

Potentially virulent strains and high colony counts of group A β -haemolytic streptococci in pharyngitis patients having a delayed recovery or a complication

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T serotyping and M and exotoxin genotyping were performed on strains from 367 streptococcal pharyngitis patients in Dutch general practice. Potentially virulent strains of group A β -haemolytic streptococci, such as *T1M1* and *T3M3* subtypes and strains expressing *speA* and *speC*, and a high number of colony counts did not occur more frequently in the 166 (45%) more seriously ill patients, defined as those who showed a delayed recovery or a complication.

Introduction

Pharyngitis caused by β -haemolytic group A streptococci (GAS) is commonly seen in general practice; it is a mostly self-limiting disease, but some patients experience delayed recovery or serious complications, such as quinsy. As in any infection, the microbe and the host both play a role in the pathogenesis. It is not clear, however, whether specific streptococcal factors can predict clinical deterioration, especially in ambulatory pharyngitis patients without underlying disease. More severe streptococcal diseases and streptococcal complications, such as invasive streptococcal disease and acute rheumatic fever, are associated with certain bacterial characteristics. For example, strains from very ill patients, e.g. those with toxic shock syndrome, are more likely to express *T1M1* and *T3M3* subtypes or to have an *speA* exotoxin gene.^{1–4} If potentially virulent strains and exotoxin A production do play a role in the pathogenesis of invasive streptococcal infections, it could be worth examining the role of these streptococcal markers in pharyngitis patients having a delayed recovery or complications. High GAS colony counts may also be of influence, since this characteristic has been shown to be significantly associated with active disease in our studies on acute sore throat.^{5,6} The association we found, however, is still under debate.⁷ For this spin-off study, all 367 GAS-positive patients who participated in our main studies^{5,6} were included; 166 of these patients (45%) still had complaints at day 5 or developed a complication. We investigated the frequency

of four potential markers in this group: (i) *T1M1* and *T3M3* subtypes; (ii) *speA* exotoxin gene; (iii) *speC* exotoxin gene; and (iv) a high GAS colony count.

Materials and methods

Patient selection

All 280 GAS-positive adult patients with an acute sore throat (≤ 7 days) who participated in our penicillin-intervention trial⁶ were included. Eighty-seven GAS-positive children, described in our first report,⁵ were also included. These patients were allocated randomly to one of three regimens: (i) penicillin V for 7 days; (ii) penicillin V for 3 days followed by placebo for 4 days; or (iii) placebo for 7 days.

Endpoints

Patients kept a diary to record the degree of pain and impairment of daily activities. They were considered to have a delayed recovery if they still perceived pain or a severe impairment of their daily activities at day 5. A complication was noted if the treatment code had to be broken because of a deteriorating condition, most often an (imminent) quinsy.⁶ As no serological investigation was carried out, the complications we found could only be suspected to be post-streptococcal.

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Bacteriology

Each throat sample was cultured semiquantitatively on 5% sheep blood agar and an ssA agar plate (Becton Dickinson, Leiden, The Netherlands). Both types of agar plate were incubated anaerobically at 35°C for ≤48 h. Pinpoint catalase-negative, Gram-positive cocci in chains were serogrouped. β -Haemolytic streptococci were classified according to whether there was no growth, sporadic growth (1–10 colonies) or 1+ ('low'), 2+ ('medium') or 3+ ('high') colony counts (growth into the initial, second, and third inoculation area, respectively). Serogroup A isolates were investigated at the National Institute of Public Health and the Environment, Bilthoven, The Netherlands, for T-serotyping and M and exotoxin gene A and C typing as described previously.⁵

Data analysis

Associations were determined using odds ratios with 95% confidence intervals, computed by logistic regression analysis. *P* values of ≤0.05 were considered significant. The following possible confounding baseline characteristics were examined in a bivariate model: age; sex; smoking habits; asthma/chronic obstructive pulmonary disease (COPD) or diabetes mellitus; a history of tonsillectomy; a documented history of pharyngitis in the previous 6 months; number of household members; system of healthcare insurance (as an indicator of socioeconomic status); urbanization; absence from school or work; prior duration of pain; and degree of pain and impairment of daily activities. To adjust for confounders, baseline characteristics with a *P* value of ≤0.20 in the bivariate analysis were entered into a multivariate model, together with treatment allocation and the four streptococcus-related factors. These four factors were (i) *TIMI* and *T3M3* versus all other subtypes; (ii) *speA* exotoxin gene present versus absent; (iii) *speC* exotoxin gene present versus absent; and (iv) colony counts of 3+ versus colony counts of less than 3+.

The multivariate logistic regression analyses tested the association between the streptococcus-related factors and the two endpoints [delayed recovery versus normal recovery (patients with a complication were excluded) and, in a separate analysis, complications versus normal recovery (patients with delayed recovery were excluded)]. A final multivariate logistic regression analysis tested the association between the streptococcus-related factors and a combination of the two endpoints.

Results

A total of 367 GAS-positive sore throat patients (53% of all 690 selected sore throat patients, mean age 25 years, s.d. 12 years) were included. Of these patients, 135 (37%) had a delayed recovery and 31 (8%) suffered from a complication (26 had imminent quinsy and one each had

quinsy, scarlet fever, impetigo, erysipelas and transient polyarthritides). Of the 367 patients, 117 were allocated to the 7 day penicillin treatment, 119 to the 3 day penicillin treatment and 131 to placebo. In the 7 day penicillin group, 32 (27%) patients had a delayed recovery and three (3%) a complication; the corresponding figures were 44 (37%) and 7 (6%), respectively, in the 3 day penicillin group. In the placebo group, 59 (45%) patients had a delayed recovery and 21 (16%) a complication.

Placebo treatment (*P* < 0.001), increasing age (*P* < 0.001) and a high initial grade of pain (*P* = 0.01) were associated with a delayed recovery, while placebo treatment (*P* < 0.001), increasing age (*P* = 0.05) and a low number of household members (*P* = 0.05) were associated with a complication.

High colony-count streptococci were isolated in 329 (90%) patients; *TIMI* was isolated in 15 (4%), *T3M3* in 24 (7%), *speA* exotoxin gene in 90 (25%) and *speC* exotoxin gene in 217 (59%) patients. The *speA* exotoxin gene was isolated in all but one of the *TIMI* and *T3M3* strains. Neither of these streptococcal characteristics was associated with a delayed recovery or a complication of GAS pharyngitis after adjustment for treatment allocation and the confounding baseline characteristics mentioned above (Table). The combination of both endpoints, i.e. delayed recovery and complications, resulted in similar findings.

Discussion

Streptococcus-related factors, such as *TIMI* and *T3M3* subtypes and exotoxin A and C genes, which play a role in invasive streptococcal disease, were detected in a group of 367 patients suffering from streptococcal pharyngitis. However, in this general-practice-based patient group, these factors did not occur more frequently in the more seriously ill pharyngitis patients (defined as those showing a delayed recovery or a complication).

Our results should be interpreted with caution, as the subgroups having *TIMI* or *T3M3* and colony counts of less than 3+ were small, thus resulting in wide confidence intervals. Furthermore, the bacteriological methods employed were not conventional at the time of the study. These methods have now been absorbed into a recent issue of the *Manual of Clinical Microbiology*, however.⁸ In our 'low-risk' population of pharyngitis patients, the streptococcal factors were not associated with virulence. In contrast, the same streptococcal factors did show such an association in a population having invasive streptococcal infections: in a nationwide surveillance in The Netherlands,⁹ the prevalence of *TIMI* and *T3M3* subtypes was 48% in patients with streptococcal toxic shock syndrome and 21% in patients without the syndrome, while the prevalence of *speA* exotoxin gene was 59% and 33% and that of the *speC* exotoxin gene 34 and 48%, respectively. During this surveillance, only 11% of the isolates cultured from patients who retrospectively did not match the defini-

Numbers of streptococci in pharyngitis patients

Table. Association between characteristics of *S. pyogenes* and a delayed recovery^a or complication^b in the course of pharyngitis [odds ratios (OR) with 95% confidence intervals (95% CI), 367 patients]

Streptococcal characteristics	Delayed recovery		Complication	
	OR ^c	95% CI	OR ^d	95% CI
<i>TIMI/T3M3</i>	1.72	0.65–4.53	3.67	0.54–25.09
<i>speA</i> exotoxin gene	0.52	0.26–1.03	0.32	0.07–1.43
<i>speC</i> exotoxin gene	1.19	0.70–2.00	1.82	0.69–4.78
Colony counts of 3+	0.59	0.27–1.28	2.63	0.31–22.64

^aDefined as a patient still complaining at day 5 of sore throat and/or impaired daily activities.

^bDefined as a broken treatment code due to a complication with a worsening condition.

^cAdjusted for treatment allocation, age and initial grade of pain (the 31 patients with complications were excluded).

^dAdjusted for treatment, age and number of household members (the 135 patients with delayed recovery were excluded).

tion of invasive streptococcal disease consisted of *TIMI* or *T3M3* subtypes.⁹ We found the same prevalence of these subtypes in our sore throat population in about the same period and in a well-defined area. Host-related factors play an important role in determining whether or not an infection with *Streptococcus pyogenes* will lead to invasive disease. This disease is often (in about half of cases) associated with an underlying disorder, such as diabetes mellitus, COPD or malignancy, and usually occurs in an older age group than streptococcal pharyngitis.^{3,4,9} However, because the proportion of *TIMI* and *T3M3* strains in invasive streptococcal disease is much higher than that in non-invasive disease, the virulence properties of these strains, promoting invasion and toxicity, also appear to play a role. In our sore throat patients, however, potentially virulent strains did not predict a deterioration in the course of streptococcal pharyngitis. High GAS colony counts did not predict such a deterioration either, although in a previous study we found them to be associated with active disease when compared with healthy controls.⁵ In conclusion, unless there is a local epidemic of invasive streptococcal disease¹⁰ or rheumatic fever, general practitioners do not need to look for potentially virulent strains in patients suffering from streptococcal pharyngitis.

References

- Schellekens, J. F., Schouls, L. M., van Pelt, W., Esveld, M. & van Leeuwen, W. J. (1998). Group A streptococci: a change in virulence? *Netherlands Journal of Medicine* **52**, 209–17.
- Stevens, D. L., Tanner, M. H., Winship, J., Swartz, R., Ries, K. M., Schlievert, P. M. *et al.* (1989). Severe group A streptococcal infections associated with a toxic shock-like syndrome and scarlet fever toxin A. *New England Journal of Medicine* **321**, 1–7.

- Johnson, D. R., Stevens, D. L. & Kaplan, E. L. (1992). Epidemiologic analysis of group A streptococcal serotypes associated with severe systemic infections, rheumatic fever, or uncomplicated pharyngitis. *Journal of Infectious Diseases* **166**, 374–82.

- Efstratiou, A. (2000). Group A streptococci in the 1990s. *Journal of Antimicrobial Chemotherapy* **45**, Topic T1, 3–12.

- Zwart, S., Ruijs, G. J., Sachs, A. P., van Leeuwen, W. J., Gubbels, J. W. & de Melker, R. A. (2000). Beta-haemolytic streptococci isolated from acute sore-throat patients: cause or coincidence? A case-control study in general practice. *Scandinavian Journal of Infectious Diseases* **32**, 377–84.

- Zwart, S., Sachs, A. P., Ruijs, G. J., Gubbels, J. W., Hoes, A. W. & de Melker, R. A. (2000). Penicillin for acute sore throat: randomized double blind trial of seven days versus three days treatment or placebo in adults. *British Medical Journal* **320**, 150–4.

- Bisno, A. L., Gerber, M. A., Gwaltney, J. M., Kaplan, E. L. & Schwartz, R. H. (1997). Diagnosis and management of group A streptococcal pharyngitis: a practice guideline. *Clinical Infectious Diseases* **25**, 574–83.

- Ruoff, K. L., Whiley, R. A. & Beighton, D. (1999). Streptococcus. In *Manual of Clinical Microbiology*, 7th edn (Murray, P. R., Baron, E. J., Tenover, F. C. & Tenover, R. H., Eds), pp. 283–96. ASM Press, Washington, DC.

- Schellekens, J. F., Schouls, L., van Silfhout, A., Elzenaar, K., Brunings, H., Ten Broek, H. *et al.* (1995). The resurgence of group A streptococcal disease; characteristics of invasive infections in the Netherlands, 1993–1995. *Nederlands Tijdschrift voor Medische Microbiologie* **4**, 78–83.

- Cockerill, F. R., MacDonald, K. L., Thompson, R. L., Roberson, F., Kohner, P. C., Besser-Wiek, J. *et al.* (1997). An outbreak of invasive group A streptococcal disease associated with high carriage rates of the invasive clone among school-aged children. *Journal of the American Medical Association* **277**, 38–43.

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