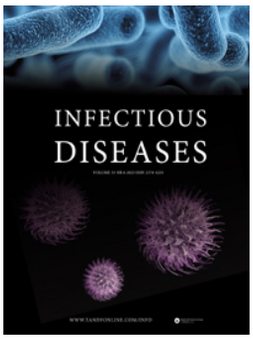

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ORIGINAL ARTICLE

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Presence of *Streptococcus pyogenes* in the throat in invasive Group A Streptococcal disease: a prospective two-year study in two health districts, Finland

Ville Kailankangas[a,b]*, Johanna Vilhonen[c,d,e]*, Kirsi Gröndahl-Yli-Hannuksela[f], Kaisu Rantakokko-Jalava[g], Tapio Seiskari[h], Kari Auranen[i], Emilia Lönnqvist[f], Mirva Virolainen[f], Hanne-Leena Hyyryläinen[j], Jarmo Oksi[c,d], Jaana Syrjänen[a,b] and Jaana Vuopio[f,g,j]

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ABSTRACT

Purpose: *Streptococcus pyogenes* (Group A *Streptococcus*, GAS) is an important human pathogen that can cause severe invasive (iGAS) infections. Throat carriage has been assumed to possibly lead to hematogenous seeding. Retrospective studies may estimate the incidence of throat carriage in iGAS patients inaccurately. In this study we aimed to gather data on the presence of GAS in the throat among iGAS patients in a prospective setting.

Methods: We conducted a prospective clinical study covering iGAS infections in adult patients in two university hospitals in Finland from June 2018 to July 2020. Recruited patients' throats were swabbed for culture and isothermal amplification tests (IAT) to search for GAS. The study was registered at ClinicalTrials.gov as ID NCT03507101.

Results: We enrolled 45 patients. Throat swabs were obtained from 39/45 (87%) patients. Ten patients (22%) had a positive IAT for GAS. They were statistically significantly more likely to be male (9/10 [90%] vs 13/29 [45%], $p = .024$). Several different *emm* types caused the iGAS infections.

Conclusions: GAS was frequently observed in throat swabs of patients with iGAS infection. This may suggest that hematogenous seeding from the nasopharynx is a possible portal of entry.

KEYWORDS
Group A *Streptococcus*
invasive infection
incidence
portal of entry
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Background

Streptococcus pyogenes, or Group A *Streptococcus* (GAS), is an important human pathogen and remains among the top 10 infectious causes of mortality [1]. It may colonize the throat, other mucous membranes or the skin asymptomatically but is also responsible for various infectious diseases. Most commonly these are mild to moderate infections of the throat and tonsillae, or of the skin, such as impetigo and cellulitis [2]. GAS also causes invasive infections, such as septicemia, necrotizing soft-tissue infection (NSTI) and streptococcal toxic shock syndrome (STSS), which are severe and life threatening even when properly treated and may result in adverse sequelae [3]. In high-income countries, the case fatality rate (CFR) of invasive GAS (iGAS) infections is 10–20%, while the most severe forms STSS and NSTI have a CFR as high as 30–50% [4–7].

The worldwide annual incidence of iGAS infections is estimated to be over half a million cases, with wide regional differences in incidence ranging from 46/100,000 in indigenous populations in the US and Australia to 3–4/100,000 in high-income countries [2,6,8,9]. Finland has seen a surge of iGAS cases in recent years with a peak incidence of 6.8/100,000 in 2018 [10].

Factors contributing to disease severity in iGAS infections are poorly documented. Several virulence factors and immune evasion mechanisms of GAS have been documented [11]. The quintessential virulence factor is the M protein, a superantigen that helps in evasion of phagocytosis. Sequencing of the *emm* gene, that encodes the M protein, is used for classification of GAS strains [12]. Recombination events in certain *emm* types, namely, *emm*1 and *emm*89, have been shown to increase virulence and invasiveness and have led to global dissemination of new variants [13–15].

The role of the pharynx as a portal of entry in iGAS infections has been demonstrated in animal models [16], but invasive dissemination from the throat in humans remains putative. Patients with an iGAS infection generally receive intravenous antimicrobials promptly, rendering negative any throat swab taken after GAS is confirmed. In an earlier prospective study of iGAS infections, 2.2% of adult cases and 19.8% of pediatric cases had pharyngotonsillitis at least four weeks prior to invasive infection based on questionnaire data and electronic health records (EHR) [17]. Blagden et al. reported that 3.4%, 2.1% and 0.7% of iGAS cases had, according to their EHR, recently experienced or were experiencing a non-invasive GAS infection, influenza or another upper respiratory tract infection, respectively [5].

In recent years, advances in nucleic acid amplification technologies have enabled more rapid detection and identification of pathogens in clinical samples. Nucleic acid amplification tests, while seeking to confirm the presence of nucleic acid targets in a sample, are not dependent on the viability of the pathogen producing the nucleic acid [18]. Therefore they are not as liable to produce false negatives with recent antimicrobial exposure. Isothermal amplification tests (IATs) are modern variants of molecular testing with simplified technology and simplified specimen preparation to enhance clinical utility. Commonly used technologies in IATs are loop-mediated isothermal amplification (LAMP) and helicase-dependent amplification (HDA) [18].

In this prospective, two-year clinical study covering iGAS cases treated in two university hospitals in Finland, we sought to gather further evidence on the presence of GAS in the throat in iGAS disease using modern molecular technologies.

Materials and methods

Data collection on incidence of iGAS

Invasive GAS infections (only isolations from blood and cerebrospinal fluid [CSF]) have been notifiable in Finland according to the Communicable Diseases Act since 1995. All clinical microbiology laboratories are required to report cases where GAS is isolated from blood or CSF and send the corresponding GAS isolate to the strain collection of the National Infectious Disease Register (NIDR) maintained by the Finnish Institute of Health and Welfare (THL). Data on yearly incidences of iGAS cases were acquired from NIDR [10].

Patient enrollment and samples taken

The study was conducted as a prospective observational study at two tertiary care Finnish hospitals: Tampere University Hospital in Pirkanmaa Health District (Hospital 1) and Turku University Hospital in the Hospital District of Southwest Finland (Hospital 2) between June 2018 and July 2020. Both hospitals have a catchment population of approximately half a million people. A case was defined as a culture positive finding of GAS from a normally sterile site (blood, CSF, pleural fluid, peritoneal fluid, synovial fluid, deep tissue sample) in a patient over 18 years of age. When GAS was isolated by the clinical microbiological laboratory from any of these sites in

either of the participating hospitals, the laboratory contacted the infectious diseases physician (VK or JVi) who recruited the patients, interviewed them and obtained a throat swab using a single eSwab kit (Copan diagnostics inc.). Sampling was done swabbing both tonsillar regions and the back of the throat. Three to four months after recruitment each patient was invited for a follow up visit. The throat swabs were sent to the Turku University for culture and IAT. Background data (Table 1) were obtained from the interview and electronic patient records with the patients' consent.

All data were compiled to a REDCap-database accessible only by the researchers. Data analysis was performed using study subject codes without possibility to reveal personal identification.

Definitions

The patients' underlying characteristics were classified according to the Charlson Comorbidity Index (CCI). The CCI was further divided into four categories, 0 score is 0, 1–2 scores is 1, 3–4 scores is 2 and $\geq$ 5 is 3 [19]. Obesity was defined as Body Mass Index (BMI) $\geq$ 30 kg/m^2.

Table 1. Demographics and clinical characteristics of the iGAS cases included in the study ($n = 45$).

Demographics and clinical characteristics	*n* (%)
Sex male	27 (60.0)
Age mean (SD)	55.07 (20.31)
Charlson class	
0	9 (20.0)
1	19 (42.2)
2	6 (13.3)
3	11 (24.4)
Healthcare-acquired	3 (6.7)
Housing type	
Independently at home	37 (82.2)
At home with help	5 (11.1)
Long-term care facility	3 (6.7)
Underlying conditions	
Obesity (BMI $\geq$ 30 kg/m^2)	14 (31.1)
Hypertension	14 (31.1)
Diabetes mellitus	8 (17.8)
Atrial fibrillation	8 (17.8)
Congestive heart failure	5 (11.1)
Pulmonary disease	5 (11.1)
Coronary artery disease	4 (8.9)
Peripheral artery disease	4 (8.9)
Cirrhotic liver disease	0 (0)
Chronic kidney disease	
CKD1 or unknown status	37 (82.2)
CKD2	3 (6.7)
CKD3	4 (8.9)
CKD4	1 (2.2)
CKD5	0 (0)
Any malignancy	6 (13.3)
Immunodeficiency	
HIV/AIDS	0 (0)
Immunosuppressive medication	1 (2.2)
Alcohol overconsumption	7 (15.6)
On-going smoking	13 (28.9)
No underlying disease	18 (40.0)

Note: Data represent no. (%) of the group.

Microbiological tests

The GAS isolates from the study subjects originally identified by either of the clinical microbiology laboratories involved (Fimlab and Turku University Hospital Clinical Microbiology) were sent to University of Turku for storage and *emm* typing. The throat swabs collected from the patients were also sent to University of Turku for culture and IAT.

Ten µl of the throat swabs (eSwab) were cultured on blood agar plates (BD) and inspected for beta-hemolytic colonies. Any suspected GAS colonies were identified with a Lancefield agglutination test.

The throat swabs were tested for presence of GAS nucleic acids by two commercial IATs, Solana GAS assay (Quidel, US), a helicase-dependent assay, and ID NOW Strep A 2 assay, that utilizes loop-mediated isothermal amplification (Abbott, US). The tests were used according to the manufacturers' instructions. In short, the eSwab liquid media were vortexed and 50 µl and 200 µl were used for testing with the Solana and the ID NOW, respectively. The Solana test system has a short initial (5 min at 95 °C) lysis step. No further sample preparations were performed. The sensitivities and specificities for as reported by the manufacturers are 98.2% and 97.2% for Solana [20] and 98.5% and 93.4% for ID NOW [21]. These values are based on comparison to throat culture on antibiotic naïve tonsillitis patients. The target gene in Solana® assay is a highly conserved *sdaB* gene, which encodes for DNAaseB protein, a well-characterized antigen produced by *S. pyogenes* [20]. The manufacturer of the ID NOW™ Strep A 2 does not specify the target, but it has been previously described as the cell envelope proteinase A (*cepA*) gene [22]. Methodologically the selected assays differ from traditional PCR as they do not require a thermal cycler and the turnaround time is much faster. Two different IATs with different technology were chosen to increase yield since IATs have not been studied in patients with ongoing antimicrobial exposure.

emm typing

THL has *emm* typed invasive GAS strains since 2005, and data on *emm* types from 2018 to 2020 were acquired from NIDR [10]. The isolates collected during our study were *emm* typed at the University of Turku according to the guidelines of the Centers for Disease Control and Prevention (CDC) [23].

Statistical analysis

Statistical analyses were performed with the IBM SPSS Statistics for Windows version 27 (IBM Corp., Armonk, NY). Categorical data were analyzed by Fisher's exact test. The normality of continuous variables was checked by histograms. A two-sided $p < .05$ was considered statistically significant.

Results

Epidemiology and patient enrollment

Figure 1 presents the incidences of iGAS infections in Finland and in the two study hospital districts during years 2010–2020. The incidence increased steeply in 2015–2018. The incidence at Hospital 2 decreased during the study period, which slowed the enrollment, whereas at Hospital 1 the incidence remained at a higher level resulting in better patient enrollment (Figure 2).

Altogether 45 patients were enrolled, 30 from Hospital 1 and 15 from Hospital 2 (Figure 3), representing 44% of all iGAS cases in these two hospital districts during the recruitment period. The COVID-19 pandemic caused a premature halt in the patient enrollment in March 2020. Enrollment was resumed in May 2020 and one further patient was enrolled in July 2020 before enrollment concluded, two months later than originally planned to make up for the missed months.

Clinical characteristics and disease severity

Details of clinical characteristics and infection foci and disease severity are summarized in Tables 1 and 2. Of the 45 patients 27 (60%) were male and 18 (40%) female. The mean and median age was 55 years. The most common underlying conditions were obesity (31%), hypertension (31%), diabetes mellitus (18%) and atrial fibrillation (18%), while 40% of the patients had no previously diagnosed chronic conditions.

The most common infectious foci were soft tissue infection on intact skin (38%) and wound infection (13%). Five of the 45 (11%) cases had NSTI and 9/45 (20%) cases had several infection foci. One patient had a verified preceding diagnosis of GAS tonsillitis and developed a very mild iGAS-disease course, possibly representing a transient tonsillitis-related bacteremia. Twelve cases of the 45 (27%) required surgical intervention, while the ICU admission rate was 29% and in-hospital mortality 16% (Table 2).

Eight of the 45 patients (18%) died before the follow-up visit: four within a week from hospital admission and an additional three later during hospitalization. One patient died at a nursing facility. The mean duration of hospitalization was 20 days (median 10 days) until discharge, referral to a secondary center for convalescence and rehabilitation or death.

emm type distribution

Altogether 43 iGAS isolates from 45 subjects were available for *emm* typing. Two isolates from Hospital 1 were lost before delivery to University of Turku. The most prevalent *emm* types among the recruited patients were *emm*1.0 (8/43, 19%), *emm*1.25 (6/43, 14%), *emm*28 (6/43, 14%) and *emm*89 (10/43, 23%).

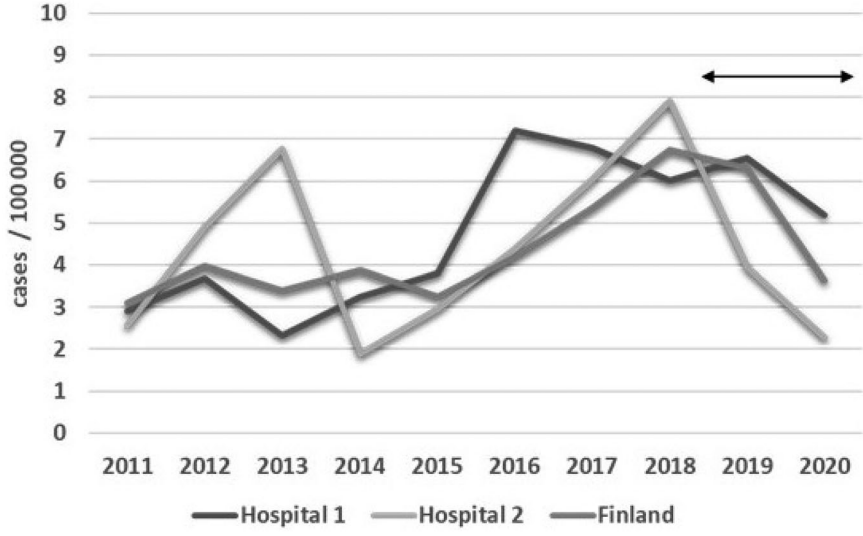

Figure 1. Incidence per 100,000 of iGAS infections in Finland, at Hospital 1 and at Hospital 2 during 2011–2020 according to the National Infectious Disease Register. The arrow indicates the study period.

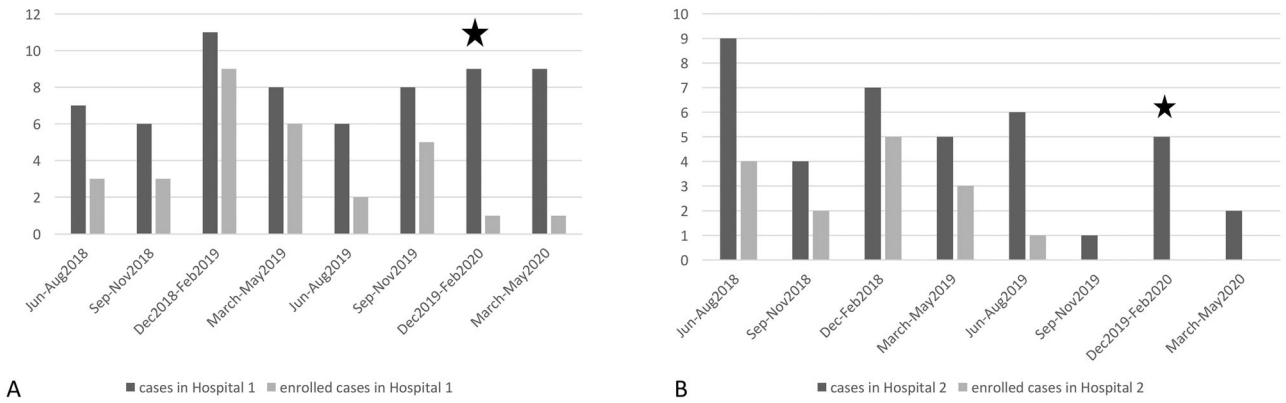

Figure 2. The enrolled cases and all occurred cases during the recruitment period (A) at Hospital 1 and (B) at Hospital 2. The asterisks indicate the beginning of the COVID19-pandemic.

The distribution of *emm*1.0 was 17% (5/30) at Hospital 1 and 20% (3/15) at Hospital 2, while *emm*1.25 represented 17% (5/30) of the cases at Hospital 1 and 7% (1/15) at Hospital 2. Likewise, *emm*28 and *emm*89 caused 7% (2/30) and 27% (8/30) of the cases at Hospital 1 and 27% (4/15) and 13% (2/15) of the cases at Hospital 2, respectively. The *emm* type distributions among all iGAS cases at Hospital 1, Hospital 2 and in all of Finland during years 2018–2020 are shown in Figure 4.

Of the eight *emm*1.0 cases, 6/8 (75%) were skin and soft tissue infections (SSTI), with 1/8 having concurrent endocarditis. SSTI was most common also among the *emm*1.25 cases (4/6, 67%), whereas among the *emm*28 cases, 3/6 (50%) were NSTIs. The *emm*89 cases had 4/10 (40%) SSTIs.

Isothermal amplification test results

Throat swabs were obtained from 39/45 (87%) patients. One patient died before sampling, one was too uncooperative due to advanced dementia, and four were intubated at the time of enrollment.

All throat swabs remained culture negative for GAS. All patients had received broad spectrum beta-lactam antimicrobials for a minimum of two days prior to swabbing. Ten of the 39 patients (22%) had a positive IAT. Five patients tested positive for GAS with both IAT tests (ID Now and Solana), and a further three patients with only the ID Now test and two patients with only the Solana test, respectively.

We compared the 10 cases with a positive IAT to the 29 IAT negative cases. The six cases with no throat swab available were excluded from this analysis.

Patients with a positive IAT were more likely to be male (9/10 [90%] vs 13/29 [45%], $p = .024$). Of the seven patients who died during hospitalization, three had a positive IAT, one was IAT negative and three had no available throat swab. Four out of 10 patients with a positive IAT required invasive mechanical ventilation while none of those with a negative IAT did (40% vs 0%, $p = .003$). However, four patients were intubated and on mechanical ventilation already at the time of enrollment and thus could not have their throats swabbed. All four of the wound infections were in the IAT negative group. There was no significant difference in mean age, underlying illnesses or *emm* type distribution between the groups. See Table 3 for a more detailed description.

The mean duration of hospitalization for IAT positive and IAT negative groups were 19 days and 20 days, respectively.

Discussion

The portal of entry in iGAS infections often remains undetermined. Invasion and dissemination from the nasopharynx has been shown to be possible in an animal model [16] and is assumed to occur in humans. In a small prospective study from Greece, only one (1/46, 2.2%) adult iGAS case and 19 (19/96, 19.8%) pediatric cases had prior streptococcal pharyngotonsillitis. This was according to the study questionnaire and EHR, so they were not systematically throat cultured for the study itself [17]. In our study 22% of patients were tested positive for GAS with IAT test, which is more common than prior GAS findings in the abovementioned study [17]. To our knowledge, there are no earlier

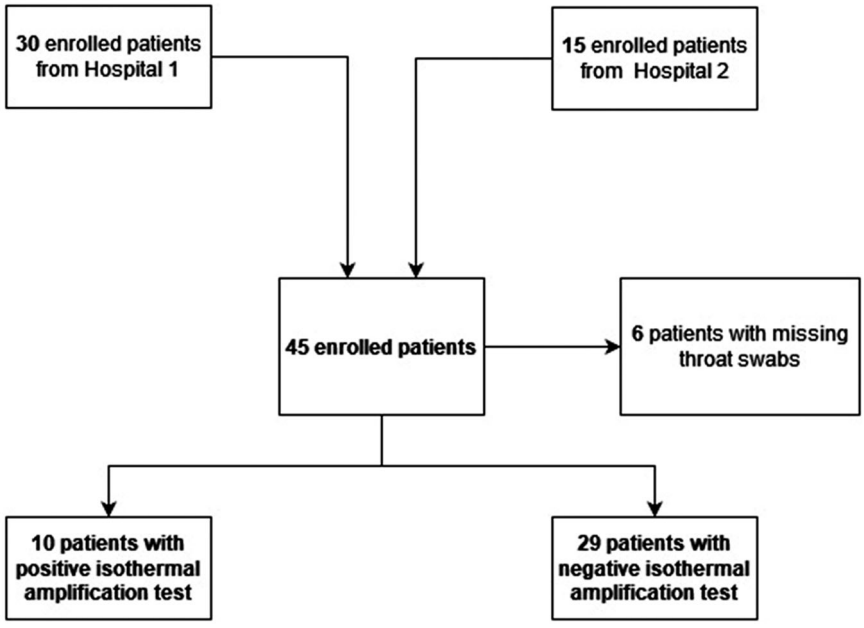

Figure 3. The flow chart of the study population. IAT: isothermal amplification test.

Table 2. Infection foci and disease severity of iGAS cases included in the study ($n = 45$).

Infection foci and disease severity	*n* (%)
Infection focus	
SSTI with or without wound	25 (55.6)
Respiratory tract	10 (22.2)
Arthritis, spondylitis, epidural abscess	8 (17.8)
NSTI	5 (11.1)
Septicemia only	3 (6.7)
Disease severity	
Need for vasoactive drug	12 (26.7)
NIV requirement	9 (20.0)
IMV requirement	6 (13.3.)
ICU admission	13 (28.9)
RRT requirement	1 (2.2.)
Outcome	
In-hospital mortality	7 (16)

Note: Data represent no. (%) of the group. Other includes one endocarditis, one peritonitis and one gynecological infection. SSTI: skin and soft tissue infections; NSTI: necrotizing skin and soft tissue infections; NIV: non-invasive ventilation; IMV: invasive mechanical ventilation; ICU: intensive care unit; RRT: renal replacement therapy.

studies using IAT or any other non-culture based method in pharyngeal swabs among patients with iGAS.

The high number of patients with a positive GAS IAT (10/39, 26%) in the throat in our study suggests a link between previous throat colonization and invasive disease, especially in male patients. None of these 10 patients had evidence of a wound infection, but two used intravenous drugs. At least in the other eight, the nasopharynx is a plausible candidate for the portal. They also seemed to have a more severe disease course, needing invasive mechanical ventilation (IMV) more often and having a higher in-hospital mortality, although this finding is significantly confounded by the fact that four of the six patients with no available throat swab also needed IMV and three of them died during hospitalization. If we were to assume them to be IAT negative, there would be no difference between the groups in this regard. It is unclear why males were more predominant in the IAT positive group, though this may be due to small sample size. It is also possible that the presence of *S. pyogenes* in the throat was a result of bacteremia and not causative to it. As all our samples were taken after the onset of bacteremia, there is no way to differentiate between correlation and causality. It may be possible that the presence of GAS in the throat simply indicates a more severe disease course and more widespread infection, which could alternatively explain the preponderance of the need for IMV and higher in-hospital mortality among IAT positives.

In earlier studies, many host factors, e.g. heart diseases, diabetes, malignancy, obesity, alcohol abuse and conditions affecting the skin have been associated with iGAS infections [6,24,25]. The same clinical characteristics were also seen in our study population. However, the proportion of patients without any underlying condition (40%) was higher than in previous reports (20–26%) [4,6,24,26]. The in-hospital mortality of 13% is accordant with previous estimates for iGAS infections in Europe, Australia and the US [4,6,8,9], but slightly higher than previously reported in Finland (8–10%) [7,26]. These discrepancies are likely due to small sample size.

The most prevalent *emm* types among the recruited patients reflected national *emm* type distribution and earlier international reports [27,28]. The iGAS epidemic caused by *emm*1 in Finland during 2017–2018 was also seen in our hospital districts as increased numbers of

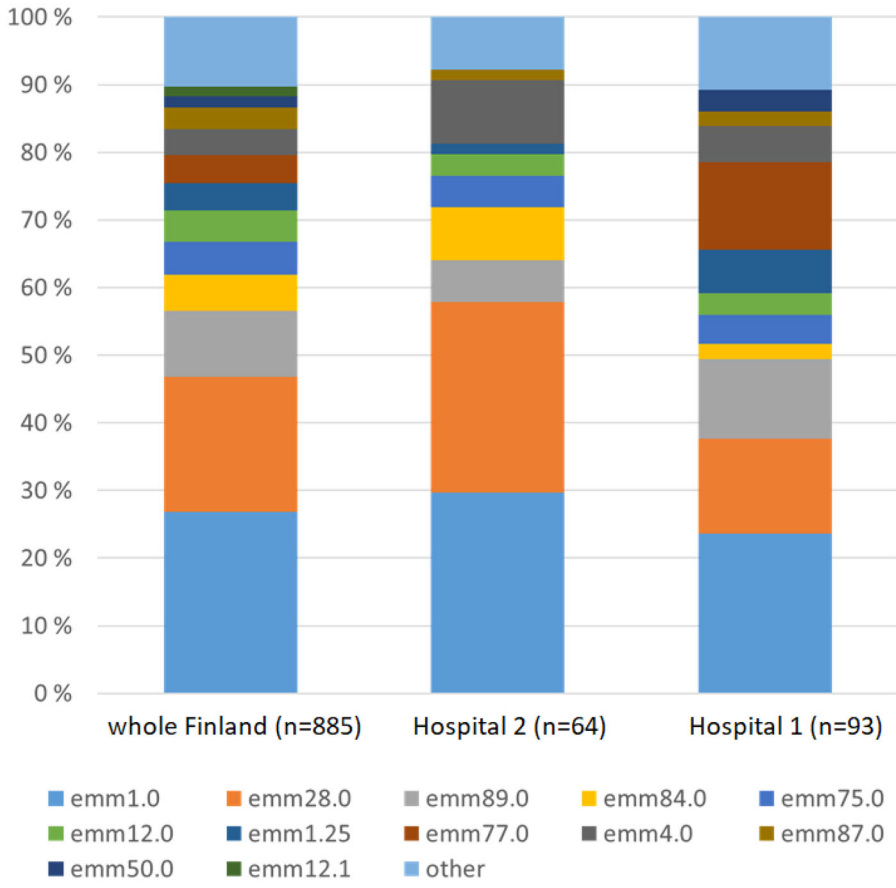

Figure 4. *emm* type distribution of iGAS cases in Finland, Hospital 2 and Hospital 1, 2018–2020.

iGAS cases [26]. The *emm*28 cases had more severe presentations with 3/6 (50%) being NSTIs. The same finding was reported in an earlier Finnish study in which a high proportion of severe iGAS cases were caused by *emm*28 [26]. No clear correlation with IAT findings with any *emm* type was observed. As the IAT assays used only tested for the presence of target nucleic acids, and all cultures were negative, no *emm* typing of the throat swabs was possible.

Isothermal amplification tests are molecular methods for rapid detection of GAS directly from a throat swab specimen. They are therefore not as vulnerable to prior antimicrobial therapy [29]. A recent meta-analysis of GAS specific IATs for pharyngitis reported the overall sensitivity as 97.5% and specificity as 95.1% [30]. However, these are based on comparing with throat cultures on antimicrobial naïve patients and therefore cannot be generalized for patients with prior antimicrobial exposure. To our knowledge IAT has not been previously tested in patients with recent or ongoing antimicrobial exposure. The bacterial load of GAS in the throat swabs was predicted to be very low, as all patients had ongoing antimicrobial treatment by the time of sampling. All the throat swabs remained culture negative for GAS. Two different IAT assays were selected to maximize the yield given these circumstances. Partly discordant results from ID NOW and Solana might be due to several factors. The same phenomenon has been reported previously [31,32]. The assays differ in their technology and target genes for nucleic acid amplification but also in the test sensitivity and specificity. Each throat swab was tested simultaneously with both assays. Cross contamination from other samples is very unlikely due to the slow patient recruitment pace but cannot be ruled out. Possible genetic differences in the GAS bacteria of the patients and the target sequence in the primers and probes of the two assays could also cause the discordance. To wit, given the fact that all swabs were culture negative, it may be assumed that the IAT positives represented dead bacteria, and as such the targets for the primers may have been partially fragmented and present only in small amounts, thus perhaps explicating this discrepancy. The difference of sampling volumes between the testing systems is in our opinion unlikely to be a factor as there was no clear favoring of either system among the positives.

Our study has some limitations. The sample size was small. The COVID-19 pandemic affected patient

Table 3. Comparison of the iGAS cases in the IAT positive and IAT negative groups.

	IAT positive ($n = 10$)	IAT negative ($n = 29$)	p Value
Age mean (SD)	53.40 (17.0)	53.90 (20.77)	.946
Sex male	9 (90.0)	13 (44.8)	.024
Charlson class			.639
0	2 (20.0)	5 (17.2)	
1	6 (60.0)	13 (44.8)	
2	0 (0)	5 (17.2)	
3	2 (20.0)	6 (20.7)	
Obesity			.693
BMI $\geq$ 30 kg/m2	2 (20.0)	10 (34.5)	
Current smoker	5 (50.0)	6 (20.7)	.109
IVDU (on-going)	2 (20.0)	4 (13.8)	.636
No underlying disease	5 (50.0)	11 (37.9)	.711
Infectious focus			.534
Wound infection	0 (0)	4 (13.8)	
SSTI without wound	6 (37.5)	10 (62.5)	
NSTI	1 (10.0)	3 (10.3)	
Laboratory values mean (95% CI)			
CRP on admission (mg/ml)	192.8 (127.2–258.4)	194.54 (149.79–239.30)	
Highest in-hospital CRP-value (mg/l)	331.4 (225.6–437.2)	304.38 (263.10–345.66)	
Leucocytes on admission (E9/l)	15.25 (11.7–18.8)	16.60 (13.49–19.71)	
Highest in-hospital leukocytes-value (E9/l)	22.7 (17.0–28.4)	20.35 (17.09–23.62)	
ICU admission	5 (50.0)	5 (17.2)	.087
NIV	3 (30.0)	4 (13.8)	.344
Vasoactive drug	4 (40.0)	6 (20.7)	.244
IMV	4 (40.0)	0 (0)	.003
In-hospital mortality	3 (30.0)	1 (3.4)	.045
emm-type			.154
*emm*1.0	1 (10.0)	7 (24.1)	
*emm*1.25	2 (20.0)	1 (3.4)	
*emm*1.3	0 (0)	1 (3.4)	
*emm*4	0 (0)	1 (3.4)	
*emm*22	2 (20.0)	0 (0)	
*emm*28	1 (10.0)	4 (13.8)	
*emm*89	1 (10.0)	8 (27.6)	

Note: Data represent no. (%) of the group. p value: comparison of the groups with Fisher's exact test except the means of age with T-test. IAT: isothermal amplification test; BMI: body mass index kg/m^2; IVDU: intravenous drug user; SSTI: skin and soft-tissue infection; NSTI: necrotizing soft-tissue infection; ICU: intensive care unit; NIV: non-invasive ventilation; IMV: invasive mechanical ventilation; CI: confidence interval.

recruitment leading to smaller enrollment than originally projected. However, almost half of all iGAS patients occurring during the study period were enrolled to our study. In addition, as all our patients had preceding antimicrobial exposure, it is possible that there were false negative IATs. The strength of our study is its prospective design. Severely ill patients are not routinely throat swabbed upon admission, so retrospective studies may fail to elucidate this issue adequately.

There is some heterogeneity in current guidelines on treatment of culture positive GAS pharyngitis. Finnish guidelines [33] recommend antimicrobial treatment whenever GAS is confirmed in pharyngitis, while in the UK antimicrobial treatment is generally discouraged and microbiological diagnostics are not considered necessary [34]. In our study, only three of the recruited patients had sought a medical consultation due to throat soreness, and thus at least 85% of these iGAS infections would have occurred regardless of GAS pharyngitis antimicrobial guidelines. In the future, if a vaccine effective in eradicating throat carriage becomes available, avoiding these potentially fatal invasive infections may become possible.

Conclusions

The presence of GAS in the throat was frequently observed among patients with iGAS infection. This may point to the nasopharynx serving as a portal of entry in these patients. The presence of GAS in the throat was more common among male patients.

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Ethical approval

The study protocol was approved by the Regional Ethics Committee of the Expert Responsibility Area of Tampere University Hospital and local research permissions were obtained from both research hospitals accordingly (permission numbers R18062, T05/026/18). The study was registered at ClinicalTrials.gov as ID NCT03507101. This study was conducted in accordance with the Declaration of Helsinki. Written consent was obtained from all study participants or from next of kin for sedated or intubated patients.

Author contributions

The material is original and has not been published elsewhere. Parts of this study were presented as a poster at the Lancefield International Symposium for Streptococci and Streptococcal Diseases in Stockholm, Sweden, June 2022. VK, JVi, KG, JO, JS and JVu were involved in the conception and design of the article. VK and JVi recruited and interviewed the patients. KG, KR-J, TS, KA and EL did further analysis and interpretation of the data. VK and JVi drafted the paper. All authors were involved in revising. All authors have approved the paper for publication and agree to be accountable for all aspects of the work.

Disclosure statement

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. VK reports a lecture fee from Thermofisher, as well as support from Pfizer and MSD to attend virtual conferences. JVu reports a lecture fee from the Finnish Medical Association and has acted as book editor for Finnish Medical Society Duodecim. JO has been a scientific advisor (review panel or advisory committee) to GlaxoSmithKline, MSD Finland and Pfizer and received lecture honoraria from Biocodex, Gilead, GlaxoSmithKline and MSD Finland, Orion and Roche. JS reports lecture fees from Roche and support for attending ESID meeting from Grifols and EACS meeting from Gilead Sciences Finland. KR-J reports a lecture fee from Hologic and unpaid scientific secretary post at NordicAST board. The remaining authors report no conflicts of interest.

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Data availability statement

The datasets generated during the current study are not publicly available as they contain health related data but limited datasets (without any identifiable, person-related data) are available from the corresponding author on reasonable request.

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