SERUM ANTIBODIES TO THE PNEUMOCOCCAL SURFACE PROTEINS PhtB AND PhtE IN FINNISH INFANTS AND ADULTS

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Abstract: We examined naturally acquired antibodies to pneumococcal vaccine candidate proteins PhtB and PhtE in children during their first 2 years of life. Prior culture-confirmed pneumococcal exposure was shown to induce the development of anti-PhtB and -PhtE antibodies. The anti-PhtB or -PhtE antibody concentrations were not significantly associated with a decreased risk of subsequent pneumococcal acute otitis media.

Key Words: *Streptococcus pneumoniae*, pneumococcal protein, Pht, protein vaccine, AOM

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Streptococcus pneumoniae (pneumococcus) continues to be a leading cause of morbidity and mortality worldwide. The 2 current pneumococcal vaccines commercially available are based on serotype-specific capsular polysaccharides. Although generally safe and efficacious, these vaccine formulations have certain shortcomings, and thus complementary or alternative approaches are being searched.

Several pneumococcal proteins are considered as potential vaccine candidates. Among these is a previously found family of similar surface-exposed proteins capable of protecting mice against fatal pneumococcal infection.[1–3] On the basis of a conserved histidine-triad motif (HXXHXH) repeated several times in these proteins, different research groups have designated the protein family with various names: Pht for pneumococcal histidine triad (PhtA, -B, -D and -E),[1] and Php for *S. pneumoniae* histidine protein (PhpA, -B, -C).[2] These proteins have also been named BVH-proteins.[3]

In the present study, we measured the development of serum antibodies to PhtB (PhpA/BVH-11) and PhtE (BVH-3) among healthy Finnish children and adults participating in the Finnish Otitis Media (FinOM) Cohort Study.[4,5] We examined the relationship between prior culture-confirmed pneumococcal exposure (carriage or disease) and the anti-PhtB and -PhtE antibody concentrations at the ages of 6, 12, 18 and 24 months. Further, we evaluated the association between serum anti-PhtB and -PhtE antibodies at 12 and 18 months and the risk of subsequent pneumococcal carriage and acute otitis media (AOM).

MATERIALS AND METHODS

The Study Population and Clinical Samples. The study population of the FinOM Cohort Study consisted of 329 healthy Finnish infants and their mothers.[4,5] The children were followed from 2 to 24 months of age for pneumococcal carriage and AOM. Bacterial cultures of the nasopharyx and middle ear fluid samples were taken during 10 scheduled and unscheduled (when sick) visits to the study clinic. Serum samples for antibody measurements were obtained at the ages of 6, 12, 18 and 24 months. In addition, one serum sample was obtained from the mothers at the first study clinic visit when the children were 2 months old.

The present study included consecutive serum samples of 50 infants taken at 6, 12, 18 and 24 months of age and serum samples of 90 mothers (unrelated to the subset of 50 children). The individuals were randomly selected from the whole FinOM Cohort Study population based on the availability of the serum samples. In addition, all available samples collected at 12 (N = 255) and 18 months (N = 255) were used for the risk analyses.

Recombinant Antigens. The recombinant Pht antigens were received from Shire Biologics Inc. (Quebec, Canada; presently GSKBio). The PhtB and PhtE antigens comprised the carboxyl-ends of the proteins (amino acids 565–838 and 408–1039, respectively) and were produced in *E. coli* as previously described.[3]

Serological Method. Total Ig (IgA + IgG + IgM) antibody concentrations to PhtB and PhtE in serum samples were measured by enzyme immunoassay (EIA). Wells of the microtiter plates (Costar 3591, Cambridge, MD) were coated with PhtB or PhtE with a coating concentration of 1 μg of antigen in 1 mL of 0.05 M carbonate buffer (pH 9.6) pipetted 100 μL per well and the plates were incubated overnight at +22°C. The wells were blocked by pipetting 150 μL of 0.5% skim milk in phosphate-buffered saline (PBS) (M/PBS) per well, and the plates were incubated for 1 hour at +37°C. The samples were diluted in M/PBS (starting at 1:100) and pipetted 150 μL per well in duplicates and incubated for 2 hours at +37°C. Alkaline phosphatase-conjugated (AFOS) conjugated AffiniPure goat antihuman IgA + IgG + IgM (Jackson ImmunoResearch Laboratories Inc, West Grove, PA) was diluted 1:6000 in M/PBS buffer, pipetted 100 μL per well and incubated for 2 hours at +37°C. Finally, 1 mg/mL *p*-nitrophenyl phosphatate disodium (Sigma Chemicals, St. Louis) substrate solution in carbonate buffer (pH 9.8) was added 100 μL per well and incubated for 1 hour at +37°C. Optical densities were measured at the 405 nm wavelength with an EIA reader (Multiscan, Labsystems, Helsinki, Finland). The results are given as microgram per millilitre; the URV/1 reference serum received from Shire Biologics Inc. has been assigned to contain 87.2 and 23.1 μg/mL of anti-PhtB of -PhtE specific antibodies, respectively.

Statistical Methods. The results are reported as geometric mean concentrations (GMC) with 95% confidence intervals (CI). Statistical comparisons were carried out on log-transformed data using Student's *t* test. A logistic regression model was used to evaluate the anti-PhtB and -PhtE antibodies at 12 and 18 months of age as risk factors for asymptomatic pneumococcal carriage 6 months later. An extended version of the Cox proportional hazard model was used to estimate the relative risk (RR) of pneumococcal AOM in the following 6 months at the age intervals 12–18 and 18–24 months in relation to the anti-PhtB and -PhtE antibody concentrations at the beginning of the age interval.

RESULTS

The Development of Anti-Pht Antibodies in Relation to Age and Prior Pneumococcal Exposure. The development of anti-PhtB and -PhtE antibodies with age had similar kinetics, although the GMCs of anti-PhtB were slightly higher when compared with anti-PhtE. The percentage of samples with detectable anti-PhtB and -PhtE, as well as the GMCs, decreased between the ages of 6 and 12 months and started to increase thereafter (Table 1). At the age of 24 months, the GMCs of infants were still significantly lower than those of adults ($P < 0.001$). The development of anti-Pht antibodies was strongly related to prior exposure to pneumococcus (Fig. 1); the GMCs were significantly higher in the group of children with prior culture-proven pneumococcal carriage or disease (pnc^+) than in the

TABLE 1. The Geometric Mean Concentrations (GMC) With 95% Confidence Intervals (CI) of Serum Anti-PhtB and Anti-PhtE Antibodies in Children at the Ages of 6, 12, 18 and 24 Months and in Adults

Age	N	Anti-PhtB		Anti-PhtE	
		Positive, %	GMC, μg/ml (95% CI)	Positive, %	GMC, μg/ml (95% CI)
6 months	50	98	1.56 (1.25–1.94)	90	0.63 (0.50–0.80)
12 months	50	86	1.09 (0.75–1.58)	74	0.56 (0.39–0.80)
18 months	48	90	2.26 (1.44–3.54)	83	1.30 (0.82–2.05)
24 months	50	98	4.35 (3.03–6.23)	98	2.03 (1.37–3.00)
Adults	90	100	16.98 (14.07–20.49)*	100	7.15 (5.75–8.90)†

*significant difference in the GMC between infants and adults ($P < 0.001$, Student's T-test).
†signifcant difference in the GMC between infants and adults ($P < 0.001$, Student's T-test).

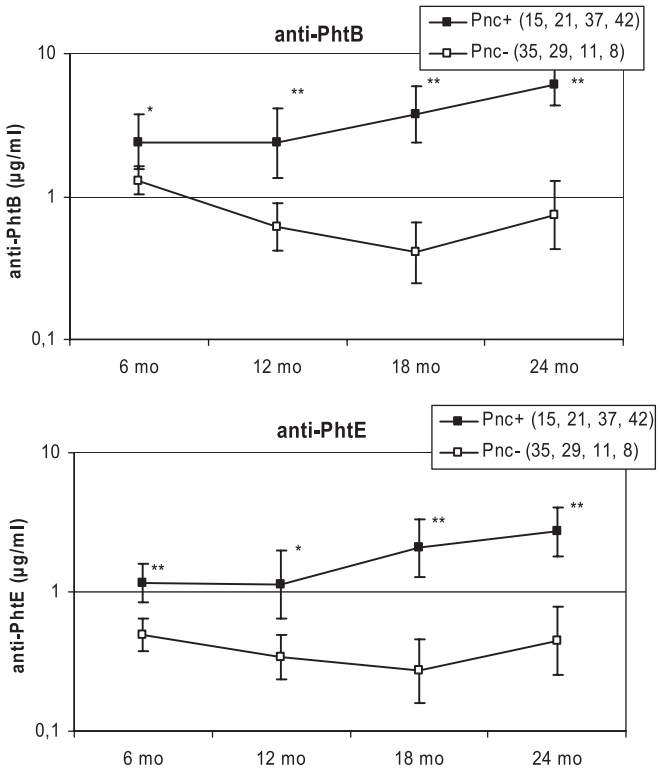

FIGURE 1. Development of serum anti-PhtB and -PhtE antibodies during the first 24 months of life in the pnc$^-$ and pnc$^+$ children. Numbers in parentheses indicate the number of samples analyzed at each age point. During the follow-up, the proportion of pnc$^+$ children increased, whereas the proportion of pnc$^-$ children decreased. Geometric mean concentrations with 95% confidence intervals are given. *$P <$ 0.05 and **$P < 0.001$ for pnc$^+$ versus pnc$^-$ children (Student's *t* test).

children with cultures negative for pneumococci (pnc$^-$) ($P <$ 0.001–0.05).

Anti-Pht Antibodies and the Risk of Subsequent Pneumococcal Carriage. The anti-Pht antibodies were associated with pneumococcal carriage 6 months later: the higher the anti-PhtB or -PhtE antibody concentration at 12 or 18 months, the higher was the odds of asymptomatic pneumococcal carriage. The odds ratios for an increase of one log unit in anti-PhtB or -PhtE concentration were 1.31 (1.07–1.59) and 1.27 (1.01–1.60) at 12 months, respectively, and 1.26 (1.03–1.53) and 1.10 (0.90–1.33) at 18 months, respectively.

Anti-Pht Antibodies and the Risk of Subsequent Pneumococcal AOM. Neither the anti-PhtB nor the anti-PhtE antibody concentration was significantly associated with the risk of subsequent pneumococcal AOM at 12 months [RR 1.0 (0.85–1.18) and RR 0.89 (0.71–1.13), respectively], or at 18 months [RR 0.84 (0.65–1.08) and RR 0.84 (0.64–1.10), respectively], even though a tendency toward a lower risk of pneumococcal AOM with higher concentration of anti-Pht antibodies was seen.

DISCUSSION

The objective of the present study was to characterize the development and role of serum antibodies to pneumococcal vaccine candidate proteins PhtB and PhtE in children during their first 2 years of life.

The majority of the serum samples in the subset of 50 children were positive for anti-Pht antibodies already at the age of 6 months. Both the percentage of positive samples and the GMCs decreased in the age interval 6–12 months and started to increase after the age of 12 months. The decrease in the antibody concentrations suggests that part of the anti-Pht antibodies measured at 6 months were of maternal origin. Although the anti-Pht concentrations increased with age, the antibody concentrations in children at the age of 24 months were still significantly lower than in adults.

The anti-PhtB and -PhtE concentrations were associated with prior pneumococcal exposure. This confirms that the Pht antigens used in EIA recognize antibodies are induced by pneumococci of different serotypes (data not shown), consistent with the existence of the PhtB and PhtE proteins among pneumococci.[3] In the pnc$^-$ children (prior cultures negative for pneumococci), the GMCs of anti-Pht antibodies decreased between the ages of 6 and 12 months, whereas in the pnc$^+$ children [prior culture(s) positive for pneumococci] the GMCs of anti-Pht antibodies stayed constant during the same age period. This suggests that the production of anti-Pht antibodies started already during the first year of life.

AOM is a mild but extremely common disease during childhood and its most common causative bacterium is the pneumococcus.[5,7] The relationship between the serum antibodies and the risk of pneumococcal AOM has been previously evaluated in the FinOM Cohort Study samples with several pneumococcal virulence-associated proteins: PsaA,[8] PpmA,[9] NanA[10] and PspA[11]. The results of these prior studies are consistent with the findings of the present study. The importance of serum antibodies in protection against a local infection such as AOM may be minor when compared with mucosal antibodies. We have previously reported that the presence

of salivary but not serum antibodies to PspA is associated with a lower risk of subsequent pneumococcal AOM at 18 months of age.[11]

We could not find any association between the serum anti-PhtB or -PhtE antibodies and protection against pneumococcal carriage at 12 and 18 months. On the contrary, the higher antibody concentrations were associated with a slightly increased risk of pneumococcal carriage 6 months later. A similar observation was earlier made with anti-PsaA and the possible explanations for the finding have been previously discussed by Rapola et al.[8] It is important to note that there are also many other antibody specificities besides anti-PhtB and -PhtE in the blood of children which may have an effect on the risk of pneumococcal carriage and AOM. Further, recent studies using animal models suggest that natural immunity, at least against pneumococcal colonization, may be elicited by acquired cellular immunity independently from antibodies.[12,13]

The studies on development or existence of anti-Pht in humans are sparse. Adults have been shown to naturally produce antibodies to PhtB and PhtE.[3] Young Filipino infants produce antibodies to PhtD[14] and antibodies to PhtA and PhtD are also detected in convalescent-phase sera of children with culture-confirmed pneumococcal bacteremia.[1] The results of the present study clearly confirm the immunogenicity of the PhtB and PhtE proteins in young children.

The studies on natural immunity against *S. pneumoniae* and its virulence-associated antigens offer important background information for pneumococcal vaccine development. We have now shown that pneumococcal proteins PhtB and PhtE are immunogenic in young children. The key issue for the Pht vaccine development is to understand the functional mechanisms of these antibodies as well as the concentrations of antibodies induced by vaccination. In young children, the anti-Pht antibodies induced by an optimally designed vaccine are expected to reach higher concentrations than antibodies induced by natural pneumococcal exposure.

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REFERENCES

1. Adamou JE, Heinrichs JH, Erwin AL, et al. Identification and characterization of a novel family of pneumococcal proteins that are protective against sepsis. *Infect Immun*. 2001;69:949–958.
2. Zhang Y, Masi AW, Barniak V, Mountzouros K, Hostetter MK, Green BA. Recombinant PhpA protein, a unique histidine motif-containing protein from *Streptococcus pneumoniae*, protects mice against intranasal pneumococcal challenge. *Infect Immun*. 2001;69:3827–3836.
3. Hamel J, Charland N, Pineau I, et al. Prevention of pneumococcal disease in mice immunized with conserved surface-accessible proteins. *Infect Immun*. 2004;72:2659–2670.
4. Syrjanen RK, Kilpi TM, Kaijalainen TH, Herva EE, Takala AK. Nasopharyngeal carriage of *Streptococcus pneumoniae* in Finnish children younger than 2 years old. *J Infect Dis*. 2001;184:451–459.
5. Kilpi T, Herva E, Kaijalainen T, Syrjanen R, Takala AK. Bacteriology of acute otitis media in a cohort of Finnish children followed for the first two years of life. *Pediatr Infect Dis J*. 2001;20:654–662.
6. Reference deleted in proof.
7. Pelton SI. Otoscopy for the diagnosis of otitis media. *Pediatr Infect Dis J*. 1998;17:540–543.
8. Rapola S, Jantti V, Eerola M, Makela PH, Kayhty H, Kilpi T. Anti-PsaA and the risk of pneumococcal AOM and carriage. *Vaccine*. 2003;21:3608–3613.
9. Bogaert D, Holmlund E, Lahdenkari M, et al. Development of antibodies against the putative proteinase maturation protein A in relation to pneumococcal carriage and otitis media. *FEMS Immunol Med Microbiol*. 2006;46:166–168.
10. Simell B, Jaakkola T, Lahdenkari M, et al. Serum antibodies to pneumococcal neuraminidase NanA in relation to pneumococcal carriage and acute otitis media. *Clin Vaccine Immunol*. 2006;13:1177–1179.
11. Simell B, Melin M, Jaakkola T, et al. Antibodies to PspA families 1 and 2 in saliva but not in serum of children were associated with a lower risk of pneumococcal AOM. In: *5th International Symposium on Pneumococci and Pneumococcal Diseases, Alice Springs, Australia, April 2–6, 2006*. [Abstract PO12. 02].
12. McCool TL, Weiser JN. Limited role of antibody in clearance of Streptococcus pneumoniae in a murine model of colonization. *Infect Immun*. 2004;72:5807–5813.
13. Malley R, Trzcinski K, Srivastava A, Thompson CM, Anderson PW, Lipsitch M. CD4+ T cells mediate antibody-independent acquired immunity to pneumococcal colonization. *Proc Natl Acad Sci USA*. 2005;102:4848–4853.
14. Holmlund E, Quiambao B, Ollgren J, et al. Development of antibodies to pneumococcal proteins PhtD, CbpA and LytC in Filipino pregnant women and their offspring in relation to pneumococcal carriage. In: *5th International Symposium on Pneumococci and Pneumococcal Diseases, Alice Springs, Australia, April 2–6, 2006*. [Abstract PO6. 25].

EPIDEMIOLOGIC STUDY ON KAWASAKI DISEASE IN BEIJING FROM 2000 THROUGH 2004

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Abstract: A hospital-based survey of Kawasaki disease was performed in all 45 hospitals with in-patient beds in Beijing during the 5-year period from 2000 through 2004. A total of 1107 patients were enrolled, with an annual incidence varying from 40.9 to 55.1 per 100,000 children <5 years of age. The incidence of coronary complications was 20.6% in the acute stage, and 6.9% in the 1–2 month follow-up.

Key Words: Kawasaki disease, epidemiology, incidence, season, Beijing

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Reported incidences of Kawasaki disease (KD) differ considerably throughout the world.[1–8] Japan has the highest incidence with a mean annual incidence of 138.8 and 151.2 per 100,000 children <5 years of age between 2001 and 2002.[1] The incidences vary widely from 3.7 to 47.7 per 100,000 <5-year-old in the United States, Europe and Australia.[2,3] We performed a survey in Beijing from 1995 through 1999,[4] and reported an annual incidence of 18.2 to 30.6 per 100,000. This second survey was performed to further evaluate the epidemiologic pictures of KD in Beijing from 2000 through 2004.

METHODS

A survey questionnaire form and diagnostic guideline for KD were sent to all children's hospitals and hospitals with pediatric in-patient beds throughout Beijing and its suburbs. The study method is similar to that of our previous study[4] and those in the bi-annual national epidemiologic studies in Japan.[1] The fifth edition of revised KD diagnostic guideline was used for the diagnosis of KD.

In each hospital, pediatric hospitalizations for patients <18 years of age with an International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) code for KD (446.1) listed as the major or the second diagnosis on the discharge record were selected for our analysis. Physicians were asked to review the medical records and to fill out the questionnaire form for all their patients with KD diagnosed during the 5-year period from January 2000 through December 2004.

Two senior pediatricians were designated to ensure investigators' compliance with the study protocol. The diagnosis was further verified by 2 senior pediatricians in our institution after all forms were collected. A coronary complication was defined as presence of dilatation or aneurysm of coronary artery evaluated by echocardiography based on the age-adjusted normal references derived from 185 normal children.[9]

Annual incidence was calculated by dividing the number of patients by the population data obtained from the Census Office of Beijing Municipality. Confidence interval (CI) was calculated as appropriate. SPSS 11.0 for Windows (SPSS Inc., Chicago) was used for the analysis. $P < 0.05$ was considered statistically significant.

RESULTS

All 45 hospitals with in-patient bed responded to the survey. A total of 1567 potential patients were reported. Of them, 460 (29.4%) who did not satisfy our inclusion criteria were excluded. The remaining 1107 patients were enrolled as study subjects. Based on population census data in Beijing from 2000 through 2004, the annual incidence of KD varied from 40.9 to 55.1 per 100,000 children <5 years of age, with an average annual incidence of 49.4 (95% CI, 46.6–52.4) per 100,000 children <5 years of age (Table 1). There was a trend of increase in incidence rates of KD from 2000 through 2004 in girls and total children ($P = 0.019–0.002$, Fig. 1). For boys, the trend of increase did not reach significance ($P = 0.224$). When we put these data on incidences and those reported from our previous study[4] together, there was a clear trend of increase in incidences in boys, girls and total children (χ^2 197.8–271.2, $P < 0.0001$).

Ages varied from 1 month to 13.8 years with an average of 2.6 ± 2.2 years. The peak age was 1 year. There were 716 boys and 391 girls, with a male to female ratio of 1.83:1. There were 2 peaks in the spring and summer during each of the 5-year periods. The lowest occurrence was in December and January. In 2000 and 2004, in addition to the 2 peaks in the spring and summer, there was another peak occurring in October in 2000, and November and December in 2004.

TABLE 1. Numbers of Children With Kawasaki Disease by Year and by Gender

Year	Boys		Girls		Total	
	Number	Incidence*	Number	Incidence	Number	Incidence
2000	124	51.9	70	29.8	194	40.9
2001	141	65.3	73	35.1	214	50.5
2002	146	64.8	60	28.8	206	47.5
2003	148	67.3	73	37.5	221	53.3
2004	157	60.3	115	49.3	272	55.1
Total	716	61.7	391	36.2	1107	49.4
χ^2	—	5.689	—	17.006	—	11.781
P	—	0.224	—	0.002	—	0.019

*Incidence was number of cases per 100,000 children under 5 yr of age.

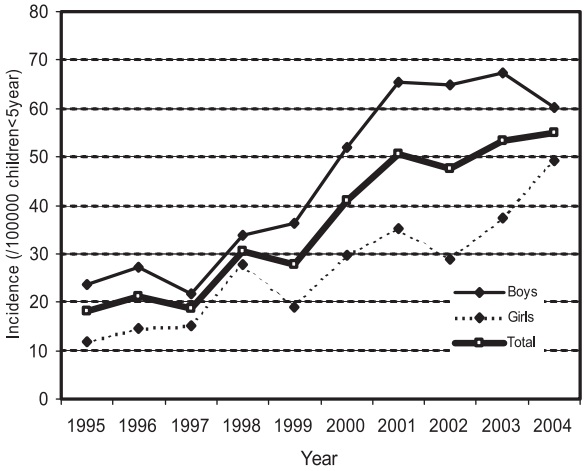

FIGURE 1. Annual incidences of Kawasaki disease in Beijing from 1995 through 2004 by gender and year of onset. The data of 1995 through 1999 was from a previous study.[4]

Of the 1107 patients included, 1104 (99.7%) had data on recurrence. Fifteen had recurrence, with a recurrence rate of 1.4% (95% CI, 0.8%–2.3%). Two had a family history of KD.

Data on coronary complications were obtained in 1106 children. In the acute stage, 228 had coronary abnormalities (20.6%). Coronary abnormality was more common in boys than girls (23.9% vs. 14.6%, χ^2 13.6, $P < 0.001$). The incidence of coronary complications was 22.2% in 2000, 27.6% in 2001, 22.8% in 2002, 18.0% in 2003, and 14.4% in 2004. There was trend of declining in the 5 years' period ($\chi^2 = 14.4$, $P = 0.006$).

Coronary aneurysm occurred in 48 patients (4.3%; 95% CI, 3.2%–5.7%). Of them, 32 (4.5%) were boys and 16 (4.1%) were girls with no significant difference between them ($\chi^2 = 0.082$, $P = 0.775$). Giant coronary aneurysm was identified in 4 children (0.4%).

No death was reported in the acute stage of the disease. Data on 1–2 month follow-up were obtained in 288 patients. Twenty (6.9%) patients still had coronary abnormalities. No other complications or death were reported.

DISCUSSION

Up to now, there have been limited epidemiologic data of KD from China. The annual incidence of KD in this study is higher than the rates in our previous report from 1995 through 1999. The difference might be ascribed to the increased recognition of KD in Beijing and its suburbs. After the first KD survey, many workshops and symposiums were organized by us and others to further train pediatricians for their diagnosis and treatment of KD. Pediatricians were probably more aware of KD in the last 5 years than before. The second possibility would be that the incidence rate truly increased as indicated from Japanese studies. We demonstrated a trend of increasing incidence in our previous survey. Thus, we speculate that the incidence increased in Beijing during the last 10-year period. The recent survey reporting an incidence of 66% from Taiwan supports this point.[6]

Although the incidence increased in Beijing, it is still lower than rates reported from Japan.[1] A recent survey in Hong Kong reported an incidence of 39 per 100,000 from 1994 through 2000.[5] Even the highest rate from the more recent report from Taiwan[6] is much lower than the incidence rates in Japan. This might hint that

environmental or genetic factors play an important role in the differences in incidence of KD, although we may not completely exclude the possible role of medical care and socioeconomic factors.

In this survey we identified 2 peaks in the spring and summer in the seasonal distribution that are similar to our previous survey.[4] Interestingly, we found a third peak in the monthly distribution curve in 2000 and 2004. The third peak was in the winter when KD is more common in the United States,[2,8] the United Kingdom and Japan.[1] This indicates that there might be some changes of risk factors of KD in Beijing during these years. Further epidemiologic studies are needed to clarify the factors.

REFERENCES

1. Nakamura Y, Yanagawa H. The worldwide epidemiology of Kawasaki disease. *Prog Ped Cardiol*. 2004;19:99–108.
2. Belay ED, Holman RC, Clarke MJ, et al. The incidence of Kawasaki syndrome in West Coast health maintenance organizations. *Pediatr Infect Dis J*. 2000;19:828–832.
3. Gardner-Medwin JM, Dolezalova P, Cummins C, et al. Incidence of Henoch-Schonlein purpura, Kawasaki disease, and rare vasculitides in children of different ethnic origins. *Lancet*. 2002;360:1197–1202.
4. Du ZD, Zhang T, Liang L, et al. Epidemiologic picture of Kawasaki disease in Beijing from 1995 through 1999. *Pediatr Infect Dis J*. 2002; 21:103–107.
5. Ng YM, Sung RY, So LY, et al. Kawasaki disease in Hong Kong, 1994 to 2000. *Hong Kong Med J*. 2005;11:331–335.
6. Chang LY, Chang IS, Lu CY, et al. Epidemiologic features of Kawasaki disease in Taiwan, 1996–2002. *Pediatrics*. 2004;114:e678–e682.
7. Harnden A, Alves B, Sheikh A. Rising incidence of Kawasaki disease in England: analysis of hospital admission data. *Br Med J*. 2002;324:1424–1425.
8. Newburger JW, Takahashi M, Gerber MA, et al. Diagnosis, treatment, and long-term management of Kawasaki disease: a statement for health professionals from committee on rheumatic fever, endocarditis, and Kawasaki disease, council on cardiovascular disease in the young, American Heart Association. *Pediatrics*. 2004;114:1708–1733.
9. Jin H, Liang YC, Zhang XC. The echocardiographic study on coronary artery in normal children. *Chin J Pediatr*. 1988;26:257–259 (in Chinese).

VALGANCICLOVIR FOR CONGENITAL CMV INFECTION: A PILOT STUDY ON PLASMA CONCENTRATION IN NEWBORNS AND INFANTS

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Abstract: The pharmacokinetics of valganciclovir were studied in 8 infants ranging in age from 4 to 90 days (mean 20 days). We suggest that doses of 15 mg/kg given twice daily may be suitable for neonates and young infants.

Key Words: valganciclovir, newborns, pharmacokinetics, congenital, CMV

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Congenital cytomegalovirus (CMV) infection is one of the most common cause of acquired sensorineural hearing loss in children. It is also a quite frequent, often underestimated, cause of neurologic dysfunction with an impact on the everyday life of affected children. Sound evidences of the efficacy of any treatment on the progression of CMV infection are lacking. A randomized study showed that intravenous ganciclovir (IV-GCV) for 6 weeks might prevent, in symptomatic infants, subsequent hearing deterioration.[1] However, IV-GCV administration causes prolonged hospitalization and risks of infections of the central venous catheter.

Valganciclovir (V-GCV), the monovalyl ester of ganciclovir (GCV), is an oral prodrug of GCV approved for the treatment of CMV retinitis in adults with human immunodeficiency virus infection. V-GCV is also indicated for the prevention of CMV disease in kidney and heart transplant patients.[2] The availability of an oral antiviral drug may be particularly useful in infants and children. However, pharmacokinetic data on V-GCV in children (particularly in newborns), are still lacking, apart 3 case reports in a 6-year-old girl[3] and in 2 newborns.[4,5] Here we report plasma GCV concentrations after V-GCV oral administration in newborns and infants with symptomatic congenitally-acquired CMV infection.

METHODS

Case Definition. Infants admitted to our centre for congenital infections with signs which may suggest congenital CMV infection (thrombocytopenia, petechiae, elevated liver enzymes, seizures, intracranial calcifications, microcephalia), were tested by quantitative polymerase chain reaction (qPCR) to detect CMV DNA in whole blood, plasma and urine. Congenital infection was defined by the detection of CMV DNA in samples obtained within 2 weeks of life.

Treatment. Antiviral treatment was undertaken after informed consent from the parents. IV-GCV was initially administered, as an induction treatment, to all children as 5 mg $\cdot$ kg^{-1} $\cdot$ dose^{-1} twice daily (bid) for 1 week.[6] Because the parents refused consent to introduce a central line for IV-GCV treatment, during the subsequent 5 weeks of treatment V-GCV was given by dividing the 450 mg tablets. V-GCV results in 40–60% bioavailability for GCV.[5,7] Thus, 15 mg/kg V-GCV dose once daily (qd) was orally given to 4 children (group A). Because preliminary data on blood samples showed very low levels of GCV in these children, 15 mg $\cdot$ kg^{-1} $\cdot$ dose^{-1} bid of V-GCV were administered to the following 4 children (group B). For safety reasons, laboratory tests for renal impairment and complete blood count with absolute neutrophil count were performed weekly.

Plasma GCV and Valganciclovir Concentrations. For ethical reasons, a complete pharmacokinetics assessment was not performed. Plasma samples were drawn immediately before the administration of IV-GCV or V-GCV (C_{min}) and at the presumed C_{max}, ie, 1 hour after IV-GCV or 1.5 hour after V-GCV administration (according to the results of previous studies performed in children).[5] Samples were obtained after multiple administrations to reach a steady-state. Thus, blood samples were scheduled at day 3 (during the IV-GCV week treatment) and at day 10 (after 3 days of oral V-GCV treatment).

Plasma GCV concentrations were determined by high-performance liquid chromatograph (HPLC) (lowest sensitivity limit 0.05 mg/L) with PerkinElmer Series 200 HPLC connected to a PerkinElmer Series 200 UV detector. A Beckman Ultrasphere C_{18} 4.6 $\times$ 150 mm 5-μm column preceded by a 5-μm 10 $\times$ 3 mm precolumn was used for the analysis, at a flow-rate of 1.2 mL/min. The mobile phase consisted of a 0.02 mol/L potassium dihydrogenphosphate (pH 3.5). The UV detector emission were set at 250 nm. Acyclovir was employed as an internal standard, and drug and internal standard were isolated by solvent extraction. An aliquot (0.5 mL) was pipetted into a polypropylene tube and acetonitrile (0.5 mL) added. The mixture was vortex mixed briefly and then centrifuged at 1200*g* for 15 minutes. The supernatant was evaporated to dryness, the residue was reconstituted in water and injected into the HPLC. In this system, GCV and the internal standard had retention

times of 7.2 and 9.2 minutes, respectively. This assay was linear (r = 0.995) over the concentration range of 0.05–10 μg/mL.[8]

Virologic Evaluations. Real-time qPCR was performed in urine samples (COBAS AMPLICOR CMV MONITOR Test, Roche Diagnostics, Branchburg, NJ). Urine samples were drawn before starting treatment, after 1 week with IV-GCV treatment, twice during V-GCV treatment (at 3 and 5 weeks) and 3 weeks after the suspension of treatment. Mean values of urine CMV DNA during V-GCV treatment in each infant were calculated to better describe the antiviral effect of treatment during the 5-week period with oral V-GCV. Results were expressed as $\log_{10}$ copies/mL.

Statistical Analysis. Differences in plasma GCV concentrations and age at start of treatment, reported as median and range, were evaluated by means of the Mann–Whitney *U* test. CMV DNA values were expressed as mean and standard deviations and differences were calculated by means of the *t* test for independent or coupled samples, as appropriate. *P* values less than 0.05 were considered significant. Analysis was performed using SPSS for Windows (version 11.0).

RESULTS

Eight infants (5 males) showing severe CNS CMV-related features were treated. Two infants had neutropenia (considered CMV-related because it developed before starting antiviral treatment) and one had thrombocytopenia. IV-GCV treatment was started at the median age of 20 (range: 4–90) days. Neutropenia represented the only overwhelming side effect during treatment in one infant in each group.

Plasma GCV Concentrations. Validation data for accuracy and precision were: coefficient of variation between 1% and 4%; intraday and interday accuracy was in the range of 92–100.8%. Plasma GCV C_{max} was 1.95 (0.98–3.96) μg/mL after administration of IV-GCV, whereas after V-GCV administration was 0.42 (0.40–0.74) μg/mL in group A and 3.1 (2.5–3.9) μg/mL in group B (respectively IV-GCV vs. group A: $P = 0.003$ and IV-GCV vs. group B not significant; group A vs. group B: $P = 0.03$).

Individual values of GCV plasma C_{min} and presumed C_{max} in group A and in group B patients, after V-GCV administration are reported in Figure 1.

Change in Urine CMV Load With Treatment. Mean and standard deviation urine CMV DNA values before treatment in the whole study group was 6.9 ± 1.32 copies/mL. Viruria significantly decreased at 3.0 ± 0.59 copies/mL during IV-GCV treatment ($P < 0.001$). During V-GCV treatment, viruria was significantly lower in group B when compared with group A (respectively 2.66 ± 0.51 vs. 3.72 ± 0.71 copies/mL, $P = 0.049$). When comparing viruria during IV-GCV versus V-GCV treatment within each group, there was no significant difference between mean urine CMV DNA copies/mL nor in group A (respectively 2.95 ± 0.37 vs. 3.72 ± 0.71; P = NS) nor in group B (3.05 ± 0.82 vs. 2.66 ± 0.51; P = NS). Mean urine CMV DNA after treatment rebounded at 4.96 ± 0.97 in group A and at 4.55 ± 1.84 in group B (group A vs. group B: P = NS). No child had undetectable levels of CMV DNA in urine during antiviral treatment.

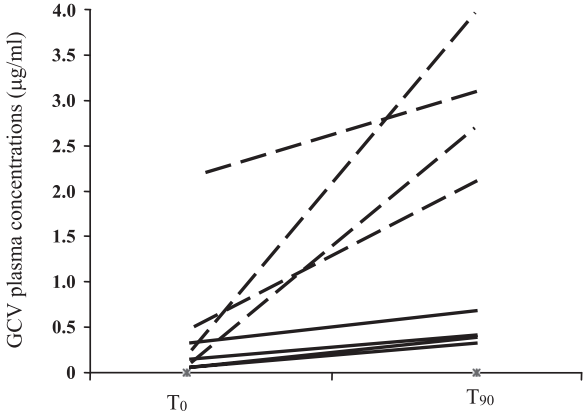

FIGURE 1. Plasma GCV concentrations at T0 (immediately before V-GCV administration) and at T90 (90 minutes after V-GCV administration) in group A (solid lines) and in group B (dotted lines) infants.

DISCUSSION

To our knowledge, this is the first study, apart 2 single-case reports,[4,5] which investigate plasma V-GCV concentrations in newborns and infants. Schulzke et al[7] administered V-GCV to a newborn up to 56 mg · kg^{-1} · day^{-1}. Meine Jansen et al[4] gave a newborn a dose ranging from 280 to 850 mg/m^2 bid, that is a dose of 70 mg bid, in a 1-month-old child measuring 0.25 m^2. In our observational study, we administrated to the first 4 newborns a V-GCV dosage of 15 mg/kg qd. This dose was estimated taking in account a 43% bioavailability for GCV reported by Burri et al in a 6-year-old girl.[3] However, this dosage resulted in very low plasma GCV concentrations after 90 minutes from administration. Therefore, in the subsequent 4 cases, we gave the same dosage (15 mg/kg) bid, achieving a significant increase of plasma GCV concentration.

The goals of antiviral treatment are suppression of viral replication and urine CMV DNA undetectability. Virologic evaluations in our patients showed that both V-GCV dosages resulted in a partial suppression of viruria, which had been significantly reduced by a 1-week treatment with IV-GCV. Notably, the bid dosing achieved significantly lower CMV DNA in urine. No patient showed a complete viral clearance, however, only exceptionally undetectable viruria was reported in other studies after 6-weeks IV-GCV administration.[9,10]

Our findings suggest that a 15 mg/kg dose bid of V-GCV might be suitable in newborns and infants. Further pharmacokinetics studies should be performed to establish the optimum dosage in this age group. V-GCV was easily administered to our patients with a good compliance by children and their parents. V-GCV was also well tolerated, with only a mild neutropenia in 2 children, which resolved soon after the suspension of treatment. A syrup preparation might be even more convenient for managing therapy at home and suitable for dosage adjustments according to increasing body weight.

Our study has some limitations. We carried out a pilot study on a small number of subjects, and only 5 were newborns, preventing us from stratifying results by age. In addition, we did not perform a complete pharmacokinetic assessment, useful because newborns and infants may have slower gastrointestinal absorption than older children and adults. However, we believe that our study may stimulate further studies in newborns and infants, in the attempt to found the optimal dosage with the higher effect and the lower rate of hematological adverse events.

REFERENCES

1. Kimberlin DW, Lin CY, Sanchez PJ, et al. Effect of ganciclovir therapy on hearing in symptomatic congenital cytomegalovirus disease involving the central nervous system: a randomized, controlled trial. *J Pediatr*. 2003;143:16–25.
2. Ayala E, Greene J, Sandin R, et al. Valganciclovir is safe and effective as pre-emptive therapy for CMV infection in allogenic hematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2006;37:851–856.
3. Burri M, Wiltshire H, Kahlert C, et al. Oral valganciclovir in children: single dose pharmacokinetics in a six-year-old girl. *Pediatr Infect Dis J*. 2004;23:263–266.

4. Meine Jansen CF, Toet MC, Rademaker CM, et al. Treatment of symptomatic congenital cytomegalovirus infection with valganciclovir. *J Perinat Med*. 2005;33:364–346.
5. Schulzke S, Buhrer C. Valganciclovir for treatment of congenital cytomegalovirus infection. *Eur J Ped*. 2006;165:575–576.
6. Michaels MG, Greenberg DP, Sabo DL, Wald ER. Treatment of children with congenital cytomegalovirus infection with ganciclovir. *Pediatr Infect Dis J*. 2003;22:504–508.
7. Martin DF, Dierra-Madero J, Walmsley S, et al. A controlled trial of valganciclovir as induction therapy for cytomegalovirus retinitis. *N Engl J Med*. 2002;346:1119–1126.
8. Loregian A, Gatti R, Palù G, De Palo EF. Separation methods for acyclovir and related antiviral compounds. *J Chromatogr B Biomed Sci Appl*. 2001;764:289–311.
9. Whitley RJ, Cloud G, Gruber W, et al. Ganciclovir treatment of symptomatic congenital cytomegalovirus infection: results of a phase II study : National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group. *J Infect Dis*. 1997;175:1080–1086.
10. Tanaka-Kitajima N, Sugaya N, Futatami T, et al. Ganciclovir therapy for congenital cytomegalovirus infection in six infants. *Pediatr Infect Dis J*. 2005;24:782–785.

PANTOEA AGGLOMERANS SEPTICEMIA IN THREE NEWBORN INFANTS

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Abstract: *Pantoea* infections are rare in humans, especially in neonates. Infections are usually associated with plant thorn injury or outbreaks traced to contaminated parenteral nutrition, intravenous anesthetics or packed erythrocytes. Between 1st of January 1994 and 1st of June 2005, 125 of 6383 patients (2%) in a 24-bed level III NICU became colonized with *Pantoea agglomerans*. Three newborns exhibited late-onset *Pantoea agglomerans* septicemia and died. Sporadic cases of *Pantoea agglomerans* septicemia have not been reported in neonatal intensive care so far.

Key Words: neonate, nosocomial septicemia, *Pantoea agglomerans*

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Although *Pantoea agglomerans* has been reported in osteomyelitis, septic arthritis and bloodstream infections traced to contaminated parenteral nutrition, intravenous anesthetics and packed erythrocytes, neonatal septicemia involving this Gram-negative rod is rare.[1–6] During a 137-month period, 3 patients in a level III neonatal intensive care unit (NICU) developed *Pantoea agglomerans* septicemia and died.

CASE REPORTS

Patient A, a term girl, birth weight 3810 g, was diagnosed as having pulmonary atresia with intact ventricular septum. Epoprostenol infusion was started. She received endocarditis prophylaxis for balloon vavulotomy at day 7. At day 10, cardiac surgery had to be postponed because of a right-sided hematothorax and hypovolemic shook, complications of a central venous catheter insertion. A chest-tube was inserted and the infant received fluids and packed erythrocytes through a femoral line. Her condition stabilized.

On day 12, she became febrile with leukocytosis with a marked shift to the left and thrombocytopenia (Table 1). She was admitted to the NICU. She was intubated and ventilated, needed circulatory support and showed signs of diffuse intravascular coagulation (DIC). Amoxicillin/clavulanic acid and netilmicin were started. Blood cultures grew *Pantoea agglomerans*. CSF-cultures were not performed. Despite antibiotics her respiratory and circulatory condition deteriorated. After 72 hours, the antibiotic regimen was changed to vancomycin and cefotaxime while blood cultures remained positive for *Pantoea agglomerans*. No abdominal focus nor a catheter related thrombosis were present. Although the organism was susceptible to all antimicrobials tested except amoxicillin, the infants condition further deteriorated and she died at the age of 17 days. The focus for *Pantoea aglomerans* sepsis remained unknown; cultures of sputum, urine and feces were negative at the time of positive blood cultures. Cultures of parenteral nutrition were not performed. Postmortem examination revealed a thoracic spinal abscess which might explain the ongoing sepsis.

Patient B, a boy, was born at 29 + 6 weeks gestation after prolonged rupture of membranes, birth weight 1795 g. He was admitted to the NICU and received antibiotics (amoxicillin and gentamicin) for suspected septicemia. Cultures remained negative. He was doing well until 5 days of age when his condition rapidly deteriorated. He was intubated and ventilated, needed circulatory support and showed signs of DIC (Table 1). Antibiotic therapy was changed to amoxicillin and cefotaxime. Twenty-

TABLE 1. Clinical Features and Laboratorium Characteristics in Patients With Neonatal *Pantoea* Sepsis

Case	Birth Weight (g)	Gestational Age (wks)	Age at Onset of Sepsis (d)	Clinical Condition	Respiratory Distress	Shock	DIC	Lowest Platelet Count (10×10 9/L)	Lowest White Cell Count (10×10 9/L)	Highest CRP (mg/L)	Outcome
1 (Patient A)	3810	40	12	CHD	+	+	+	6	17.4	187	Death
2 (Patient B)	1795	29	5	RDS	+	+	+	6	10.7	143	Death
3 (Patient C)	630	28	20	IUGR, RDS	+	+	+	4	1.6	334	Death
4	950	26	11	RDS	+	+	+	86	2.6	—	Death
5	3300	40	4	Asphyxia	+	+	+	19	6.4	—	Alive
6	1700	33	180	Pneumonia	+	+	+	29	8.6	—	Death
7	1500	32	3	RDS	+	+	+	33	6.1	—	Death
8	3200	40	5	VACTERL	+	+	−	53	7.3	—	Death
9	1670	36	5	IUGR	+	+	+	23	1.9	—	Death
10	2000	36	4	Asphyxia	+	+	+	25	3.7	—	Death
11	1200	26	5	RDS	+	+	+	13	4	—	Death

Our Patients (1–3) and Patients by Van Rostenberghe et al (4–11).

DIC, disseminated intravascular coagulation; RDS, respiratory distress syndrome; VACTERL, vertebral, anal, cardiac, tracheoesophageal fistula, renal and limb anomaly; CHD, congenital heart disease.

four hours after his deterioration blood cultures grew *Pantoea agglomerans*. CSF-culture remained negative. Although the organism was susceptible to gentamicin and cefotaxime, the infants condition deteriorated. Amoxicillin/clavulanic acid and tobramycin were added to the antibiotic regimen. A central venous catheter was inserted for parenteral nutrition. The infant developed massive intracerebral bleeding and died at the age of 8 days. The focus for *Pantoea agglomerans* remained unknown; cultures of feces, urine and sputum were negative as were cultures of the mother's milk and the intralipid infusion fluid. Postmortem examination was not performed

Patient C, was born at 28 + 3 weeks by cesarean section because of fetal distress, birth weight 630 g. She received continuous positive airway pressure (CPAP) for respiratory distress syndrome and antibiotics (amoxicillin and gentamicin) for suspected septicemia for 7 days. A central venous catheter was inserted for parenteral nutrition. Feeding problems resulting from immature bowel syndrome were present.

By 12 days of age surveillance cultures revealed gastrointestinal colonization with *Pantoea agglomerans* susceptible to all antimicrobials tested. At day 18, the infant exhibited apnea, bradycardia and feeding problems and was treated with amoxicillin/clavulanic acid and gentamicin for suspected necrotizing enterocolitis. After initial stabilization, her condition deteriorated 48 hours later. She was intubated and ventilated, needed circulatory support and showed signs of DIC. Leukopenia and thrombocytopenia were present (Table 1). Amoxicillin and gentamicin susceptible *Pantoea agglomeran* was isolated from previous blood cultures as were coagulase negative staphylococci. CSF-culture remained negative. Antibiotic therapy was changed to vancomycin and ceftotaxime. In the next 48 hours her condition did not improve. The central venous catheter was removed, a new catheter was inserted and cefotaxime was changed to meropenem therapy. Breastmilk cultures were negative. Despite maximal supportive therapy the infants' condition gradually deteriorated and she died at 34 days of age. Postmortem examination was not performed.

DISCUSSION

Pantoea is a Gram-negative noncapsulated, nonspore forming rod. It is a member of the *Enterobacteriaceae.* The genus *Pantoea* forms a monophyletic unit closely related to *Erwinia*. The most prominent species of the genus is *Pantoea agglomerans*, formally known as *Enterobacter agglomerans* and earlier referred to as *Erwinia herbicola.*[1,2] It can be isolated from plants, human feces and animal feces.

Pantoae agglomerans infection is rare in humans and is usually associated with plant thorn arthritis or synovitis.[1] Nosocomial septicemia has been traced to outbreaks related to contaminated parenteral nutrition, intravenous anesthetics and packed erythrocytes.[2–5,7] So far there have only been 4 reports of nosocomial infections in neonates and infants.[5–8]

Between 1st of January 1994 and 1st of June 2005, 125 of 6383 patients (2%) in a 24-bed level III NICU became colonized with *Pantoea agglomerans.* These colonization's were not clustered. Molecular typing (random amplification of polymorphic DNA with enterobacterial repetitive intergenic consensus primers) was performed.[9] Two blood culture isolates (patient B and C) were compared with isolates from 5 colonized neonates and another blood isolate from a 16-year-old boy. Isolates were different except for isolates of 2 colonized newborns.

The use of broad spectrum antibiotics is known to increase the risk of colonization with *Enterobacteriaceae*[2] and gastrointestinal colonization could possibly be a reservoir for the organism. Three patients in our NICU developed culture proven late-onset septicemia. Only one of them was previously colonized with *Pantoea agglomerans*. Suspected NEC with increased intestinal permeability favoring the passage of *Pantoea agglomerans* into the blood could possibly explain for septicemia in this patient.

As previously reported *Pantoea agglomerans* in general seems to be noninvasive. However, if introduced directly into blood, it is as virulent as any other common Gram-negative rod.[2] All newborns exhibited respiratory failure, tachycardia, hypotension and DIC. The clinical presentation was compatible with the presentation reported during an outbreak in a Malaysian neonatal intensive care unit (Table 1).[8] Although the organism was susceptible to all antimicrobials tested, except amoxicillin in patient A, their condition gradually worsened despite maximal supportive therapy and they died of ongoing sepsis. The lack of response to seemingly appropriate antimicrobial therapy could be consistent with a focus of infection like an abscess, an infected thrombus, a contaminated central venous catheter or contaminated parenteral nutrition.[2,5] In patient A, a focus for the ongoing sepsis was present. In patients C, a focus was suspected and in patient B there was no identified focus.

Compared with other reports mortality in our patients was high, in part at least because of the high-risk population.[2,10] High case fatality was also reported during the outbreak in a Malaysian neonatal intensive care unit resulting from infected parenteral nutrition solutions.[5,8]

REFERENCES

1. Kratz A, Greenberg D, Barki Y, et al. Pantoea agglomerans as a cause of septic arthritis after palm tree thorn injury; case report and literature review. *Arch Dis Child*. 2003;88542–88544.
2. Maki DG, Rhame FS, Mackel DC, et al. Nationwide epidemic of septicemia caused by contaminated intravenous products. I. Epidemiologic and clinical features. *Am J Med*. 1976;60:471–485.
3. Bennet SN, McNeil MM, Lee MP, et al. Postoperative infections traced to contamination of an intravenous anesthetic, propofol. *N Eng J Med*. 1995;3333:147–154.
4. Arduino MJ, Bland LA, Tipple MA, et al. Growth and endotoxin production of *Yersinia enterocolitica* and *Enterobacter agglomerans* in packed erythrocytes. *J Clin Microbiol*. 1989;27:1483–1485.
5. Habsah H, Zeehaida M, van Rostenberghe H, et al. An outbreak of *Pantoea* spp. in a neonatal intensive care unit secondary to contaminated parenteral nutrition. *J Hosp Infect*. 2005;61:213–218.
6. Elliott TS, Ispahani P, Cowlishaw WA. Gram-negative bacillary meningitis in neonates: a glimmer of therapeutic success. *J Antimicrob chemother*. 1986;17:245–250.
7. Cicchetti R, Iacobini M, Midulla F, et al. *Pantoea agglomerans* sepsis after rotavirus gastroenteritis. *Ped Infect Dis J*. 2006;25:280–281.
8. Van Rostenberghe H, Wan Pauzi WI, Habsha H, et al. The clinical picture of neonatal infection with *Pantoea* species. *Jpn J Infect Dis*. 2006;59:120–121.
9. Koo HS, Kim JS, Eom JS. Pseudo outbreak of *Pantoea* species bacteremia associated with contaminated cotton pledgets. *Am J Inf Control*. 2006;34:443–446.
10. Matsaniotis NS, Syriopoulou VP, Theodoridou MC, et al. Enterobacter sepsis in infants and children due to contaminated intravenous fluids. *Infect Control*. 1984;5:471–477.

FOMITE-TRANSMITTED COCCIDIOIDOMYCOSIS IN AN IMMUNOCOMPROMISED CHILD

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Abstract: An unusual, nonendemic case of fomite-transmitted, disseminated coccidioidomycosis in a neutropenic 3-year-old boy is presented. Accurate diagnosis of coccidioidomycosis hinges on recognition of host risk factors, clinical signs and symptoms, and effective implementation of diagnostic studies. Timely diagnosis and treatment is critical for improved morbidity and mortality in the pediatric oncology population.

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CASE REPORT

Invasive fungal infections are a major cause of morbidity and mortality in pediatric oncology patients. A case of fomite-transmitted, disseminated coccidioidomycosis in a neutropenic 3-year-old boy is presented, highlighting several potential pitfalls in the management of neutropenic fever patients.

A 3-year-old Caucasian boy, undergoing treatment of bilateral Wilms' tumor, presented with a 1-day history of fever, diarrhea, and emesis in the setting of neutropenia. A regimen of empiric broad-spectrum antibiotics was administered and 5 days later expanded to include liposomal amphotericin B (Ambisome), as the physical examination did not suggest an etiology for his symptoms. Laboratory tests included a white blood cell count of $<400/mm^3$ with an absolute neutrophil count of zero and 100% lymphocytes. Hemoglobin was 8.6 g/dL. The platelet count was $34{,}000/mm^3$. Liver associated enzymes were trending downward from a prior chemotherapy-associated hepatitis with alanine aminotransferase of 125 U/L and aspartate aminotransferase of 127 U/L. Multiple aerobic and anaerobic blood cultures, viral hepatitis studies and stool studies for *C. difficile* and bacterial culture were all negative.

The appearance of unique dermatologic findings, the finding on noncontrast chest computed tomography of bilateral pneumonia with left pleural effusion and a new pulmonary nodule, and sonographic diagnosis of typhlitis complicated his 14 day ICU course. Skin findings included an erythematous indurated plaque on his left, proximal posteriolateral thigh, and sharply demarcated erythema and edema of the distal fingers. Skin biopsy and bronchoscopy were considered, but not performed. Gradually, the dermatologic, gastrointestinal and pulmonary findings remitted. With clinical improvement and no other identifiable source of infection, the patient's antimicrobial therapy was discontinued after receiving 3 weeks of antibacterial and antifungal coverage.

The day following discontinuation of antimicrobial therapy, 1 of the patient's fungal blood cultures grew *Coccidioides immitis*, which was confirmed by rRNA probe. As the patient became more active, he had difficulty bearing weight and complained of left leg pain. Magnetic resonance imaging demonstrated a solid, enhancing lesion in the left vastus lateralis muscle. The primary service opted to manage conservatively without biopsy and continue empiric treatment with antifungal therapy, given patient improvement and consistency of the vastus lateralis lesion with *Coccidioides immitis* infection.

DISCUSSION

Detailed review of the patient's social history revealed that the patient lived in the Washington DC metropolitan area, and had never traveled to regions endemic for *Coccidioides immitis*. The patient's grandparents had visited from Arizona one week before the patient received chemotherapy, and 17 days before onset of symptoms. The parents reported specific contact with 2 of the Grandfather's items. The child helped carry an old, "dirty and dusty" suitcase, and played with a pair of everyday-use shoes that had been worn in an outdoor construction site at the grandparent's home. Upon learning of the diagnosis, the grandfather disposed of the shoes and suitcase, so microbiological testing could not be performed. We postulate that these items served as the mode of transmission of this geophilic fungi.

Having received 20 days of liposomal amphotericin B, the patient was continued on oral fluconazole (Diflucan) therapy. High dose fluconazole (12 mg/kg/d) was chosen because of the extent of this patient's illness, the need for ongoing chemotherapy, and its increased efficacy in severe disseminated and meningeal coccidioidomycosis.[1] Acute and convalescent serum *Coccidioides* complement fixation titers were negative ($<$1:2). Six months after infection, the patient has undergone multiple rounds of chemotherapy without recurrence. The patient's therapy is planned for at least one year, although ongoing immunosuppression may require lengthening treatment.

Disseminated coccidioidomycosis in an immunocompromised host outside of endemic areas is rarely described in the published literature, and is typically caused by reactivation of past infection.[2,3] Three such pediatric cases presenting in Germany, New York, and Illinois were discussed by MacDonald et al.[2] The latter 2 patients died with underlying diagnoses of ALL and Hodgkin lymphoma, respectively, and with unknown exposures to *C. immitis*. The patient from Germany had lived his first two and a half years in Arizona, and was the first reported immunocompromised, pediatric patient to survive disseminated coccidioidomycosis caused by a reactivated, latent infection.

This patient represents an unusual, nonendemic case of fomite-transmitted, disseminated coccidioidomycosis. Transmission of *Coccidioides immitis* by fomites has been previously reported in only a limited number of publications.[4–8] Dry *C. immitis* spores can remain viable for 6 months if stored at temperatures less than 37°C, and then transfer from inanimate objects to humans through inhalation.[6] Albert and Sellers discuss a case of coccidioidomycosis in a Georgian man who probably acquired the disease from bales of cotton originating from the San Joaquin Valley. The authors also review other reports of transmission of *C. immitis* by fomites, including fruit, sisal, grain, oat hay and animal products.[6] Rothman et al further emphasize the reality and danger of fomite transmission with a review of cases of coccidioidomycosis associated with exposure to Native American relics, cotton balls, dusty clothing, and powdered cloth waste termed "flock."[7] Desai et al present a more recent case series of patients with coccidioidomycosis. The etiology of one patient's infection is explained by an occupational history of exposure to fomites, including graphite, from the endemic region of Mexico.[8]

The case presented herein emphasizes the need of an extended and thorough history of potential exposures to opportunistic pathogens in pediatric oncology patients. This should include detailed travel, social and environmental history. Moreover, physicians should be cautious in narrowing the breadth of diagnostic and therapeutic interventions despite a negative history, if the clinical course is consistent with the disease. In the absence of a positive blood culture, invasive diagnostic procedures may be necessary because serologic tests, the most common way of diagnosing *Coccidioides immitis*, may be less accurate in immunocompromised patients.[9] Clinicians should understand that coccidioidomycosis can present outside of its endemic areas and poses a serious risk of mortality in the pediatric oncology popula-

tion. Timely diagnosis and treatment of disseminated coccidioidomycosis is critical for clinical improvement.

REFERENCES

1. Saitoh A, Homans J, Kovacs A. Fluconazole treatment of coccidioidal meningitis in children: two case reports and a review of the literature. *Pediatr Infect Dis J*. 2000;19:1204–1208.
2. MacDonald N, Steinhoff MC, Powell KR. Review of coccidioidomycosis in immunocompromised children. *Am J Dis Child*. 1981;135: 553–556.
3. Kotton CN, Marconi VC, Fishman JA, et al. Coccidioidal meningitis after liver transplantation in a non-endemic region: a case report. *Transplantation*. 2006;81:132–134.
4. Eckmann BH, Schaefer GL, Huppert M. Bedside interhuman transmission of coccidioidomycosis via growth on fomites. An epidemic involving six persons. *Am Rev Respir Dis*. 1964;89:175–185.
5. Bennett HD, Milder JW, Baker LA. Coccidioidomycosis-possible fomite transmission. *J Lab Clin Med*. 1954;43:633–636.
6. Albert BL, Sellers TF Jr. Coccidioidomycosis from fomites. *Arch Intern Med*. 1963;112:253–261.
7. Rothman PE, Graw RG, Harris JC. Coccidioidomycosis: Possible fomite transmission: a review and report of a case. *Am J Dis Child*. 1969;118: 792–801.
8. Desai SA, Minai OA, Gordon SM, et al. Coccidioidomycosis in non-endemic areas: a case series. *Respir Med*. 2001;4:305–309.
9. Pappagianis D. Serologic studies in coccidioidomycosis. *Semin Respir Infect*. 2001;16:242–250.

INVASIVE *CHAETOMIUM* INFECTION IN TWO IMMUNOCOMPROMISED PEDIATRIC PATIENTS

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Abstract: The majority of fungal infections are caused by species such as *Candida* and *Aspergillus*. Other rare and emerging opportunistic fungal infections are on the increase. Risk factors for such infections include receipt of antimicrobial agents, chemotherapy, immunosuppression secondary to hematopoietic stem cell or solid organ transplantation, neutropenia, presence of indwelling intravascular catheter, prior hemodialysis, or previous fungal colonization. We present here the first 2 reports of fatal and invasive *Chaetomium* infections in pediatric patients. The first case occurred in a child with acute myeloid leukemia (AML) and the other in a child with hemophagocytic syndrome (HSP).

Key Words: *Chaetomium*, child, fungal, immunocompromised

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CASE 1

A 12-year-old male was diagnosed with AML (M4 monosomy 7) in July 2001. He achieved remission after completion of 2 phases of induction chemotherapy in Aug 2001. He was admitted 4 days later because of fever, vomiting and dehydration. There was no significant clinical finding except oral stomatitis. Complete blood count showed white blood cells 0.285×10^9, hemoglobin 99 g/L, platelets 46×10^9 and absolute neutrophils count (ANC) zero. The patient treated with empiric antibacterial therapy for fever and neutropenia. One week later the child started to complain of headache, right facial pain, right periorbital swelling and nasal discharge. Nasal swab culture grew *Aspergillus flavus*. Computed tomography (CT) scan of the paranasal sinuses showed opacification of the ethmoid, maxillary and frontal sinuses with some mucosal thickening. CT scan of the brain was normal. He was treated with amphotericin B and itraconazole. The neutropenia improved, but he started to have respiratory distress. CT scan of the chest showed multiple nodular cavitary lesions. Fine needle aspirate of the lung tissue grew *Aspergillus terrus*. Antral wash out done 1 week later grew also *Aspergillus* species. The patient continued to be febrile and his repeated CT scan after the addition of itraconazole and amphotericin B showed progression of the cavitary lesion, so treatment was changed to liposomal amphotericin B (Ambisome) 5 mg/kg/d. Itraconazole was continued considering dual infection with *Aspergillus flavus* and *A. terrus*. There was no serum itraconazol concentration obtained during treatment.

On the 10th day of the combination antifungal therapy, the patient developed high-grade fever, headache, vomiting and decreased consciousness. CT scan of the brain showed ventriculomegaly with ependymal enhancement and a small lesion at the foramen of Monro. External ventricular drain was inserted. Cerebrospinal fluid (CSF) analysis showed WBC 7 per mm^3, polymorphonuclear cells 54%, lymphocytes 46%, glucose 5 mmol/L and protein 8 g/L. CSF fungal culture was positive for *Chaetomium atrobrunneum.* Despite increasing the dosage of ambisome to 10 mg/kg/d and itraconazole iv (125 mg twice per day for 3 days, then once daily), the CSF was still positive. Voriconazole was not used because it was not available at that time. Follow-up CT showed loculated fluid collection that required the insertion of left occipital, right and left frontal external ventricular drain. The child continued to have persistent positive growth of *Chaetomium atrobrunneum* from the CSF and continued to have respiratory distress with progressive pneumonitis. A trial to obtain one of the new generation triazoles was not successful. The patient died on day 75 of admission with a final diagnosis of disseminated fungal infection with *Aspergillus* and *Chaetomium* infection. No autopsy was done.

CASE 2

A 4-month-old male infant was referred to our institution in October 2002 as a case of aplastic anemia with hepatosplenomegaly and pancytopenia. Investigations including bone marrow biopsy were inconclusive and he was discharged. The child was readmitted in November 2002 with fever, respiratory distress and neutropenia.

Bone marrow biopsy showed no evidence of hemophagocytic syndrome. Despite receiving broad spectrum antibiotic coverage that include pipracillin/tazobactam, gentamicin and vancomycin for 20 days, the patient continued to be neutropenic (ANC zero) but afebrile. Ten days later, the clinical condition deteriorated and he developed respiratory distress, coughing and a high-grade fever. CT scan of the chest showed picture of necrotizing pneumonia in the form of hypodense lesions with cavitation in the middle lobe. Empiric therapy with amphotericin B (1 mg/kg/d iv) was started. Bacterial, fungal and mycobacterial cultures of broncoalveolar lavage fluid were negative. Ultra sound guided needle aspiration of the spleen supported the diagnosis of hemophagocytic syndrome. Bacterial, fungal and mycobacterial cultures of splenic tissue were negative. CT scan of the brain showed global atrophic changes. CSF culture was negative.

The patient's clinical and radiologic conditions deteriorated during the following 3 weeks with consolidation involving most of the right lung tissue. A lung biopsy was done on day 96 after admission and histopathology showed necrotic tissue infiltrated by

multiple fungal hyphae without branching. Therapy was changed to liposomal amphotericin B 6 mg/kg iv on the same day. The patient's clinical condition deteriorated further and he was mechanically ventilated. Antifungal coverage was changed to itraconazole 10 mg/kg/d every day. After 2 weeks of intubation lung tissue grew *Chaetomium* species and voriconazole 6mg/kg every12 hours for 2 doses followed by 4 doses of 4mg/kg every 12 hours was started. Because his primary disease did not respond to chemotherapy for 2 weeks no further therapy was offered. He died on day 120 of admission. Autopsy was not done.

DISCUSSION

Chaetomium is a member of the subphylum Ascomycotina (family Chaetomiaceae), which has characteristic ascocarps. These are covered with thick-walled, pale to dark brown, elaborately branched or coiled hairs or setae. Some of the species are found in warm dry, cellulose rich media, such as on animal dung, straw, seeds, plant debris, birds' feathers and many other substrates. The ascospores, which are very hardy, are easily dispersed and distributed by insects and animals. These filamentous fungi bring about different clinical syndromes involving immunocompromised and immunocompetent patients. Twelve cases of disseminated *Chaetomium* infections have been described in the medical literature. The first documented case of pheohyphomycosis brain infection caused by C*haetomium atrobrunneum* was reported in October 1989[1] and the latest report was invasive mycotic infections caused by *Chaetomium perlucidum* in 2 patients. The first patient with acute myelogenous leukemia had *Chaetomium* infection involving the brain, lung and myocardium. The second case was in an immunocompetent patient with chronic bronchiectasis involving the lung.[2] Reported cases of invasive C*haetomium* infections are summarized in Table 1.[2,3] None of the previously reported cases occurred in children.

Invasive *Chaetomium* infections have been reported in severely immunocompromised patients such as hematologic malignancy, renal transplant, patients on peritoneal dialysis, and after organ transplantation. Brain abscesses in intravenous drug users have also been reported.[2] Cutaneous lesions, oncomycosis and sinusitis caused by *Chaetomium* species can develop in immunocompetent patients.[4,5]

The most commonly identified species in human infections has been *C. globosum*, followed by *C. atrobrunneum*. *Chaetomium strumarium* can cause primary brain infection. *C. atrobrunneum* caused a systemic infection which disseminated to the brain. It is believed that *C. atrobrunneum* was also the cause of a primary fatal brain abscess originally attributed to *C. globosum.*[2,6]

Little information exists on the susceptibility of *Chaetomium* to the conventional antifungal agents. Guarro et al[7] in 1995 studied the susceptibility of 24 strains of *Chaetomium* species (mainly *C. globosum*, *C. nigrocolor*, *C. atrobrunneum* and *C. funicola*) that were resistant to flucytosine and fluconazole. Itraconazole, ketoconazole and miconazole demonstrated inhibitory activity. None of these agents, including amphotericin B, demonstrated fungicidal activity. Serena et al[2] in 2003 investigated the in vitro antifungal susceptibilities of novel antifungal agents of 19 strains belonging to 3 species of *Chaetomium* (*C. globosum*, *C. atrobrunneum* and *C. nigricolor*) and 1 strain of the species *Achaetomium strumarium*. A modification of the NCCLS reference microdilution method (M38-A) was used to evaluate the in vitro activities of 3 triazoles (ravuconazole, voriconazole, albaconazole) and echinocandin (micofungin). Micofungin was not active, while the geometric mean minimum inhibitory concentration and minimum effective concentration of the 3 triazoles were less than 0.5 and 0.4 μg/mL, respectively.

To our knowledge these are the first 2 pediatric patients with invasive C*haetomium atrobrunneum infection.* In our first case, *Aspergillus* was isolated from the nose, antral wash out and lung tissue culture and *Chaetomium* was isolated from the CSF only. Buston et al[14] reported that the sporulation of *Chaetomium* is enhanced by the presence of *Aspergillus*, which excretes such compounds as sugar phosphate and phosphoglyceric acid. In the 2 cases, *Chaetomium* infection was the major factor contributing to the deterioration of the patient's clinical conditions.

TABLE 1. Summary of Reported Cases of Invasive *Chaetomium* Infections

Patient or Reference	Age (yr)/Sex	Underlying Medical Condition[a]	Site of Infection	Species	Treatment	Outcome
Patient 1	12/M	Leukemia	CSF	*C. atrobrunneum*	Liposomal AMB, Itraconazole	Death
Patient 2	4/M	Hemophagocytic syndrome	Lung	*C. atrobrunneum*	AMB, liposomal, AMB, Itraconazole, Variconazole	Death
Ref. 13	45/F	Leukemia/umbilical cord blood transplant	Multiple organs	*C. perlucidum*	L-AMB	Death
Ref. 13	78/F	Asthma/chronic bronchiectasis	Lung	*C. perlucidum*	RML lobectomy	Cure
Ref. 6	19/F	Lymphoma/autologus BMT	Lung pleura	*C. globosum*	Tienamycin, vancomycin, amikacin, AMB	Death
Ref. 8	19/M	AML	Lung	*Chaetomium sp.*	Liposomal AMB	Death
Ref. 9, 10	31/M	Multiple myeloma/allogenic BMT	Brain, lung	*C. atrobrunneum*	AMB, ITC	Death
Ref. 11	28/M	IVDU	Brain	*C. strumarium*	Oxacillin, cefotaxime, metronidazole	Death
Ref. 11	25/M	IVDU	Brain	*C. strumarium*	Amoxicillin, chloramphenicol, acyclovir, AMB, rifampin, isoniazid	Death
Ref. 11	20/M	IVDU	Brain	*C. strumarium*	Ceftriaxone, penicillin, acyclovir	Death
Ref. 12	24/M	ALL	Lung	*C. globosum*	AMB	Death
Ref. 1	32/M	Renal transplant	Brain	*C. atrobrunneum*	Unknown	Death
Ref. 5	73/F	None	Left maxillary sinus	*Chaetomium sp.*	Infundibulectomy	Cure
Ref. 3	34/M	Chronic myeloid leukemia/bone marrow transplant	Axillary and cervical lymph nodes	*C. globosum*	AMB	Cure

BMT indicates bone marrow transplant; RML, right middle lobe; IVDU, intravenous drug user; AML, acute myelogenous leukemia; ALL, acute lymphocytic.

REFERENCES

1. Anandi V, Jacob John, T, Walter A, et al. Cerebral Phaeohyphomycosis caused by *chaetomium globosum* in a renal transplant recipient. *J Clin Microbiol.* 1989;27:2226–2229.
2. Serena C, Ortoneda M, Capilla J, et al. In vitro activities of new antifungal agents against *Chaetomium* inoculum standardization. *Antimicrob Agents Chemother*. 2003;47:3161–3164.
3. Teixeira ABA, Trabasso P, Moretti-Branchini ML, et al. Phaeohyphomycosis caused by *Chaetomium globosum* in an allogeneic bone marrow transplant recipient. *Mycopathologia*. 2003;156:309–312.
4. Schulze H, Aptroot A, Grote-Metke A, et al. *Aspergillus fumigatus* and *Chaetomium homopilatum* in a leukemic patient. Pathogenic significance of chaetomium species. *Mycoses*. 1997;40(Suppl 1):104–109.
5. Aru A, Munk-Nielsen L, Federspiel BH. The soil fungus *Chaetomium* in the human paranasal sinuses. *Eur Arch Otorhinolaryngol*. 1997;254: 350–352.
6. Lesire V, Hazouuard E, Dequin PF, et al. Possible role of *Chaetomium globosum* in infection after autologous bone marrow transplantation. *Intens Care Med*. 1999;25:124–125.
7. Guarro J, Soler L, Rinaldi MG. Pathogenecity and antifungal susceptibility of C*haetomium* species. *Eur J Clin Microbiol Infect Dis*. 1995; 14:613–618.
8. Yeghen T, Fenelon L, Campbell CK, et al. *Chaetomium* pneumonia in patient with acute myeloid leukemia. *J Clin Pathol*. 1996;49:184–186.
9. Guppy KH, Thomas C, Thomas K, et al. Cerebral fungal infections in the immunocompromised host: a literature review and a new pathogen—*Chaetomium atrobrunneum*: case report. *Neurosurgery*. 1998;43:1463–1469.
10. Thomas C, Mileusnic D, Carey R, et al. Fatal *Chaetomium cerebritis* in a bone marrow transplant patient. *Hum Pathol*. 1999;30:874–879.
11. Abbott SP, Sigler L, McAleer R, et al. Fatal cerebral mycoses caused by the ascomycete *Chaetomium strumarium*. J Clin Microbiol. 1995;33: 2692–2698.
12. Hoppin EC, McCoy E, Rinaldi M. Opportunistic mycotic infection caused by *Chaetomium* in a patient with acute leukemia. *Cancer*. 1983;52:555–556.
13. Baron MA, Sutton DA, Veve R, et al. Invasive mycotic infections caused by *Chaetomium perlucidum*, a new agent of cerebral phaeohyphomycosis. *J Clin Microbiol*. 2003;41:5302–5307.
14. Buston HW, Khan AH. The influence of certain micro-organisms on the formation of perithecia by *Chaetomium globosum*. *J Gen Microbiol*. 1956;14:655–660.
15. Bokhary HA, Parvez S. Fungi inhabiting household environment in Riyadh, Saudi Arabia. *Mycopathologia*. 1995;130:79–87.

ERRATUM

There is an error in the manuscript referenced as "Madhi SA, Kohler M, Kuwanda L, Cutland C, Klugman KP. Usefulness of C-reactive protein to define pneumococcal conjugate vaccine efficacy in the prevention of pneumonia. *Pediatr Infect Dis J.* 2006;25:30–36". The units reported for C-reactive protein measurement in the manuscript was "mg/dl", whereas in fact it should have been "mg/l".