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# Serologic responses in patients with invasive group a streptococcal disease

Ville Kailankangas[1,2,*], Kirsi Gröndahl-Yli-Hannuksela[3], Johanna Vilhonen[4], Kaisu Rantakokko-Jalava[3,5], Tapio Seiskari[6], Emilia Lönnqvist[3], Jarmo Oksi[4,7], Jaana Syrjänen[1,2], Jaana Vuopio[3,8]

[1] Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland
[2] Department of Internal Medicine, Tampere University Hospital, Tampere, Finland
[3] Institute of Biomedicine, University of Turku, Turku, Finland
[4] Department of Infectious Diseases, Turku University Hospital, Turku, Finland
[5] Department of Clinical Microbiology, Laboratory Division, Turku University Hospital, Turku, Finland
[6] Department of Clinical Microbiology, Fimlab Laboratories, Tampere, Finland
[7] Faculty of Medicine, University of Turku, Turku, Finland
[8] Finnish Institute for Health and Welfare (THL), Helsinki, Finland

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ABSTRACT

*Objective:* To investigate kinetics of anti-streptolysin O (ASO) and anti-deoxyribonuclease B (ADB) and an in-house enzyme immunoassay (EIA) for IgG and IgA in acute and convalescent invasive group A Streptococcal (iGAS) infection.

*Materials and methods:* We recruited iGAS cases from two Finnish hospitals, between 2018 and 2020. Serum was obtained, on average 2 days, (timepoint A), 9 days (timepoint B) and 3 months (timepoint C) from admission. The sera were analyzed for ASO, ADB, and with EIA for IgA and IgG against the causative strain and the three most common *emm* types in Finland (*emm*1, *emm*28, *emm*89).

*Results:* Forty-five cases were recruited, with sera available from 42, 35 and 26 at timepoints A, B and C respectively. At timepoint A, 33% were above the upper limit of normal (ULN) for ASO, and 40% for ADB, but 52% for at least one. At timepoint B, 74% were above ULN for ASO, 79% for ADB, and 91% for at least one. EIA results did not differ between the causative and pooled strains except for IgA at timepoint A.

*Conclusions:* ASO and ADB have utility in iGAS disease with unavailable culture confirmation. EIA results suggest emm type cross-reactivity, with possible vaccine development implications.



## Background

*Streptococcus pyogenes*, or Group A *Streptococcus* (GAS), is an important human pathogen causing infectious diseases of varying degrees of severity, ranging from mild and self-limiting to life-threatening invasive GAS (iGAS) infections. Apart from infections, it commonly persists as an asymptomatic colonizer [1].

GAS has several secretory virulence factors. Among these is streptolysin O, a toxin mediating lysis and apoptosis of neutrophils and macrophages [2]. GAS strains also produce four deoxyribonucleases, that enhance immune evasion [3]. The most common of these deoxyribonucleases is the B type [4]. Commercial assays for antibodies against streptolysin O and deoxyribonuclease B have been in clinical use for decades [5].

Of these assays, the anti-streptolysin O (ASO) is the most widely used. Antibodies against streptolysin O are usually demonstrated at 1 week after infection, with peak titers at three to 6 weeks after infection [5]. The rate of decline is less clear, but some patients have been shown to have indefinitely elevated titers [5]. ASO titers typically elevate more in upper respiratory tract infections and less in skin infections, such as pyoderma and impetigo [5].

The anti-deoxyribonuclease B test (ADB) usually shows elevated titers during the second week after infection, with peak titers occurring at 6 to 8 weeks and usually remaining elevated longer than ASO. Skin infections cause a pronounced ADB reaction, as opposed to ASO [5].

* Corresponding author: Ville Kailankangas, Tampere University Hospital, Infectious Disease Unit, FM2, 6th floor, PO box 2000, Tampere 33521, Finland.
*E-mail address:* ville.kaila@fimnet.fi (V. Kailankangas).

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In this paper, we aim to deepen our understanding of antibody dynamics among patients with invasive GAS infections of varying severity in a prospective setting. We specifically wanted to explore the serological response and the kinetics of the response across different stages of the disease and different levels of disease severity,

## Materials and methods

### *Study design and patient enrollment*

The study design has been described previously [6]. Briefly, the study was conducted as a prospective observational study at two tertiary care Finnish hospitals: Tampere University Hospital in Pirkanmaa Health District and Turku University Hospital in the Hospital District of Southwest Finland between June 2018 and July 2020. A case was defined as a culture positive finding of *S. pyogenes* (henceforth 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. After recruitment, serum samples were taken at the next convenient time, usually the next morning (timepoint A). Second serum samples (timepoint B) were taken 5 to 7 days later. Three to 4 months after recruitment a third serum sample representing a recovered state (timepoint C) was obtained. The timepoints were selected to represent different phases of the disease and timed to also benefit another arm of this same study focusing on transcriptomics [7]. All collected samples were sent to the University of Turku for storage and further analysis. Background data was obtained from the interview and electronic patient records with the patients' consent as described previously [6]. The GAS isolates originally identified from the blood culture by a clinical microbiology laboratory were sent to the University of Turku for storage and *emm* typing according to the guidelines of the Centers for Disease Control and Prevention (CDC) [8].

Data analysis was performed using study subject codes without possibility to reveal personal identification.

### *Serological testing*

The serum samples were analysed at HUSlab (Helsinki University Hospital Diagnostic Center) to detect antibodies against streptolysin O (ASO) and deoxyribonuclease B (ADB) using the nephelometric method. The assays' detection thresholds were 60 U/ml and 100 U/mL, respectively. The ASO and ADB assays do not differentiate between antibody classes. Samples that had antibody titer below the threshold of detection were rounded down to half the detection limit (30 U/mL for ASO and 50 U/mL for ADB) for statistical purposes. The commonly used upper limits of normal (ULN) titers for ASO and ADB are $<$200 U/mL and $<$280 U/mL [9].

The serum samples were also tested with an in-house EIA-test for IgA and IgG antibodies, both against the causative GAS isolate, and against a pool of three GAS isolates, namely *emm* types 1.0, 28.0 and 89.0. The method has been described previously [10]. Briefly, whole bacterial cell lysates were used as coating antigens. Overnight cultures of GAS isolates were lysed and pooled for GAS strains: MGAS5005 (*emm*1, ATCC strain BAA-947), MGAS6180 (*emm*28, ATCC strain BAA-1064), and MGAS27520 (*emm*89, a Finnish clinical isolate kindly acquired from the Finnish Institute for Health and Welfare). These strains represent the most common *emm* types in bacteremic GAS infections in Finland at the time of the study [11]. For the causative GAS isolates, culturing and lysis was performed similarly to the pooled GAS isolates.

Microtiter plates (Nunc-Immuno Maxisorp, ThermoFisher) were coated with lysed bacteria at +4°C overnight. The next day, after blocking the wells with 1% BSA in PBS + 0,05% Tween (PBST), serum samples diluted to 1:100 in PBST were added and incubated at 37°C for 2 hours. Secondary IgG (1:30 000 Anti-Human IgG (Fc specific) −Alkaline Phosphatase, A9544 Sigma) and IgA antibodies (1:10 000 Anti-Human IgA (a-chain specific)-Alkaline Phosphatase from goat, A9669 Sigma) were incubated at 37°C for 1 hour. Phosphatase substrate (1 mg/ml pNPP, Sigma, S0942) in diethanolamine buffer (Reagena) was added and incubated at 37°C for 30 minutes. After stopping the reaction with 3 M NaOH, absorbance (optical density, OD) was measured at 405 nm.

### *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 are 1, 3-4 scores are 2, and $\geq$5 is 3 [12]. Obesity was defined as Body Mass Index (BMI) $\geq$ 30 kg/m$^2$. Severe disease was defined as requiring intensive care or leading to death. Fold-change was measured by dividing the titer or optical density of timepoint B with the titer or optical density of timepoint A.

### *Statistical analysis*

Statistical analyses were performed with IBM SPSS Statistics for Windows version 29.0 (IBM Corp., Armonk, NY). Categorical data were analyzed by Fischer's exact test. Unpaired, nonparametric Mann-Whitney's U test was used to calculate differences between antibody titers across categories. One-way ANOVA within-subjects was used to calculate the differences of antibody titers across timepoints. Pearson's bivariate correlation was used to check for correlations between antibodies of different classes and timepoints. A $p$-value of $<$0.05 was considered significant.

## Results

### *Patient enrollment*

Forty-five patients were enrolled altogether. Out of the three possible samplings (timepoints A, B or C), serum samples were obtained from 42 at timepoint A, 35 at timepoint B and 26 at timepoint C. Twenty-four cases had serum samples available at all three timepoints, while 34 cases had only timepoint A and B samples, and one case only timepoint A and C samples. Timepoint A was on average 4.5 days after the onset of fever, with a maximum delay of 11 days. Timepoint B was on average 7 days after timepoint A.

### *Clinical characteristics and disease severity*

Details of clinical characteristics and infection foci are described in our previous article [6]. Of the 45 patients, 27 (60%) were male and 18 (40%) female. The mean and median age was 55 years (range 21-96 years). Twenty percent (9/45) of patients were in CCI class 0, 42% (19/45) in class 1, 13% (6/45) in class 2 and 24% (11/45) in class 3. Soft-tissue infection (non-necrotizing or necrotizing) was the most common infection focus (30/45, 67%).

Thirteen of the 45 cases (29%) needed intensive care. Eight of the 45 patients (18%) died before timepoint C: four within a week from hospital admission, and an additional three later during hospitalization. One patient died over a month after the acute phase.

The most common causative *emm* types were *emm*89.0 (22,2%), *emm*1.0 (17,8%), *emm*1.25 (13,3%), and *emm*28.0 (13,3%). For a more detailed outline see [6].

### *ASO and ADB results*

The titers of both ASO and ADB had a marked, statistically significant elevation from timepoint A to B (median titers 119 U/mL

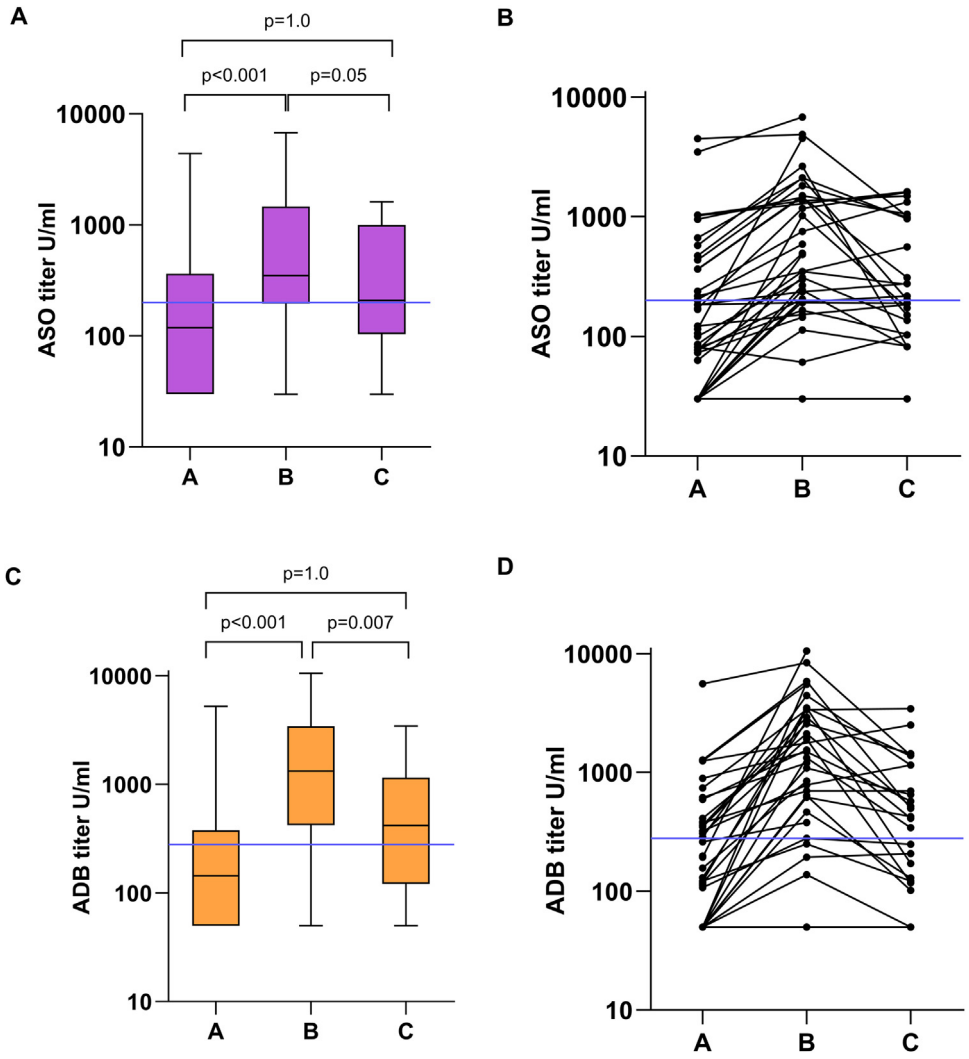


**Figure 1.** Anti-streptolysin O (ASO) and anti-deoxyribonuclease B (ADB) antibody titers at three timepoints measured (A, B and C). (A) Box plot of ASO titers at the three timepoints with outliers and the upper limit of normal marked. T-bars indicate 95% confidence interval, line within the boxes indicates median. (B) Line graphs of individual ASO titers at each timepoint. Titers below threshold of detection were rounded to 30 U/mL. (C) Box plot of ADB titers at the three timepoints with outliers and the upper limit of normal marked. T-bars indicate 95% confidence interval, line within the boxes indicates median. (D) Line graphs of individual ADB titers at each timepoint. Titers below threshold of detection are rounded to 50 U/mL. Blue horizontal line in figures A-D indicates upper limit of normal.

and 349 U/mL for ASO, respectively, and 144 U/mL and 1330 U/mL for ADB, respectively), but tended towards the timepoint A titers again at timepoint C (median 210 U/mL for ASO and 418 U/mL for ADB). However, high degree of variation was observed in both ASO and ADB concentrations at all timepoints for both antibodies (standard deviation 860 for ASO and 880 for ADB at timepoint A; 1528 for ASO and 2531 for ADB at timepoint B, and 540 for ASO and 818 for ADB at timepoint C) (Figure 1). There was a strong correlation between ASO and ADB titers at timepoint A, but not at the other two timepoints (Figure 2, Supplementary Table 1a). Two serum samples at timepoint B were insufficient for both assays so only ASO titers were measured.

There was no significant difference between titers of either ASO or ADB antibodies at any timepoint versus disease severity as defined by need for intensive care or death. However, the fold-change of ADB between timepoint A and B was significantly greater for cases with a severe disease course (mean 7.6 vs 22.8, $p = 0.009$) than the others. For ASO no such difference was seen. Men had statistically significantly more robust titers of ADB at timepoint B as opposed to women (mean 2861 U/mL vs 945 U/mL, $p = 0.018$). ADB titers at timepoint A and ASO titers at timepoints A and B had a moderate correlation with Charlson comorbidity score, but not with age at any timepoint (Supplementary Table 1b). The fold-change of ADB titers from timepoint A to B had a moderate positive correlation with higher age ($p = 0.034$). The delay from onset of fever to timepoint A did not correlate with the titers of either ASO or ADB, nor with their fold-change from timepoint A to B.

To correlate our findings with the commonplace clinical usage of ASO and ADB tests we used the commonly accepted ULN. At timepoint A, 33% of the cases were above the ULN for the ASO titer and 40% for the ADB titer, with three cases being above the ULN only for the ASO titer, and seven only for the ADB titer. Taken together, 52% (22/42) were above the ULN for at least one of the two. At timepoint B 74% were above the ULN for the ASO titer and 79% for the ADB titer, with four being only above the ULN for the ASO titer and five only for the ADB titer. Ninety-one percent (32/35) were above the ULN for at least one of the two. The ratio of cases above the ULN decreased at timepoint C, being 54% and 58% for the ASO and the ADB titer respectively. The differences in the percentage of cases above the ULN were statistically significant between all timepoints for the ASO titer ($p < 0.001$ between A and B, $p = 0.031$ between B and C and $p < 0.001$ between A and C). For

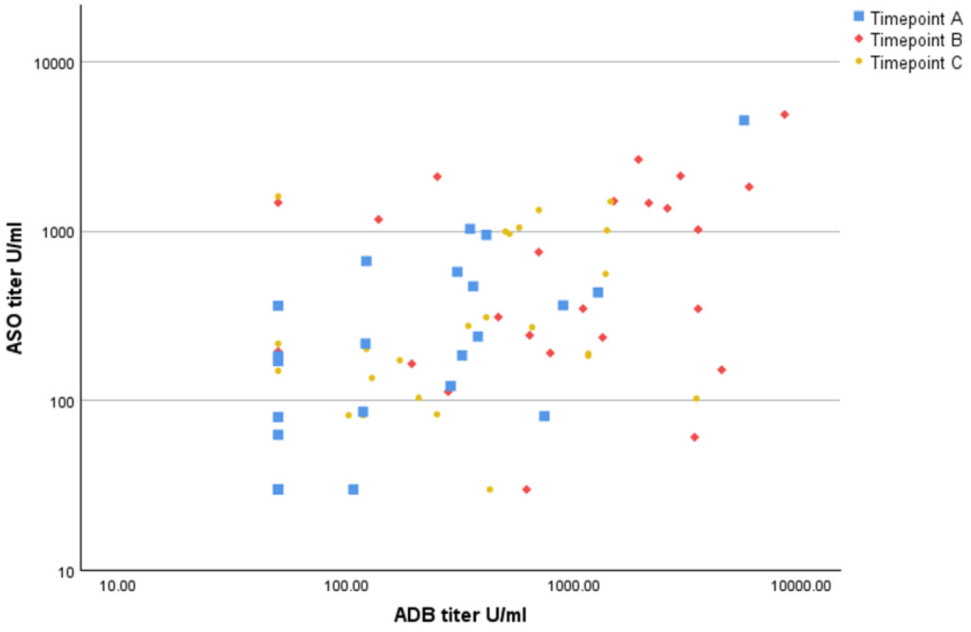


**Figure 2.** Correlation of anti-streptolysin O (ASO) versus anti-deoxyribonuclease B (ADB) titers at each timepoint. Titers below threshold of detection were rounded to 30 U/mL for ASO and 50 U/mL for ADB. Correlation is significant only at timepoint A (Pearson correlation 0.846, $p < 0.001$).

the ADB titer, the rate of being above the ULN differed significantly between timepoint A and B ($p < 0.001$) and A and C ($p < 0.001$), but not between B and C ($p = 0.073$).

*EIA results*

All the available serum samples were analyzed with in-house EIA analysis for antibodies against the causative GAS isolate of the case in question, and a combination of three common GAS *emm* types. There was a statistically significant difference between mean ODs against the causative strain and pooled strains only at timepoint A and only for IgA (mean 0.65 vs causative strain, 0.80 vs pooled strains, $p = 0.009$, Figure 3). Both IgG and IgA ODs correlated statistically significantly between the causative and pooled strains at all timepoints (all $p < 0.001$) (Figure 3). The correlation was not markedly different when only looking at the cases with a causative *emm* type not included in the pool.

There was also a statistically significant difference across both antibody classes in pairwise comparisons of ODs between timepoints A and B, with timepoint B ODs being higher (Figure 4). In pairwise comparisons between timepoints B and C, the difference was not significant for IgG antibodies against the causative strain but was significant for IgG against the pooled strains ($p = 0.024$) and IgA for both, with timepoint C being lower ($p = 0.001$ for both). The difference between the ODs at timepoints A and C was significant for IgG ($p = 0.016$ for the causative strain, $p = 0.003$ for the pooled strains), but not for IgA (Figure 4). Similarly to ASO and ADB, there was no significant difference in ODs between the cases with severe or nonsevere disease at any timepoint. However, the fold-change of IgG against the causative strain between timepoints A and B was significantly higher among the cases with severe disease (1.26 vs 1.67, $p = 0.014$). This was not seen in IgG against the pooled strains, nor in either IgA. As with ASO and ADB, the delay from onset of fever to sampling did not correlate with the mean ODs or the fold-change.

The EIA results showed a statistically significant correlation with the ASO and ADB antibodies at timepoint A for the IgG class antibodies, but mainly for the pooled strains for IgA (ASO and ADB at timepoint A, only ADB at timepoint B, but both pooled and causative strains for ASO at timepoint C) (Supplementary Table 2, Supplementary Figures 1-4). However, unlike ASO and ADB, there was no correlation with Charlson comorbidity score at any timepoint for either antibody, nor was there a correlation with age.

## Discussion

Antibodies against important GAS virulence factors streptolysin O and deoxyribonuclease B have been known and in clinical use for decades [5]. Immunity against GAS infections is understood to be complex and operating on different levels, with different antibodies conferring protection against colonization, infection, and systemic invasion [13]. ASO and ADB antibodies are also believed to demonstrate GAS exposure, and there is evidence of age-related increases in their titers [13]. The antibodies have neutralizing activity against their respective virulence factors, but the protective effect is limited [13].

Studies on seroreactivity in invasive GAS infections are less common than studies on the serology of pharyngitis and immunological sequelae of GAS, but an Australian study from 2024 explored serological cross-reactivity between invasive beta-hemolytic streptococcal infections [14], and an earlier Swedish study also dealt with the seroresponses of invasive GAS [15].

The current understanding of the kinetics of ADB and ASO antibodies is that their titers start increasing a week after the infection, and that ASO is more related to upper respiratory tract infections, whereas ADB to skin infections [5]. The gold standard for measuring ASO and ADB titers has been the neutralization assay [5], but to gain a better understanding of the clinical relevance of our findings, we chose to perform the analyses with the nephelometric method, as it is the method used in Finnish clinical practice.

In our study, over half the cases had a titer above the commonly used ULN for at least one of the two antibodies at timepoint A, despite it only being on average 4.5 days after the onset of fever. By timepoint B nearly all were above the ULN. The individual variance for both was great, however, and the medians for both were below the ULN at timepoint A. The titers were highest at timepoint B for both antibodies, and at timepoint C levels were generally not statistically significantly different from the

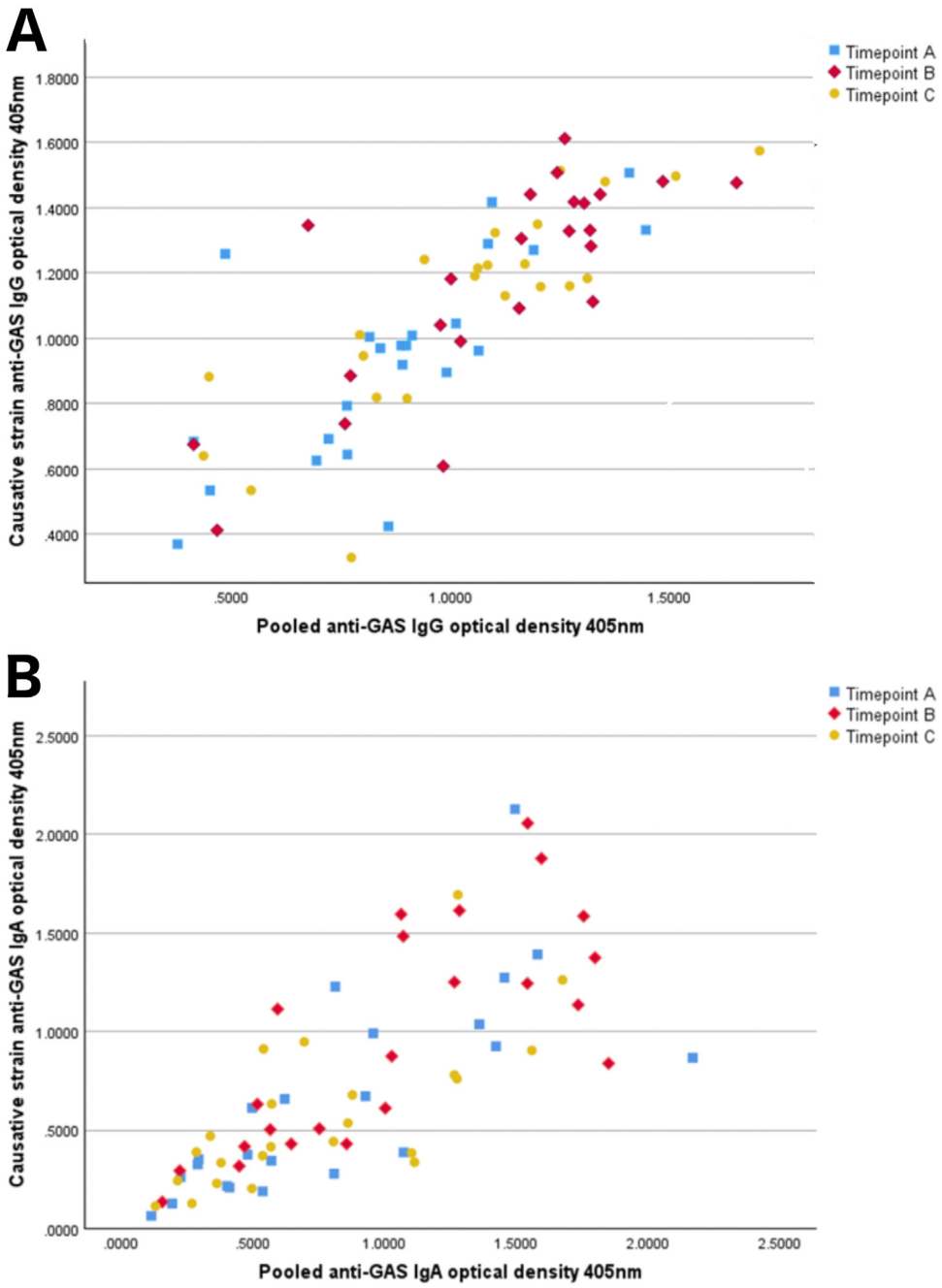


**Figure 3.** Correlations of the EIA results of anti-GAS IgG (A) and IgA (B) responses against the causative GAS strain and a pool of pool of *emm*1, *emm*28 and *emm*89 at each timepoint. Antibodies against the causative *emm* type are on the Y-axes, while antibodies against the pooled *emm* types are on the X-axes. All correlations are clearly significant ($p < 0.001$).

titers at timepoint A. However, there was a marked amount of variance in the antibody titers at timepoint C, which represents the recovered state. While soft-tissue infections were the most common focus, all patients had a severe invasive systemic disease, and as such clearly increased titers for both antibodies were equally common.

For ASO, the mean titers at timepoint C were still over two times above the ULN, and the median level was slightly above the ULN. With ADB, the mean and median titers were more clearly above the ULN at three to 4 months after the onset of the disease. The severity of the disease or age did not explain why some cases had strongly elevated titers remaining at timepoint C. This finding correlates with an earlier study in pediatric tonsillitis cases, where ASO and ADB titers remained elevated for up to over a year post infection [16].

Interestingly, age did not correlate with the mean titers or ODs, except for a moderate positive correlation in the fold-change of ADB titers from timepoint A to B. Charlson comorbidity score did, however, especially at timepoint A, with cases with more comorbidities having higher titers. This may represent such cases having a smoldering inflammatory state due to their comorbidities. It is unlikely that it would be explained by having had more prior encounters with GAS, as the correlation would then likely also be clearer with higher age. The higher fold-change seen with older cases can be seen to be demonstrative of a memory response though. In a recent Australian study age specific ULNs for ASO and

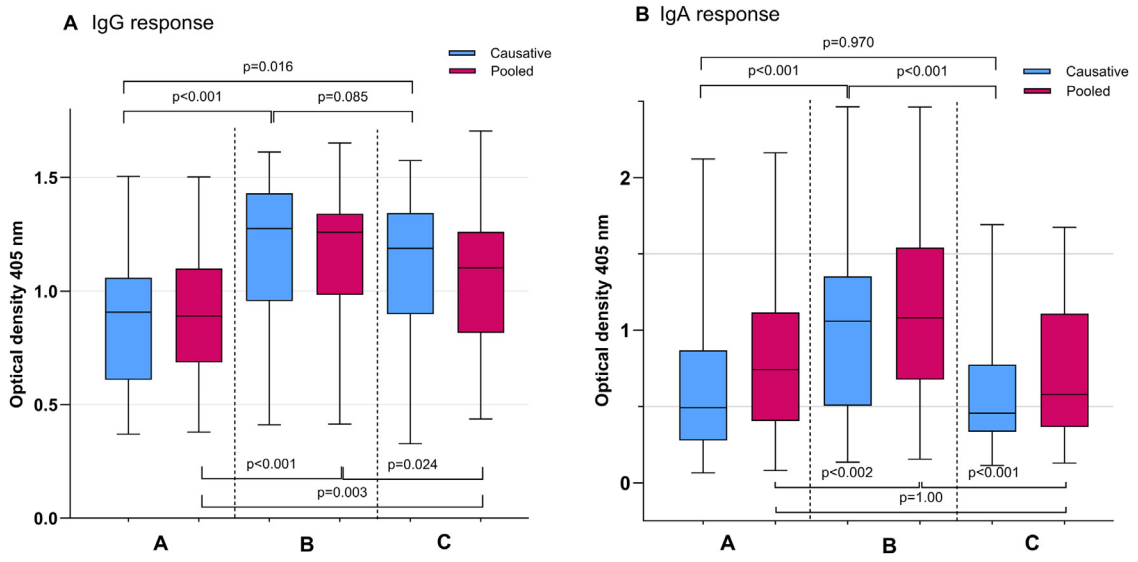


**Figure 4.** Box plots of the EIA results at each timepoint. (A) Box plot of optical density at 405 nm when measuring IgG against both the causative strain (blue) and the pooled strains (*emm*1, *emm*28, *emm*89, purple) at the three timepoints with outliers marked. T-bars indicate 95% confidence interval, line within the boxes indicates median. (B) Box plot of optical density at 405 m when measuring IgA against both the causative strain and the pooled strains (*emm*1, *emm*28, *emm*89) at the three timepoints with outliers marked. T-bars indicate 95% confidence interval, line within the boxes indicates median.

ADB titers were highest at age 10-14, at 249 U/mL for ASO antibodies and 422 U/mL for ADB antibodies [17], which demonstrates that baseline titers do not rise linearly with age.

In an experimental GAS pharyngitis model, higher baseline ADB levels seemed to be protective against more severe disease course [18]. Unfortunately, due to our recruitment protocol, we cannot know the baseline titers of our cases. In our study, the mean ADB levels at timepoint A did not differ between the severe and non-severe cases, but the severe cases had a significantly higher fold-change from timepoint A to B. This could be indicative of the non-severe cases having higher baseline titers but is speculative with no baseline samples.

The temporal trends in the EIA results were similar to the ASO and ADB antibody titers. The IgA against both the causative strain and the pooled strains did not correlate statistically significantly with the ASO and ADB titers at any timepoint, which is likely due to the latter two also consisting of IgG and IgM class antibodies.

The EIA results did not differ when testing against the causative strain and a pool of common *emm* types. This is in line with the earlier Swedish study suggesting antibody responses against *S. pyogenes* to be less type specific than previously thought [15]. There is also evidence that some cross immunity might extend beyond Lancefield groups in invasive beta-hemolytic streptococcal infections [15]. The finding, that the responses were similar against both the causative strain and pooled strains may also point to the response being mainly against conserved antigens, such as streptolysin O and DNase B, instead of type-specific ones. This preference for conserved antigens of GAS in the serological response and the apparent cross-reactivity may bring hope to the search for a GAS vaccine.

The main clinical utility of GAS serology has been to ascertain GAS as the pathogen especially when suspecting GAS related immune sequelae such as rheumatic fever or post-streptococcal glomerulonephritis. They are of limited use for uncomplicated infections but can sometimes be helpful in complicated cases. Pair serum showing a rise in titers is regarded as definitive proof of preceding streptococcal infection [5]. Our findings are aligned with this. According to our data, in invasive infections ASO and ADB titers do not distinguish clearly between soft tissue infections and other foci. Nor are they clearly dependent on severity. The titers are seen to increase quickly, suggesting a memory response, but the elevation is transient in most cases. GAS serology can thus still have clinical utility in helping confirm suspicion of GAS as the causative pathogen in a suspected invasive infection without culture confirmation and as such help with antibiotic stewardship. Using both tests together seemed to improve sensitivity in our cohort. However, no formal sensitivity and specificity analysis is possible, as our study protocol did not have a negative control group.

The main weakness of our study is the small sample size limiting the power of our findings. Another weakness is the lack of baseline samples, due to the study protocol triggering recruitment only when invasion of GAS had already happened. This also leads to a delay from the onset of symptoms to sampling, but the delay did not significantly affect the results in our study population. Furthermore, the usage of heated bacterial lysate as a target antigen in in-house EIA method might affect the epitope repertoire that it contains compared to usage of live bacteria. At the time, the multiplex serological assay described by Whitcombe and colleagues [19] was not available, and the lack of specificity in the EIA presented is a clear limitation. In the future, repeating a prospective study of iGAS cases and analyzing with said multiplex could be interesting and shed further light on the host immune response. Also, of interest would be observing the behavior of target vaccine antigens in iGAS.

## Conclusions

A significant serological response of ASO and ADB antibodies was evident in most cases within the first week of iGAS infection. In a systemic, invasive infection, ASO and ADB did not differ markedly in relation to infectious foci. IgG and IgA class antibodies against GAS behaved similarly, both against a lysate of the causative *emm* strain and a pool of three common *emm* strains, pointing towards cross-reactivity and a preference for conserved GAS antigens. IgG levels remained elevated after 3 months, while IgA levels reverted to baseline on average. GAS serology thus has continued clinical utility in select situations, and the apparent cross-reactivity of cell-surface antigens between *emm* types is likely useful in vaccine development.

## Ethics approval and consent to participate

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 on 24-Apr-2018. This study was conducted in accordance with the Declaration of Helsinki. Informed, written consent was obtained from all study participants, or from next of kin for sedated or intubated patients.

## Clinical trial

Not applicable; this was not a clinical trial.

## Consent for publication

Not applicable.

## Availability of data and material

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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## Declaration of competing interest

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

VK has received coverage for congress travel/accommodation expenses from Pfizer and coverage for congress travel/accommodation expenses from the Nordic Society of Clinical Microbiology and Infectious Diseases to present a poster at their Annual meeting, as well as coverage for congress travel/accommodation expenses from the Finnish Infectious Diseases Society and the Finnish Infectious Diseases Research Society to present posters at the Lancefield Symposium. JO has been a scientific advisor (advisory committee) to AstraZeneca, Biocodex, and Pfizer; received lecture honoraria from Biocodex, GlaxoSmithKline, and Tillotts; and received coverage for congress travel/accommodation expenses from Gilead and Unimedic Pharma. JVu has received coverage for congress travel/accommodation expenses from the Nordic Society of Clinical Microbiology and Infectious Diseases to give an honorary lecture at their Annual meeting (no lecture fee).

## CRediT authorship contribution statement

**Ville Kailankangas:** Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Kirsi Gröndahl-Yli-Hannuksela:** Conceptualization, Formal analysis, Investigation, Methodology, Resources, Writing – review & editing, Visualization. **Johanna Vilhonen:** Conceptualization, Investigation, Writing – review & editing, Methodology. **Kaisu Rantakokko-Jalava:** Resources. **Tapio Seiskari:** Resources. **Emilia Lönnqvist:** Resources. **Jarmo Oksi:** Conceptualization, Methodology, Writing – review & editing. **Jaana Syrjänen:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing. **Jaana Vuopio:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2026.108891.

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