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Aetiology of febrile pharyngitis in children: Potential of myxovirus resistance protein A (MxA) as a biomarker of viral infection

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Summary Objectives: Besides group A streptococcus (GAS), microbial causes of pharyngitis in children are not well known. We aimed to document the viral and bacterial aetiology of pharyngitis and to assess the pathogenic role of viruses by determining the myxovirus resistance protein A (MxA) in the blood as a marker of interferon response.

Methods: In this prospective observational study, throat swabs and blood samples were collected from children (age 1–16 years) presenting to the emergency department with febrile pharyngitis. Microbial cause was sought by bacterial culture, polymerase chain reaction, and serology. Blood MxA level was determined.

Results: A potential pathogen was detected in 88% of 83 patients: GAS alone in 10%, GAS and viruses in 13%, group C or G streptococci alone in 2% and together with viruses in 3%, and viruses alone in 59% of cases. Enteroviruses, rhinoviruses, and adenoviruses were the most

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frequently detected viruses. Blood MxA levels were higher in children with viral (880 [245–1250] $\mu\text{g/L}$; median [IQR]) or concomitant GAS-viral (340 [150–710] $\mu\text{g/L}$) than in those with sole GAS (105 [80–160] $\mu\text{g/L}$) infections.

Conclusions: Detection of respiratory viruses simultaneously with elevated blood MxA levels supports the causative role of viruses in the majority of children with pharyngitis.

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Introduction

Acute pharyngitis accounts for a substantial portion of visits to physicians in outpatient setting.^{1–4} Group A streptococcus (GAS) is the only common bacterial cause of pharyngitis justifying antimicrobial treatment according to current guidelines.^{5,6} However, overuse of antibiotics for pharyngitis occurs both in children and adults.^{7–10} Non-GAS pharyngitis is suggested to be mainly a viral infection,^{11–13} but only a few comprehensive studies have evaluated the microbiological aetiology of the disease in the paediatric population.^{14–18} There is a lack of studies addressing the viral aetiology of pharyngitis in children and adolescents by molecular diagnostic methods.

The prevalence of asymptomatic streptococcal carriage in the oropharynx complicates the causal interpretation of GAS findings in children with symptomatic pharyngitis.^{19,20} In addition, the significance of respiratory virus detection by polymerase chain reaction (PCR) has been questioned because of frequent virus findings in asymptomatic subjects.^{21,22} Among patients with positive viral PCR results, low cycle threshold (CT) and serological response can indicate true infections. An alternative approach distinguishes between viral and bacterial infections by differences in the biomarker blood concentrations or host gene expression patterns.^{23–25}

The aim of this study was to document the microbial causes of acute pharyngitis in children and adolescents in an outpatient setting and to evaluate the causative role of viruses by determining myxovirus resistance protein A (MxA) and other biomarker levels.

Materials and methods

Study design and patients

This prospective observational study was conducted at the Department of Emergency Services, Turku University Hospital, Turku, Finland from November 25, 2013 through January 31, 2015. The Department of Emergency Services (ED) serves as a walk-in clinic with approximately 25,000 yearly visits by patients under the age of 17. Children or adolescents aged 1–16 years with acute febrile pharyngitis were eligible for the study. There were no exclusion criteria in the study. The study protocol and inclusion criteria were introduced to ED physicians who diagnosed acute pharyngitis, defined as exudates or intensive redness in the tonsils/oropharynx and fever (body temperature ≥ 38 °C measured at the ED or reported by the parents during the current illness episode). The patients were treated according to the judgement of the attending ED physician. Local guidelines recommend oral phenoxymethylpenicillin as the

first line and cephalexin as the second line antimicrobial therapy for microbiologically proven GAS pharyngitis.

At enrolment, the patient's symptoms and their duration, clinical examination findings, preceding illnesses and vaccinations, and underlying conditions were recorded, and oropharyngeal swab samples and blood sample were obtained. Symptom diary cards were given to the parents of the participating children. Each family was contacted by telephone after the enrolment visit to record the duration of the patient's symptoms. Follow-up calls were continued every second day until the fever and soreness of the throat had resolved. All patients were invited to a follow-up visit approximately 2–4 weeks after the enrolment. At the follow-up visit, possible symptoms and clinical findings were recorded, and an oropharyngeal swab sample and blood sample for paired serology were obtained.

The Ethics Committee of the Hospital District of Southwest Finland approved the study protocol. The legal guardians of all participating children and adolescents and the adolescent patients themselves gave their written, informed consent.

Collection of oropharyngeal samples

At enrolment, oropharyngeal samples were collected in standardized order by rubbing the swabs against both tonsils. Throat swabs were handled as follows: 1) The sample for bacterial culture was collected by a flocced swab that was put immediately after the collection in a tube with liquid transport media (ESwab, Copan, Brescia, Italy) and transferred to the Department of Clinical Microbiology, Turku University Hospital. 2) The sample for virus PCR was collected by a flocced swab (Copan, Brescia, Italy) and transferred in a dry, clean tube to the Department of Clinical Virology, Turku University Hospital. At the follow-up visit, a study physician collected a flocced swab sample for virus PCR.

Bacterial analyses

Fifty microlitres from the vortexed ESwab was inoculated on streptococcal-selective blood agar, on standard blood, McLeod, and Fastidious anaerobe agars as well as in a streptococcal-selective broth, followed by subculture on streptococcal-selective blood agar. Beta haemolytic streptococci and other colonies of interest were identified by standard methods, including MALDI-TOF (Bruker Daltonics, Bremen, Germany).

Anti-streptolysin O (ASO) antibody levels were determined using the RapiTex[®] ASL kit (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) according to the manufacturer's instructions.

Viral analyses

The pharyngeal swab was suspended into phosphate-buffered saline, and nucleic acids were extracted using the NucliSENS easyMag (bioMérieux, Boxtel, The Netherlands) automated extractor. The Anyplex II RV16 multiplex PCR system (Seegene, Seoul, Korea) was used for the detection of influenza A and B viruses, respiratory syncytial virus (RSV) groups A and B, adenovirus, metapneumovirus, coronaviruses 229E, NL63, and OC43, parainfluenza virus types 1 to 4, rhinoviruses, enteroviruses, and bocavirus. In addition, a laboratory-developed PCR assay was used for the detection of rhinoviruses and enteroviruses.²⁶ All amplifications were performed using Rotor-Gene 6000 or Qiagen Q (Qiagen, Hilden, Germany) instruments.

All enterovirus- and adenovirus-positive specimens were subjected to genotyping by PCR amplification of the partial gene region of the enterovirus VP1 protein region²⁷ or the adenovirus hexon protein.²⁸ The amplicons were purified with the ExoSAP protocol and sequenced at GATC Biotech (Constance, Germany), and the obtained sequences were analysed with the NCBI Basic Local Alignment Tool.

Paired serum samples were stored at -70°C until analysed. Commercial test kits were used for the detection of IgG and IgM antibodies to *Mycoplasma pneumoniae* (Lab-systems Diagnostics, Vantaa, Finland) and Epstein–Barr virus (EBV) (Vidas, bioMérieux, Marcy l'Étoile, France) according to the manufacturers' instructions. IgG antibodies against adenovirus, enteroviruses, influenza A and B viruses, parainfluenza virus types 1, 2, and 3, and RSV were analysed by *in-house* enzyme immunoassays routinely used in the virus diagnostic laboratory.^{29,30} A μ -capture enzyme immunoassay was used to measure enterovirus-specific IgM antibodies. A mixture of coxsackievirus A16, coxsackievirus B3 and echovirus 11 antigens was used in IgG and IgM tests for enteroviruses.³¹

Biomarkers

Whole blood, plasma, and serum samples for biomarker level determinations were collected during the enrolment visit by antecubital venepuncture. The white blood cell count and plasma levels of C-reactive protein (CRP) and procalcitonin (PCT) were determined in the hospital central laboratory. Whole blood samples for MxA measurement were transported to the laboratory where samples were diluted 1:20 in hypotonic buffer and stored at -70°C until the EIA analysis was performed as described earlier.³² Serum samples for tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) level determination were stored at -70°C until the analysis by ELISA (Human TRAIL, Quantikine, R&D Systems Inc., Minneapolis, USA) according to the manufacturer's protocol. Optical density was determined using a microplate reader set to 450 nm with wavelength correction at 570 nm.

Data analyses

For the statistical comparison, all patients enrolled in the study were classified in two etiological groups: Patients with GAS (diagnosis based on bacterial culture) or GAS-viral

pharyngitis were classified as GAS, and patients with all other aetiologies, or with undetermined aetiology, were classified as non-GAS. The groups were compared by the χ^2 or Mann–Whitney U tests. The areas under the receiver operating characteristic (ROC) curve were determined for biomarkers. Furthermore, patients were classified according to their microbial findings in six separate groups for the descriptive analysis. All analyses were performed using IBM SPSS Statistics, version 22 (IBM Corp., Armonk, NY, USA).

Results

Patient population

The study population comprised 83 children and adolescents with febrile pharyngitis (Table 1). The median age of the children was 5.5 years (interquartile range, 3.2–12.2), 37 (44.6%) were girls, and none of them were admitted to

Table 1 Characteristics of 83 patients with febrile pharyngitis.

Characteristic	No. (%)
Age group, y	
1–4	40 (48.2)
5–8	16 (19.3)
9–12	10 (12.0)
13–16	17 (20.5)
Underlying conditions	
None	70 (84.3)
Asthma	9 (10.8)
Other ^a	4 (4.8)
Symptoms at presentation	
Fever ($\geq 38^{\circ}\text{C}$)	83 (100.0)
Sore throat ^b	70 (94.6)
Cough	38 (45.8)
Rhinitis	35 (42.2)
Rash	8 (9.6)
Conjunctivitis	6 (7.2)
Diarrhoea	8 (9.6)
Time between fever onset and study enrolment	
0 to <1 day	29 (34.9)
1 to <3 days	32 (38.6)
3 to <7 days	18 (21.7)
≥ 7 days	4 (4.8)
Number of previous pharyngitis episodes reported by parents	
None	58 (69.9)
1	11 (13.3)
2–3	5 (6.0)
4–5	6 (7.2)
Missing information	3 (3.6)
Clinical findings (reported by the physician)	
Oropharyngeal/tonsillar exudates	47 (57)
Intensive oropharyngeal redness	61 (74)
Cervical lymphadenopathy	51 (61)

^a Includes cardiological (n = 1) and neurological (n = 1) conditions, ankylosing spondylitis (n = 1), and vitiligo (n = 1).

^b Number of applicable patients n = 74.

hospital. Five children (6.0%) had received antibiotics within 5 days before enrolment.

Microbial aetiology

A potential causative agent was detected in 73 (88.0%) patients: GAS alone in 8 (9.6%), GAS together with virus in 11 (13.3%), group C or G β -haemolytic streptococci (GCS, GGS) alone in two (2.4%), GCS or GGS together with virus in three (3.6%), and one or more viruses alone in 49 (59.0%) cases (Fig. 1). One of the GAS cases was diagnosed by enrichment culture only, and the remaining 18 were detected by standard culture on a streptococcal-selective blood agar plate. Other bacteria found by throat culture were considered to be non-pathogenic (Supplemental Table 1). There was no significant difference in the mean initial serum ASO levels between the GAS and non-GAS patients. Five patients showed a 2-fold ASO increase in paired serum samples: Three of them were GAS positive, one of them was GCS positive, and one was negative for streptococci by throat culture. Overall, paired serum samples were available in 14/19 patients with GAS.

The most frequently detected viruses were enteroviruses (in 25.3% of the children), rhinoviruses (21.6%), and

adenoviruses (15.7%). The most common types of enteroviruses were coxsackievirus types A6 and A16. Adenoviruses were typed as C2 in seven patients and as C1 in two patients. EBV IgM antibodies were detected in five patients. However, when the duration of symptoms, the presence of IgG antibodies, and the results from paired serum samples were considered, acute infection caused by EBV was confirmed only in two (2.4%) patients. *M. pneumoniae* IgM antibodies were detected together with IgG antibodies in the serum of 10 patients, seven of whom were 14–16 years old. Paired serum samples were available in four of these patients. None of them showed ≥ 2 -fold increase in their *M. pneumoniae* IgG antibody levels. A β -haemolytic streptococci or virus was detected in 9 of these 10 patients.

The diagnosis of a virus infection was made by PCR in 49 patients. In sixteen patients, a PCR diagnosis was confirmed by an antibody response in the paired serum samples. Multiple virus detection occurred in 15 patients with a virus infection, in four patients with a GAS-virus infection, and in one patient with a GCS-virus infection. The majority of enterovirus (13/21), rhinovirus (13/18), adenovirus (10/13), and all coronavirus OC43 (4/4) findings were detected together with other potential pathogens. By using semi quantitative information from PCR CT values combined

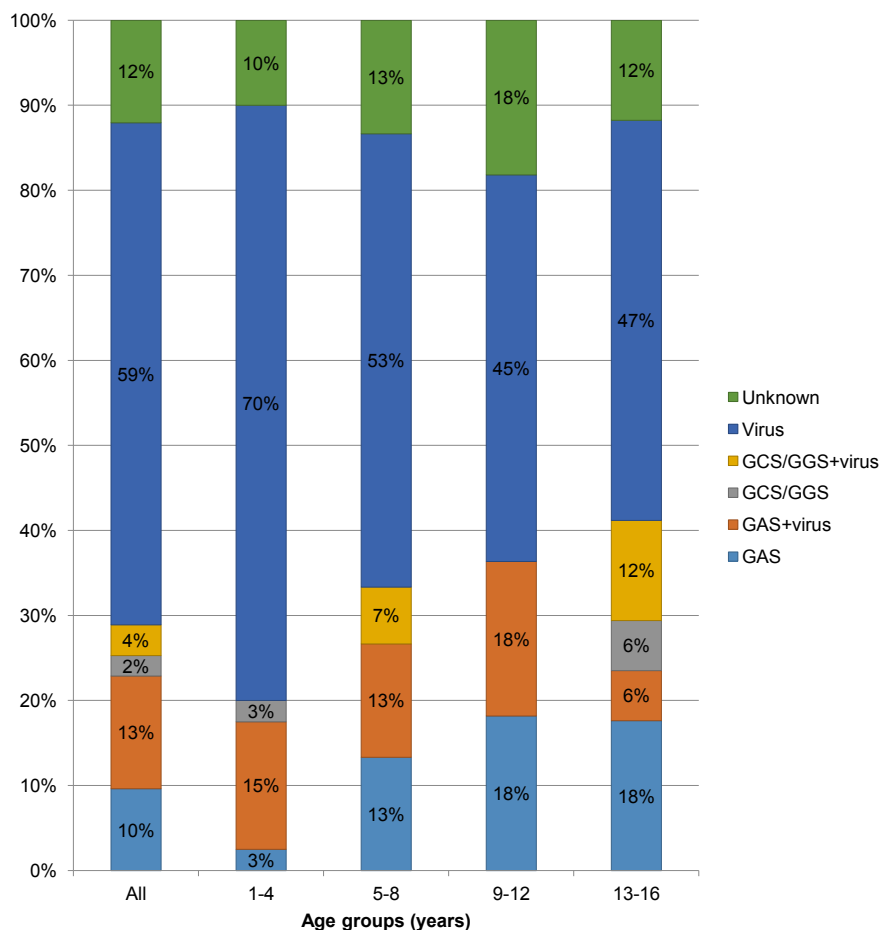


Figure 1 Aetiology of febrile pharyngitis in different age groups (n = 83). The vertical axis (Y) displays the percentage of microorganisms detected: group A streptococcus, GAS (light blue); GAS + virus (orange); group C streptococcus, GCS or group G streptococcus, GGS (grey); GCS/GGS + virus (yellow); virus (dark blue); microbiological aetiology unknown (green). The horizontal axis (X) represents different age groups included in the study (all, 1–4, 5–8, 9–12 and 13–16 years old).

with serological results, we were able to plausibly estimate the most important virus in most patients with multiple virus findings (Supplemental Table 1). These results suggest that despite their frequent co-detection with other viruses, enteroviruses and adenovirus were often also the most probable pharyngitis pathogens. Viruses caused the majority of cases in all age groups, but a viral aetiology was particularly common in the age group of one to four years (Fig. 1).

Clinical presentation

The occurrence of selected symptoms and clinical findings in patients with GAS and non-GAS pharyngitis are presented in Table 2. No clinical scoring system was used in the clinical management of the patients, but the Mclsaac scores were determined retrospectively for all eligible patients ($n = 66$). None of the symptoms, clinical findings, or the Mclsaac score were specific for a GAS or non-GAS aetiology of febrile pharyngitis.

Biomarkers

The white blood cell count and CRP, PCT, MxA, and TRAIL levels were measured in the blood, plasma or serum of all pharyngitis patients. The MxA/CRP ratio was calculated. The levels of all biomarkers, except that of PCT, were significantly different between GAS and non-GAS patients (Table 2). However, none of the biomarkers was accurate in discriminating GAS from non-GAS aetiology (see Supplemental Figs. 1–6). Based on our previous study, we used 175 $\mu\text{g/L}$ as a cut-off level for increased blood MxA concentration.³² Blood MxA levels were elevated in 79.4% of patients with virus findings, and remained low in 90.0% of patients with streptococcal pharyngitis without virus detection. Blood MxA levels were also elevated in most of the patients (90.0%) without a confirmed microbiological diagnosis (Fig. 2). Viruses, but no bacteria, were identified in all five patients with recent antibiotic exposure. These

patients had also markedly elevated blood MxA levels indicating an acute virus infection.

Follow-up visit

In total, 57 patients (69%) returned for a scheduled follow-up visit after 10–40 days (median 18 days, interquartile range 17–21). At the follow-up visit, a throat swab was taken from 56 and blood sample from 52 children. Fever and throat soreness of the initial pharyngitis episode had resolved in all patients. Two patients presented to the follow-up visit with a relapsed GAS pharyngitis and another of them was febrile. None of the other patients were febrile on the follow-up visit but 29/57 (51%) of them reported milder respiratory symptoms. Overall, viruses were detected in 37/56 (66.1%) patients. Virus detection was more common in the symptomatic 24/29 (82.8%) than in the asymptomatic 13/27 (48.1%) patients. Blood MxA levels were lower in virus positive patients at the follow-up visit (110 [70–218] $\mu\text{g/L}$; median [IQR]) than they were during the febrile pharyngitis episode (780 [180–1190] $\mu\text{g/L}$).

Discussion

In this study, viruses had a dominant role in the aetiology of febrile pharyngitis. A possible causative agent could be detected in 88.0% and virus in 75.9% of patients, whereas in earlier studies from the era before PCR, viruses were detected in 11–42% of children or adolescents with pharyngitis or tonsillitis.^{16–18} Furthermore, an elevated blood MxA level, as a marker of type I or type III interferon production, demonstrated an active innate immune response against acute virus infection in the majority of patients with a detected virus.

We detected group A β -haemolytic streptococci less frequently than earlier reported.¹⁹ This is partly explained by the fact that half of the children in this study were

Table 2 Comparison of the clinical parameters and biomarkers in GAS and non-GAS pharyngitis.

	GAS (n = 19)	Non-GAS (n = 64)	P^a
Clinical findings			
Cough or rhinitis – no. (%)	9 (47.4)	41 (64.1)	0.19
Oropharyngeal/tonsillar exudates – no. (%)	14 (73.7)	33 (52.4)	0.10
Intensive oropharyngeal/tonsillar redness – no. (%)	17 (89.5)	44 (69.8)	0.09
Cervical lymphadenopathy – no. (%)	12 (63.2)	39 (66.1)	0.82
Mclsaac score ^b – mean (95% CI)	3.8 (3.4–4.3)	3.5 (3.2–3.8)	0.13
Biomarkers			
WBC – median [IQR], E9/L	12.9 [9.5–16.0]	9.0 [5.9–12.1]	0.005
CRP – median [IQR], mg/L	32 [12–45]	9.5 [4–31]	0.01
PCT – median [IQR], $\mu\text{g/L}$	0.16 [0.09–0.26]	0.16 [0.10–0.40]	0.65
MxA – median [IQR], $\mu\text{g/L}$	170 [100–400]	810 [213–1198]	<0.001
TRAIL – median [IQR], pg/mL	82 [66–142]	137 [74–185]	0.04
MxA ($\mu\text{g/L}$)/CRP (mg/L) ratio – median [IQR]	4.7 [2.5–17.2]	67.5 [14.0–156.9]	<0.001

Abbreviations: GAS, group A streptococcus; WBC, white blood cell count; IQR, interquartile range; CRP, C-reactive protein; PCT, procalcitonin; MxA, myxovirus resistance protein A; TRAIL, tumour necrosis factor (TNF)-related apoptosis-inducing ligand.

^a Univariate analysis by the χ^2 or Mann–Whitney U test.

^b Number of eligible patients $n = 66$.

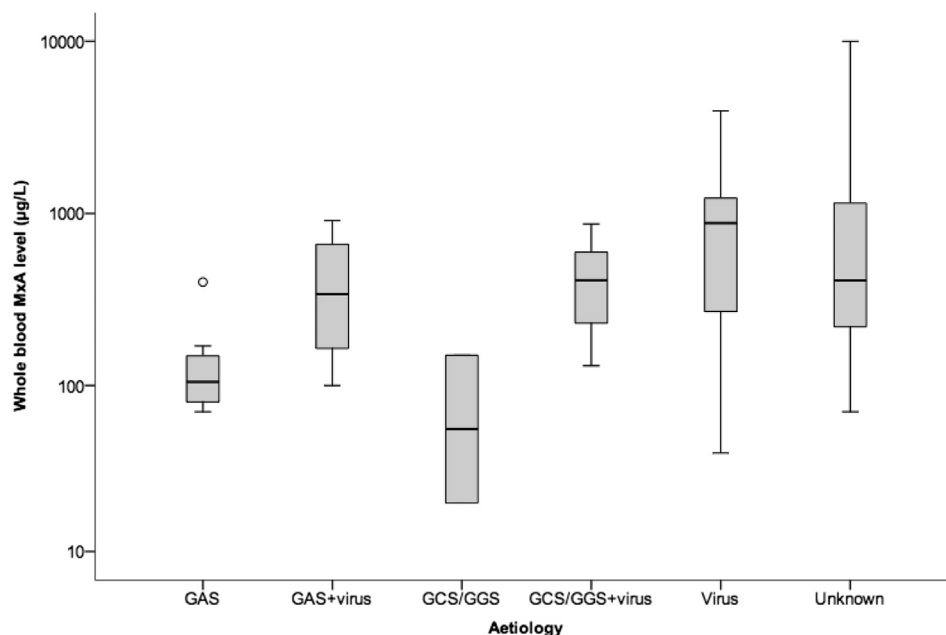


Figure 2 Boxplot graph (median, interquartile range [boxes], 95% confidence interval [error bars], and outliers [open circles]) represents the blood myxovirus resistance protein A (MxA) levels in the different aetiological groups of patients with febrile pharyngitis ($n = 83$). The vertical axis (Y) displays the MxA protein concentration ($\mu\text{g/L}$) in whole blood. Scale of the Y axis is logarithmic. The horizontal axis (X) represents different aetiological groups: group A streptococcus, GAS; GAS + virus; group C streptococcus, GCS or group G streptococcus, GGS; GCS/GGS + virus; virus; unknown.

younger than five years old. Group C and G β -haemolytic streptococci were detected only infrequently and often together with viruses. It has been suggested that *M. pneumoniae* is a major pharyngitis pathogen in children.¹⁵ In our study, the detection rate of *M. pneumoniae* IgM antibodies was 12%. However, considering the duration of the patients' symptoms, the presence of other potential pathogens, the presence of IgG antibodies, and the missing titre changes in the paired sera, the clinical significance of *M. pneumoniae* IgM antibody findings remain unclear.

Respiratory viruses can be detected by PCR in many asymptomatic individuals as well, and therefore, their role as etiologic agents can be argued.^{21,22,33} We and others have shown earlier that a MxA response is strongly associated with a symptomatic viral infection.^{32,34} Indeed, in the present study, most patients with virus findings had elevated levels of MxA protein in their blood. In contrast, at the follow-up visit when the patients were asymptomatic or had only minor, non-febrile respiratory symptoms, their blood MxA levels remained low. Our finding suggests that at the enrolment they had a true, symptomatic virus infection rather than coincidental virus detection. Still, all but one patient with febrile pharyngitis positive for group A, C, or G β -haemolytic streptococci without virus detection demonstrated no MxA response. Interestingly, nine of ten patients without a confirmed microbiological diagnosis had increased MxA levels. This finding suggests that the majority of these patients might have had an infection caused by an undetected virus.

Enteroviruses were the most often detected pathogens in this study. This observation is probably a result of two facts: First, the use of a PCR assay instead of viral culture in diagnostics increases the detection rate of enteroviruses.

Second, epidemiological factors influence the results. During autumn 2014, there were substantially more reported enterovirus infections in Finland than in the two previous years, especially in children younger than 10 years old.³⁵

Viruses were detected together with β -haemolytic streptococci in 14 patients. Rhinoviruses and adenoviruses were the most frequent virus findings in these patients. Because detection of rhinovirus in asymptomatic subjects is not uncommon, and because adenovirus can persist in tonsillar tissue, the pathogenic role of these viruses is not always clear.^{33,36,37} However, 11 out of 14 of the patients had an MxA response, which suggests that these patients either had a viral-bacterial co-infection or they had a virus infection and an incidental GAS carriage.

Biomarkers of bacterial infection have limited clinical utility in distinguishing between GAS and non-GAS pharyngitis.^{38–40} We found a significant difference in the blood levels of WBC, CRP, MxA, TRAIL, and the MxA/CRP ratio between patients with GAS and non-GAS illness, but their analytical performance measured by the AUC was poor. MxA could not discriminate between a GAS and non-GAS aetiology of pharyngitis, mainly because streptococcal and viral co-infection induced an MxA response comparable to that of virus infection. Because GAS can be detected relatively easily in throat swabs, there is probably no clinical need for a biomarker in the routine diagnostics of GAS pharyngitis. Here, we studied the biomarkers primarily to assess the pathogenic role of detected viruses or bacteria, not to evaluate their performance in differential diagnostics in clinical practice. However, the MxA level and MxA/CRP ratio remained low in patients with sole streptococcal infection and increased in the majority of patients with a virus infection. Therefore, they could be helpful

tools in the differential diagnosis of viral and bacterial infections in other clinical settings. In our study, blood level of TRAIL, another virus specific biomarker, was less accurate in differentiating between viral and bacterial aetiology.

This study had several limitations. First, this was a single-centre study and, therefore, the results might not be fully generalizable to other centres. Moreover, patient recruitment continued only for 14 months, and epidemiological variation might have influenced the etiological outcomes. Additionally, the sample size was rather small. Several different ED physicians carried out the preliminary recruitment of the patients, which could have influenced the diagnostic accuracy of pharyngitis. However, our setting pragmatically reflects how the clinicians identify febrile pharyngitis. Furthermore, we could possibly have increased the detection rate of viruses by collecting also nasopharyngeal swab samples.^{41,42} Our rationale for sole oropharyngeal sampling was that we were investigating pharyngeal infection and that nasopharyngeal sampling might have increased the detection of viruses with a questionable role in causing pharyngeal symptoms.

Conclusions

Viruses are the most common cause of febrile pharyngitis in children and adolescents. This study reinforces the current practice of sparing patients with non-GAS illness from antibiotic treatment. Biomarker measurements seem clinically irrelevant in the diagnostics of pharyngitis. Nevertheless, blood MxA has potential as a biomarker of acute virus infection.

Conflicts of interest

There are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jinf.2017.01.002>.

References

- Nash DR, Harman J, Wald ER, Kelleher KJ. Antibiotic prescribing by primary care physicians for children with upper respiratory tract infections. *Arch Pediatr Adolesc Med* 2002;**156**:1114–9.
- Charles J, Pan Y, Britt H. Trends in childhood illness and treatment in Australian general practice, 1971–2001. *Med J Aust* 2004;**180**:216–9.
- Hing E, Hall MJ, Xu J. National Hospital Ambulatory Medical Care Survey: 2006 outpatient department summary. *Natl Health Stat Report* 2008:1–31.
- Danchin MH, Rogers S, Kelpie L, Selvaraj G, Curtis N, Carlin JB, et al. Burden of acute sore throat and group A streptococcal pharyngitis in school-aged children and their families in Australia. *Pediatrics* 2007;**120**:950–7.
- Shulman ST, Bisno AL, Clegg HW, Gerber MA, Kaplan EL, Lee G, et al. IDSA Clinical practice guideline for the diagnosis and management of group A streptococcal pharyngitis: 2012 update by the infectious diseases society of America. *Clin Infect Dis* 2012;**55**:1–17.
- Pelucchi C, Grigoryan L, Galeone C, Esposito S, Huovinen P, Little P, et al. Guideline for the management of acute sore throat. *Clin Microbiol Infect* 2012;**18**(Suppl. 1):1–28.
- Dooling KL, Shapiro DJ, Van Beneden C, Hersh AL, Hicks LA. Overprescribing and inappropriate antibiotic selection for children with pharyngitis in the United States, 1997–2010. *JAMA Pediatr* 2014;**168**:1073–4.
- Barnett ML, Linder JA. Antibiotic prescribing to adults with sore throat in the United States, 1997–2010. *JAMA Intern Med* 2014;**174**:138–40.
- Kronman MP, Zhou C, Mangione-Smith R. Bacterial prevalence and antimicrobial prescribing trends for acute respiratory tract infections. *Pediatrics* 2014;**134**:e956–65.
- Fleming-Dutra KE, Hersh AL, Shapiro DJ, Bartoces M, Enns EA, File Jr TM, et al. Prevalence of inappropriate antibiotic prescriptions among US ambulatory care visits, 2010–2011. *JAMA* 2016;**315**:1864–73.
- Bisno AL. Acute pharyngitis. *N Engl J Med* 2001;**344**:205–11.
- Bisno AL. Acute pharyngitis: etiology and diagnosis. *Pediatrics* 1996;**97**:949–54.
- Herath VC, Carapetis J. Sore throat: is it such a big deal anymore? *J Infect* 2015;**71**:S101–5.
- Hsieh TH, Chen PY, Huang FL, Wang JD, Wang LC, Lin HK, et al. Are empiric antibiotics for acute exudative tonsillitis needed in children? *J Microbiol Immunol Infect* 2011;**44**:328–32.
- Esposito S, Blasi F, Bosis S, Droghetti R, Faelli N, Lastrico A, et al. Aetiology of acute pharyngitis: the role of atypical bacteria. *J Med Microbiol* 2004;**53**:645–51.
- Putto A. Febrile exudative tonsillitis: viral or streptococcal? *Pediatrics* 1987;**80**:6–12.
- McMillan JA, Sandstrom C, Weiner LB, Forbes BA, Woods M, Howard T, et al. Viral and bacterial organisms associated with acute pharyngitis in a school-aged population. *J Pediatr* 1986;**109**:747–52.
- Glezen WP, Clyde WA, Senior RJ, Sheaffer CI, Denny FW. Group A streptococci, mycoplasmas, and viruses associated with acute pharyngitis. *JAMA* 1967;**202**:455–60.
- Shaikh N, Leonard E, Martin JM. Prevalence of streptococcal pharyngitis and streptococcal carriage in children: a meta-analysis. *Pediatrics* 2010;**126**:e557–64.
- DeMuri GP, Wald ER. The group A streptococcal carrier state reviewed: still an enigma. *J Pediatric Infect Dis Soc* 2014;**3**:336–42.
- Jartti T, Jartti L, Peltola V, Waris M, Ruuskanen O. Identification of respiratory viruses in asymptomatic subjects: asymptomatic respiratory viral infections. *Pediatr Infect Dis J* 2008;**27**:1103–7.

22. Jansen RR, Wieringa J, Koekkoek SM, Visser CE, Pajkrt D, Molenkamp R, et al. Frequent detection of respiratory viruses without symptoms: toward defining clinically relevant cutoff values. *J Clin Microbiol* 2011;**49**:2631–6.
23. Oved K, Cohen A, Boico O, Navon R, Friedman T, Etshtein L, et al. A novel host-proteome signature for distinguishing between acute bacterial and viral infections. *PLoS One* 2015; **10**:e0120012.
24. Mejias A, Suarez NM, Ramilo O. Detecting specific infections in children through host responses: a paradigm shift. *Curr Opin Infect Dis* 2014;**27**:228–35.
25. Tsalik EL, Henao R, Nichols M, Burke T, Ko ER, McClain MT, et al. Host gene expression classifiers diagnose acute respiratory illness etiology. *Sci Transl Med* 2016;**8**:322ra11.
26. Österback R, Tevaluoto T, Ylinen T, Peltola V, Susi P, Hyypiä T, et al. Simultaneous detection and differentiation of human rhinovirus and enteroviruses in clinical specimens by real-time PCR with locked nucleic acid probes. *J Clin Microbiol* 2013;**51**:3960–7.
27. Nix WA, Oberste MS, Pallansch MA. Sensitive, seminested PCR amplification of VP1 sequences for direct identification of all enterovirus serotypes from original clinical specimens. *J Clin Microbiol* 2006;**44**:2698–704.
28. Ylihäsälä M, Harju E, Arppe R, Hattara L, Hölsä J, Saviranta P, et al. Genotyping of clinically relevant human adenoviruses by array-in-well hybridization assay. *Clin Microbiol Infect* 2013; **19**:551–7.
29. Koskinen P, Vuorinen T, Meurman O. Influenza A and B virus IgG and IgM serology by enzyme immunoassays. *Epidemiol Infect* 1987;**99**:55–64.
30. Vuorinen T, Meurman O. Enzyme immunoassays for detection of IgG and IgM antibodies to parainfluenza types 1, 2 and 3. *J Virol Methods* 1989;**23**:63–70.
31. Lönnrot M, Korpela K, Knip M, Ilonen J, Simell O, Korhonen S, et al. Enterovirus infection as a risk factor for beta-cell autoimmunity in a prospectively observed birth cohort: the Finnish Diabetes Prediction and Prevention Study. *Diabetes* 2000;**49**:1314–8.
32. Toivonen L, Schuez-Havupalo L, Rulli M, Ilonen J, Pelkonen J, Melen K, et al. Blood MxA protein as a marker for respiratory virus infections in young children. *J Clin Virol* 2015;**62**:8–13.
33. Heinonen S, Jartti T, Garcia C, Oliva S, Smitherman C, Anguiano E, et al. Rhinovirus detection in symptomatic and asymptomatic children: value of host transcriptome analysis. *Am J Respir Crit Care Med* 2016;**193**:772–82.
34. Engelmann I, Dubos F, Lobert PE, Houssin C, Degas V, Sardet A, et al. Diagnosis of viral infections using myxovirus resistance protein A (MxA). *Pediatrics* 2015;**135**:e985–93.
35. Jaakola S, Lyytikäinen O, Huusko S, Salmenlinna S, Pirhonen J, Savolainen-Kopra C, et al. *The Institution of Health and Welfare, Finland. Report on communicable diseases in Finland*, ISBN 978-952-302-481-6. Available at: <http://urn.fi/URN> [Accessed 7 August 2016].
36. Johnston SL, Sanderson G, Pattermore PK, Smith S, Bardin PG, Bruce CB, et al. Use of polymerase chain reaction for diagnosis of picornavirus infection in subjects with and without respiratory symptoms. *J Clin Microbiol* 1993;**31**:111–7.
37. Garnett CT, Talekar G, Mahr JA, Huang W, Zhang Y, Ornelles DA, et al. Latent species C adenoviruses in human tonsil tissues. *J Virol* 2009;**83**:2417–28.
38. Christensen AMG, Thomsen MK, Ovesen T, Klug TE. Are procalcitonin or other infection markers useful in the detection of group A streptococcal acute tonsillitis? *Scand J Infect Dis* 2014;**46**:376–83.
39. Elsammak M, Hanna H, Ghazal A, Edeen FB, Kandil M. Diagnostic value of serum procalcitonin and C-reactive protein in Egyptian children with streptococcal tonsillopharyngitis. *Pediatr Infect Dis J* 2006;**25**:174–6.
40. Putto A, Meurman O, Ruuskanen O. C-reactive protein in the differentiation of adenoviral, Epstein-Barr viral and streptococcal tonsillitis in children. *Eur J Pediatr* 1986;**145**:204–6.
41. Benkouiten S, Gautret P, Belhouchat K, Drali T, Nougairede A, Salez N, et al. Comparison of nasal swabs with throat swabs for the detection of respiratory viruses by real-time reverse transcriptase PCR in adult Hajj pilgrims. *J Infect* 2015;**70**:207–10.
42. Dawood FS, Jara J, Estripeaut D, Vergara O, Luciani K, Corro M, et al. What is the added benefit of oropharyngeal swabs compared to nasal swabs alone for respiratory virus detection in hospitalized children aged <10 years? *J Infect Dis* 2015;**212**:1600–3.