



Detection of group A streptococcus in children with confirmed viral pharyngitis and antiviral host response

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Abstract

Our aim was to study the detection of group A streptococcus (GAS) with different diagnostic methods in paediatric pharyngitis patients with and without a confirmed viral infection. 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. A confirmed viral infection was defined as a positive virus diagnostic test (nucleic acid amplification test [NAAT] and/or serology) together with an antiviral immune response of the host demonstrated by elevated (≥ 175 $\mu\text{g/L}$) myxovirus resistance protein A (MxA) blood concentration. Testing for GAS was performed by a throat culture, by 2 rapid antigen detection tests (StrepTop and mariPOC) and by 2 NAATs (Simplexa and Illumigene). Altogether, 83 children were recruited of whom 48 had samples available for GAS testing. Confirmed viral infection was diagnosed in 30/48 (63%) children with febrile pharyngitis. Enteroviruses 11/30 (37%), adenoviruses 9/30 (30%) and rhinoviruses 9/30 (30%) were the most common viruses detected. GAS was detected by throat culture in 5/30 (17%) with and in 6/18 (33%) patients without a confirmed viral infection. Respectively, GAS was detected in 4/30 (13%) and 6/18 (33%) by StrepTop, 13/30 (43%) and 10/18 (56%) by mariPOC, 6/30 (20%) and 9/18 (50%) by Simplexa, and 5/30 (17%) and 6/18 (30%) patients by Illumigene.

Conclusion: GAS was frequently detected also in paediatric pharyngitis patients with a confirmed viral infection. The presence of antiviral host response and increased GAS detection by sensitive methods suggest incidental throat carriage of GAS in viral pharyngitis.

What is Known:

- The frequency and significance of GAS-virus co-detection are poorly characterised in children with pharyngitis.
- Detection of a virus and the antiviral host response likely indicates symptomatic infection.

What is New:

- Group A streptococcus (GAS) was detected in 17–43% of the children with confirmed viral pharyngitis depending on the GAS diagnostic method.
- Our results emphasize the risk of detecting and treating incidental pharyngeal carriage of GAS in children with viral pharyngitis.

Keywords GAS · Group A streptococcus · MxA · Myxovirus resistance protein A · Pharyngitis · *Streptococcus pyogenes*

Abbreviations

ASO	Anti-streptolysin O	GAS	Group A streptococcus
CI	Confidence interval	IQR	Interquartile range
CRP	C-reactive protein	MxA	Myxovirus resistance protein A
ED	Emergency department	NAAT	Nucleic acid amplification test
		RADT	Rapid antigen detection test
		RT-PCR	Reverse-transcription polymerase chain reaction

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Introduction

Acute pharyngitis caused by group A streptococcus (GAS, *Streptococcus pyogenes*) is a common reason for antibiotic prescribing [1, 2]. It has been estimated that the proportion of pharyngitis cases caused by GAS is 24–37% in children [3, 4] and that pharyngitis is associated with substantial antibiotic overuse [5]. Furthermore, only few studies have incorporated modern virological methods [6, 7], and the possibility of a GAS-virus co-detection in children with pharyngitis has thus been overlooked.

The clinical significance of the GAS-virus co-detection in pharyngitis patients is unclear. This is a result of the relatively high asymptomatic oropharyngeal carriage rate of GAS and the frequent detection of respiratory viruses by nucleic acid amplification tests (NAATs) in asymptomatic subjects [3, 4, 8]. We have recently shown that finding a virus in children with febrile pharyngitis is often accompanied by an antiviral host response demonstrated by an elevated blood myxovirus resistance protein A (MxA) level, suggesting a causative role for a virus in these children [7]. An MxA response is mediated by type I and III interferons and correlates with fever and other symptoms [9]. In addition, the emerging use of novel, highly sensitive rapid antigen detection tests (RADTs) and NAATs could further increase the detection rate of GAS [10, 11]. Therefore, we hypothesized that GAS can be detected in part of the children with confirmed viral pharyngitis, likely reflecting an asymptomatic pharyngeal carriage.

Objective

We used culture, two RADTs and two NAATs to study the occurrence of GAS in paediatric pharyngitis patients with or without a viral infection confirmed by both a positive virus detection and an antiviral MxA response. We postulate that the symptoms in children with a viral infection fulfilling this definition are mostly caused by a virus and not by GAS.

Materials and methods

This was part of a prospective observational study on the aetiology and diagnosis of febrile pharyngitis in children and adolescents [7]. The study was done at the Department of Emergency Services of Turku University Hospital, Turku, Finland, between November 25, 2013, and January 31, 2015. Inclusion criteria were age 1–16 years, fever during the acute illness episode and acute pharyngitis defined as oropharyngeal exudate and/or intensive

redness. Pharyngitis was diagnosed, and a treatment decision was made by the attending emergency department (ED) physician. In the original cohort, the study size was estimated to be sufficient to demonstrate the wide spectrum of viral agents capable of causing pharyngitis. All patients were invited to a follow-up visit 2–4 weeks after the initial visit to collect paired serum samples. In this study, only children with diagnostic test results available by both GAS RADT methods and by both GAS NAAT methods were included.

Confirmed viral infection was defined as a positive virus diagnostic test (NAAT and/or serology) together with the antiviral immune response of the host demonstrated by an elevated (≥ 175 $\mu\text{g/L}$) blood MxA concentration. The threshold was chosen based on the findings in an earlier study where a blood MxA concentration ≥ 175 $\mu\text{g/L}$ best differentiated symptomatic viral infections from asymptomatic conditions in children [9].

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.

Sample collection and GAS RADTs

At the enrolment, blood samples for biomarker measurement and serology and oropharyngeal swab samples for culture, RADT and NAATs were collected. From each child, four throat swabs were collected in a standardized order by rubbing the swabs against both tonsils: (1) A flocced swab was used to collect the sample for bacterial culture and transferred in a tube with liquid transport media (ESwab, Copan, Brescia, Italy) to the Division of Clinical Microbiology, Turku University Hospital for bacterial culture and GAS NAAT analysis. (2) A flocced swab (Copan) was used to collect the sample for virus NAAT and transferred in a dry, clean tube to the Laboratory of Diagnostic Virology of Turku University Hospital for analysis. (3) The swab sample for an immunochromatographic GAS RADT was collected, and the test was performed immediately at the ED according to the manufacturer's instructions (StrepTop, AllDiag, Strasbourg, France). (4) The sample for automated GAS RADT was collected by a flocced swab (Copan) and transferred in a dry, clean tube to the Central Laboratory of Turku University Hospital, where the test was immediately performed according to the manufacturer's instructions (mariPOC, ArcDia International Ltd., Turku, Finland).

Throat culture, virus diagnostics and biomarker measurements

Bacterial culture, virus NAAT and serology and blood MxA measurements were performed as described in more detail earlier [7]. Briefly, throat culture for GAS was performed by inoculating transport media on streptococcal selective blood agar plate including colistin-oxolinic acid, as well as in a streptococcal-selective broth, followed by subculture on streptococcal-selective blood agar. Beta-haemolytic streptococci were identified by standard methods, including the Lancefield antigen test (PathoDextra Strep Grouping Kit, Thermo Scientific, Basingstoke, UK) and/or matrix-assisted laser desorption and ionization time-of-flight mass spectrometry (Bruker Daltonics, Bremen, Germany). Respiratory virus diagnostics were performed by a multiplex respiratory virus real-time–reverse-transcription polymerase chain reaction (RT–PCR) test (Anyplex II RV16; Seegene, Seoul, Korea), by an in-house–real-time RT–PCR assay for rhinoviruses and enteroviruses and by serological methods (Epstein-Barr virus, adenovirus, enteroviruses, influenza A and B viruses, parainfluenza virus types 1, 2 and 3 and respiratory syncytial virus) mainly from paired serum samples [7]. Plasma C-reactive protein (CRP) and procalcitonin levels were determined in the hospital central laboratory by routine methods. Whole blood samples for MxA measurement were transported to the laboratory where samples were diluted 1:20 in hypotonic buffer and stored at $-70\text{ }^{\circ}\text{C}$ until the enzyme immunoassay analysis was performed as described earlier [9, 12, 13].

GAS serology

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

GAS NAATs

Molecular methods tested were Simplexa GAS assay using 3 M Integrated Cycler (Focus Diagnostics, Cypress, CA, USA) and Illumigene GAS assay (Meridian Bioscience, Paris, France). Tests were performed as per the manufacturer's instructions except that the swab samples were replaced with 50 μl of the Eswab transport media.

Data analysis

A confirmed viral infection was defined as a positive virus detection (NAAT and/or serology) in a child with a documented antiviral innate immune response (blood MxA

concentration $\geq 175\text{ }\mu\text{g/L}$). Sensitivities, specificities, positive and negative predictive values and test accuracies with 95% confidence intervals (CI) were calculated for each GAS diagnostic assay in comparison with the throat culture. The percentage of the GAS detection rate and the occurrence of virus co-detection and elevated blood MxA levels in GAS-positive pharyngitis patients with 95% CIs were calculated separately for each GAS diagnostic assay. Nominal data were analysed by χ^2 or Fisher's exact tests. IBM SPSS Statistics, version 27 (IBM Corp., Armonk, NY, USA) was used for analysis.

Results

In total, 83 children (median age 5.5 years, interquartile range (IQR) 3.2–12.2) with febrile pharyngitis were recruited. The final study population included 48 children (median age 8.2 years, IQR 3.4–12.5) with specimens available for GAS RADT and NAAT analysis by all four methods. Throat culture was positive for GAS in 11/48 (23%) of the final study population and in 19/83 (23%) of all recruited children, showing that our study sample was comparable to the larger patient population with febrile pharyngitis in terms of GAS occurrence.

Viruses were detected in 36/48 (75%), elevated blood MxA levels in 36/48 (75%) and both in 30/48 (63%) children, comprising the group of confirmed viral infection. Enteroviruses, adenoviruses and rhinoviruses were the most frequently detected viruses. More than 1 virus was detected in 13/48 (27%) children. All virus findings are presented in detail in Table 1. Cough and/or rhinitis were present in 27/48 (56%) children. Median (IQR) C-reactive protein (CRP) plasma levels in children with confirmed and no confirmed viral infection were 10 (5–14) mg/L and 34 (10–45) mg/L, and procalcitonin levels were 0.1 (0.1–0.3) $\mu\text{g/L}$ and 0.1 (0.1–0.3) $\mu\text{g/L}$, respectively. When CRP results were combined with MxA results as a MxA ($\mu\text{g/L}$)/CRP (mg/L) ratio, the median (IQR) levels were in the group of confirmed viral aetiology 97 (65–187) and in the group of no confirmed viral aetiology 6 (3–21).

GAS was detected by throat culture in 5/30 (17%) with and in 6/18 (33%) without confirmed viral infection ($p = 0.288$). The detection rate of GAS (proportion of positive samples of all tested) was lower among children with confirmed viral infection than among children without it, regardless of the GAS test (Table 2). ASO antibodies were detected at enrolment in 1/5 (20%) children with confirmed viral infection and GAS-positive throat culture and in 2/6 (33%) without confirmed viral infection and GAS positive throat culture. Paired serum samples were collected from 31/48 (65%) children for a median of 18.5 days (IQR

Table 1 Virus detection in pharyngitis patients with and without demonstrable antiviral host response

	Blood MxA concentration $\geq 175 \mu\text{g/L}$ ($n = 36$)	Blood MxA concentration $< 175 \mu\text{g/L}$ ($n = 12$)
Virus detection positive, n (%)	30 (83)	6 (50)
Virus findings, n		
	Rhinovirus	Human coronavirus NL63 2
	Influenza A virus	Rhinovirus 2
	Adenovirus	Bocavirus and rhinovirus 1
	Enterovirus	Enterovirus 1
	Human metapneumovirus	
	Parainfluenza virus 3	
	Adenovirus and human coronavirus OC43	
	Epstein-Barr virus	
	Respiratory syncytial virus B	
	Influenza A virus and adenovirus	
	Enterovirus and parainfluenza virus 3	
	Enterovirus, parainfluenza virus 3, and rhinovirus	
	Enterovirus and rhinovirus	
	Enterovirus and parainfluenza virus 2	
	Human metapneumovirus and enterovirus	
	Enterovirus and adenovirus	
	Adenovirus, enterovirus, and rhinovirus	
	Enterovirus, adenovirus, and rhinovirus	
	Adenovirus and rhinovirus	
	Respiratory syncytial virus A, adenovirus, enterovirus, and human coronavirus OC43	
No viruses detected, n (%)	6 (17)	6 (50)

MxA myxovirus resistance protein A

17–21) apart. Only one child showed \geq fourfold increase in ASO levels and in this case throat culture was negative for GAS.

When the diagnostic performances of GAS RADTs and NAATs were compared with throat culture, distinct test features could be seen; StrepTop (an immunochromatographic RADT) had a sensitivity of 82% and a specificity of 100%. MariPOC (an automated RADT) had a 100% sensitivity and 68% specificity. The two GAS NAATs Simplexa and Illumigene had a sensitivity of 91% both and a

Table 2 Group A streptococcus (GAS) test results of 48 children with pharyngitis with and without confirmed viral infection

GAS diagnostic method	Confirmed viral infection ($n = 30$) ^a , GAS detected		No confirmed viral infection ($n = 18$) ^a , GAS detected		p^b
	n	% (95% CI)	n	% (95% CI)	
Throat culture	5	17 (6–27)	6	33 (20–47)	0.288
StrepTop	4	13 (4–23)	6	28 (15–40)	0.265
mariPOC	13	43 (29–57)	10	56 (41–70)	0.412
Simplexa	6	20 (9–31)	9	50 (36–64)	0.030
Illumigene	5	17 (6–27)	6	33 (20–47)	0.288

CI confidence interval

^aConfirmed viral infection: virus detected and blood myxovirus resistance protein A concentration $\geq 175 \mu\text{g/L}$

^b χ^2 or Fisher's exact test

specificity of 86% and 97%, respectively. In this study, the overall accuracy was highest with StrepTop and Illumigene assays (Table 3).

Discussion

The most important finding in this study is that GAS can be frequently detected also in children with pharyngitis of a confirmed viral aetiology. Depending on the diagnostic method, GAS was identified in 13–43% of them. By using the novel concept of confirmed viral infection defined as a virus finding and an antiviral immune response of the host as indicated by elevated blood MxA level, we aimed to minimize the risk of labelling clinically insignificant virus findings by NAAT as true infections. Blood MxA level increases due to type I and III interferon production as part of the innate immune response triggered by a symptomatic viral infection. Therefore, MxA is a promising and a relatively simple biomarker that has proven to be a useful addition to virological methods in epidemiological or diagnostic studies [7, 9, 14–16].

Some pre-clinical and ex-vivo data suggest that also GAS infection could trigger type I interferon response [17–19]. However, the role of interferon production in clinical GAS pharyngitis remains unknown. In our cohort, GAS was detected by throat culture in 11 children. Any

Table 3 Diagnostic performance of four different group A streptococcus (GAS) assays in comparison with throat culture (n = 48)

	GAS positive samples, n (%)	Performance, % (95% CI)				
		Sensitivity	Specificity	PPV	NPV	Accuracy ^a
Throat culture	11 (23)					
RADTs						
StrepTop	9 (19)	82 (71–93)	100	100	95 (89–100)	96 (90–100)
mariPOC	23 (48)	100	68 (54–81)	48 (34–62)	100	75 (63–87)
NAATs						
Simplexa	15 (31)	91 (83–99)	86 (77–96)	67 (53–80)	97 (92–100)	88 (78–97)
Illumigene	11 (23)	91 (83–99)	97 (93–100)	91 (8–99)	97 (93–100)	96 (90–100)

CI confidence interval PPV positive predictive value, NPV negative predictive value, RADT rapid antigen detection test, NAAT nucleic acid amplification test

^aAccuracy = the percentage of correctly classified samples

virus was co-detected in 6/11 (55%) of these GAS-infected patients. Blood MxA level was elevated ($\geq 175 \mu\text{g/L}$) in 5/6 (83%) of these patients and in none of the GAS positive but virus negative ($n = 5$) patients. This preliminary finding suggests that GAS pharyngitis without concomitant viral infection does not induce a systemic interferon response that would complicate the use of blood MxA level as a biomarker for acute viral infection. Furthermore, earlier data on CRP in differentiating GAS from other aetiologies of pharyngitis does not support its use [21, 22]. Our data on combining viral and bacterial biomarkers in pharyngitis patients is promising and in line with our recent findings in hospitalized febrile children [20].

There were 6/36 (17%) children with high blood MxA levels ($\geq 175 \mu\text{g/L}$) without any viruses detected. The reason for this can only be speculated, but it seems possible that some of them had a viral infection that we were not able to diagnose. There were 6/12 (50%) children with virus finding among those with low blood MxA levels ($< 175 \mu\text{g/L}$). As stated before, viruses can often be detected also in asymptomatic children by NAAT methods [8]. In these asymptomatic infections, host response, including interferon/MxA response, differs from that of symptomatic infection [9, 20]. This could be the case also with the virus-positive but MxA-negative children in our cohort.

Our results are in line with another pragmatic study in children with pharyngitis by Shapiro et al. [21]. They found that 53% of the children diagnosed with GAS pharyngitis had at least one clinical sign or symptom (cough, rhinitis, ulcers/vesicles on their mucosa or conjunctival injection) suggestive of viral infection. In our study, 5/11 (45%) of the children with throat culture-positive GAS pharyngitis had a confirmed viral infection. Thus, our study with a comprehensive virological workup completes the earlier clinical findings and confirms the role of viruses as the most probable cause of illness in children with GAS-virus co-

detection. In children from high-income countries, the rate of asymptomatic pharyngeal GAS carriage is estimated to be around 10% [3, 4]. In other words, detection of GAS most likely reflects an asymptomatic pharyngeal carriage and contributes to the risk of unnecessary antibiotic prescribing.

Another notable observation is the variation in the sensitivity and specificity of different diagnostic GAS assays in comparison with the throat culture. All four assays showed relatively good sensitivity, 82–100%, but the specificity between different assays was more variable, 68–100%. The exceptionally high detection rate of mariPOC GAS RADT is in line with an earlier study of this assay [10]. Technology in GAS NAATs is based either on RT-PCR, such as Simplexa GAS assay or isothermal amplification, such as Illumigene GAS assay. In settings where the burden of rheumatic fever and other post-streptococcal sequel is high, antibiotic treatment is recommended for all suspected GAS infections. In these settings, highly sensitive GAS assays could reduce unnecessary antibiotic use [22]. In other settings, the potential impact of the increased analytical sensitivity of GAS NAATs on the antimicrobial prescription rate for pharyngitis is of concern [11, 23]. All these findings emphasize the importance of proper validation of novel diagnostic methods and highlight the role of clinical guidelines in use of them.

Our study is subject to limitations. Due to the small size of the study population, our findings should be interpreted as preliminary observations. It is also worth noticing that stringent diagnostic criteria for pharyngitis were not used in this study. Thus, children with symptoms suggestive of viral infection were not categorically excluded if the attending ED physician diagnosed acute pharyngitis. Our study design was pragmatic and based on how pharyngitis is diagnosed, and how these children are treated at the actual site of care. Furthermore, the collection of four throat swabs might theoretically influence the results despite the standardised sampling protocol.

Conclusions

Our data show that GAS can be frequently detected in paediatric pharyngitis patients with a viral infection confirmed by microbiological diagnosis together with the demonstration of an antiviral innate immune response. When the GAS diagnosis was based on a highly sensitive assay instead of the throat culture, co-detections were even more frequent. These findings emphasize the risk of detecting and treating asymptomatic pharyngeal carriage of GAS, highlight the importance of diagnostic stewardship in children with pharyngitis and call for larger studies to examine the GAS-virus co-detections.

Authors' contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Lauri Ivaska, Jussi Niemelä, Kirsi Gröndahl-Yli-Hannuksela, Niina Putkuri, Tytti Vuorinen, Matti Waris, Kaisu Rantakokko-Jalava and Ville Peltola. The first draft of the manuscript was written by Lauri Ivaska, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets generated during the current study are not publicly available due to privacy protection but are available from the corresponding author on a reasonable request.

Code availability Not applicable.

Declarations

Ethics approval The Ethics Committee of the Hospital District of Southwest Finland approved the study protocol.

Consent to participate The legal guardians of all participating children and adolescents and the adolescent patients themselves gave their written, informed consent.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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