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Invasive *Streptococcus anginosus* group infection—does the species predict the outcome?

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SUMMARY

Objective: To determine whether there is an association between the species of *Streptococcus anginosus* group (SAG) bacteria and the clinical outcome.
Methods: Isolates from invasive infections caused by SAG bacteria at our institution between January 2004 and February 2009 were identified phenotypically to the taxonomic level of species. Clinical data from the medical records of the patients from whom these isolates were recovered were obtained retrospectively and analyzed.
Results: Patients with invasive *Streptococcus intermedius* infections had a significantly longer hospital stay than patients infected with *S. anginosus* (p = 0.024) and a significantly higher 30-day all-cause mortality than patients infected with *Streptococcus constellatus* (p = 0.049).
Conclusion: Identification of SAG bacteria to the taxonomic level of species may be of prognostic importance.

1. Introduction

The *Streptococcus anginosus* (SAG) group of bacteria (previously known as the *Streptococcus milleri* group) includes *Streptococcus anginosus*, *Streptococcus intermedius*, and *Streptococcus constellatus*. These organisms are commensal flora of the human oropharynx and gastrointestinal tract, but they also have the capacity to cause severe invasive infections with a propensity for dissemination and abscess formation.[1] Historically, *S. anginosus* has been found to be the species most frequently isolated from clinically significant specimens and *S. intermedius* the least common.[2–5]

Identification of SAG bacteria to the taxonomic level of species is not routinely performed in most microbiology laboratories. This is in part because currently available phenotypic identification methods are often unable to provide precise results, but also because SAG bacteria are predictably susceptible to penicillin[6–8] and therefore speciation is generally not considered necessary for patient management. We reviewed invasive infections due to SAG bacteria at our institution over a 5-year period to determine whether there was an association between the species identified and the presenting clinical syndrome, disease severity, and outcome.

2. Materials and methods

2.1. Clinical data collection

Invasive SAG infections that occurred over a 5-year period (January 2004 to February 2009) at the Sir Charles Gairdner Hospital (a 650-bed adult tertiary referral center in Perth, Western Australia) were identified retrospectively from a database of sterile site isolates maintained at PathWest Laboratory Medicine, Queen Elizabeth II Medical Centre, Perth, Western Australia. The medical records of patients from whom isolates were obtained were reviewed. An invasive SAG infection was defined as a case where SAG bacteria were cultured from a specimen(s) obtained from a normally sterile site; cultures of upper respiratory tract swabs, wound swabs, urine, and sputum were excluded.

The following demographic and clinical data were obtained from the medical records: age at diagnosis, sex, presenting symptoms, co-morbidities (Charlson index),[9] details of recent surgery, vital signs, white cell count, C-reactive protein, renal and liver function (using the most abnormal values obtained within the

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first 24 h of hospitalization), and length of hospital stay. The Simplified Acute Physiology II Score (SAPS II) was calculated for each patient as an estimate of illness severity at presentation.[10] Thirty-day mortality was determined from the hospital clinical information system that is linked to the Western Australian Registry of Deaths.

Invasive SAG infections were classified into the following specific clinical syndromes: bacteremia (primary or secondary), bone and/or soft tissue infection, central nervous system infection, intra-abdominal infection, respiratory infection, and infective endocarditis. Primary bacteremia was defined as a blood culture yielding SAG bacteria without an identifiable source. Secondary bacteremia was defined as a positive blood culture for SAG bacteria in the setting of an identifiable primary focus of infection.

2.2. Laboratory methods

Isolates that had previously been identified as SAG in the routine diagnostic microbiology laboratory were recovered from −80 °C storage in glycerol. Thawed suspensions were cultured on horse blood agar (HBA) and incubated at 37 °C in 6% CO_2 for 48 h and checked for purity. Colony features typical of SAG were observed, including the presence of a caramel odor, hemolysis, and cellular morphology on Gram stain, and representative colonies were subcultured onto HBA and re-incubated for 24 h in 6% CO_2. Subsequent testing to exclude non-SAG bacteria was performed using the catalase test, rapid biochemical tests, and Lancefield grouping using a commercial latex particle agglutination test.

Isolates provisionally identified as SAG bacteria were subsequently analyzed in three separate phenotypic identification systems routinely used in diagnostic microbiology laboratories: the API-20 and Rapid-ID 32 Strep test strips and the VITEK GPI card/VITEK 2 system (bioMérieux, France). If two or three of the identification systems identified the isolate as a particular species within the SAG at ≥50% probability, then the isolate was classified as that species.

2.3. Statistical methods

Medians were compared using the Student's *t*-test or the Wilcoxon non-parametric test when appropriate. Similarly, percentages were compared with the Pearson's Chi-square test or Fisher's exact test. Multiple group comparison was done with the Kruskal–Wallis test. A *p*-value of less than 0.05 was considered significant. All statistical analyses were performed using the PASW Statistics 18.0 software package (SPSS Inc., Chicago, IL, USA).

3. Results

Seventy-six isolates were stored in the sterile site culture collection as SAG bacteria over the period of the study. Of these, two were found not to be SAG bacteria and five isolates were deemed not to be invasive isolates. Ten isolates represented second or subsequent isolates from the same patient and three isolates contained two different species of SAG bacteria. Data presented hereafter are for the 56 discrete episodes of invasive infection due to a single species of SAG bacteria. Of these, 31 (55.4%) were identified as *S. anginosus*, 16 (28.6%) as *S. constellatus*, and nine (16.0%) as *S. intermedius*.

Twenty-three of the 56 specimens (41%) that contained a single species of SAG bacteria also cultured other bacteria: 13/31 (42%) in the *S. anginosus* group, 9/16 (56%) in the *S. constellatus* group, and 1/9 (11%) in the *S. intermedius* group. The majority of these isolates (15 out of 23) were cultured from intra-abdominal specimens and were commonly mixed with anaerobes and *Enterobacteriaceae* (data not shown). Two episodes of co-infection with *S. constellatus* and *Staphylococcus aureus* occurred in patients with bone and soft tissue infection.

When the demographic and clinical features of patients with invasive *S. anginosus*, *S. intermedius*, and *S. constellatus* infections were compared (Table 1), a male predominance was noted in all three groups and all were of similar mean age. As expected, intra-abdominal (48%) and respiratory infections, including lung abscesses and empyema (23%), were the most commonly identified clinical syndromes. Bacteremia was observed in 13 cases: nine due to *S. anginosus* and four due to *S. constellatus*, with 11 secondary bacteremia and two primary bacteremia. We found that bacteremia occurred most frequently in *S. anginosus* infections, occurring in 29% of cases. Similarly, *S. anginosus* was the only species isolated in the three cases of infective endocarditis. Central nervous system infection was uncommon in this study ($n = 2$), with *S. intermedius* isolated in both of these cases. The number of positive isolates from a respiratory source was proportionally highest in the *S. intermedius* group, comprising 56% of all *S. intermedius* isolates. Mean Charlson index scores and SAPS II scores were comparable throughout all groups. The median hospital length of stay was 18 days for *S. intermedius*, 10.5 days for *S. constellatus*, and 10 days for *S. anginosus*. The median hospital

Table 1
Demographic and clinical features of patients with invasive *Streptococcus anginosus* group infections

	S. anginosus ($n = 31$), *n* (%)	*S. constellatus* ($n = 16$), *n* (%)	*S. intermedius* ($n = 9$), *n* (%)	*p*-Value
Mean age, years	49.5	53.2	59.8	NS
Male sex	18 (58)	10 (62)	6 (67)	NS
CRP (mg/l), mean (range)	206 (1.5–490)	290 (100–610)	195 (11–340)	NS
Mean Charlson score	1.2	2.1	1.8	NS
SAPS II, mean (range)	23.2 (6–63)	20.2 (0–39)	24.7 (10–42)	NS
HDU/ICU admission	9 (29)	3 (19)	3 (33)	NS
Type of infection				
Bacteremia	9 (29)	4 (25)	0 (0)	NS
Intra-abdominal	17 (55)	9 (56)	1 (11)	NS
Bone/soft tissue	4 (13)	3 (19)	1 (11)	NS
Respiratory	5 (16)	3 (19)	5 (56)	NS
CNS	0 (0)	0 (0)	2 (22)	NS
Endocarditis	3 (10)	0 (0)	0 (0)	NS
LOS (days), median (range)	10.0 (1–40)	10.5 (4–199)	18 (8–37)	$p = 0.024$[a]
Dead at 30 days	3 (10)	0 (0)	2 (22)	$p = 0.049$[b]

CRP, C-reactive protein; SAPS II, Simplified Acute Physiology II Score; HDU, high dependency unit; ICU, intensive care unit; CNS, central nervous system; LOS, length of stay; NS, not significant.

[a] Statistical significance achieved when comparing *S. anginosus* and *S. intermedius*.

[b] Statistical significance achieved when comparing *S. constellatus* and *S. intermedius*.

length of stay of patients with *S. intermedius* infection was significantly longer than that of patients with *S. anginosus* infection ($p = 0.024$).

Fifty-one of 56 (91%) patients with invasive SAG infection were alive at 30 days following hospital admission. The five patients who died did so during their initial hospitalization. Three deaths occurred in the *S. anginosus* group and two in the *S. intermedius* group. None of the 16 patients infected with *S. constellatus* died. Thirty-day mortality in patients with *S. intermedius* infection was significantly higher than that of patients with *S. constellatus* infection ($p = 0.049$).

4. Discussion

Previous studies have suggested that different species within the SAG bacteria are associated with specific clinical syndromes, although the data are somewhat conflicting. A review of 153 clinical isolates of SAG bacteria found that *S. anginosus* was the predominant strain isolated in intra-abdominal infections, whereas *S. intermedius* was associated with central nervous system infections, particularly brain abscesses.[2] Two later studies confirmed the association of *S. anginosus* with the gastrointestinal tract, however also discovered that *S. intermedius* was isolated more frequently from purulent head and neck specimens.[3,4] It has also been suggested that there is an association between *S. constellatus* and thoracic infection.[11] These associations between species of SAG bacteria and specific clinical syndromes, however, were not demonstrated in a recent review of 245 clinical isolates of SAG bacteria. The only association the authors found was a higher relative representation of *S. constellatus* among blood culture isolates.[12] Our study did not demonstrate any statistically significant correlation between species and site of infection.

There is a paucity of data in the published literature evaluating clinical outcomes of patients with invasive SAG bacterial infections, in particular the morbidity and mortality attributable to individual subspecies. Pooled data of seven studies of 215 patients with SAG bacteremia found an all-cause mortality rate of 16%.[12] Three of these studies looked at the mortality associated with each of the three species of SAG bacteria.[12–14] Jacobs et al. found that of 19 cases of SAG bacteremia, 15 were caused by *S. anginosus*, of which five died.[13] Similarly, an Israeli study of 28 episodes of SAG bacteremia found that 19 were caused by *S. anginosus* and that the three deaths that occurred came from this cohort.[12] In contrast, a review of 30 cases of SAG bacteremia performed by Casariego and colleagues found that the three patients who died were infected with *S. constellatus*. Only two of the 30 patients were infected with *S. anginosus*, neither of whom died.[14]

To our knowledge, our study represents the largest evaluation of clinical outcomes of patients with invasive SAG bacterial infections reported in the literature. We were unable to demonstrate any statistically significant difference between the three species of SAG bacteria with regards to the severity of infection on presentation (as represented by SAPS II) or likelihood of admission to the intensive care unit/high dependency unit. In contrast, 30-day mortality and median length of hospital stay were significantly higher/longer in the *S. intermedius* group than in patients infected with *S. constellatus* and *S. anginosus*, respectively. This is in spite of *S. anginosus* and *S. constellatus* being much more common causes of bacteremia than *S. intermedius*.

This study has several important limitations. Firstly, it was a retrospective single-center study with a relatively small sample size. Secondly, a significant proportion of invasive SAG episodes in this study were polymicrobial, although this reflects what is commonly observed in clinical practice. Thirdly, we did not collect data on antimicrobial therapy or other interventions that may affect patient outcome (e.g., surgical drainage of abscesses), however there should be no reason why this should differ between SAG species.

The significantly longer length of stay and higher 30-day mortality of patients infected with *S. intermedius* in this study suggests that identification of SAG bacteria to the taxonomic level of species is of potential prognostic importance. Prospective studies are needed to ascertain whether infection with a specific species of SAG is a predictor of infection severity and clinical outcome.

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