RANDOMIZED, CONTROLLED TRIAL EFFICACY OF PNEUMOCOCCAL CONJUGATE VACCINE AGAINST OTITIS MEDIA AMONG NAVAJO AND WHITE MOUNTAIN APACHE INFANTS

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Abstract: We report the phase III trial efficacy of 7-valent pneumococcal conjugate vaccine against clinical and culture proven otitis media (OM) among Navajo and White Mountain Apache infants. Efficacy was −0.4% (95% CI: −19.4 to 15.6) for clinically-diagnosed OM, 5.1% (95% CI: −51.5 to 40.6) for severe OM, and 64% (95% CI: −34% to 90%) for vaccine serotype pneumococcal OM suggesting that this vaccine is efficacious for pneumococcal OM in this high risk population.

Key Words: *Streptococcus pneumonia*, pneumococcal conjugate vaccine, American Indian, otitis media, randomized clinical trial, pneumococcus

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Acute otitis media (AOM) is the most common manifestation of pneumococcal disease; certain American Indian populations are known to have high rates of acute and chronic suppurative otitis media (OM).[1] Four randomized controlled trials among infants have assessed the efficacy of 7- or 11-valent pneumococcal conjugate vaccines (PCV) against OM outcomes.[2–6] Although study definitions and methodologies varied in these studies, efficacy against clinically-diagnosed OM ranged from −1% to 33% and against vaccine serotype OM from 56% to 66%.

We present here the efficacy analysis of culture proven and clinically-diagnosed OM among participants in a large, phase III, efficacy trial of PCV among American Indian infants.

METHODS

A double-blind, community-randomized phase III clinical efficacy trial of a 7-valent PCV (serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F, PnCRM7, Wyeth Vaccines, Pearl River, NY) compared with control vaccine (MnCC, meningococcal group C conjugate vaccine, Wyeth Vaccines, Pearl River, NY) was conducted among Navajo and White Mountain Apache children from April 1997 through August 2000. This trial methodology has been previously described,[7] and results published for immunogenicity,[8] invasive pneumococcal disease efficacy,[9] and nasopharyngeal colonization efficacy.[10]

At trial completion, a retrospective chart review was conducted to determine the efficacy of PnCRM7 against various clinically-defined pneumococcal diseases, which were secondary outcome measures of the trial. Those children who were <7 months of age at receipt of first dose of study vaccine, had received 3 doses by 12 months of age, and had a minimum of 30 days between each dose were considered eligible for this chart review and OM efficacy analyses. We randomly selected 944 of the 4476 children chart review eligible infants. The chart review sample size was determined by logistic feasibility and expected frequency of healthcare events. With 1000 charts, 1.5 person-years of follow-up per chart (−10% for vaccination delays), and a baseline incidence of 1 OM episode per person-year, there was over 80% power to detect a reduction of 15% in OM incidence, with 5% type I error.

We evaluated every medical visit made by study children through 2 years of age. OM visits, as documented by the patients' treating physician were recorded. We counted a visit as a new OM episode if any of the following were recorded as the diagnosis and it was more than 21 days since the preceding OM visit: OM, AOM, bilateral OM, chronic OM, OM with perforation, otorrhea, pressure-equalizing tube placement, perforated tympanic membrane, serous OM, and bullous myringitis. We further subcategorized an episode as AOM if the clinical diagnosis was either "acute otitis media" or "bilateral otitis media." We also subcategorized an OM episode as severe if there were 3 or more OM visits for the episode. A child's first medical visit for OM was considered their first episode. OM visits occurring less than 21 days after the immediately prior otitis-related visit and visits noted as a follow-up to a previous otitis-related visit were counted as follow-up visits, not as OM episodes.

The primary endpoint for this substudy was the efficacy of PnCRM7 in preventing clinically-diagnosed episodes of OM among American Indian children <2 years of age who were scheduled to receive 3 doses of vaccine at 2, 4, and 6 months of age plus a booster dose at 12–15 months of age.

Analyses were done using 2 subgroups of the 944 children whose charts were reviewed: primary efficacy group and per-protocol group (Fig. 1, available online). The primary efficacy subgroup included 856 children who were found to have strictly met the chart review criteria. This group included 6 children who mistakenly received mixed regimens of PnCRM7 and MnCC vaccine; 850 received the vaccine to which they were randomized. The per-protocol subgroup included 803 children who received the first vaccine dose on or after 42 days of age, received the third dose by 365 days of age, received the booster dose between 365 and 480 days of age, and the booster dose was separated from the primary series by at least 60 days. Time at risk for the per-protocol subgroup began 14 days after the third vaccine dose. Time-at-risk for the primary efficacy group began immediately after the first vaccine dose.

In children with spontaneously draining ears, the treating clinicians were encouraged to send a sample of the ear fluid for bacterial culture. Those specimens which grew *Streptococcus pneumoniae* were sent for serotyping using the Quellung method (Arctic Investigations Program, Centers for Disease Control and Prevention, Anchorage, AK).

The incidence rate ratio (IRR) was calculated as the ratio of the rate of OM episodes in the PnCRM7 vaccine arm compared with that in the control (MnCC) vaccine arm. Efficacy was calculated as [1-IRR]. Poisson regression analyses used sandwich variance estimation to account for within-community correlation. All analyses were performed using STATA Version 7.0 statistical software.

RESULTS

Four hundred twenty-four children were randomized to PnCRM7 and 432 to MnCC vaccine. The groups did not differ by demographic variables (gender, birth weight, number of children <6

TABLE 1. Efficacy of PnCRM7 Against Clinical Episodes of Otitis Media in American Indian Children

	PnCRM7 Vaccine (Study Group)			MnCC Control Vaccine (Control Group)			Efficacy	
	No. Events	Person-Years	Rate	No. Events	Person-Years	Rate	Point Estimate	95% CI
Per-protocol								
All otitis media	785	523.9	1.5	816	546.7	1.5	−0.4	−19.4 to 15.6
Acute OM	706	523.9	1.3	736	546.7	1.3	−0.1	−20.8 to 17.1
Severe episodes								
No. medical visits	70	523.9	0.1	77	546.7	0.1	5.1	−51.5 to 40.6
PE tube insertion	11	523.9	0.02	16	546.7	0.03	28.3	−225.3 to 84.2
Primary efficacy								
All otitis media	1189	765.8	1.6	1171	779.3	1.5	−3.3	−21.4 to 12.0
Acute OM	1092	765.8	1.4	1059	779.3	1.4	−4.9	−25.4 to 12.2
Severe episodes								
No. medical visits	106	765.8	0.1	125	779.3	0.2	13.7	−34.1 to 44.5
PE tube insertion	13	765.8	0.02	17	779.3	0.02	22.2	−255.7 to 83.0

years of age living in the household with the study subject, history of breast-feeding, tribal affiliation, and receipt of the influenza vaccine) (data not shown).

The 856 subjects eligible for the primary efficacy group analysis contributed 1544.5 person-years of observation and 3490 OM medical visits during the time at risk resulting in 4.08 OM medical visits/child, and 2.3 OM medical visits/person-year. There were 2360 OM episodes observed for a rate of 1.5 OM episodes/person-year of observation. Restricting the analysis to only AOM, there were 2151 AOM illness episodes for an incidence of 1.4 AOM episodes/person-year.

Efficacy Analyses. Among children vaccinated per-protocol, there were 785 OM episodes among those receiving PnCRM7 and 816 episodes in those receiving MnCC, resulting in an efficacy against OM episodes of −0.4% (95% CI: −19.4 to 15.6) (Table 1). Restricting the analysis to AOM episodes, there were 706 events in the PnCRM7 arm and 736 events in the MnCC arm, for an efficacy against AOM of −0.1% (95% CI: −20.8 to 17.1). Efficacy against severe OM episode was 5.1% (95% CI: −51.5 to 40.6) and against pressure equalizing (PE) tube insertion was 28.3% (95% CI: −225.3 to 84.2). Efficacy estimates for each of these categories was somewhat lower for the primary efficacy group (Table 1).

Serotype Analysis. There were 51 spontaneously draining OM episodes which grew *S. pneumoniae* among the 8292 children enrolled in the efficacy trial. Of these pneumococcal OM episodes, 23 isolates were collected for serotyping yielding 11 vaccine serotypes among children who enrolled in the trial at <7 months of age. Three of these cases occurred among infants who had received PnCRM7 [all 19F], and 8 among those receiving MnCC [19F(3), 18C(2), 4(2), 14(1)], for an efficacy of 64% (95% CI: −34% to 90%) for preventing vaccine serotype pneumococcal OM. Our data and that of others[2,4] suggest a differential efficacy for serotype 19F. Therefore, the analysis was repeated excluding serotype 19F isolates resulting in an efficacy of 100% (95% CI: 14%–100%).

DISCUSSION

This study confirms the high burden of AOM in Navajo and White Mountain Apache children. Our study showed an incidence of 1.4 episodes of AOM per person-year of follow-up among children <2 years of age. By comparison, the FinOM Trial control subjects had an incidence of 1.24 episodes of AOM per person-year when followed actively for all otitis episodes.[3] The Kaiser trial had a total of 73,041 OM visits in a study population of 37,868.[8] This yields a rate of 1.8 visits per child, which is less than half the visit rate in our study population of 4.08 OM visits/child.

We did not demonstrate PnCRM7 efficacy in preventing clinically-diagnosed episodes of OM (all types of OM diagnoses) or AOM episodes. Although the point estimates were not statistically significant, we showed a 5.1% efficacy against severe OM episodes when defined as 3 or more visits for a given episode. Furthermore, vaccine efficacy against episodes with PE tube insertion was 28.3% although not statistically significant. In the subpopulation of children in whom ear isolates were collected and serotyped, there was a 64% efficacy of the PnCRM7 vaccine in preventing vaccine-type episodes of AOM. Our data suggest a lower efficacy of the 19F serotype; excluding this serotype there was 100% efficacy against vaccine serotype spontaneously draining OM events. Although these various OM efficacy results were not statistically significant, they are very similar to the estimates in other trials.

Surveillance data just before the initiation of the vaccine trial revealed that 50% of invasive pneumococcal disease isolates in Navajo children were a vaccine serotype.[11] By contrast, 85% of invasive isolates in the Northern California Kaiser Permanente population were caused by vaccine serotypes.[1] If this serotype distribution difference also existed among OM cases, the lower vaccine serotype coverage in our population could partially explain the lower efficacy of PnCRM7 in preventing clinically-defined episodes of OM among Navajo and White Mountain Apache children.

In summary, this analysis has demonstrated that PnCRM7 is likely to be efficacious against vaccine serotype pneumococcal OM, severe OM, and PE tubes among Navajo and White Mountain Apache infants who are at high risk for OM, although the lower bound of the confidence intervals were less than zero. There was no demonstrated efficacy of PnCRM7 vaccine against physician diagnosed clinical episodes of OM, likely because the diagnosis is nonspecific for pneumococcal OM. PCV are an important contribution to the reduction in pneumococcal disease syndromes among American Indian children. PCV with broader serotype coverage would likely provide significant additional benefit for further disease reduction.

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REAL-TIME POLYMERASE CHAIN REACTION DETECTION OF *BORDETELLA PERTUSSIS* DNA IN ACELLULAR PERTUSSIS VACCINES

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Abstract: Using 2 real-time polymerase chain reaction (PCR) assays for *Bordetella pertussis*, 2 of 5 acellular pertussis vaccines were found to contain *B. pertussis* DNA. Because residual DNA in vaccines can cause environmental contamination, the administration of acellular pertussis vaccines to patients should be physically separated from the collection of patients' specimens for testing of *B. pertussis* DNA by real-time PCR.

Key Words: *B. pertussis*, vaccines, real-time PCR, environmental contamination, Tdap

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Bordetella pertussis, the etiologic agent of whooping cough, continues to be an important cause of morbidity in the United States with 25,616 cases of pertussis and 39 deaths reported in 2005 despite high vaccination coverage rates.[1] By 1944, pediatric diphtheria and tetanus toxoids and whole cell pertussis vaccines (DTP) were routinely endorsed for children by the American Academy of Pediatrics. Because of side effects of the DTP vaccines, less reactogenic pediatric acellular pertussis vaccines (DTaP) were developed and replaced all pediatric DTP vaccines in 1997.[1,2] Two tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis vaccines (Tdap) were licensed in the United States for adolescents and adults in 2005; BOOSTRIX (GlaxoSmithKline, Research Triangle Park, NC) for persons aged 10–18 years and ADACEL (Sanofi Pasteur, Swiftwater, PA) for persons aged 11–64 years.[1,2] In the United States, acellular pertussis vaccines have entirely replaced whole cell vaccines.

Specific diagnosis of pertussis based on clinical symptoms and laboratory detection of the etiologic agent can be challenging. Among the diagnostic methods to detect *B. pertussis*, culture is the most specific but sensitivity is typically low, depending upon the stage of disease, time, and technique of specimen collection, transport, and culture conditions.[3] Culture is sensitive for young children with classic pertussis symptoms, but its sensitivity is low for adolescents and adults with atypical symptoms. In addition, culture does not provide rapid diagnosis. Real-time PCR assays can be fast (2–24 hours), specific (86–100%), and sensitive (70–99%) and have been widely used to detect *B. pertussis*.[3] However, the highly sensitive PCR is also prone to false positives because the smallest amount of contaminating DNA can be amplified. For example, positive results with a single-target PCR assay such as the insertion sequence IS*481*, have led to a false diagnosis of pertussis as the etiology of some respiratory outbreaks.[4–8]

In real-time PCR, the cycle threshold (Ct) value is the cycle number where the fluorescence crosses the threshold line. Typically a slower encounter with the threshold (or a higher Ct value) means that a smaller amount of DNA template is present in the specimen. Previously, whole cell vaccines were found to contain pertussis DNA and were the source of contamination in sensitive assays such as PCR.[7] Our objective was to determine if the acellular vaccines for pertussis currently administered in the United States contain *B. pertussis* DNA rendering these vaccines as a potential source of environmental contamination.

METHODS

Vaccines. Seven vaccines including a nonpertussis (control) vaccine from each company were received directly from the 2 manufacturers and analyzed in this study for the presence of *B. pertussis* residual DNA. These vaccines were the 2 licensed adolescent/adult Tdap vaccines (BOOSTRIX [GlaxoSmithKline] and ADACEL [Sanofi Pasteur]), 3 licensed childhood DTaP vaccines (INFANRIX [GlaxoSmithKline], DAPTACEL [Sanofi Pasteur] and Tripedia [Sanofi Pasteur]), and 2 nonpertussis "control" vaccines (adult Td DECAVAC [Sanofi Pasteur] and hepatitis A Havrix [GlaxoSmithKline]).

Culture. One hundred microliter aliquots from 2 vials of each vaccine were plated in duplicate at 37°C, under high humidity on Regan-Lowe medium containing charcoal agar (Oxoid, Basingtoke, UK) and 10% defibrinated horse blood for 10 days.

DNA Methods. DNA extractions were performed on MagNA Pure LC equipment using MagNA Pure LC DNA Isolation Kit III (Roche Applied Science, Indianapolis, IN). A volume of 100 μL from each vaccine vial underwent DNA extraction in duplicate. Negative water controls were placed between each vaccine sample.

Real-Time PCR Assays. Two real-time PCR assays (IS*481* and *ptxS*1) were performed using the AB7000 (Applied Biosystems,

Inc., Foster City, CA) in a total volume of 25 μL in a 96-well plate. The amplification mixture contained 4 μL of extracted DNA using the TaqMan Universal PCR Master Mix (Applied BioSystems) with 300 nM of each primer and probe for IS*481* target and 700 nM of each primer and 300 nM probe for *ptxS*1 target. The PCR protocol consisted of a hold for 2 minutes at 50°C; enzyme activation for 10 minutes at 95°C; and 45 cycles of amplification for 15 seconds at 95°C and 1 minute at 60°C for the IS*481* assay (1 minute at 57°C for the *ptxS*1 assay), with the intensity of fluorescence measured at the end of each cycle. Positive controls were tested in duplicate in each run and nontemplate control samples (PCR grade water) were placed between specimens. Each DNA extract was tested in duplicate with both PCR assays.

RESULTS

Cultures from all vaccine vials were negative after 10 days of incubation. Thus, no viable *B. pertussis* was found in any of the vaccines.

Two pertussis-containing vaccines were strongly positive, based on the low Ct values with both the IS*481* (Ct = 16.48 and 16.93) and the *ptxS*1 (Ct = 25.57 and 25.95, respectively) targets. The low Ct values with both targets indicate numerous copies of DNA present in the specimen and confirm the presence of *B. pertussis* DNA in the vials. A different lot number of 1 positive vaccine had been tested previously and had also been found to be strongly positive for *B. pertussis* DNA using the real-time PCR assays with 2 targets. All the other vaccines, including the control vaccines and water samples extracted, were negative for both PCR targets. Additional vials of the control vaccines without pertussis antigens and pertussis vaccines that were negative in the first 2 vials were extracted in duplicate, tested and found to be negative.

DISCUSSION

Real-time PCR assays are extremely sensitive methods for the detection of *Bordetella* DNA. The 2 specific real-time assays used in this study can detect as few as 5 genomic equivalents of DNA at the lower limit of detection. The *B. pertussis* genome has 238 copies of the insertion sequence IS*481,*[9] and thus, real-time PCR results with high Ct values (Ct >35) and extremely low levels of DNA must be interpreted cautiously. Samples for which PCR testing is positive at both targets IS*481* and *ptxS*1 have *B. pertussis* DNA. The low Ct values for both targets in the PCR assays obtained from vaccine vials in this study indicate that multiple copies of *B. pertussis* DNA were found in these vials. In addition, residual *B. pertussis* DNA was detected in 2 different lots of one of the PCR positive vaccines.

In a previous study, environmental contamination from whole cell pertussis vaccines was found to be the cause of positive PCR results from patient specimens.[7] Environmental investigation was performed in rooms at 2 different clinics where the vaccine was administered to the patients. *B. pertussis* PCR-positive material was detected on laboratory benches, steel tables, staff's clothes, and skin of the hands of the staff in the vaccination room at both clinics. In the clinic where the vaccine administration and specimen collection for *B. pertussis* were separated by a great distance, patient specimens were culture negative and PCR-negative. However, in the clinic where the vaccinations were performed in closer proximity to the examination room, 91% of the patient specimens were PCR-positive and 66% of the specimens were culture-negative. In this situation, the whole cell pertussis vaccine was the cause of the contamination. The presence of DNA in whole cell vaccines is predictable. Depending on the method of antigen preparation in the acellular pertussis vaccines, the presence of residual DNA might also be predictable.

In any clinical laboratory, unexpected sources of contamination either from the environment or from the laboratory can generate false-positive results from patient specimens. Because there is no interlaboratory standardization of PCR for pertussis, contamination in the laboratory or at the time of specimen collection can cause pseudo-outbreaks.[5,8,10] The increase in the number of high cycle positive-PCR results using the target IS*481* has confounded the diagnosis of pertussis. Many of the standardization issues for pertussis PCR diagnostics will be assessed in a clinical validation study that is in progress at the Centers for Disease Control and Prevention.

In the current study the strong positive real-time PCR results from 2 of the vaccines suggests that the administration of pertussis vaccines to individuals should be in a separate room far removed from collection of specimens to be used for the diagnosis of *B. pertussis* by PCR. The results of this study do not provide any evidence that safety, efficacy, immunogenicity of the vaccines should be affected by the presence of DNA nor should the presence of the low levels of DNA cause any adverse effects from these vaccines. However, if precautions are not taken, the presence of *B. pertussis* DNA in acelluar pertussis vaccines could be a potential source of environmental contamination and thus cause contamination of patient specimens.

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FOSCARNET SALVAGE THERAPY FOR ACYCLOVIR-RESISTANT VARICELLA ZOSTER
REPORT OF A NOVEL THYMIDINE KINASE MUTATION AND REVIEW OF THE LITERATURE

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Abstract: The authors describe an acyclovir-resistant varicella zoster virus infection in a pediatric patient after hematopoietic stem cell transplant, the use of foscarnet as salvage therapy, and review the literature to clarify the pediatric experience with foscarnet in this setting. A novel thymidine kinase mutation is described, along with a new phenotypic assay for characterizing acyclovir resistance in varicella zoster virus.

Key Words: acyclovir resistance, VZV, foscarnet

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Varicella zoster virus (VZV) causes significant morbidity and mortality in immunosuppressed children. After pediatric hematopoietic stem cell transplant (HSCT), VZV disease is common and often atypical.[1–3] Acyclovir (ACV)-resistant VZV is increasingly reported in immunocompromised populations, posing therapeutic dilemmas. Foscarnet is a therapeutic option in many of these cases—although few publications include pediatric patients. We report a pediatric HSCT patient with severe ACV-resistant zoster caused by an ACV-resistant VZV with a novel thymidine kinase (TK) mutation and the successful use of foscarnet salvage therapy.

CASE PRESENTATION

A 10-year-old girl with Fanconi anemia, presented 1 year after a second, matched unrelated donor HSCT with painful vesicular skin lesions on her left lower back, clustered in a dermatomal band. She was afebrile and exhibited no signs of systemic infection. There was a history of VZV infection in early childhood supported by a VZV seropositive status pretransplant. She had received several months of ACV prophylaxis after each HSCT because of poor engraftment. A clinical diagnosis of herpes zoster was made, oral ACV (80 mg/kg/d) was initiated, and several lesions were cultured yielding VZV. Unfortunately, despite 2 weeks oral ACV therapy, new vesicles continued to develop over her trunk and lower extremities in a multidermatomal pattern, prompting hospital admission and high-dose (45 mg/kg/d) intravenous ACV.

After 1 week of intravenous ACV, new lesions continued to develop (culture positive for VZV) and ACV resistance was suspected. Therapy was switched to intravenous Foscarnet (120 mg/kg/d) while the VZV isolates were referred to the National Microbiology Laboratory (Winnipeg) for ACV resistance testing.

Response to intravenous foscarnet was dramatic. New lesions ceased to appear and, within 5 days, all previous lesions were healing. Foscarnet therapy was continued for 4 weeks and was well tolerated. However, 1 week after stopping foscarnet, new lesions appeared and foscarnet was restarted. Again, the response to therapy was rapid and an additional 4-week course was planned. Unfortunately, the patient developed bacterial sepsis related to a central venous access device and died.

RESISTANCE TESTING

Genotypic ACV resistance testing was done by polymerase chain reaction amplification of the VZV TK (open reading frame, ORF 36) and DNA polymerase (ORF 28) genes using the following primer sequences: ORF 36 Forward = 64692-64711 (5′-AAACGAGTGTGGCAACGTTG-3′),

ORF36 Reverse = 65950-65931 (5′-GGCGGGATTAAAGGATGTGG-3′),

ORF 28 Forward = 46853-46874 (5′-GTTATATTTCCGGGGAAATCTG-3′), ORF 28 Reverse = 50763-50742 (5′-TAAACGGGTATTACATATGCGG-3′). Amplicons of 1258 bp (ORF 36) and 3910 bp (ORF 28) were then sequenced.

Sequences were compared with wild-type and ACV-resistant VZV strains. A novel 2 base-pair (TA at nt375-6) deletion in the TK gene was detected—resulting in a frame shift and truncated TK protein. The mutated protein diverged from the wild-type TK sequence after AA-125 and was truncated from 361 amino acids (normal protein) to 162. No mutations were detected in the DNA polymerase gene.

In addition to the genotyping data, a phenotypic assay was developed to assess the mutant VZV strain for ACV susceptibility/resistance. The Merck vaccine strain (Varivax) was used as the wild-type strain with respect to ACV susceptibility. A 50 $TCID_{50}$ inoculum of the clinical VZV isolate and the vaccine strain were added to MRC-5 cells (ATCC CRL-2222) in 24-well tissue culture plates, and adsorbed for 1 hour at 35°C. Minimal essential media containing 3-fold serial dilutions of ACV (acycloguanosine, A4669, Sigma, Oakville, Ontario) was added to the wells and incubated for 5 days at 35°C. ACV concentrations were 0, 1, 3, 9, 27, 81, and 243 μM. Total DNA was harvested from each well using Qiamp extraction (Qiagen, Mississauga, Ontario). VZV was quantified by real-time polymerase chain reaction and standardized by comparison to β-globin as described previously.[4] Viral strains were tested at each ACV concentration in quadruplicate. Two-way variance analysis indicated a statistically significant difference between the wild type and clinical isolates at 1, 3, 9, and 27 μM ACV concentrations (Fig. 1)—confirming the genotyping data's evidence of ACV-resistance.

DISCUSSION

ACV-resistant VZV, is rare in immunocompetent individuals but has been described in immunocompromised patients.[5–9] Forty-four clinical isolates of ACV-resistant VZV are described in the literature; largely in patients with acquired immunodeficiency syndrome (AIDS) (Table 1 available online).[10–28] Clinically, ACV-resistant VZV infection has been defined by the persistence of lesions despite 10 days of therapy.[25,30] Saint-Leger et al[26] suggest that persistent lesions at day 10 may simply indicate the need for prolonged ACV in immunocompromised patients and that treatment failures after 21 days of therapy are more predictive of ACV resistance in this setting.

Laboratory detection of ACV resistance is a specialized process. Among phenotypic assays, plaque reduction remains the gold standard. Unfortunately, this assay can take weeks to perform and is influenced by cell lines, viral inoculum size, antiviral concentration, and user interpretation, making interassay comparison difficult. Phenotypic assays also have a limited ability to discriminate small proportions of ACV-resistant viruses within a larger heterogeneous population.[22,27] In addition, passage of resistant virus in antiviral free cells during initial isolation removes selective pressure and may allow wild-type subpopulations to reemerge as the dominant population, masking resistant strains.[31]

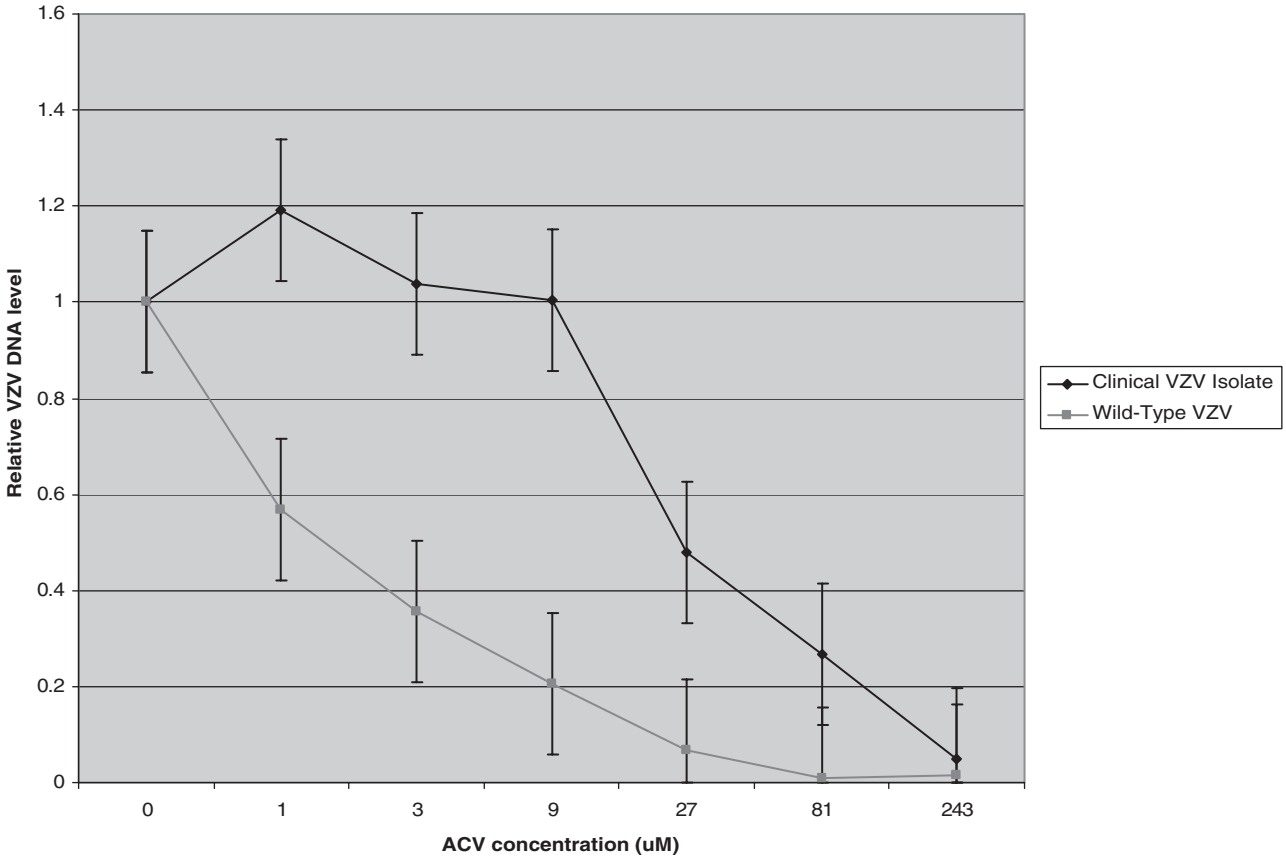

FIGURE 1. Acyclovir sensitivity profile for a VZV clinical isolate (diamond) and a wild-type (square) VZV strain. Viral quantification data are expressed as a percentage of the no drug level. Error bars indicate 95% confidence intervals.

In genotypic assays, amplification and sequencing of a target gene allows comparison with wild-type virus to detect changes responsible for resistant phenotypes. Genotyping is rapid, more sensitive than phenotypic assays, and allows detection of resistant viruses present in low numbers within a mixed population. Resistance mutations are well characterized for viruses such as human immunodeficiency virus and cytomegalovirus, but less data is available for herpes simplex virus and VZV.[17,31]

The initial step in ACV phosphorylation to its active triphosphorylated form is catalyzed by viral TK.[2,13,32,33] TK gene mutations leading to a nonfunctional, truncated or absent protein (TK^N), or mutations resulting in decreased production of TK (TK^P), account for 95% of ACV-resistant mutants.[5,31] Rare DNA polymerase mutations account for others. TK gene mutations occur most frequently at "mutagenic hot spots" in the adenosine triphosphate and nucleoside-binding sites.[31] The 2 base-pair (T-A at nt375-6) deletion in the TK gene detected in our case is, to our knowledge, novel. VZV isolates have been reported, however, with TK gene additions or deletions leading to similarly truncated proteins and the TK^N phenotype seen in this case.[13,22,29,31]

Our patient responded rapidly to foscarnet—the drug of choice for ACV-resistant VZV strains.[30,32] A pyrophosphate analogue, foscarnet directly inhibits viral DNA polymerase without requiring phosphorylation to be active.[10] Renal toxicity and availability only as an intravenous drug limits its prophylactic role in patients with recurrences of ACV-resistant VZV.[12]

Recently, emergence of foscarnet-resistant strains have been reported.[34] Cidofovir, an acyclic nucleoside phosphonate that does not require TK for conversation to the active form, is often effective against both TK-deficient and foscarnet-resistant mutants.[33,35]

CONCLUSIONS

Our case illustrates the need for clinicians to have an index of suspicion for ACV-resistant VZV in immunocompromised patients with VZV infections—particularly those with prior ACV exposure. Laboratory testing for ACV resistance is a specialized procedure but is valuable both for augmenting clinical diagnoses and for understanding the molecular epidemiology of the problem. The phenotypic characterization of the novel mutation we described (TA at nt375-6) supports previous reports that a single mutation in the TK gene can confer ACV resistance. Foscarnet can be a life-saving alternative for patients with ACV-resistant VZV infections.

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LIPOSOMAL AMPHOTERICIN B ASSOCIATED WITH SEVERE HYPERPHOSPHATEMIA

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Abstract: We report 4 patients who developed hyperphosphatemia while receiving liposomal amphotericin B to treat an invasive fungal infection. Resolution of the hyperphosphatemia occurred after transition to amphotericin B lipid complex. This phenomenon may occur more commonly in patients with mild to moderate renal insufficiency.

Key Words: antifungal, invasive fungal infection, amphotericin B, hyperphosphatemia

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The standard treatment of invasive fungal infections in children is the polyene amphotericin B deoxycholate or one of its lipid-formulations, as they have a broad-spectrum of activity against *Candida* spp., *Aspergillus* spp., *Fusarium* spp., and fungi in the Zygomycetes class.[1,2] These agents can cause hypokalemia and hypomagnesemia secondary to renal tubule injury.[3] Hyperphosphatemia may be an under-recognized problem with administration of liposomal amphotericin B deoxycholate (AmBisome, Astellas Pharma US, Inc., Deerfield, IL). We report 4 children who developed hyperphosphatemia while receiving liposomal amphotericin B.

CASE 1

An 11-year-old boy with high-risk pre-B-cell acute lymphoblastic leukemia (ALL) in remission was admitted with fever and neutropenia. Although his induction chemotherapy had been complicated by mild acute renal insufficiency (peak creatinine 1.68

mg/dL), by admission the serum creatinine had normalized. Evaluation revealed a left temporal brain abscess and a cavitary pulmonary lesion. Excisional biopsy of the brain lesion yielded Aspergillus fumigatus by fungal culture. Because of the extent of the brain lesion, combination antifungal therapy was initiated with high-dose liposomal amphotericin B (10 mg/kg/d), voriconazole (12 mg/kg/d), and caspofungin (50 mg/m^2/d). The 24 hour MICs were amphotericin = 0.25 mcg/mL, voriconazole = 0.25 mcg/mL, and caspofungin = 0.5 mcg/mL.

Three days after starting liposomal amphotericin B therapy, the patient's serum phosphate increased from a baseline of 4.6 mg/dL to 13.2 mg/dL (normal range, 2.5–4.5 mg/dL). During the next 10 days, the patient's phosphate ranged from 6.4 to 13.7 mg/dL, with most values above 8 mg/dL (Fig. 1). He experienced a mild prerenal state related to fluid restriction (baseline creatinine 0.56 mg/dL, peak 1.64 mg/dL), but hyperphosphatemia persisted despite normalization of creatinine. Although the patient's serum calcium remained normal, he developed conjunctival injection, thought to be caused by calcium-phosphate deposition.[4]

When liposomal amphotericin B was suspected as a potential cause of the hyperphosphatemia, it was discontinued. Amphotericin B lipid complex (ABELCET, Enzon Pharmaceuticals, Inc., Bridgewater, NJ) was initiated in the same dosage (10 mg/kg/d). The patient's phosphate normalized immediately.

CASE 2

A 13-year-old boy with high-risk ALL in remission was admitted with fever and neutropenia. Despite several days of vancomycin and ceftazidime therapy, he remained febrile. Computed tomography revealed multiple scattered hypodense lesions within his liver, consistent with possible invasive fungal infection.[5] Biopsy was not attempted because of coagulopathy. Liposomal amphotericin B therapy was initiated at 5 mg/kg/d. During the next month, the patient's serum phosphate was intermittently elevated. Despite dietary restriction and administration of phosphate binders (sevelamer), the patient's phosphate remained in the range of 4–8.7 mg/dL (Fig. 1). During this period, he had mild, intermittent renal insufficiency (baseline creatinine 0.6 mg/dL, peak 1.1 mg/dL).

To test the association of hyperphosphatemia and liposomal amphotericin B, this drug was stopped and hyperphosphatemia resolved. Amphotericin B lipid complex therapy was then initiated at 5 mg/kg/d and escalated to 7.5 mg/kg/d. Serum phosphate values remained normal (1.6–5.8 mg/dL), despite removal of dietary phosphate restriction and discontinuation of sevelamer.

CASE 3

A 16-year-old girl was admitted with severe systemic lupus erythematosis and lupus nephritis. She required aggressive immunosuppressive therapy including high-dose corticosteroids, cyclophosphamide, and rituximab. After this, she developed a biopsy-proven cutaneous angioinvasive fungal infection with broad branching hyphae thought to be caused by *Mucor* spp., and high-dose liposomal amphotericin B therapy (10 mg/kg/d) was initiated. At the time the invasive fungal infection was diagnosed, the patient had severe renal insufficiency and was receiving continuous renal replacement therapy. Phosphate values remained normal (3–4.1 mg/dL). After 8 days, continuous renal replacement therapy was replaced with intermittent hemodialysis. Despite receiving complete phosphate restriction, the serum phosphate immediately increased (10.3–12.4 mg/dL) (Fig. 1).

Two days later, continuous renal replacement therapy was restarted and the serum phosphate quickly normalized (3.7–4.4 mg/dL). Liposomal amphotericin B was replaced with amphotericin B lipid complex at an equivalent dosage (10 mg/kg/d). Two weeks later, continuous renal replacement therapy was again replaced with intermittent hemodialysis. While receiving amphotericin B lipid complex, the patient did not develop hyperphosphatemia on intermittent hemodialysis (1.9–5.4mg/dL).

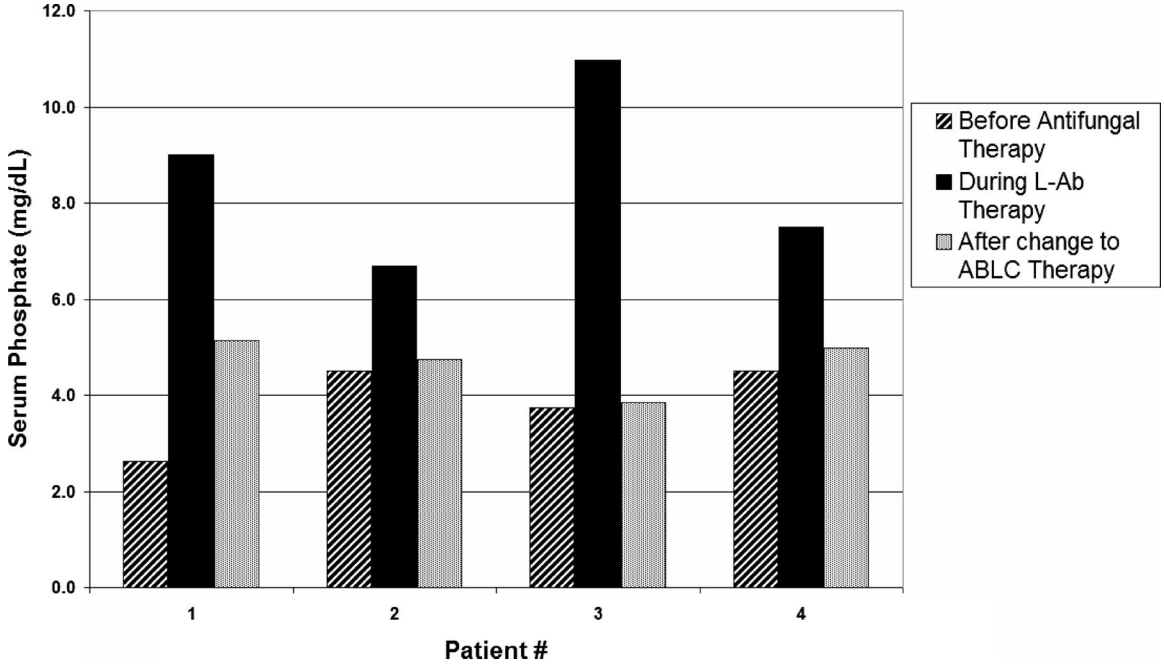

FIGURE 1. Average serum phosphate for each patient before antifungal therapy was initiated, while the patient was receiving liposomal amphotericin B, and while receiving amphotericin B lipid complex.

CASE 4

An 18-year-old male with acute myeloblastic leukemia (AML) in the second complete remission was admitted to undergo an allogeneic bone marrow transplant. Treatment of his primary AML disease 3 years earlier had been complicated by multiple organ failure requiring mechanical ventilation and hemodialysis. He had had multiple positive endotracheal tube cultures that grew both *Aspergillus* spp. and *Candida albicans* and computed tomography showed dense consolidations of the bilateral lower lobes, consistent with probable fungal pneumonia.[5] He was treated with amphotericin B lipid complex (3 mg/kg/d) for 6 days, caspofungin (50 mg/d) for 28 days, and voriconazole (300 mg BID) for approximately 6 months. Complete remission of the AML was achieved, his invasive fungal infection resolved, and his renal function normalized.

Given his history of invasive fungal infection, secondary antifungal prophylaxis with voriconazole (300 mg PO BID) was started during the high-risk neutropenic period after his bone marrow transplant.[6] The patient developed elevated serum transaminase values, raising concern about voriconazole toxicity. For this reason, his prophylaxis was changed to liposomal amphotericin B (5 mg/kg/d).

Although the patient's serum phosphate values were normal (range, 3–4.5 mg/dL) before the start of liposomal amphotericin B, on day 1 of L-Ab, a >90% increase in the serum phosphate (7.7 mg/dL) was observed and sustained for 3 days (Fig. 1); creatinine values were normal (0.5 mg/dL). The patient was switched to amphotericin B lipid complex (5 mg/kg/d) resulting in normalization of his serum phosphate level within 3 days, with continued normal values for the duration of his 28-day prophylaxis.

DISCUSSION

Liposomal amphotericin B is just as effective as conventional amphotericin when used as empiric therapy for patients with prolonged fever and neutropenia, but has a more favorable side effect profile than conventional amphotericin with regards to fever and nephrotoxicity.[7] Although electrolyte disturbances such as hypokalemia and hypomagnesemia are well-documented with amphotericin therapy,[3] hyperphosphatemia has been not been extensively noted. We describe 4 patients who developed significantly elevated serum phosphate associated with liposomal amphotericin B administration, which resolved when liposomal amphotericin B was replaced with amphotericin B lipid complex.

The phosphate load of the liposomal formulations comes from the phospholipid carrier rather than the amphotericin. Liposomal amphotericin B contains 37 mg of inorganic phosphate per 50 mg of amphotericin B administered.[8] Amphotericin B lipid complex, by comparison, delivers 6.8 mg of inorganic phosphate for each 50 mg of amphotericin (personal communication, Enzon Pharmaceuticals, Inc.) Additionally, liposomal amphotericin B is highly protein bound and has slow tissue penetration, which may result in higher phosphate availability.

One other case of hyperphosphatemia associated with liposomal amphotericin B administration has been reported. An 8-year-old girl with normal renal function received liposomal amphotericin B at 25 mg/kg/d to treat a brain abscess caused by *Mucor*, *Aspergillus*, and *C. albicans*. One week into her therapy she had a phosphate level of 19 mg/dL. Liposomal amphotericin B was changed to itraconazole, resulting in normalization of serum phosphate levels.[8] This patient and 2 of our patients received liposomal amphotericin B at a dose higher than the standard range. A recent study demonstrated that such dosages are not more efficacious and are associated with more frequent side effects.[9] Hyperphosphatemia, however, can occur at standard dosages of liposomal amphotericin B, as evidenced by our 2 other patients.

All 4 of our patients had prior renal injury and likely had residual renal dysfunction, suggesting that diminished renal excretory function may contribute to liposomal amphotericin B associated hyperphosphatemia. However, creatinine clearance was not statistically different between the period of liposomal amphotericin B administration and the period of amphotericin B lipid complex administration. This implies that resolution of hyperphosphatemia was because of substitution of amphotericin B lipid complex for liposomal amphotericin B, rather than improved renal function.

It has been suggested that the hyperphosphatemia associated with liposomal amphotericin B administration represents pseudohyperphosphatemia because of interference of liposomal amphotericin B with the Beckman analyzer technique.[10] Our institution uses Beckman analyzers, however, as the amphotericin B molecule is identical in both liposomal amphotericin B and amphotericin B lipid complex, any interference must be because of compositional or physical differences between the lipid formulations.

Clearly, further investigation is necessary to determine the clinical relevance of the hyperphosphatemia associated with liposomal amphotericin B. Even if liposomal amphotericin B causes pseudohyperphosphatemia, therapy may still need to be altered so as to avoid masking true causes of hyperphosphatemia. In situations where discontinuation of polyene therapy is not an option, an alternative approach may be to administer amphotericin B lipid complex in place of liposomal amphotericin B, as they have similar efficacy.[11] This adjustment may be required more frequently in the setting of reduced renal function.

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CUTANEOUS LEISHMANIASIS TREATED WITH AZITHROMYCIN IN A CHILD

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Abstract: This report describes the case of a 10-year-old boy with cutaneous leishmaniasis presumed to be caused by *Leishmania major* and successfully treated with oral azithromycin. Clinical studies using azithromycin for the treatment of cutaneous leishmaniasis are reviewed.

Key Words: leishmaniasis, cutaneous, leishmaniasis, old world, azithromycin, child

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Treatment of cutaneous leishmaniasis (CL) is not well standardized. Topical drugs or devices, and oral, intramuscular, or intravenous medications, have been reported to be effective, but their effectiveness varies with the causative species.[1] Azithromycin, a macrolide antibiotic, seems promising in vitro and in mice, especially against *Leishmania major*.[2]

We report the case of a 10-year-old boy who had old world CL and was successfully treated with this antibiotic.

CASE REPORT

NT was a previously healthy 10-year-old boy. He was born in France and lived in Marseilles, southern France, in a well-known focus of *Leishmania infantum* visceral leishmaniasis. During summer 2004, he traveled to northern Algeria (Bejaia geographical area) to visit relatives. In January 2005, he noticed an extending nodule in his left cheek. He was treated with several courses of topical fusidic acid unsuccessfully. In December 2005, he was referred to our universitary hospital.

At admission, the child seemed healthy. Weight was 60 kg. Hepatosplenomegaly, lymph node enlargement, and pallor were not present. A crusty nodule 1 cm in diameter was present on the left cheek. The blood count and the chemistry panel were unremarkable; white blood cells, platelet counts, and hemoglobin were normal. Erythrocyte sedimentation rate was 38 mm, C-reactive protein was less than 7 mg/L, and blood fibrin 3.82 g/L.

Bacterial culture of the lesion isolated *Staphylococcus epidermidis* and fungal culture was negative. Microscopic examination of a skin biopsy found a nonspecific inflammatory reaction with mononuclear cell infiltrate, and in some places, nonspecific granulomatous reaction. Polymerase chain reaction for *Mycobacterium* sp. using internal transcribed spacer gene was negative. CL was clinically suspected but the first biopsy failed to reveal parasites by direct examination, culture in RPMI medium, or specific polymerase chain reaction. Although enzyme-linked immunosorbent assay (ELISA) serology was negative, Western blot for leishmaniasis was positive. A second biopsy was performed in May 2006: direct examination and culture in RPMI medium were negative, but a real time quantitative polymerase chain reaction targeting kinetoplast DNA and using 2 taqman probes specific for *L. infantum* and *L. major*[3] was positive. The polymerase chain reaction (PCR)-estimated parasitic load was 2.2 *Leishmania* per million nucleated cells numbered by means of a quantitative PCR for human albumin gene.

The child was treated with azithromycin 500 mg/d for 10 consecutive days per month in June, July, and September 2006. The treatment was well tolerated. The cheek lesion rapidly improved, as shown in Figure 1. In February 2007, 5 months after the last course of azithromycin, the lesion had resolved with scar formation. There was no evidence of relapse, nor signs of visceral leishmaniasis.

DISCUSSION

Azithromycin concentrates in tissues, especially in macrophages that are infected by *Leishmania* parasites, and can reach concentrations 100 to 200 times higher than in serum.[2] Its oral

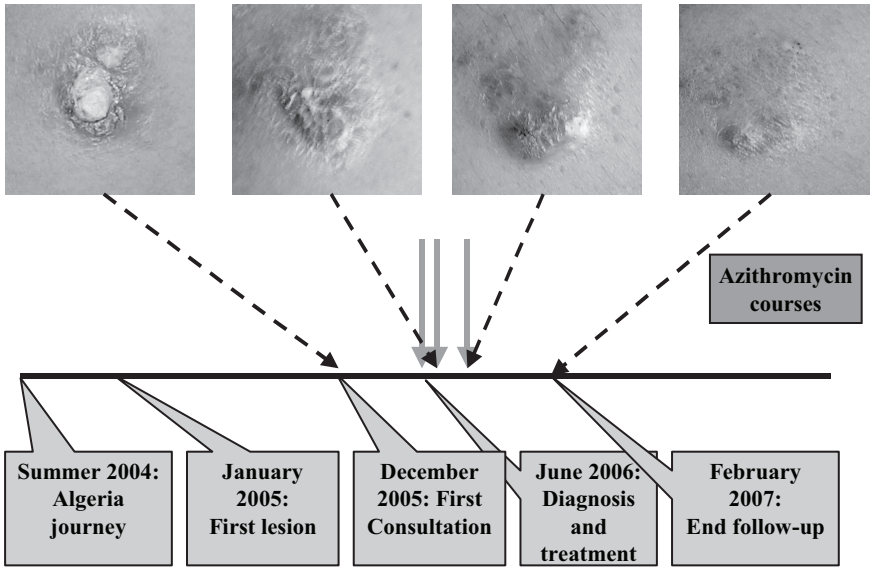

FIGURE 1. Evolution of the child's cutaneous leishmaniasis with azithromycin treatment.

administration, its long half-life, and its safety in children are advantages for the treatment of leishmaniasis. Azithromycin is effective for decreasing *L. major* promastigotes count in cell-free culture, and *L. major* amastigotes count in macrophages culture.[2] It has been effective in susceptible mice infected with *L. major*.[2] Two small series[4,5] of *L. braziliensis* infected patients have been described: azithromycin (500–1000 mg/d, for 2 to 10 d/mo, and a maximum of 4 months of treatment) cured 85% of the patients.

Two other studies using azithromycin in the treatment of patients with old world CL (*L. major* and *L. tropica*) have recently been published.[6,7] Both reported that azithromycin is not effective for the purpose, but the observations were not conclusive. The first study[6] included 21 patients, mainly adults (mean age 21.4 years), treated with 500 mg/d for 3 weeks. The diagnosis was clinically done, then "laboratory-confirmed" (unspecified tests). Only subjects with lesions lasting less than 4 months were included. Cultures were not mentioned but the causative species was said to be *L. major* in this geographic area (Isfahan, Iran). Patients were evaluated only at the end of treatment (21 days) and were not followed for a longer time. At the end of treatment, 23.5% improved but were only "partially cured." In that series, the short duration of follow-up may have missed patients who would have been cured with a longer period of observation. The second study,[7] which took place in Syria (*L. tropica* and *L. major*), collected data of 43 infected people; 27.9% of the patients were 2 to 12 years old. Only patients with lesions lasting less than 6 months and without any specific treatment in the previous 3 months were included. CL was confirmed by direct smear examination before inclusion, but culture or specific polymerase chain reaction was not performed. Young children received azithromycin 10 mg/kg/d, and adults and children more than 12 years of age received 500 mg/d for 10 days in all the patients. Treatment cycles were repeated monthly for a maximum of 3 courses, when the lesions failed to respond clinically. None of the adults showed improvement of their cutaneous lesions. However, 10 of the 12 children less than 13 years of age improved. The lesions of these children were nonulcerative papulonodules present less than 8 weeks before treatment.

In our reported pediatric case, the lesion was nonulcerative too, but it had been noticed 16 months before diagnosis. Leishmaniasis was clinically suspected, but direct smears or biopsies examinations were negative, probably because of the small number of parasites. ELISA serology was negative, which is common in cutaneous leishmaniasis. Immunoblot detects low levels of specific antibodies. In our patient who lived in an *L. infantum* endemic area, the positivity of immunoblot might also reflect asymptomatic carriage of this species. CL was confirmed by specific polymerase chain reaction, but the species was not identified. The strain did not grow in culture indeed, probably because of the low parasitic load in the sample. The child might have been infected with *L. infantum* (the only species that is endemic in the south of France where he lived) or with *L. major* (endemic in the north of Algeria, where he had traveled). In France, *L. infantum* strains isolated in the Marseilles area (Provence focus) preferentially induce visceral leishmaniasis, when compared with strains isolated in the Pyrénées-Orientales focus.[8] In our case consequently, the probability of a *L. major* infection was high. The long duration of evolution before azithromycin treatment (16 months) prevents clear evidence for the effectiveness of this antibiotic, because most of *L. major* lesions self-heal within several months. However, the cutaneous lesion clearly seemed to improve between the second and the third course of treatment, and later, during the follow-up.

In this report, a rapid improvement after azithromycin treatment has been noticed in a child suffering from old world CL. The case illustrates the need of controlled clinical trial to evaluate the efficacy of azithromycin in *L. major* infected children, who seem to be good responders in previous studies.

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TRANSMISSION OF *MYCOBACTERIUM MARINUM* FROM FISH TO A VERY YOUNG CHILD

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Abstract: *Mycobacterium marinum* causes tuberculosis in fish and can cause skin infections in humans who swim in contaminated water or who have direct contact with infected fish. We report the case study of an 18-month-old girl with *M. marinum* abscesses, who acquired the infection through indirect contact with a contaminated bucket. Appropriate cleaning of aquarium equipment is very important, especially with young children in the household.

Key Words: *Mycobacterium marinum*, child, transmission
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Mycobacterium marinum first described by Aronson in 1926,[1] is one of the nontuberculous mycobacteria. Skin disease, known as "swimming pool granuloma" or "fish tank granuloma," is the clinical presentation with ulcers, nodules, and abscesses as the prominent signs. Infection is most commonly acquired through abraded skin while taking care of aquaria with tropical fish or bathing in water where contaminated fish live.[1,2] It is a rare cause of skin infections in young children. We present a very young child who had not been in direct contact with fish, but nevertheless developed a skin infection with *M. marinum*.

CASE REPORT

An 18-month-old girl was presented to the surgeon with an abscess for 4 weeks, located on her right elbow. Treatment of the abscess with broad spectrum antibiotics had not been successful. Surgical drainage was performed after which she developed 3 similar lesions on her right arm (Figure available online). She was otherwise well without signs of disseminated infection. Cultures, taken during surgery, eventually grew *M. marinum* and we treated her successfully with rifampin and clarithromycin for 6 months. In 2 years of follow-up, some residual scarring remains but there have not been recurrences of skin lesions. The girl had been born with a Tetrology of Fallot, corrected at the age of 1 month, with some perioperative complications. A second cardio surgical intervention, performed after the cure from the *M. marinum* infection, passed uneventfully. She did not have a history of remarkable infections, and in her blood we found a normal white blood cell count, with a normal differentiation. Total lymphocyte counts were normal for age, as were the counts for T-cells (CD3, $CD3^+4^+$, $CD3^+8^+$), B-cells ($CD19^+$), and natural killer (NK) cells ($16^+/56^+$) as measured by standard flow cytometry. Whole blood cultures stimulated with phytohemagglutinin (PHA) and cytokine responses [interferon (IFN)-γ), tumor necrosis factor (TNF)-α), and interleukin (IL)-10] measured in the supernatant with standard enzyme-linked immunosorbent assay (ELISA) techniques did not reveal any deficiencies compared with results in a healthy age-related control.

Shortly before the abscesses appeared, she had scratched mosquito bites on her forearm and they seemed to have become infected. Because of these infected mosquito bites, she had bathed with bicarbonate of soda in a bucket that was also used for fish during aquarium clean-ups. Her arm was held in the bucket by her father during these "therapeutic baths." Her father, who had eczema, had developed abscess-like lesions on his hands several weeks before. These lesions appeared after he had done a clean-up of the aquarium, a household job he normally does not do because of his eczema. Father had been treated with antibiotics by the family physician and a dermatologist. Routine microbiologic cultures of the abscess material from father were sterile and specific mycobacterial cultures done by us (after 5 weeks of clarithromycin) did not grow mycobacteria. The family had an aquarium with tropical fish placed high in a bookcase, which the child could not possibly reach and she had never had direct contact with the fish. Some fish in the aquarium died when father developed his abscesses.

We collected and investigated the remaining 6 fish in the aquarium. One of the neon tetras (*Paracheirodon innesi*) had clear granulomatous lesions in the liver and the spleen. Plating of these organs resulted in the isolation of large numbers of *M. marinum*. No mycobacteria could be isolated from the remaining fish. Amplified fragment length polymorphism (AFLP) analysis, performed as described previously,[3] showed that the *M. marinum* strain isolated from the infected neon tetra was identical to the strain isolated from the lesion of our patient (results not shown).

DISCUSSION

M. marinum is found worldwide in salt and fresh water.[2,4] The clinical presentation of disease in humans is that of a primary painless papule or nodule at the site of inoculation, appearing after a median time of 16–21 days (range: 0–292 days) from inoculation.[2,4] In some cases, these nodules can develop as ulcers or abscesses. About 20% of the time the nodule is accompanied by lesions proximally along the lymphatic vessels in a sporotrichoid form.[2] Dissemination to deeper structures (eg, arthritis, osteomyelitis) has been described, but is exceptionally rare and limited to immune compromised patients.[5] Diagnosis is traditionally made through a positive Lowenstein-Jensen culture,[2] but is complicated by the fact that *M. marinum* does not grow at 37°C. Detection of mycobacterial DNA by polymerase chain reaction (PCR) (16S ribosomal DNA) or by PCR restriction analysis looking for the heatshock protein 65 kD gene has recently been introduced and can be positive within 2 days.[6,7]

In immune competent patients, spontaneous but slow recovery occurs during a period of 1–6 years. Antibiotics may accelerate natural recovery and prevent progression to deep infections. There are no well conducted studies about the type and duration of optimal antibiotic treatment. Most studies have been done with rifambutin and ethambutol, but trimethoprim, clarithromycin, and tetracyclines also show activity in vitro against *M. marinum.*[8] Duration of treatment varies between 6 weeks and 6 months for superficial lesions and 6 to 18 months for deeper infections. Surgical intervention is necessary to obtain cultures and histology, and may play a role in curative therapy, specifically for deep infections.[8]

We did an extensive literature search for reports of children less than 5 years of age with a *M. marinum* infection and found that, since 1983, 10 such young children have been described. In the case histories of 2 of these children, there is mention of bathing in the same bath where the fish tanks were cleaned and from the other 8 children we only know that there was a fish tank in the family house and/or that the family was very active in fishing and camping.[5,9,10] Van der Sar et al[3] recently showed that the *M. marinum* strains can be divided into 2 virulent strains ("clusters"), and strains from cluster 1 show increased pathogenicity for humans and zebra fish and are capable of causing severe and persistent infections. The strain identified in this study belongs to cluster 1.

Twenty to thirty years ago, most reported *M. marinum* infections were related to swimming-pools ("swimming pool granuloma"); but these days this seems to be a rare event (2.6% of reported cases).[4] This decline is probably the result of improved chlorination of swimming pools. Dailloux et al[11] showed that *M. marinum* disappeared from a swimming pool after increasing the chlorine concentration to more than 0.2 mg/L, but for other mycobacteria, Le Dantec et al[12] reported that standard chlorine disinfectants might not be sufficient. The fact that adequate cleaning procedures need to be determined for specific circumstances is further emphasized by a recent report on a *Mycobacterium fortuitum* outbreak in nail care salons.[13]

In our patient of 18 months we proved an infection with *M. marinum* and we showed with AFLP analysis that fish from the family aquarium was infected with the same virulent strain. We know that the child had not been in direct contact with the fish and we assume that the child has been infected through the bucket used for the fishes during aquarium clean-ups in which she had "arm baths" for her infected mosquito bites. Although we were not able to prove infection with *M. marinum* in the father, we find it very likely that he too had a *M. marinum* infection on his hand. If this is true, we cannot rule out that the father has functioned as an intermediate, although human to human transmission of *M. marinum* has never been described. We learned from our literature review that incidental cases of very young children in relation to bathing in presumed contaminated bathtubs have been reported. Appropriate care of all aquarium equipment should be advocated.

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HEMOSIDERIN-LADEN MACROPHAGES IN THE CEREBROSPINAL FLUID OF A NEONATE AFTER TRAUMATIC LUMBAR PUNCTURE

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Abstract: Macrophages in cerebrospinal fluid are described as indicators of pathology. We present findings from the lumbar puncture of a child without neurologic disease. Cerebrospinal fluid obtained after an initial, traumatic lumbar puncture attempt included a high proportion of macrophages, some containing erythrocyte fragments and hemosiderin. This suggests that although macrophages may indicate pathology, they can also accumulate after traumatic lumbar puncture.

Key Words: neonate, cerebrospinal fluid, macrophage, lumbar puncture

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The presence and analysis of cells in cerebrospinal fluid (CSF) may provide valuable diagnostic information when evaluating for possible central nervous system infection or trauma. Macrophages are infrequently found in CSF; they can be classified by the type of material they have phagocytosed. Erythrophages contain red blood cells and fragments of red blood cells. These macrophages can be found shortly after subarachnoid hemorrhage. Siderophages contain hemosiderin, a product formed by the degradation of hemoglobin from within red blood cells. These cells are identified several days after subarachnoid hemorrhage and can persist for weeks or months.[1] In contrast to the expected finding of these macrophages several days after hemorrhagic disease, we report a neonate without identified intracranial pathology who had macrophages, some containing hemosiderin and erythrocyte fragments, 24 hours after a traumatic lumbar puncture.

CASE

The patient was a 12-day-old, born at 39 weeks gestational age to a healthy mother after a normal pregnancy by an uncomplicated repeat cesarean section. On day of life 4, the mother first noted that the baby was having low amplitude, nonrhythmic twitching movements of the extremities. These lasted about 30 seconds and could be stopped with gentle pressure. They occurred more frequently after the patient was moved or touched. He remained alert and responsive throughout the events. These increased in frequency until day of life 11, when they occurred about 10 times per day. He was brought to his pediatrician's office, where an episode of twitching was observed. The child was sent to an emergency room for evaluation. There he was afebrile with normal vital signs. The medical and neurologic examinations were normal. Several further twitching events occurred in the emergency room. Lorazepam was administered because of concern for seizures; this did not affect the events. Initial evaluation included normal blood count, computerized tomography of the brain, electrocardiogram, urinalysis, and urine toxicology screening test. Serum electrolytes were normal, with the exception of calcium 6.6 mg/dL. This was treated with calcium gluconate. A lumbar puncture was attempted, but only a small amount of bloody fluid was obtained.

The patient was transferred to our institution's neonatal intensive care unit for further evaluation. Computerized tomography images of the brain were reviewed by our pediatric neuroradiologists and confirmed normal. Lumbar puncture was successfully repeated 24 hours after the initial attempt. CSF analysis demonstrated 6 white blood cells/μL, 184 red blood cells/μL, glucose 46 mg/dL, and protein 157 mg/dL. The leukocyte differential was notable for 58% macrophages, some of which were hemosiderin-laden (Fig. 1). Gram stain, CSF cultures, and CSF herpes simplex virus (HSV) polymerase chain reaction (PCR) were negative. Likewise, surveillance nasopharyngeal, conjunctival, skin, and rectal HSV, and blood cultures were negative. Serum electrolytes within the first 24 hours of admission were notable for continued hypocalcemia (calcium 7.4 mg/dL at time of transfer), requiring intravenous (IV) calcium and magnesium for correction. The infant was gradually weaned off IV fluids and onto formula without difficulty. The infant's twitching movements, which had continued after transfer, became less frequent with calcium correction and stopped completely after ionized calcium levels stabilized in the normal range. Random intact parathyroid hormone and vitamin D 25-hydroxy values were normal. Ionized and serum calcium remained stable off IV supplements. The patient was diagnosed with transient hypoparathyroidism, with no further treatment required. Long-term electroencephalographic monitoring demonstrated a normal background and captured multiple typical twitching episodes. These had no electrographic correlate, and therefore were not seizures.

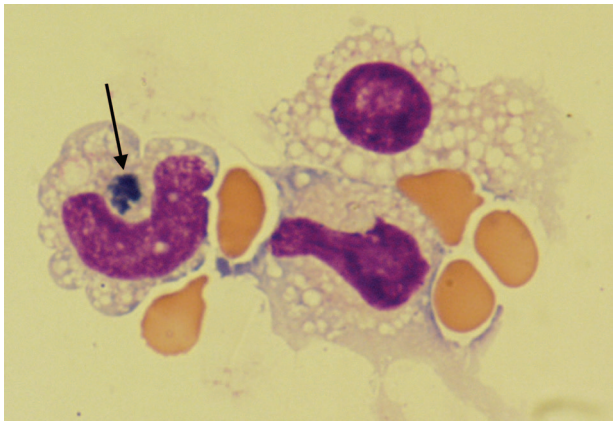

FIGURE 1. One hundred power image of red blood cells being phagocytosed by macrophages that contain hemosiderin (arrow) from previously degraded red blood cells.

DISCUSSION

The patient's movements were not thought to be related to intracranial pathology, and the neonate was considered to be neurologically normal, except for having undergone a traumatic lumbar puncture attempt. The initial lumbar puncture attempt may have introduced blood into the CSF, which led to macrophage accumulation during the 24 hours before the second lumbar puncture was performed.

Lymphocytes and monocytes, with a few polymorphonuclear cells, are the normal leukocyte differential in noninfected infants.[2–6] Some studies have suggested macrophages can constitute up to 59% of leukocytes in noninfected neonatal CSF.[6] Fat laden macrophages have been reported in infants with meningitis or ventriculitis, with higher numbers associated with poorer outcome.[7] Likewise, hemosiderin laden macrophages have been reported several days after intraventricular hemorrhage or other intracranial hemorrhage.[1,8,9] In our patient, the traumatic lumbar puncture may explain the finding. Initial lumbar punctures are often traumatic in neonates and require repetition. Finding CSF macrophages after a traumatic lumbar puncture, even within 24 hours of the initial lumbar puncture attempt, may be caused by the initial lumbar puncture and not by underlying neurologic pathology.

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BRAIN ABSCESS SECONDARY TO DENTAL BRACES

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Abstract: We report a case of *Haemophilus aphrophilus* brain abscess presenting 6 weeks after application of dental braces in an adolescent patient with a formerly undetected patent foramen ovale. Neither brain abscess nor invasive *H. aphrophilus* infection has previously been associated with dental braces. Application or tightening of dental braces may cause bacteremia and invasive disease from oral commensals.

Key Words: brain abscess, braces, orthodontics, *Haemophilus aphrophilus*, patent foramen ovale

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Consent has been obtained for publication of this case from the patient and his parents.

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A 12-year-old boy was admitted to the Royal Children's Hospital Melbourne, Australia, with a history of 3 days of headache, marked photophobia, vomiting, and lethargy. He had no fever, neck stiffness, diarrhea, or focal neurologic symptoms. Six weeks before presentation, dental braces had been applied to his upper and lower teeth. The braces had been tightened 4 weeks later, at which time he also had symptoms of an upper respiratory tract infection. His medical history was unremarkable and his immunizations were up-to-date. He had no known cardiac disease and was an international-standard competitive skier.

Examination on admission was normal except for photophobia, marked irritability, and unusually aggressive behavior. An MRI scan showed a ring-enhancing lesion in the right temporal lobe, consistent with an intracerebral abscess (Fig. 1). The abscess was surgically drained and the patient was treated with intravenous flucloxacillin (200 mg/kg/d), cefotaxime (200 mg/kg/d), and metronidazole (22.5 mg/kg/d). His symptoms resolved rapidly, but his course was complicated by reaccumulation of the abscess 5 days later that required a repeat drainage procedure.

Culture of pus from the first drainage procedure grew profuse *Haemophilus aphrophilus*. The isolate was susceptible to penicillin and cefotaxime. Pus from the second drainage procedure was sterile. Echocardiography showed a structurally normal heart with no vegetations, but a bubble test revealed a small patent foramen ovale. The patient's antibiotic treatment was changed to intravenous ceftriaxone (100 mg/kg/d) and oral metronidazole (22.5 mg/kg/d), and he completed a total of 6 weeks of therapy. At review 2 weeks after completion of therapy, he had made a good recovery. There was some persistent impulsivity and irritability, but no other neurologic symptoms or signs.

DISCUSSION

Placement of dental braces involves attaching wire to the lingual or buccal surface of the teeth with both glue and circumferential metal bands. The American Heart Association advises antibiotic prophylaxis against endocarditis for placement but not tightening of braces in patients at moderate to high risk of infective

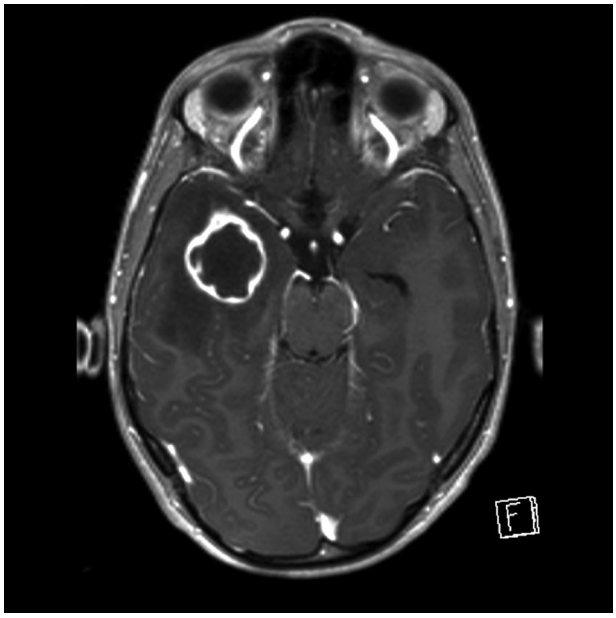

FIGURE 1. Magnetic resonance image of the patient's brain. Postcontrast, T1 weighted, axial multiplanar reformation image showing a ring-enhancing temporal-lobe lesion with hypodense core and surrounding cerebral edema.

endocarditis.[1] Application of braces is associated with bacteremia with oral organisms.[2]

H. aphrophilus is a Gram-negative bacillus. Its name derives from the Greek for blood-loving and foam-loving, referring to its preferred in vitro growth conditions of blood-derived X, but not V, factor and high CO_2 levels. The bacterium is a ubiquitous colonizer of the oral cavity, especially of dental plaque.[3] It is a well-described cause of infective endocarditis, one of the "HACEK" group of bacteria.[4] Invasive disease has also been described in a number of other sites including brain abscess, meningitis, sinusitis, otitis media, pneumonia, empyema, skin and soft tissue infections, and bone and joint infections.[5] Infective endocarditis from oral flora after similar intraoral procedures, such as tongue piercing, has been reported.[6]

Patent foramen ovale is a defect of the atrial septum, which is not hemodynamically significant and usually remains closed during normal respiration but opens to allow right-to-left interatrial blood flow during periods of increased intrathoracic pressure, such as the Valsalva maneuver. It is a common incidental finding, present in up to 25% of healthy adults.[7] Endocarditis and brain abscess in conjunction with patent foramen ovale have been described infrequently considering the common nature of this defect. The lesion is not listed by the American Heart Association as an indication for prophylactic antibiotics in bacteremia-prone procedures.[1]

This is the first reported case of a brain abscess or of any invasive *H. aphrophilus* infection after the application and tightening of braces. We postulate that brain abscess formation in this patient was a consequence of a transient bacteremia resulting from manipulation of dental braces, and that the presence of a patent foramen ovale enabled bacteria to bypass the normal physiologic filter of the lung and enter the cerebral circulation. This case is a reminder that, even without known risk factors, procedures in the mouth can lead to bacteremia and invasive infection at distant sites.

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