them, took microbiological samples, and performed CBCT imaging. Oral paracetamol (1000 mg × 1–3 per day) was allowed for symptom relief.

The second visit was scheduled to be on the workday closest to the 10th day of symptoms. We performed the same procedures as on enrollment. Furthermore, we took a nasal mucosal biopsy. In the case of abnormal CBCT findings in the maxillary sinus, we performed a sinus puncture and took microbiological samples from the aspirate.

The same author (T.J.A.) did all the examinations. The radiological, microbiological, and histopathological analyses were done blinded to the clinical data.

Clinical Data

Background Information. We collected information on age, sex, education, smoking, and prior medical history.

Recording of Symptoms. The participants recorded the presence of acute symptoms and graded their severity as 0 (no) to 10 (worst possible)[5] in the diary daily for 21 days. The following symptoms were documented: facial pain/pressure, nasal blockage, clear nasal discharge, purulent nasal discharge, postnasal discharge, and reduction/loss of smell. The patients filled in the diary from memory for the days they had had symptoms prior to enrollment. We summed up the grades for each symptom to obtain a total symptom score (0–60) for each day. Furthermore, we calculated the average symptom score by adding the scores for each day from the onset of symptoms to the second visit and dividing the sum by the number of days.

Physical Examination. We looked for nasal mucosal edema and discharge by using a head light and a speculum. We used a 2.7-mm 30-degree rigid TruView nasal endoscope (Olympus, Hamburg, Germany) to grade mucosal swelling, discharge, and polyps from 0 to 2 points on each side, blinded to the symptom scores. We then aggregated these scores to form the total endoscopic score (0–12).[14]

Cone-Beam CT of the Paranasal Sinuses. We performed CBCT with an open Cone Beam 3D system (Scanora 3D, Soredex Inc., Tuusula, Finland). We used standard resolution, 8 mA current, and small-sized fields of view (height 6.0 cm, diameter 6.0 cm) with a voxel size of 0.20 mm. This way we could see the coronal slices of the maxillary, ethmoidal, and sphenoidal sinuses and the ostiomeatal complex. The dose to the head was measured with a phantom to 0.008 mSv per each tomography examination.[15]

Two otolaryngologists (T.J.A., O-P.A.) graded the paranasal sinuses, as described earlier by Lund and Kennedy,[14] with some
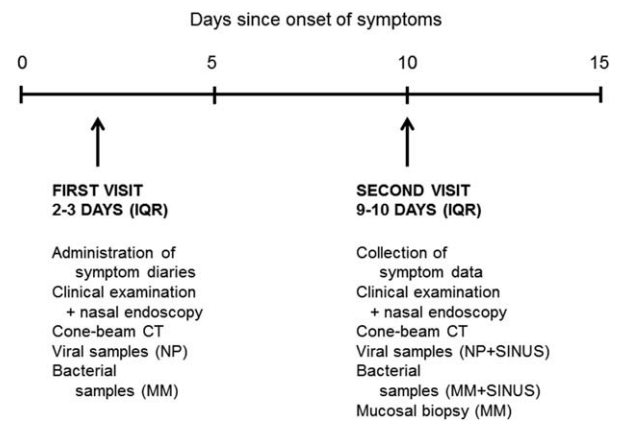

Fig. 1. Protocol of the study of acute rhinosinusitis among 50 military recruits. IQR = interquartile range; MM = nasal middle meatus; NP = nasopharynx; SINUS = maxillary sinus.

modifications: grade $0, \leq 3$-mm mucosal thickening; grade $1, > 3$-mm mucosal thickening; and grade 2, air–fluid level, gas bubbles, or total opacification. The ostiomeatal complex was graded as 0 (open) or 2 (occluded). We determined the total CBCT score (0–16) by summing up the grades of each sinus and ostiomeatal complex on both sides.

Puncture of Maxillary Sinus. If we saw an air–fluid level, gas bubbles, or total opacification in the maxillary sinus in the CBCT scans taken on the second visit, we punctured the sinus through the inferior meatus under topical anesthesia with a 2-0 G needle.

Microbiological Specimen and Analyses

Multiplex Respiratory Virus Polymerase Chain Reaction From Nasopharynx and Sinus Aspirates. We took samples for multiplex respiratory virus PCR from the nasopharynx and the sinus aspirates (supporting material) with an ultrathin minitip flocked swab (Copan Diagnostics Inc., Murietta, CA). The samples were analyzed by a multiplex qualitative PCR assay. Searches were conducted for 12 respiratory viruses (adenovirus; bocavirus; human metapneumovirus; influenza A and B viruses; coronavirus; picornavirus group (entero and rhinovirus); parainfluenza 1, 2, 3, and 4 viruses; and respiratory syncytial virus). An RVP Fast assay (xTAG respiratory virus panel) was performed, as described earlier by Jokela et al (2012).[16] Briefly, total nucleic acid from samples stored at −70°C was isolated by a MagNA Pure robot (Roche Diagnostics Ltd. Basel, Switzerland), and the extracts were instantly subjected to the RVP Fast assay (Luminex Molecular Diagnostics Inc., Toronto, Canada).

Bacterial Cultures From Nasal Middle Meatus and Sinus Aspirates. We took the middle meatus bacterial samples under endoscopic control with a cotton-tipped aluminum sterile bent swab (Deltalab, Spain), which was stored in an M40 Amies Agar Gel transsystem tube (Copan Diagnostics Inc.). No topical anesthesia was used.

For sinus punctures, we applied 2 to 3 ml of sterile sodium chloride solution into the maxillary sinus through the puncture needle and aspirated the contents with a 10-ml syringe, avoiding contamination. We injected the aspirates into a Portagerm bottle (bioMérieux SA, France) (Supporting Material). The swab samples were analyzed using aerobic bacterial cultures, and the aspirates were analyzed using both aerobic and anaerobic bacterial cultures on sheep blood, chocolated blood, and fastidious anaerobic blood agar plates in aerobic and anaerobic

atmospheres at 35°C for 48 hours to find common respiratory bacterial pathogens, which were identified at the species level.

Broad-Range Bacterial 16S rDNA Polymerase Chain Reaction From Sinus Aspirates. We took samples for bacterial PCR from the sinus aspirate with an ultrathin minitip flocked swab. To identify bacterial sequence types, PCR targeting conserved regions of the gene that encodes 16S rDNA was performed. We extracted DNA using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) with blood and body fluid spin protocols according to the manufacturer's instructions. Furthermore, clone libraries from control samples were used to exclude bacterial sequence types derived from the reagents used. The extracted DNA samples were amplified by PCR, as described earlier,[17] using broad-range 16S rDNA primers fD1mod[18] and 16S RR-B1[19] (Supporting Material).

Scanning Electron Microscopy Samples for Detection of Biofilm Formation and Cilia Destruction. We took an endoscopically guided biopsy with a 3-mm punch (Fokkens Biopsy Forceps; Explorent, Tuttlingen, Germany) from the mucosa of the middle turbinate after topical anesthesia (Supporting Material). The biopsy failed in two cases. Biofilms and ultrastructure of the mucosal epithelium were visualized with a scanning electron microscope (Zeiss Ultra plus FESEM, Field Emission Scanning Electron Microscopy, Oberkocken, Germany). Biofilm findings were categorized by a trained evaluator (T.T.) as absent (clearly normal ciliary epithelium with abundant cilia and without a covering matrix) or other (epithelium covered with microbial structures or other unspecified matrix). The presence and structure of epithelial cilia were recorded.

Statistical Analysis

For descriptive data, we calculated means with ranges or medians with interquartile (IQR) ranges, depending on the normality of the data. We used the χ^2 test and when appropriate, Fisher's exact test, for categorical data comparison. Two-sided P values $< .05$ were considered significant. Significant associations between various viral, bacterial, and cilia findings and demographic characteristics were studied further by cross-tabulation and calculation of risk ratios (RR) and 95% confidence intervals (CIs). We used a nonparametric Mann-Whitney U test to compare continuous variables. All of the viral and bacterial samples were analyzed per patient.

RESULTS

Study Population

Of the 66 patients offered participation, 51 consented and were enrolled. One patient with pneumonia was excluded. Seven (14%) who did not return the diary were excluded from the analyses of symptoms. The mean age of the participants was 20 years (range 18–23), and 48 (96%) were males. Twenty-two (44%) smoked. The median duration of acute symptoms was 2 days (IQR 2–3) on the first visit (early phase) and 10 days (IQR 9–10) on the second visit (later phase).

Clinical Findings During the ARS Episode

Nasal symptoms and abnormal nasal findings were common on both visits, although both the symptoms and nasal endoscopy scores declined during the study period (Table I). Fifty-eight percent of the patients both during the early and later phase had abnormal imaging findings in several paranasal sinuses. We performed maxillary sinus punctures on 20 (40%) participants.

Multiplex Respiratory Virus PCR Assay

During the study period, 42 participants (84%) had respiratory virus nucleic acid detected in the nasopharynx. The most frequent viruses were influenza A virus (n = 20), adenovirus (n = 20), and picornavirus group (n = 21). In 23 (46%) cases, nucleic acid from more than one virus was found.

During the early phase of ARS, 78% of the participants had respiratory virus nucleic acid in their nasopharynx; the proportion declined to 62% during the later phase (Table II). In 25 (50%) cases, the same virus nucleid acid persisted (Supporting Material).

Out of the 20 participants who had their sinus punctured during the later phase, 12 (60%) cases were found to have a virus nucleic acid in the sinus. The distribution of different viruses was similar to that in the nasopharynx (Table II). In five (25%) cases, a virus nucleic acid was the sole pathogen found in the sinus.

Bacterial Cultures and Bacterial 16S rDNA Polymerase Chain Reaction

During the study period, 36 (72%) of the subjects had pathogenic bacteria (*H. influenzae, S. pneumoniae, S. aureus, M. catarrhalis*) in the nasal middle meatus, detected by bacterial culture. We found nontypeable *H. influenzae* (NTHi) in the middle meatus in 28 (56%) of the participants during the early phase and in 24 (48%) of the participants during the later phase (Table II). In 23 cases, NTHi persisted. Other pathogenic bacteria were detected more rarely.

Among patients with a sinus aspirate sample during the later phase, the culture revealed eight (40%) cases of NTHi and no other pathogenic bacteria. In seven of these cases, a virus was also found in the sinus.

Bacterial 16S rDNA PCR of the sinus aspirate revealed altogether 53 different bacterial-sequence types from 20 patients (Supporting Material). Of these sequence types, three represented known respiratory pathogens: *Haemophilus influenzae* (7 patients, all found in the bacterial culture), *Streptococcus pneumoniae* (1 patient, not found in the bacterial culture), and *Neisseria meningitidis* (1 patient, not found in the bacterial culture).

Mucosal Biopsy From the Nasal Middle Meatus

We found a normal ciliary epithelium in the middle meatus in 26 (54%) cases. Abnormal cilia were associated with smoking (RR 2.5, 95% CI 1.3–4.7) but not with any microbiological finding or clinical outcomes. In 39 (78%) participants, the presence of microbial biofilm could be excluded (Supporting Material). The biopsy failed in two cases because of patient dizziness.

Association Between Viral and Bacterial Findings

The type and persistence of the nasopharyngeal virus did not correlate with the finding of NTHi in the

TABLE I.
Clinical Outcomes During a Single Acute Rhinosinusitis (ARS) Episode in a Cohort of 50 Military Recruits.

Outcomes	Early-phase ARS 2–3 days (IQR) from onset	Later-phase ARS 9–10 days (IQR) from onset
Symptoms*		
Nasal blockage	43 (100)	39 (91)
Nasal discharge	42 (98)	41 (93)
Facial pain/pressure	27 (63)	16 (37)
Reduction/loss of smell	37 (86)	29 (67)
Mean (SD) symptom score[†]	20.6 (8.0)	14.6 (9.7)
Clinical Nasal Findings[‡]		
Moderate/severe mucosal edema	48 (96)	45 (90)
Obstruction	39 (78)	22 (44)
Moderate/severe amount of secretion	37 (74)	19 (38)
Postnasal discharge	11 (22)	20 (40)
Mean (SD) nasal endoscopy score[§]	5.5 (0.9)	4.4. (2.1)
Spread of ARS to Paranasal Sinuses (CBCT scans)		
Nasal cavity only	10 (20)	14 (28)
1 sinus	11 (22)	7 (14)
2 sinuses	19 (38)	21 (42)
3 sinuses	10 (20)	8 (16)
Mean (SD) CBCT score[#]	5.5 (4.1)	5.1 (4.3)

Data are No. (%) unless otherwise specified.
*Data available for 43 participants.
[†]Sum of the scores (on a scale of 0–10) for six major ARS symptoms among those 43 (86%) who returned the symptom diary.
[‡]Head lamp and nasal speculum or mirror and nasal endoscope for scoring.
[§]Based on the findings of mucosal swelling, discharge, and polyps on both sides (Lund and Kennedy[14]).
[#]Based on the findings of mucosal thickening, air–fluid level, gas bubbles, and total opacification of the maxillary, ethmoidal, and sphenoidal sinuses on both sides (Lund and Kennedy[14]).
ARS = acute rhinosinusitis; CBCT = cone-beam CT; IQR = interquartile range; SD = standard deviation.

middle meatus. In contrast, the detections of viral nucleic acid persistence or multiple viruses during the later phase were related to a finding of NTHi in the maxillary sinus (RR 17.0, 95% CI 1.0–279 and RR 4.7, 95% CI 1.3–17, respectively). Persistence of NTHi in the middle meatus tended to increase the risk of finding the same bacteria in the sinus (RR 3.5, 95% CI 0.8–16).

Correlation With Clinical Outcomes

None of the viral findings per se (type, persistence, presence in the sinus) correlated with the symptom scores (Table III) or with the spread of the disease to the sinuses (data not shown).

In contrast, subjects with a middle meatus finding of NTHi during the early phase had significantly higher mean symptom scores during the whole episode compared with those who did not have the bacteria (Fig. 2A; Table III). Furthermore, the presence of NTHi in the middle meatus correlated with a wider spread of the disease to the paranasal sinuses already in the early phase of the ARS episode (Fig. 3).

Subjects with NTHi found in the sinus had higher mean symptom scores during the latter part of the episode only (Fig. 2B). Similarly, the nasal endoscopy and imaging scores were higher in these subjects compared with those who underwent a maxillary puncture but did not have the bacteria (Table III).

DISCUSSION

The role of viruses during the development and course of the ARS episode was evident in our study; we found that most of the subjects had viral nucleic acid in the nasopharynx during the early and later phases and in the maxillary sinus during the later phase. Moreover, viral persistence and detection of multiple viruses correlated with the finding of pathogenic bacteria in the maxillary sinus. However, the viruses per se were not directly associated with the clinical outcome. In contrast, the presence of pathogenic bacteria in the nose and paranasal sinuses—H. influenzae in these subjects—modified the course of the ARS episode markedly. During the early phase of ARS, NTHi in the middle meatus correlated with wider spreading of the disease to the paranasal sinuses and with stronger symptoms during the study period. During the later phase of ARS, the presence of NTHi in the maxillary sinus was associated with prolonged symptoms.

It has been suggested that if an individual develops an ARS episode while colonized with S. pneumoniae, H. influenzae, or M. catarrhalis, the individual may develop bacterial ARS with the colonizing strain.[20] Our finding that the clinical picture of ARS was different, if NTHi was already present in the nose from the beginning of the symptoms, indicates that the nasal middle meatus of these subjects was coinfected—not colonized—with NTHi during viral ARS. It appears that the presence of

TABLE II.

Microbiological Findings During a Single Acute Rhinosinusitis (ARS) Episode in a Cohort of 50 Military Recruits.

	Early-phase ARS 2–3 days (IQR) from onset		Later-phase ARS 9–10 days (IQR) from onset		
	NP N = 50	MM	NP N = 50	MM	SINUS N = 20
Viral Findings*					
Any virus	39 (78)		31 (62)		12 (60)
No virus	11 (22)		19 (38)		8 (40)
Multiple virus	19 (38)		10 (20)		2 (10)
Only virus, no bacteria	13 (26)		8 (16)		5 (25)
Influenza A	17 (34)		10 (20)		6 (30)
Adenovirus	17 (34)		14 (28)		3 (15)
Picornavirus (entero or rhino)	19 (38)		16 (32)		3 (15)
Coronavirus	7 (14)		3 (6)		2 (10)
Other viruses[†]	1 (2)		4 (8)		0
Bacterial Findings (culture)*					
Any bacteria		33 (66)		34 (68)	8 (40)
No Bacteria		17 (34)		16 (32)	11 (55)
H. influenzae (NTHi)		28 (56)		24 (48)	8 (40)
S. pneumoniae		4 (8)		4 (8)	0
S. aureus		4 (8)		8 (16)	0
Other bacteria[‡]		4 (8)		9 (18)	0
Virus and bacteria		26 (52)		23 (46)	7 (35)
Histopathological Findings[§]					
Normal cilia				26 (54)	
No biofilm[#]				37 (77)	

Data are No. (%) unless otherwise specified.
*One patient may have more than one respiratory virus or bacterium.
[†]Parainfluenza viruses, bocavirus.
[‡]*M. catarrhalis*, streptococcus group G, *E. sakazakii*, *N. meningitidis*, *H. parainfluenzae*, citrobacter sp, serratia sp, *E. coli*, klebsiella oxytoca.
[§]Nasal mucosal biopsy, electron microscope image. Numbers do not add up to 50 because of missing information in 2 patients.
[#]Intact ciliary epithelium not covered with any matrix, bacterial structures, or artefacts examined with scanning electron microscopy.
MM = nasal middle meatus; NP = nasopharynx; NTHi = nontypable *H. influenzae*; SINUS = maxillary sinus.

TABLE III.

Association Between Viral and Bacterial Findings and Clinical Outcomes During a Single Acute Rhinosinusitis (ARS) Episode in a Cohort of 50 Military Recruits.

	Early-phase ARS, 2–3 days* From Onset			Later-phase ARS, 9–10 days* From Onset		
	Mean (SD) scores			Mean (SD) scores		
	Symptoms[†]	Nasoendoscopy[†]	Imaging[†]	Symptoms[†]	Nasoendoscopy[†]	Imaging[†]
Viral Findings During the Early Phase (NP)						
No virus (N = 11)	19.2 (8.1)	5.8 (0.9)	4.4 (4.9)	15.3 (10.2)	3.7 (2.2)	2.9 (2.6)
Any virus (N = 39)	20.9 (8.1)	5.5 (1.0)	5.8 (3.8)	14.5 (9.7)	4.6 (2.1)	5.7 (4.5)
Multiple viruses (N = 19)	20.7 (7.1)	5.7 (1.0)	6.1 (3.9)	16.6 (11.5)	5.6 (1.7)[‡]	7.0 (4.5)[‡]
Bacterial Culture Findings During the Early Phase (MM)						
No NTHi (N = 22)	17.1 (6.7)	5.6 (1.0)	3.9 (3.7)	11.7 (9.0)	4.7 (2.2)	4.3 (3.9)
NTHi (N = 28)	23.2 (8.0)[‡]	5.5 (0.9)	6.8 (3.9)[‡]	16.8 (9.8)[‡]	4.2 (2.1)	5.7 (4.6)
Bacterial Culture Findings (SINUS)[§]						
No pathogenic bacteria (N = 12)	19.9 (9.4)	5.3 (1.0)	7.8 (3.6)	15.7 (11.8)	4.8 (1.5)	7.0 (2.6)
NTHi (N = 8)	20.4 (9.0)	5.6 (0.9)	7.9 (4.6)	22.8 (9.2)	6.8 (0.9)[‡]	11.0 (2.0)[‡]

*Interquartile range.
[†]Sum of the scores (on a scale of 0–10) for six major ARS symptoms among those 43 (86%) who returned the symptom diary. Nasoendoscopy scored according to Lund and Kennedy.[14] Imaging scored from the CBCT scans, modified by Lund and Kennedy.[14]
[‡]$P < 0.05$.
[§]Among the 20 (40%) who underwent a maxillary puncture.
MM = nasal middle meatus; NP = nasopharynx; NTHi = *H. influenzae*; SD = standard deviation; SINUS = maxillary sinus.
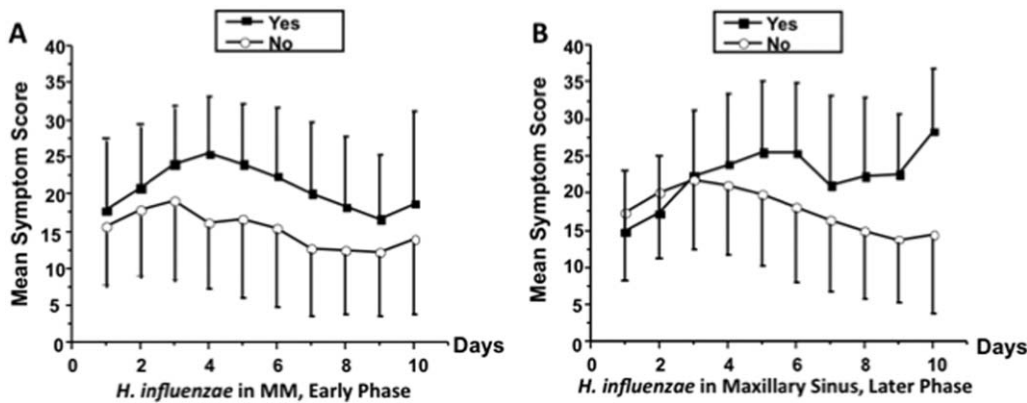
Fig. 2. Symptom scores among 50 military recruits with acute rhinosinusitis. (A) Mean total symptom scores during the whole episode are higher among the subjects who had nontypable *H. influenzae* (NTHi) in the nasal middle meatus than among those who did not during the early phase of ARS (difference in average symptom scores, *P* =.012). (B) Mean total symptom scores during the whole episode are similar, but the scores on the day of the second visit are higher among the subjects who had NTHi in the maxillary sinus than in those who did not or who did not undergo maxillary sinus puncture (difference in average symptom scores, *P* =.19; difference in total symptom score on the day of the second visit, *P* =.01). Total Symptom Score is calculated per day by adding up the scores (0–10) of facial pain/pressure, nasal obstruction, clear and purulent nasal discharge, postnasal discharge, and loss/reduction of smell (maximum score 60). Average Symptom Score (total symptom scores for each day added up from the onset of symptoms to the second visit and divided by the number of days). *P* values of the 2-tailed Mann Whitney U test. Bars represent standard deviations.

pathogenic bacteria may have a role in the pathogenesis of ARS from the start. Altogether, 56% of the subjects (28) had middle meatal NTHi during the early phase; and in six of these subjects (21%, 6/28), it led to NTHi being found in the maxillary sinus during the later phase. The active role of pathogenic bacteria in the middle meatus, with wider spreading of the disease to the paranasal sinuses, supports the theory that the ostiomeatal complex is the key area in the pathogenesis of bacterial ARS.[21–23]

Supporting our present findings, we have shown earlier in children that the presence of nasal middle-meatus pathogenic bacteria predicted delayed recovery.[24] Similarly, Kaiser et al.[25] found that adult patients with acute respiratory infection and *S. pneumoniae*, *H. influenzae*, or *M. catarrhalis* in nasopharyngeal cultures benefit from antimicrobial treatment.

The microbiome of the sinus cavities has been described earlier in patients with chronic sinusitis[26,27] In our patients with ARS, broad-range bacterial PCR did not reveal significantly more respiratory pathogens in sinus aspirate samples than in conventional bacterial culture, and biofilm structures on the ciliary epithelium of the middle meatus were rarely observed. Destruction of the ciliary epithelium was observed in 22 (46%) of the subjects, but it did not correlate with clinical outcomes. Earlier, 31% of adults have been reported to have bacterial pathogens in the nasal cavity during viral infection,[28] which is clearly lower than the present figure of 72%. Furthermore, we found NTHi to be the only culture-proven pathogenic bacterium in the maxillary sinus, whereas others have reported *S. pneumonia* and *M. catarrhalis* to be common as well.[11] The fact that our study was conducted among military recruits—among who NTHi is reported to be the most common nasal and paranasal pathogen[29,30]—probably explains these differences.

Several limitations of this study warrant further discussion. We had a relatively small sample size due to our desire to collect sequential data on multiple variables, which would have been practically impossible with a larger cohort, considering our resources. Still, the sample size enabled us to find significant associations between the microbiological findings and the clinical outcomes. Despite the short recruitment period, we managed to collect our data during at least three larger virus epidemics (influenza A, adenovirus, picornavirus). Moreover, we used acknowledged methods to score the
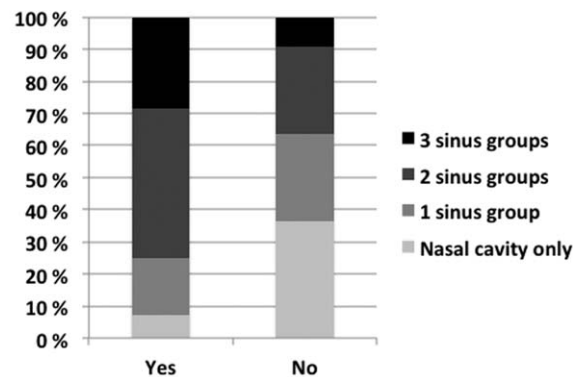
Fig. 3. Correlation of *H. influenzae* and the spread of the disease. Presence of *H. influenzae* in the nasal middle meatus during the early phase of acute rhinosinusitis (ARS) correlates with the spread of the disease to the paranasal sinuses among 50 military recruits (*P* =.026). The spread of ARS was evaluated by dividing the subjects into groups according to the number of abnormal sinus findings (mucosal thickening > 3 mm, gas bubbles, or air–liquid level on the worst side) on cone-beam CT scans. *P* values were calculated with 2-tailed χ^2 test.

clinical outcomes.[5,14] Both of these facts increase the generalizability of our findings.

However, several issues require recognition concerning the generalizability of our results. Due to the military environment, most of the recruits were men. Unfortunately, smoking was common among the recruits, but it did not correlate with the clinical outcomes. Carriage rate of nasal pathogens is known to be higher in military recruits than in other young populations,[29] but one may argue that the pathophysiology of ARS is the same in both groups. Still, the present finding of pathogenic bacteria being correlated to clinical outcomes warrants reconfirmation among other patients and bacteria.

To summarize, we found that the overwhelming majority of military recruits have viruses during all phases of an ARS episode, but in over half of the subjects, nasal and paranasal *H. influenzae* coinfection with a respiratory virus modified the symptoms, as well as the extent of sinus mucosal disease seen in CBCT scans already from the onset of the ARS episode.

CONCLUSION

Respiratory viruses are frequently found but are not necessarily associated with clinical outcomes. Nasal and paranasal *H. influenzae* coinfection results in more severe symptoms and radiological findings than viral infection alone. Broad-range bacterial PCR does not significantly add findings compared with sinus culture.

Acknowledgments

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