Group A Streptococcus Meningitis in Children

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Objective: To characterize the epidemiologic burden and the molecular determinants of group A streptococcal (GAS) meningitis among the pediatric population of the state of Paraná, Brazil.

Methods: Clinical and epidemiologic data were gathered by a compulsory notification system during the period 2003 to 2011. Bacterial identification, antibiotic resistance profile, emm-typing, pulsed-field gel electrophoresis typing and virulence profile were analyzed by a central reference laboratory. A review of published pediatric cases of GAS meningitis from the last 45 years was undertaken and compared with the Brazilian series.

Results: The incidence of GAS meningitis among the pediatric population was 0.06 cases per 100,000 children per year and was associated with a case fatality rate of 43%. Neonatal age and the presence of an associated toxic shock syndrome were identified as risk factors for death. A distant focus of infection was present in more than half of the patients in the literature and in 36% in the Brazilian case series. A high diversity of emm-types was associated with GAS meningitis in Brazil. No single virulence determinant could be associated with death.

Conclusions: GAS meningitis is associated with high mortality and with a high diversity of GAS emm-types and virulence determinants in Brazil.

Key Words: Streptococcus pyogenes, meningitis, incidence, virulence determinants, child


Meningitis caused by Streptococcus pyogenes (group A Streptococcus, GAS) is uncommon, representing fewer than 1–2% of invasive GAS disease cases in Europe and the United States, but numerous case reports of GAS meningitis have been published, predominately in children. These case reports suggest that GAS meningitis is associated with a high case fatality (17%) especially when complicated by streptococcal toxic shock syndrome. A high rate of severe neurologic sequelae has also been reported (32%).

The pathophysiology of GAS meningitis is largely unknown. Although most patients, both adults and children, present with an extrameningeal primary focus of infection, around one-third of patients do not. Several bacterial virulence determinants associated with invasive GAS infections have been identified, although none has been specifically linked to GAS meningitis. The GAS surface M protein plays a pivotal role in its virulence potential, mainly due to its antiphagocytic properties in the nonimmune host. The M protein is also the substrate of the standard molecular typing method called emm-typing. More than 200 emm-types have been described so far. Some emm-types, especially emm-type 1 and 3, have been associated with invasive infections in high-income countries. These 2 emm-types are, however, also frequent among pharyngitis cases in the same countries. However, as these emm-types belong to the most common circulating emm-types in these countries, it is not clear whether this association is due to their increased virulence potential or rather simply reflects their high frequency in the population. The virulence of GAS has also been suggested to be related to adhesion of the bacteria to the host cell wall and to the secretion of specific toxins. Superantigen toxins are potent immunostimulators of T cells and are central to the development of streptococcal toxic shock syndrome.

The purpose of this study was to report the clinical, epidemiologic and molecular characterization of cases of GAS meningitis in children during the last decade in the state of Paraná, southern Brazil. To our knowledge, this is the first population-based assessment of GAS meningitis in children. We have also undertaken a comprehensive review of all published reports of GAS meningitis, and we compare features of these reported cases to our population-based case series in Brazil.

Study Design

Notification of cases of meningitis is mandatory in the Brazilian state of Paraná, with all cases reported through an online centralized system. Once notified, clinical samples are sent to the Laboratories Division of Epidemiology and Disease Control of the Central Laboratory of the State of Paraná for microbiologic confirmation. We analyzed all cases of GAS meningitis in pediatric patients aged from birth to less than 15 years notified over a 9-year period (January 1, 2003 to December 31, 2011). A case of meningitis was defined as a symptomatic patient with GAS cultured from cerebrospinal fluid (CSF). Toxic shock syndrome was defined based on the previously published consensus definition. The study was approved by the ethics committee of Department of Health of Paraná—SESA/HT,CAAE-0157.0.429.000-11.

Study Location

Brazil has a population of over 192 million people and is classified as an upper middle-income country by the World Bank. The state of Paraná had 10,444,526 inhabitants in 2010, is located...
in the south of the country and is ranked as the 5th richest of the 26 Brazilian states based on gross domestic product per capita ($21,600 in 2010). However, wealth reparation in the state is unequal as illustrated by the 20.87% of (very) poor people. Population data for Paraná were obtained by accessing the website from the Brazilian Ministry of Health.

**Bacterial Identification and Antimicrobial Susceptibility Profile**

β-Hemolytic streptococci were phenotypically identified on blood agar and susceptibility to 0.04 U bacitracin disk. GAS were identified using a latex agglutination test containing group A specific antisera (Slidex; Biomérieux, Paris, France). GAS isolates were characterized by emm-typing and pulsed-field gel electrophoresis typing as previously described. The antimicrobial susceptibility profile of 7 antibiotics (penicillin, erythromycin, clindamycin, tetracycline, chloramphenicol, vancomycin and tigecycline) was performed to determine the minimum inhibitory concentration (MIC) by agar dilution technique as previously described. The breakpoints used for each antibiotic were those recommended by Clinical and Laboratory Standards Institute. S. pneumoniae ATCC 49619 was used as a quality control. The presence of 20 virulence genes, including 8 superantigens (speA, speC, speF, speG, speH, speJ, smz and ssa) and 12 adhesins (cna, cpa-1, fha, fbp-54, pbp, prf-1, prf-2, prf-15, sciA, sciB, sfb and sfb-2) was tested by polymerase chain reaction using primers and protocols developed by Vlaminkx et al. The presence of the cysteine protease speB was used as an internal positive control.

**Review of the Literature**

Reports of pediatric cases of GAS meningitis between 1966 and 2011 were identified by searching the Medline database and using the following keywords: “Streptococcus pyogenes,” “Group A Streptococcus,” “GAS,” “β-hemolytic Streptococcus” and “Meningitis.” Publications in English, French, Portuguese and Spanish were considered. The incomes of each country were classified according to the World Bank classification.

**Statistical Analysis**

Dichotomous data were compared using χ² analysis where appropriate, and continuous data were analyzed using the Wilcoxon rank-sum (Mann–Whitney) test where appropriate.

**RESULTS**

**Clinical and Epidemiologic Characteristics of Cases in Paraná, Brazil**

Fourteen pediatric cases of GAS meningitis were reported during the 9 years of study in 10 different cities in the state of Paraná, Brazil. Half of them were reported in large (>200,000 inhabitants) and more affluent cities whereas the 7 others were reported in smaller cities. The 6 fatal cases occurred in equal proportion in smaller and larger cities. The clinical features and laboratory findings of these children at presentation are summarized in Table 1. Higher leukocyte count and lower glucose value were observed in CSFs from patient with fatal outcomes (P = 0.014 and 0.01, respectively). The overall incidence of GAS meningitis in children aged less than 15 years was 0.06 cases per 100,000 children per year (95% confidence interval [CI]: 0.03–0.09). The yearly incidence varied from 0 to 0.15 cases per 100,000 per year.

Further clinical and demographic features of the 14 cases are presented in Table 2. One-third of the patients were aged less than 1 year with an incidence in this age group of 0.34 per 100,000 per year (95% CI: 0.10–0.59). Apart from these 14 pediatric cases, 3 adult cases were also notified during the study period (data not shown), which represents an all ages incidence of GAS meningitis of 0.02 cases per 100,000 per year (95% CI: 0.01–0.03).

Six patients (43%) died in our series. We unfortunately do not have access to the average time from becoming ill to presenting for evaluation, but 3 patients died within the first 24 hours of their admission to hospital and 3 others died within 24–48 hours after admission. Mortality was associated with age less than 1 month (relative risk [RR] 3.7, 95% CI: 1.4–9.6) and with the presence of streptococcal toxic shock syndrome (RR 9.0, 95% CI: 1.4–57.1). All of the children in our series received a combination of ceftriaxone and penicillin. Clindamycin was added for 1 patient, and no patients received intravenous immunoglobulin. A family contact with GAS was noted for 2 cases (one contact had GAS pharyngitis and the other had proven GAS pneumonia). A distant focus of infection, within the week before admission, was noted in only 5 patients (3 with acute otitis media, 1 with pneumonia and 1 with varicella), and a predisposing medical condition was observed for 1 patient (rhabdomyosarcoma and HIV positive).

**Comparison of Cases in Paraná, Brazil With Those in the Published Literature**

Our search of the literature identified 57 pediatric cases of GAS meningitis published between 1966 and 2011. Most of these cases were described in high incomes countries (48/57, 84%). The remaining 9 cases occurred in low to upper middle-income countries (Ethiopia, The Gambia, India, Nigeria and Brazil). Information about these 57 cases are compared with our series in Table 2 (complete details in Table, Supplemental Digital Content 1, http://links.lww.com/INF/B324).

There were a number of differences noted between our patients and those in the available literature. The mortality rate was 43% in our series, which is more than double that of 17% in the literature (RR 2.5, 95% CI: 1.1–5.9). However, if a severe outcome was considered as either death or neurologic sequelae then the outcome was similarly poor in both our series and the literature (43% versus 51%, respectively). There was a lower proportion of cases with a distant focus of infection in the Brazilian series (36%) compared with 62% in the published literature (RR 0.6, 95% CI: 0.3–1.2). The median age of children with GAS meningitis in our series was older (2.4 years) than that in the published literature (0.9 years), but this difference was not statistically significant (P = 0.66).

To assess the difference in clinical characteristics among poorer countries (low to upper middle-income countries) and high-income countries, we pooled the data from our study with those from the published literature (n = 71). We found that the mortality rate of GAS meningitis in poorer countries was higher (43%) than in high-income countries (13%; RR 3.3, 95% CI: 1.3–8.0).

**Bacterial Determinants**

All 14 isolates were susceptible to the 6 antibiotics tested with low MICs (MIC₅₀/MIC₉₀: 0.015/0.015 µg/mL for penicillin, 0.03/0.03 µg/mL for erythromycin and clindamycin, 2/2 µg/mL for chloramphenicol, 0.5/0.5 for vancomycin and 0.12/0.12 for tigecycline). Only 2 isolates (emm-types 83) were resistant to tetracycline (MIC of 32 µg/mL). Few data are available about the emm-types associated with pediatric case of GAS meningitis. Among the 14 cases published with accompanying emm-typing data, emm-type 1 and 3 accounted together for 43% of cases of meningitis (Table, Supplemental Digital Content 1, http://links.lww.com/INF/B324). By contrast, in our series, a total of 9 emm-types and pulsed-field gel electrophoresis pattern were detected among the 14 isolates (Table, Supplemental Digital Content 2, 2013 Lippincott Williams & Wilkins www.pidj.com | 111
TABLE 1. Clinical and Laboratory Data at Presentation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number (%) (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptoms</td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>14 (100)</td>
</tr>
<tr>
<td>Headache</td>
<td>7 (50)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>10 (71)</td>
</tr>
<tr>
<td>Bulging fontanelle</td>
<td>4 (29)</td>
</tr>
<tr>
<td>Neck stiffness</td>
<td>11 (79)</td>
</tr>
<tr>
<td>Coma</td>
<td>1 (7)</td>
</tr>
<tr>
<td>Petechiae</td>
<td>3 (21)</td>
</tr>
<tr>
<td>Median cerebrospinal fluid values</td>
<td></td>
</tr>
<tr>
<td>Leukocyte count (cells/mm³)</td>
<td>1350 (564–3584)</td>
</tr>
<tr>
<td>Neutrophils proportion (%)</td>
<td>93.5 (85–95)</td>
</tr>
<tr>
<td>Protein (mg/dL)</td>
<td>240 (190–320)</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>5.5 (2–18)</td>
</tr>
</tbody>
</table>

TABLE 2. Demographic and Clinical Characteristics of 71 Pediatric Patients With Meningitis Due to Group A Streptococci

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patient in our Series (n = 14)</th>
<th>Patient in the Literature (n = 57)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, no. of male/no. of female</td>
<td>6/14 (43)</td>
<td>24/48 (50)</td>
</tr>
<tr>
<td>Age, median years (IQR)</td>
<td>2.4 (0.25–5)</td>
<td>0.9 (0.1–6.5)</td>
</tr>
<tr>
<td>Cases &lt;1 month old</td>
<td>3/14 (21)</td>
<td>12/57 (21)</td>
</tr>
<tr>
<td>Cases &lt;1 year old</td>
<td>5/14 (36)</td>
<td>30/57 (53)</td>
</tr>
<tr>
<td>Underlying medical conditions*</td>
<td>1/14 (7)</td>
<td>11/48 (23)</td>
</tr>
<tr>
<td>Distant focus of infection at the onset</td>
<td>5/14 (36)</td>
<td>30/57 (62)</td>
</tr>
<tr>
<td>Acute otitis media</td>
<td>3/14 (21)</td>
<td>11/48 (23)</td>
</tr>
<tr>
<td>Sore throat</td>
<td>0/14 (0)</td>
<td>9/48 (18)</td>
</tr>
<tr>
<td>Varicella</td>
<td>1/14 (7)</td>
<td>4/48 (8)</td>
</tr>
<tr>
<td>Other†</td>
<td>1/14 (7)</td>
<td>14/48 (29)</td>
</tr>
<tr>
<td>Positive blood culture</td>
<td>NA</td>
<td>23/57 (40)</td>
</tr>
<tr>
<td>Complications</td>
<td>6/14 (43)</td>
<td>38/52 (73)</td>
</tr>
<tr>
<td>Seizure</td>
<td>4/14 (29)</td>
<td>23/52 (44)</td>
</tr>
<tr>
<td>Brain abscess</td>
<td>0/14 (0)</td>
<td>5/52 (10)</td>
</tr>
<tr>
<td>Other‡</td>
<td>4/14 (29)</td>
<td>20/52 (38)</td>
</tr>
<tr>
<td>Died</td>
<td>6/14 (43)</td>
<td>9/53 (17)</td>
</tr>
<tr>
<td>Neurologic sequelae</td>
<td>0/14 (0)</td>
<td>18/53 (34)</td>
</tr>
</tbody>
</table>

DISCUSSION

This study provides a reliable estimate of the incidence of GAS meningitis among Brazilian children in Paraná (0.06 per 100,000 children per year) because of the compulsory notification system in the state. However, the incidence in Paraná most likely represents an underestimation of the Brazilian national incidence because Paraná is one of the wealthier states in the country. Three other limitations are likely to underestimate this incidence as well: early treatment with antimicrobial therapy before CSF sampling, bacterial culture issues around appropriate microbiologic characterization and the passive setting of the Brazilian notification system. It is our impression that a significant proportion of cases of meningitis in rural areas in Brazil might be treated without CSF sampling (R. Torres, personal communication, August 5, 2012). All these limitations indicate that the incidence of 0.06 cases per 100,000 children per year in Paraná is a conservative estimation of the Brazilian incidence.

The reason for the high mortality rate (43%) observed in our study is not clear. It is known from previous studies that the case fatality rate in invasive GAS disease is highest in patients with streptococcal toxic shock syndrome and in the very young patients. The patients in our series all received treatment in medical centers with adequate access to intensive care therapy, however, none of the children received intravenous immunoglobulin, including those with toxic shock syndrome. One may argue that the lack of immunoglobulin treatment favored a higher mortality rate, but it is unlikely that this, on its own, could explain the high mortality rate. Our hypothesis is that a late diagnosis due to a late arrival to the hospital might be one of the explanations of this higher mortality. The higher leukocyte count and lower glucose values observed in CSFs from fatal cases are compatible with this hypothesis although it might also simply reflect their increased virulence potential.

http://links.lww.com/INF/B325. The most frequent was emm-type 12, which was identified in 3 isolates. The emm-types 1, 6 and 83 accounted for 2 isolates whereas single isolates of emm-types 66, 75, 89, 92 and 112 were recovered. No emm-type 3 isolates were detected. A strong correlation between the emm-type and the presence of a determined pattern of virulence genes was observed. However, 2 examples of isolates belonging to the same emm-type but displaying slightly different virulence determinant profiles were noted. An emm-type 1 isolate from 2009 did not carry the speC gene whereas the emm-type 1 isolate from 2003 did (Table, Supplemental Digital Content 2, http://links.lww.com/INF/B325). Similarly, emm-type 12 isolates from 2011 were characterized by the presence of 2 supplementary virulence determinants (scpJ and sfb) in comparison with the emm-type 12 isolates from 2003 and 2004. The presence of the cpa adhesin gene could not be identified in any isolate. However, all isolates carried the superantigen speG and the sciB adhesin. No virulence determinant could be associated with specific clinical output. The number of superantigens or adhesins carried by isolates associated with a case fatality was not statistically significantly different from the number carried by isolates associated with the survival of the patient.
and in South America, indicating that the predominance of emm-type 1 and 3 might not be always the rule in all epidemiologic settings.

Vlaminkx et al observed a correlation between the presence of certain genes of adhesion and the development of specific invasive infections, particularly the association between the presence of the prfA-1 gene and clinical meningitis. In our study, this association was only observed among isolates belonging to emm-type 6. Notably, this particular emm-type has been previously associated with meningitis. In agreement with other studies, the emm1 strain was characterized by a classical virulence factor pattern including the presence of fibronectin binding genes cpa-1, fba, fbp-54, sciA and sciB together with superantigens speA, speF, speG, speJ and smeZ. Most of these genes are located on bacteriophages and could, therefore, be easily transferred from one strain to the other. The presence of a particular toxin pattern appears, however, to be closely associated with emm-type suggesting that some restrictions in horizontal gene transfer are probable. Moreover, because of the multifactorial nature of GAS pathogenesis, strict correlation between virulence genes carried by a particular strain and its clinical virulence is difficult to establish.

REFERENCES


