PLACENTAL TESTING FOR *TOXOPLASMA GONDII* IS NOT USEFUL TO DIAGNOSE CONGENITAL TOXOPLASMOSIS

Denis Filisetti, MD, PhD, Vanessa Cocquerelle, Alexander Pfaff, PhD, Odile Villard, MD, PhD, and Ermanno Candolfi, MD, PhD

Abstract: We examined 785 placentas, including 51 from documented cases of congenital toxoplasmosis. *Toxoplasma* was detected in 16 placentas, including 1 in which congenital toxoplasmosis was ruled out. Placental screening had poor sensitivity (25%) but good specificity (99%), positive predictive value (93%), and negative predictive value (95%).

Key Words: congenital toxoplasmosis, *Toxoplasma gondii*, placenta, mouse inoculation, PCR

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From the Institut de Parasitologie et de Pathologie Tropicale, les Hôpitaux Universitaires de Strasbourg et Université de Strasbourg, Strasbourg, France.
The authors D. F. and V. C. contributed equally to this work.
Address for correspondence: Denis Filisetti, MD, PhD, Laboratoire de Parasitologie et Mycologie Médicale, Les Hôpitaux Universitaires de Strasbourg, 3 rue Kœberlé, F-67000 Strasbourg, France. E-mail: denis.filisetti@unistra.fr.

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Congenital toxoplasmosis (CT) results from transplacental passage of *Toxoplasma gondii* to the fetus after acute maternal infection. The fetus can be infected when symptomatic acute toxoplasmosis occurs up to 6 months before conception. HIV-infected and immunodepressed pregnant women are at a particularly high risk. Pregnant women seronegative for *Toxoplasma* living in France undergo mandatory monitoring based on monthly specific IgG and IgM assays. If maternal infection occurs, the fetus is also monitored more closely with ultrasound scans, and prenatal diagnosis is recommended. However, prenatal diagnosis lacks sensitivity, and some cases of CT are diagnosed in the neonatal period or early infancy. If CT is confirmed, early treatment can attenuate the clinical consequences. The standard treatment consists of a combination of pyrimethamine and sulfamide. As this treatment can have severe adverse effects, the diagnosis of CT must be established with confidence.

The need for placental screening is controversial. The Classification System and Case Definition of *Toxoplasma gondii* Infection in Immunocompetent Pregnant Women and their Congenitally Infected Offspring provides no guidance on interpreting the presence of the parasite in the placenta.[1] We and others have considered it to be synonymous with CT.[2] The placenta being a fetomaternal organ, neither polymerase chain reaction (PCR) nor mouse inoculation can identify the maternal or fetal source of infected cells. Mouse inoculation has the advantage of permitting parasite isolation and genotyping, but it is time-consuming, slow (1 month), and expensive. In this study, we examined the contribution of placental screening for *T. gondii* by reviewing our laboratory's files over a 7-year period, during which we tested 785 placentas.

MATERIALS AND METHODS

Materials. From January 1, 2000 to December 31, 2007, we tested 785 human placentas for *T. gondii* by mouse inoculation and/or PCR, for the diagnosis of congenital toxoplasmosis. Placentas were tested with mice inoculation when received less than 48 hours after delivery. When placentas were received more than 48 hours after delivery; or if necrotic and/or fixed with formalin: they were tested with PCR alone. Both PCR and inoculation were performed when we were not sure if the mice would survive the inoculation.

Methods. Mice were inoculated as described elsewhere.[3] Briefly, half the placenta was digested with trypsin, filtered and washed. Each milliliter of resuspended pellet was injected intraperitoneally into 1 to 6 Swiss mice. One month later anti-*Toxoplasma* IgG titers were measured with an indirect immunofluorescence assay in tail blood.

T. gondii PCR was performed as follows. DNA was extracted from 200 μL of placental pellet (QIAamp DNA Minikit, Qiagen SA, Courtaboeuf, France) and 5 to 10 μL was amplified (3 PCR reactions per placenta) using different methods over the study period. End-point PCR detecting the 18S ribosomal RNA gene[4] was used from 2000 to May 2005; end point PCR targeting the B1 gene[5] was used from 2000 to September 2003; end-point PCR targeting the AF146527 sequence was used from September 2003 to May 2007[6]; then quantitative real-time PCR alone was used from May 2007 onwards[7] with inhibitor screening and positive and negative controls.

A total of 627 placentas were studied by mouse inoculation, 128 by *T. gondii* PCR and 30 by both methods.

The diagnosis of CT was considered definitive if at least one of the following tests was positive (i) *T. gondii* PCR and/or mouse inoculation with amniotic fluid prenatally or at delivery, or in cord blood or newborn peripheral blood; (ii) different immunoblot profiles of anti-*Toxoplasma* IgG and/or IgM antibodies between the newborn and the mother at birth or within the first month of life; (iii) persistence of anti-*Toxoplasma* IgG titer 1 year after birth, or an increase in the titer during the first year of life.

RESULTS

Eight of the 627 placentas analyzed by mouse inoculation only contained *Toxoplasma* (sensitivity 1.3%) (cases 1, 2, 3, 5, 12, 13, 15, and 16). Among the 128 placentas analyzed by PCR alone, 8 contained the parasite DNA (sensitivity 6.2%) (cases 4, 6, 7, 8, 9, 10, 11, and 14). *Toxoplasma* was thus detected in 16 (2.0%) of the 785 placentas (Table 1).

The diagnosis of CT was confirmed in 13 of these 16 cases of placental positivity (Table 1). The first tests to suggest a diagnosis of CT were the following: (i) prenatal diagnosis (cases 1, 10, 12, and 15), (ii) presence of *Toxoplasma* in amniotic fluid at birth (cases 4, 5, 13, and 16), (iii) presence of *Toxoplasma* in cord blood (case 11), (iv) different anti-*Toxoplasma* IgG and/or IgM immunoblotting profiles between the mother and child at birth (cases 2, 7, and 14), or (v) during the first month of life (case 3).

The infection occurred during the first trimester in 1 case of the 16 in which the placenta was *Toxoplasma*-positive (6%) (case 14); during the 2nd trimester in 3 cases (19%) (cases 6, 11, and 15), and during the third trimester in 10 cases (59%) (cases 1, 2, 3, 4, 7, 9, 10, 12, 13, and 16). In 2 cases the maternal infection could not be dated (cases 5 and 8) (Table 1).

The diagnostic work-up for CT could not be completed in 2 of the 16 cases of placental positivity (cases 6 and 8), because the patients were lost to follow-up. Prenatal diagnosis was negative in case 6 and not performed in case 8. The newborns' anti-*Toxoplasma* IgG antibody profiles were similar to the mothers,' and anti-*Toxoplasma* IgM was absent (case 6). Neonatal blood was tested for *T. gondii* DNA in one case (case 8) and was negative.

CT was dismissed in one case (case 10) because specific IgG was negative at age 8 days, and still seronegative at 11 months without any treatment against congenital toxoplasmosis; all other

TABLE 1. Characteristics of 16 Cases of *Toxoplasma* Detection in Placenta

Characteristics	Case No.															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Maternal infection (month of pregnancy)	8	7	8	8	NK	6	8	NK	9	7	6	7	8	2	6	8
Prenatal diagnosis																
Amniotic fluid (mouse inoculation)						−				+	−	−			−	
Amniotic fluid (*Toxoplasma* PCR)	+					−				+	−	+			+	
Neonatal diagnosis: mother																
Amniotic fluid (mouse inoculation)												−	+			+
Amniotic fluid (*Toxoplasma* PCR)	+			+	+							−	+			+
Placenta (mouse inoculation)	+	+	+		+							+	+		+	+
Placenta (*Toxoplasma* PCR)				+		+	+	+	+	+	+			+		
Neonatal diagnosis: newborn																
Peripheral/cord blood at birth (*Toxoplasma* PCR)	+		−	−	−		−				+	−		−		
Peripheral/cord blood at birth (Western-blot)	≠	≠	=	≠	=	=	≠		=	≠	≠	=	≠	≠	=	≠
Serum: Western blot (<1 mo)			≠		≠											
Peripheral blood after birth (*Toxoplasma* PCR)		−		+			+		−				−			
Peripheral blood after birth (mouse inoculation)		−														
IgG increase/persistence		I	I									IP	I			
IgM (ELISA/ISAGA)	+		+	+			+			+	+			+		+
Final diagnosis: congenital toxoplasmosis	Yes	Yes	Yes	Yes	Yes	NK*	Yes	NK*	No†	Yes	Yes	Yes	Yes	Yes	Yes	Yes

*Lost to follow-up.

†Seronegative at 8th day and 11th month of life, never treated for congenital toxoplasmosis.

NK indicates not known; +, positive; −, negative; blank, not determined; ≠, different mother/newborn profiles of anti-*Toxoplasma* IgG and/or IgM antibodies; =, identical mother/newborn profiles of anti-*Toxoplasma* IgG and/or IgM antibodies; I, increase of the IgG in the first year of life; P, persistence of the IgG after 1 year of life.

tests were also negative in this patient, apart from parasite detection in placenta.

During the study period, 17 other fetuses and 21 other newborns or children were found to be congenitally infected with *Toxoplasma*, but the placentas were negative in all 38 cases. Thus, a total of 51 cases of CT were diagnosed during the study period.

Consequently, the sensitivity and specificity of placental screening were 0.25 (13/51) and 0.99 (733/734), respectively, and the positive and negative predictive values were 0.93 (13/14) and 0.95 (733/771).

DISCUSSION

T. gondii was found in the placenta in only 13 (25%) of the 51 cases of documented CT in this series. This proportion is similar to that found by Desmonts and Couvreur (25%).[3] Thus, sensitivity has not improved in more than 20 years, even with the use of PCR, although we tested only 158 placentas by PCR (20% of the 785 placentas analyzed).

Four of the 13 cases of CT in which *Toxoplasma* was found in the placenta were diagnosed antenatally (cases 1, 10, 12, and 15) and 9 at birth (cases 2, 4, 5, 7, 11, 13, 14, 15, and 16). In a previous study, we showed that parasite detection in amniotic fluid at birth was a sign of fetal infection.[6] This criterion could be added to the characterization of a CT case.[1] Case 3 was diagnosed 3 days after birth, based on the appearance of specific IgM on the Western blot of the newborn's serum. Analysis of the placenta did not contribute to the diagnosis of CT in any of the 13 cases, as other tests showed CT either before or simultaneously with the discovery of the parasite in the placenta.

Transplacental passage of *Toxoplasma* is a complex process. The presence of *Toxoplasma* in the placenta is not synonymous with fetal infection, as illustrated by case 9, in which all other tests were negative. On the other hand, failure to detect *Toxoplasma* in the placenta does not rule out the diagnosis of CT, as illustrated by the 38 cases of CT in which the placenta was negative. Previous studies have yielded similar results.[8,9]

When *Toxoplasma* detection in the placenta is the only sign of fetal infection, some authors advocate treatment if the maternal infection occurred late in pregnancy, when the risk of transmission is highest.[10] In this case, however, our patient 9 would have been treated unnecessarily, and it must be borne in mind that the standard treatment for toxoplasmosis (pyrimethamine and sulfamide) can have serious adverse effects. Moreover, when the placenta contains *Toxoplasma* but all other prenatal and neonatal tests are negative, treatment may delay definitive diagnosis, as it can lower or even transiently suppress anti-*Toxoplasma* IgG.

In conclusion, molecular and immunologic diagnostic methods applied to fetal and newborn samples have supplanted placental testing for *Toxoplasma*, which is costly and poorly sensitive. We have decided to abandon this method in our laboratory. But mouse inoculation of the placenta may still be valuable for those interested in performing genotyping studies of the parasite.

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REFERENCES

1. Lebech M, Joynson DH, Seitz HM, et al. Classification system and case definitions of *Toxoplasma gondii* infection in immunocompetent pregnant women and their congenitally infected offspring. *Eur J Clin Microbiol Infect Dis*. 1996;15:799–805.

2. Candolfi E, Pelloux H. Biological postnatal diagnosis of congenital toxoplasmosis. In: Ambroise-Thomas P, Petersen E, eds. *Congenital Toxoplasmosis*. Berlin: Springer; 2000:179–188.

3. Desmonts G, Couvreur J. L'isolement du parasite dans la toxoplasmose congénitale : intérêt pratique et théorique. *Arch Fr Pédiatr*. 1974;31:157–166.
4. Dupon M, Cazenave J, Pellegrin JL, et al. Detection of *Toxoplasma gondii* by PCR and tissue culture in cerebrospinal fluid and blood of human immunodeficiency virus-seropositive patients. *J Clin Microbiol*. 1995;33:2421–2426.
5. Pelloux H, Weiss J, Simon J, et al. A new set of primers for the detection of *Toxoplasma gondii* in amniotic fluid using polymerase chain reaction. *FEMS Microbiol Lett*. 1996;138:11–15.
6. Filisetti D, Gorcii M, Pernot-Marino E, et al. Diagnosis of congenital toxoplasmosis: comparison of targets for detection of *Toxoplasma gondii* by PCR. *J Clin Microbiol*. 2003;41:4826–4828.
7. Reischl U, Bretagne S, Kruger D, et al. Comparison of two DNA targets for the diagnosis of toxoplasmosis by real-time PCR using fluorescence resonance energy transfer hybridization probes. *BMC Infect Dis*. 2003;3:7.
8. Fricker-Hidalgo H, Pelloux H, Racinet C, et al. Detection of *Toxoplasma gondii* in 94 placentae from infected women by polymerase chain reaction, in vivo, and in vitro cultures. *Placenta*. 1998;19:545–549.
9. Ajzenberg D, Cogne N, Paris L, et al. Genotype of 86 *Toxoplasma gondii* isolates associated with human congenital toxoplasmosis, and correlation with clinical findings. *J Infect Dis*. 2002;186:684–689.
10. Fricker-Hidalgo H, Brenier-Pinchart MP, Schaal JP, et al. Value of *Toxoplasma gondii* detection in one hundred thirty-three placentas for the diagnosis of congenital toxoplasmosis. *Pediatr Infect Dis J*. 2007;26:845–846.

THE CHANGING SEROEPIDEMIOLOGY OF VARICELLA IN JAPAN

1977–1981 AND 2001–2005

Kumi Ueno-Yamamoto, MD,† Keiko Tanaka-Taya, MD, PhD,* Hiroshi Satoh, DVM, PhD,* Kazuko Araki, PhD,* Mitsuo Kaku, MD, PhD,† and Nobuhiko Okabe, MD, PhD**

Abstract: We conducted an anti-varicella-zoster virus antibody seroepidemiologic survey comparing the pre- and postvaccine eras of 1977–1981 and 2001–2005. For each period, 828 samples were measured by enzyme immunoassay test and compared. The differences from 1-year-old to high-school aged children were statistically significant. The introductions of optional varicella immunization and lifestyle changes for children were considered influencing factors.

Key Words: varicella, seroepidemiology, routine immunization

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From the *Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Japan; and †Department of Infection Control and Laboratory Diagnostics, Internal Medicine, Tohoku University Graduate School of Medicine, Japan.

Supported by the Ministry of Health, Labour and Welfare, Government of Japan.

Address for correspondence: Kumi Ueno-Yamamoto, MD, 1-23-1, Toyama, Shinjuku-ku, Tokyo, 162-8640, Japan. E-mail: kumi-u@nih.go.jp.

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Varicella is an acute, highly contagious disease caused by the varicella-zoster virus (VZV). Although mostly a mild disorder during childhood, varicella tends to be severe in infants, adults, and immunocompromised hosts. After decades, the initial VZV infection can reemerge to cause herpes zoster that occurs frequently among older adults.

A live attenuated varicella vaccine was originally developed from the Oka vaccine strain in 1974 by Takahashi et al in Japan,[1] where it was introduced in 1987 as an optional, but not a routine, immunization. The varicella vaccine is used globally, and it is currently approved in Japan for adults and healthy children who are ≥1 year of age.

In 2008, the total population in Japan was approximately 127,692,000, and comprised 7,667,000 children aged 0 to 6 years and 105,658,000 young adults/adults aged ≧19 years. Weekly case reports of varicella were aggregated from pediatric sentinels consisting of approximately 3000 pediatric facilities, about 10% of the total pediatric medical settings, under the National Epidemiological Surveillance of Infectious Diseases (NESID). According to NESID, there are about 250,000 case reports of varicella infection every year.[2]

In this study, we conducted a seroepidemiologic survey comparing 2 periods, the pre- and the postvaccine eras, to assess the influence of optional immunization and to estimate the current susceptible population.

MATERIALS AND METHODS

A total of 1656 serum specimens (844 and 812 from males and females, respectively), or 828 samples each from the 2 periods of 1977 to 1981 (period 1: prevaccine era) and 2001 to 2005 (period 2: postvaccine era), were obtained. The geographic sampling distribution was set to a 4:3:2 ratio of the total population for the Hokkaido/Tohoku/Kanto area (East), Chubu/Kinki area (Central), and Chugoku/Shikoku/Kyushu area (West). The samples from people aged 0 to 82 years were stratified into 8 age groups: <1 (infants in daycare or under parental supervision at home (home care)), 1 to 2 (daycare or home care), 3 to 6 (daycare or kindergarten), 7 to 12 (primary school), 13 to 18 (junior high and high school), 19 to 29 (young adults), 30 to 49 (adults), and ≧50 years (adults and elderly).

All specimens were obtained from the National Serum Reference Bank/Tokyo the National Institute of Infectious Diseases in Japan, where they were randomly collected from healthy Japanese individuals and stored at −80°C. Under the constitution of the Bank, these samples excluded personal information such as contact details, vaccine, and disease history. In the Bank, the maximum number of samples that could be obtained at one time was decided approximately 1000.

All samples were tested using the virus-specific IgG enzyme immunoassay kit SEIKEN varicella-zoster IgG (Denka Seiken, Tokyo, Japan), and the results were interpreted according to the manufacturer's instructions. The calculated prevalence of anti-VZV antibodies for each age group during each period were compared for their differences in proportions using the χ^2 test, or the Fisher exact test when appropriate, with a value of $P < 0.05$ considered statistically significant.

Period 2 prevalence and the 2003 national demographic statistics were used to calculate the current susceptible population.

RESULTS

The overall prevalence of antibodies to VZV was 66.5% (a 95% confidence interval (CI): 63.3%–69.7%) for period 1 and 74.2% (95% CI: 71.1%–77.0%) for period 2, and the difference in prevalence between the 2 periods was statistically significant ($P < 0.001$).

A comparison of the antibody prevalence in each age group is shown in the Figure 1. The prevalence in period 2 (2001–2005) was higher than period 1 (1977–1981) for all age groups except the <1 year olds. In particular, the differences among the 1 to 2, 3 to 6, 7 to 12, and 13 to 18-year age groups were statistically significant ($P < 0.01$). The antibody prevalence of the 3 age groups older than 19 years of age was high during both periods.

In the <1 year age group, the seroprevalence for those children 6 to 11 months of age only minutely changed from 12.3%

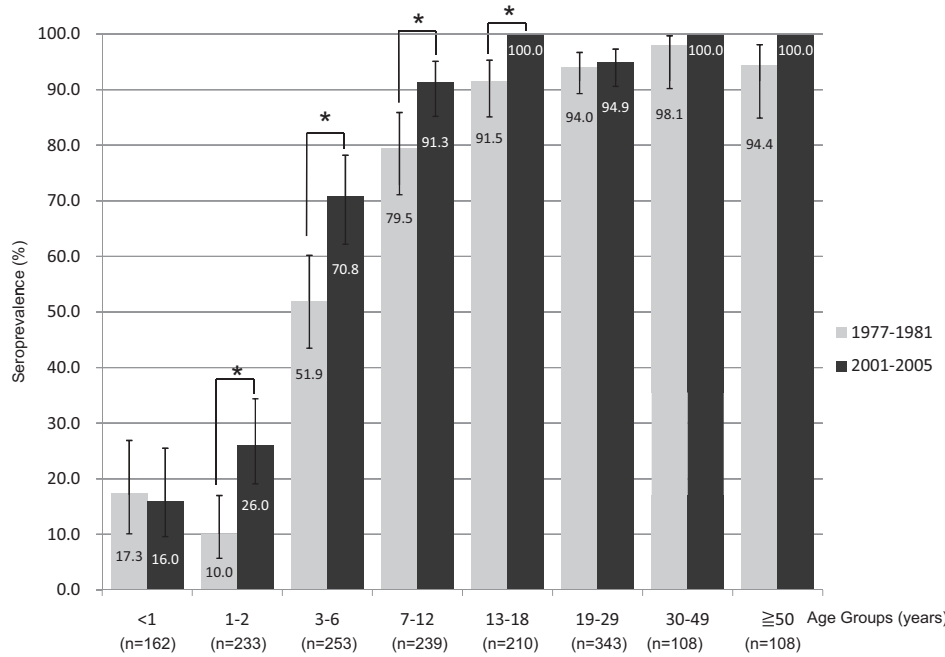

FIGURE 1. Differences in anti-VZV antibody prevalence between 1977–1981 and 2001–2005 according to age. Vertical bars indicate 95% CIs. The numbers at the top of each bar indicate the proportion of anti-VZV antibody prevalence. *$P < 0.01$.

(95% CI: 6.1%–23.2%) for period 1 (n = 57) to 12.1% (95% CI: 6.3%–22.1%) for period 2 (n = 66) (no statistical significance). The numbers of specimens for infants 0 to 5 months of age were too few to analyze: 23 and 6 in periods 1 and 2, respectively.

The susceptible population was estimated at 497,074 (range: 440,525–529,874) for those 6 to 11 months of age, and at 942,021 (498,717–1,736,274) for the 19 to 29-year age group. In addition, there were an estimated 1,724,940 (1,529,136–1,885,779) susceptible in the 1 to 2-year age group and 1,379,700 (699,300–1,030,050) in the 3 to 6-year age group.

DISCUSSION

The most noticeable difference in the seroprevalence curves for the 2 time periods was the higher prevalence in the postvaccine era (period 2) from 1-year-old children to high school-aged children. Two possibilities, the influence of the varicella vaccine and changes in lifestyle, were investigated when considering this difference.

Since licensing in 1987, the cost of varicella vaccination, approximately 6000 to 12,000 yen per dose (depends on the medical institution; cost not covered under national insurance), has been the responsibility of the individual. Although there are no national data regarding varicella vaccination, estimates on immunization coverage from limited reports place the figure at 25% to 30% in preschool-aged children.[2] The increasing seroprevalence of varicella in 4 of the age groups (1–2, 3–6, 7–12, and 13–18 year olds) for period 2 could have occurred as a result of limited vaccination. However, the reported number of varicella cases has not changed in Japan.[2] It appears that the varicella vaccine is not being used effectively under the existing national immunization policy.

Regarding children's lifestyle changes, there has been an increasing and earlier trend toward placing children in daycare versus parental care at home. In the national statistical data, although the number of children aged 0 to 6 years decreased from 12,866,000/y in period 1 to 8,154,360/y in period 2, the number of the children enrolled in daycare centers, which cater to working mothers/guardians in Japan, increased from period 1 (1,917,328/y; proportion in the 0–6-year-old group: 14.9%) to period 2 (2,073,146/y; 25.4%). In addition, the proportion of <3 year olds in daycare increased from 19.0% in period 1 to 31.0% in period 2. Concurrently, based on NESID data since 1982, the proportion of varicella cases in children aged 1 to 4 years has increased yearly, while cases in children aged 5 to 9 years have decreased.[2] Thus, varicella is occurring in younger children in Japan compared with the past, which is consistent with our results showing an increasing prevalence of varicella antibodies among the younger age groups.

According to our estimations, large numbers of not only children but also infants and young adults remain susceptible to varicella. Because Japan has a high potential for more endemic, severe cases, and deaths related to varicella, careful monitoring is needed.

Although varicella vaccination coverage remains low in Japan, the coverage of measles immunization was high in 2008, the first year of implementation of the government's measles elimination plan. Coverage was 94.3% for the first dose in children aged 12 to 23 months and 91.8% for the second dose in children aged 5 to 6 years.[3] The first priority for public health action against varicella in Japan is to gain higher coverage of varicella vaccination among children, for instance, by inoculating children against varicella at the time of measles vaccination. Immunization in older adults to prevent herpes zoster, the effectiveness of which has been confirmed[4] and recommended in the United States,[5] should be discussed as a future second step. To prevent endemic and severe cases in the susceptible population, varicella vaccine needs to be introduced as a routine immunization as soon as possible.

REFERENCES

1. Takahashi M, Otsuka T, Okuno Y, et al. Live vaccine used to prevent the spread of varicella in children in hospital. *Lancet*. 1974;2:1288–1290.
2. National Institute of infectious Diseases, Tuberculosis and Infectious Diseases Control Division, Health Service Bureau, Ministry of Health, Labour and Welfare, Government of Japan. Chickenpox 1982–2004. Infectious Agents Surveillance Reports. 2004;25:318–319.
3. Infectious Disease Surveillance Center, National Institute of Infectious Diseases. Measles containing vaccine coverage, 2008 [in Japanese]. Available at: http://idsc.nih.go.jp/disease/measles/01.html. Accessed December 14, 2009.
4. Oxman MN, Levin MJ, Johnson GR, et al. A vaccine to prevent herpes zoster and postherpetic neuralgia in older adults. *N Engl J Med*. 2005;352:2271–2284.
5. Harpaz R, Ortega-Sanchez IR, Seward JF. Advisory Committee on Immunization Practices, Centers for Disease Control and Prevention. Prevention of Herpes Zoster, Recommendation of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep*. 2008;57(RR-5):1–30.

NOSOCOMIAL TRANSMISSION OF RESPIRATORY SYNCYTIAL VIRUS IN NEONATAL INTENSIVE CARE AND INTERMEDIATE CARE UNITS

A PROSPECTIVE EPIDEMIOLOGIC STUDY

Angelika Berger, MD, Eva Obwegeser, MD,* Stephan W. Aberle, MD,† Michaela Langgartner, MD,* and Theresa Popow-Kraupp, MD†*

Abstract: To test the hypothesis that a considerable number of preterm infants acquire respiratory syncytial virus (RSV) within the hospital during the postnatal stay, a prospective epidemiologic survey was performed. Nasopharyngeal swabs were taken twice weekly for a period of 8 weeks from preterm infants, medical/nursing staff, and parents during the peak of RSV season 2007/2008 and tested for RSV by polymerase chain reaction. Of 1002 samples, only 4 tested positive (2 from a patient, 2 from staff). Sequence analyses of the G protein demonstrated that nosocomial transmission did not occurr between these individuals.

Key Words: respiratory syncytial virus, nosocomial transmission, neonatal intensive, care unit, PCR

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From the Departments of *General Pediatrics and Neonatology and †Virology, Medical University Vienna, Austria.

Address for correspondence: Angelika Berger, MD, Department of General Pediatrics and Neonatology, Medical University Vienna, Waehringerguertel 18-20, A-1090 Vienna, Austria. E-mail: angelika.berger@meduniwien.ac.at.

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At lot is known about acquisition of respiratory syncytial virus (RSV) in pediatric wards.[1–4] Major risk factors for nosocomial RSV infections are prematurity, long duration of hospital stay, poor hygienic standards, and underlying clinical conditions, such as chronic lung disease, congenital heart disease, and neurologic diseases.[3–6] Few data, however, are available on the incidence of RSV infections in neonatal intensive care units (NICUs) and neonatal intermediate care units (NIMCUs). To protect preterm infants during their first RSV season, palivizumab (Synagis), a monoclonal antibody preparation against the fusion protein F of RSV, is routinely given in Austria to all preterm infants <29 weeks gestational age and to preterm infants of 29 to 32 weeks gestational age with additional risk factors. The first injection is generally given 2 to 3 days before discharge home.

In our institution, no nosocomial RSV infection has been diagnosed within the last 10 years in a preterm infant during the first postnatal stay. By contrast, in a German study from 2001, 47% of preterm infants <28 weeks gestational age acquired RSV infections within the NICU before their first discharge home.[7] On the basis of that report, we hypothesized that routine testing for RSV might reveal a high proportion of preterm infants acquiring RSV in the hospital during the postnatal stay. Confirmation of this hypothesis would have a substantial impact on the routine care of preterm infants including palivizumab prophylaxis during the postnatal hospital stay and routine testing for RSV in cases of suspected nosocomial sepsis during RSV season.

MATERIALS AND METHODS

To test this hypothesis, repetitive sampling of nasopharyngeal swabs was performed twice weekly (Monday and Thursday) in all preterm infants admitted to the NICU and NIMCU of the Department of Neonatology, Medical University Vienna, in medical and nursing staff as well as in parents/visitors of those patients for 2 months, during the peak of the RSV season 2007/2008 (January 14 to March 6, 2008). The beginning of the study period was determined by active surveillance. As soon as more than 10% of nasopharyngeal specimens obtained from hospitalized patients tested at the Clinical Institute of Virology were positive for RSV during 2 consecutive weeks, the study was initiated.

Nasopharyngeal swabs were stored at −20 °C and processed at the Clinical Institute of Virology. Analyses for RSV were performed by highly sensitive seminested reverse transcription (RT) polymerase chain reaction (PCR) at the end of the study period. The PCR methodology used for this study has been previously described in detail.[8] To avoid false positive results as a consequence of contamination, numerous water controls were included in every run. Each positive PCR result was confirmed by testing a second portion of the original nasopharyngeal swab. In RSV positive cases, sequencing of the G protein gene was performed to determine strain relatedness to provide evidence for a link between individual cases. Testing of collected samples was performed after the end of the study period. Therefore, PCR test results had no effect on clinical routine practices.

RESULTS

A total of 1002 specimens obtained from 266 persons were analyzed. Of those, 343 samples were obtained from 81 preterm infants, 502 samples from 118 caregivers, and 157 samples from 67 visitors. Only 4 specimens were positive for RSV by PCR analysis. Two specimens were taken from 1 female 26 week gestational age 920 g birth weight preterm infant. This infant had an uneventful course at the NICU with 29 days of continuous positive airway pressure support (no mechanical ventilation, no surfactant) and gained full enteral feeds at day 19 of life. The patient was transferred to the NIMCU at day 40 of life and showed first symptoms of RSV infection (apnea and bradycardia) at day 42 of life. The patient was readmitted to our second NICU (not involved in the study), intubated after 2 days of continuous positive airway pressure support, and subsequently mechanically ventilated with conventional and high-frequency ventilation. After a complicated respiratory course, the patient ultimately died of bacterial and fungal superinfection at 36 weeks postconceptual age (72 days of life), 4 weeks after the first symptoms of RSV infection. In addition, RSV infection was confirmed in 2 staff members of the NICU (1 nurse and 1 doctor who both had mild cough and coryza). Nosocomial transmission between staff members and the patient could be ruled out by sequence analysis of the G protein. Follow-up swabs taken 3 and 5 days, respectively, after

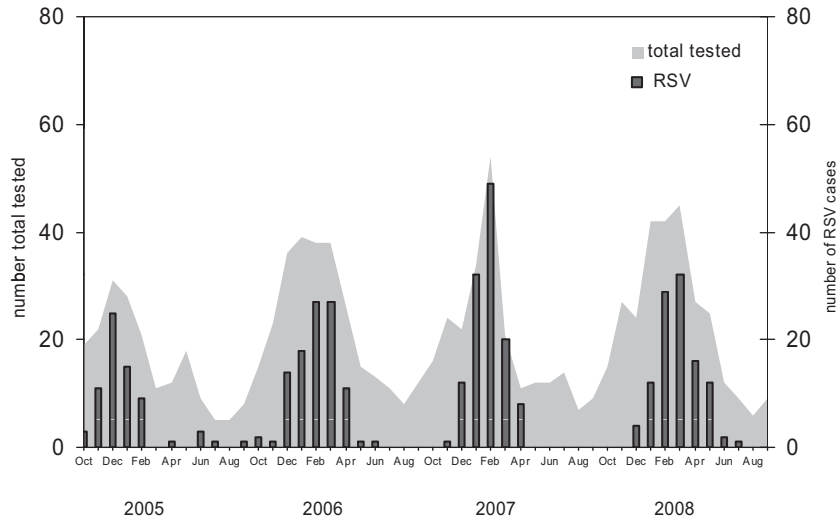

FIGURE 1. The seasonality of RSV in Austria during 2005 to 2008. Columns indicate number of samples positive for RSV, areas under curve indicate total tested samples at the Department of Virology, Medical University of Vienna.

the first specimen in both staff members were negative for RSV. No case of asymptomatic viral shedding was identified in staff members, visitors, and patients, and no further cases of RSV infection occurred throughout the study period.

DISCUSSION

The results of this study indicate a low rate of RSV-infection in the neonatal wards of the Medical University of Vienna during epidemic activity of RSV in 2007/2008. The single RSV-positive patient had a fatal outcome, however, rendering any recommendations on the routine application of palivizumab for RSV prophylaxis in preterm infants during the stay in the NICU and NIMCU precarious. It has been shown before that these infections have a considerable risk of an adverse outcome in these settings.[3,4,6]

The seminested RT-PCR methodology applied in this study provides sensitive detection of RSV infections even in patients experiencing RSV-reinfection.[8] Nasopharyngeal swabs were chosen to decrease the effect on patients as well as to increase compliance of staff and visitors. RSV detection rates of nasopharyngeal swabs have been proven slightly less optimal than nasal washes in adults in the study of Spyridaki et al[9] using multiplex real-time PCR. A highly sensitive single pathogen RT-PCR, however, was used in this study. Furthermore, there were no episodes of clinical infection not responding to antibiotic treatment in the study patients, thus rendering it unlikely that symptomatic RSV infections were missed.

The source of infection of the patient could not be identified. The mother had symptoms of respiratory disease for 5 days prior to the first symptoms in the child; however, her nasopharyngeal swab was negative for RSV and other respiratory viruses.

The low incidence of RSV-infections cannot be explained by a low epidemic activity of RSV during the study period compared with previous RSV seasons, as shown in Figure 1.[10] We assume that this very low rate of RSV neonatal infections is a consequence of constant education and subsequent high awareness by the NICU and NIMCU staff members. Furthermore, these wards are largely closed units with the majority of infants being delivered at the Obstetrical Department of our hospital.

In conclusion, the results of the present study indicate a low rate of RSV infections in the NICU and NIMCU of the Medical University of Vienna during the peak season of RSV 2007/2008. Infections acquired during the neonatal ward stay, however, can have devastating effects on preterm infants. No recommendations can be inferred from our study regarding the routine application of palivizumab prophylaxis in preterm infants within the hospital during their first postnatal stay.

REFERENCES

1. Hall CB, Geiman JM, Douglas RG Jr, et al. Control of nosocomial respiratory syncytial viral infections. *Pediatrics*. 1978;62:728–732.
2. Hall CB. Nosocomial respiratory syncytial virus infections: the "Cold War" has not ended. *Clin Infect Dis*. 2000;31:590–596.
3. Bont L. Nosocomial RSV infection control and outbreak management. *Paediatr Respir Rev*. 2009;10(suppl 1):16–17.
4. Thorburn K. Pre-existing disease is associated with a significantly higher risk of death in severe respiratory syncytial virus infection. *Arch Dis Child*. 2009;94:99–103.
5. Weisman LE. Populations at risk for developing respiratory syncytial virus and risk factors for respiratory syncytial virus severity: infants with predisposing conditions. *Pediatr Infect Dis J*. 2003;22:S33–S37; discussion S37–S39.
6. Simon A, Muller A, Khurana K, et al. Nosocomial infection: a risk factor for a complicated course in children with respiratory syncytial virus infection–results from a prospective multicenter German surveillance study. *Int J Hyg Environ Health*. 2008;211:241–250.
7. Berner R, Schwoerer F, Schumacher RF, et al. Community and nosocomially acquired respiratory syncytial virus infection in a German paediatric hospital from 1988 to 1999. *Eur J Pediatr*. 2001;160:541–547.
8. Henkel JH, Aberle SW, Kundi M, et al. Improved detection of respiratory syncytial virus in nasal aspirates by seminested RT-PCR. *J Med Virol*. 1997;53:366–371.
9. Spyridaki IS, Christodoulou I, de Beer L, et al. Comparison of four nasal sampling methods for the detection of viral pathogens by RT-PCR-A GA(2)LEN project. *J Virol Methods*. 2009;156:102–106.
10. Aberle SW, Aberle JH, Sandhofer MJ, et al. Biennial spring activity of human metapneumovirus in Austria. *Pediatr Infect Dis J*. 2008;27:1065–1068.

LOW RISK OF BACTEREMIA IN OTHERWISE HEALTHY CHILDREN PRESENTING WITH FEVER AND SEVERE NEUTROPENIA

Carlos Pérez-Méndez, MD, Cristina Molinos-Norniella, MD,* María Morán-Poladura, MD,* Eloy Fernández-Rodríguez, MD,† Cristina Suárez-Castañon, MD,* and Gonzalo Solís-Sánchez, MD**

Abstract: Thirty-eight previously healthy, well-appearing children with severe neutropenia (absolute neutrophil count less than 500/mm^3), and fever were analyzed. Blood cultures were negative in all cases; a bacterial infection was found in 2 children and it was readily diagnosed on clinical grounds at their first visit. Antibiotic therapy was started in only 14 patients. All children recovered uneventfully.

Key Words: severe neutropenia, fever, bacteremia

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From the *Servicio de Pediatría, Hospital de Cabueñes, Gijón, Spain; and †Servicio de Análisis Clínicos, Hospital de Cabueñes, Gijón, Spain.

Address for correspondence: Carlos Pérez Méndez, MD, Servicio de Pediatría, Hospital de Cabueñes, Cabueñes, s/n. 33202, Gijón, Spain. E-mail: perezmendez.carlos@gmail.com.

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Neutrophils play a critical role in the host-defense against bacterial infections. A deficiency of these cells predisposes to infection, the risk being greatest with severe neutropenia, defined by an absolute neutrophil count (ANC) less than 500/mm^3.

Neutropenia is not an uncommon finding in the evaluation of children presenting with fever and it is frequently a cause of concern for the pediatrician. Although several reports[1–7] demonstrate a very low risk of serious bacterial infection (SBI) in an otherwise healthy child with fever and neutropenia there are no clear guidelines for the management of these children.[8–10] Even less is known about the outcome of children with severe neutropenia and some experts recommend assuming a SBI in all these children.[10] The aim of this study was to determine whether previously healthy, well-appearing children who are found to be severely neutropenic during the evaluation of a febrile illness are at high risk of developing bacteremia or other systemic bacterial infections.

METHODS

The study was a retrospective medical records review of all the children younger than 14 years of age who presented to the pediatric emergency department of the Hospital de Cabueñes, in Gijon (Spain), from September 2001 to March 2009, with a history of fever (temperature >38°C), and were found to be severely neutropenic (ANC <500/mm^3) at the time of initial evaluation. Patients with previously documented malignancy or hematologic disease, and children with toxic appearance were excluded (a "nontoxic appearance" was required to be specifically stated in the clinical record). Children were also excluded from the study when a diagnosis of leukemia or other hematologic disease was apparent at the time of their first evaluation.

Outcome variables included a positive blood culture result (any growth after a period of incubation of 7 days was considered positive), diagnosis of an invasive bacterial infection, antibiotic administration after the evaluation of the patient, hospitalization, or death. The study was approved by the Institutional Review Board.

RESULTS

Fifty-four children with severe neutropenia were identified. Three children with a diagnosis of leukemia, 1 child with myelodysplastic syndrome, and 1 toxic-appearing boy who was transferred to the Intensive Care Unit upon arrival were excluded. Eleven nonfebrile children were also excluded from the study. The remaining 38 patients (22 male) are the subject of this report. Median age was 22 months (range: 1 month–12 years). Mean temperature was 39.1°C. Accompanying symptoms included upper respiratory symptoms in 17 patients, skin rash in 7 (a rash subsequently developed in 4 additional children), gastrointestinal symptoms in 2, muscle pain in 3 patients, and irritability in 2 young infants. Fever was the only symptom in 7 patients. Ten children were receiving oral medication at the time they were found to be neutropenic: 6 were taking a beta-lactam antibiotic, 2 terbutaline, 1 cotrimoxazole, and 1 clarithromycin. The mean ANC was 297/mm^3 and it was lower than 100/mm^3 in 8 cases. Mean C-reactive protein value was 4.4 mg/L and values above 50 mg/L were found only in 3 children: a 7-year-old boy with a bacterial skin superinfection complicating varicella and two 2-year-old children with rotavirus gastroenteritis. Blood cultures were sterile in all cases. Urine cultures were obtained in 8 patients and were negative in all.

A presumed bacterial infection was diagnosed in 2 patients: the aforementioned patient with skin infection complicating varicella and a 2-year-old boy with pneumonia and Epstein-Barr virus infection; localizing signs in the physical examination were evident at the time of their first evaluation in both cases and they responded to standard antibiotic therapy. Virologic studies revealed Epstein-Barr virus (3 patients), rotavirus (2), bocavirus (2), and influenza, herpesvirus 6, herpesvirus 7, adenovirus, and metapneumovirus (1 case each). An additional 11 cases had a diagnosis of viral infection made on clinical grounds: unspecific viral exanthema (5 cases), roseola infantum (3), viral myositis (2), and varicella (1).

Antibiotic therapy was started in 14 cases (in 75% of children with an ANC lower than 100/mm^3 and in 26% of children with an ANC greater than 100/mm^3). A third generation intravenous cephalosporin was used in 9 cases, ceftazidime plus amikacin in 3, and imipenem and the combination ceftazidime plus vancomycine in 1 case each. The remaining 24 children were observed with no treatment. Only 3 of the children who were previously given antibiotics received antibiotic therapy after the finding of neutropenia: the child with pneumonia and 2 children who had extremely low ANC (20 and 40 neutrophils per mm^3). There were no deaths and all the children recovered uneventfully.

DISCUSSION

Although detailed clinical guidelines have been developed for children with cancer-related neutropenia who develop fever, there is scarce information regarding the optimal management of fever and neutropenia in otherwise healthy children. In the last 2 decades, several reports have demonstrated a very low risk of infection if there is a short history of neutropenia and the child appears well with no findings on the physical examination suggesting a serious underlying disease.[1–7] Even less information is available on the risk of SBI in children with severe neutropenia, defined as an ANC less than 0.5×10^9 L. Therefore, some experts recommend assuming a bacterial infection in all these children and performing a full sepsis evaluation and treating with parenteral broad-spectrum antibiotics, pending culture results.[10] Table 1 shows the incidence of SBIs in our study together with previous reports, when these data could be estimated. In 1987, Valiaveedan et al[1] reported a series of 21 neutropenic children, 16 with severe neutropenia, and found bacteremia in 3 patients (including 2 cases by *Haemophilus influenzae*, a rare cause of bacteremia since the introduction of universal immunization). However, it is not clear whether these children had a toxic appearance. The rest of the studies, including the present one, account for a total of 142 children with fever and severe neutropenia.[2–7] Bacteremia was found in 5, including 4 children with toxic appearance and 1 case of bacteremia in a patient with chronic neutropenia. No cases of

TABLE 1. Summary of the Literature on the Incidence of Sepsis and Bacteremia in Previously Healthy Children With Severe Neutropenia (ANC <500/mm^3)

Reference	No. Patients	Patients With Positive Blood Culture, Sepsis or Meningitis	Comment
Alario,[3] 1989	15	0	
Bonadio,[2] 1989	43 (12 <100/mm^3)	3	All appeared toxic
Bonadio,[4] 1991	5	0	Infants <8 wk
Serwint,[5] 2005	11 (5 <200/mm^3)	1	Toxic appearance, leukemia
Karavanaki,[6] 2006	11	1	Chronic neutropenia
Vlacha,[7] 2007	19 (None <100/mm^3)	0	
Present study	38 (8 <100/mm^3)	0	

bacteremia were found in well-appearing children with a short history of neutropenia. In contrast to previous series, most of the patients in our study did not receive antibiotic treatment after severe neutropenia was diagnosed and all recovered uneventfully.

We are aware that the retrospective nature of our study and the long period of observation are its major limitations. Although a systematic evaluation of the general appearance of the children is not feasible in this type of studies, a description of the general appearance of the child as "well appearing" or "non toxic" in the medical record was required for their inclusion. The fact that 10 of the children were previously receiving antibiotic therapy at the time they were found to be neutropenic may be of concern since it might have been responsible for a false-negative result of the blood cultures. However, 7 of these children did not receive any further therapy and they all did well. The 3 children who were continued on antibiotics included 1 with pneumonia who would have required antibiotic therapy regardless of his neutrophil count and 2 with extremely low neutrophil counts.

Our study adds to the growing body of evidence showing that fever and neutropenia in otherwise healthy children do not put these patients at a higher risk of SBIs and suggest that this may be true for those with severe neutropenia as well. However, because few children with extremely low ANC (less than 100/mm^3) or prolonged neutropenia were included in this and previous studies, most of them have been treated empirically with antibiotics, and there have been case reports of severe *Pseudomonas* infection in previously healthy children with extremely low ANC, we agree with Sung and Johnston that a more prudent approach is warranted in these patients.[9]

REFERENCES

1. Valiaveedan R, Rao S, Miller S, et al. Transient neutropenia of childhood. *Clin Pediatr*. 1987;26:639–642.
2. Bonadio WA, Stremski E, Shallow K. Clinical characteristics of children with fever and transient neutropenia who experience serious bacterial infections. *Pediatr Emerg Care*. 1989;5:163–165.
3. Alario AJ, O'Shea JD. Risk of infectious complications in well-appearing children with transient neutropenia. *Am J Dis Child*. 1989;143:973–976.
4. Bonadio WA, Smith DS, Mathews S, et al. Clinical significance of newly documented neutropenia in febrile young infants evaluated for sepsis. *Pediatr Infect Dis J*. 1991;10:407–408.
5. Serwint JR, Dias MM, Chang H, et al. Outcomes of febrile children presumed to be immunocompetent who present with leucopenia of neutropenia to an ambulatory setting. *Clin Pediatr*. 2005;44:593–600.
6. Karavanaki K, Polychronopoulou S, Giannaki M, et al. Transient and chronic neutropenias detected in children with different viral and bacterial infections. *Acta Paediatrica*. 2006;95:565–572.
7. Vlacha V, Feketea G. The clinical significance of non-malignant neutropenia in hospitalized children. *Ann Hematol*. 2007;86:865–870.
8. Alario AJ. Management of the febrile, otherwise healthy child with neutropenia. *Pediatr Infect Dis J*. 1994;13:169–170.
9. Sung L, Johnston DL. Approach to the febrile neutropenia in the general paediatric setting. *Paediatr Child Health*. 2007;12:19–21.
10. Segel GB, Halterman S. Neutropenia in pediatric practice. *Pediatr Rev*. 2008;29:12–23.

DOWN SYNDROME AND RESPIRATORY SYNCYTIAL VIRUS INFECTION

Orli Megged, MD, and Yechiel Schlesinger, MD

Abstract: We reviewed the medical records of all children with Down syndrome (DS), hospitalized in our medical center due to infection with respiratory syncytial virus. During the 9-year study period, there were 41 hospitalizations of 39 children with DS. Mean age was 1.3 years; mean duration of hospitalization was 10.9 days. Patients with DS were older than healthy controls with respiratory syncytial virus infection and needed longer hospitalization.

Key Words: Down syndrome, respiratory syncytial virus, RSV

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From the Department of Pediatrics and Infectious Diseases Unit, Shaare Zedek Medical Center, Jerusalem, affiliated with the Hadassah-Hebrew University Medical School, Jerusalem, Israel.

Address for correspondence: Orli Megged, MD, Infectious Diseases Unit, Shaare Zedek Medical Center, P.O. Box 3235, Jerusalem 91031, Israel.
E-mail: orlimegged@yahoo.com.

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Down syndrome (DS) is the most common chromosomal abnormality among live-born infants. It is associated with variable immunologic impairments. Few studies found an increased risk of case fatality due to sepsis in DS children compared with normal hosts hospitalized with sepsis.[1,2] Another study found an increased risk for RSV infection in this population and raised the question of the need for RSV prophylaxis for these infants.[3]

The aim of this study was to evaluate the clinical characteristics of patients with DS admitted due to respiratory syncytial virus (RSV) infection.

METHODS

The study was performed in Shaare Zedek Medical Center, a 550-bed university-affiliated general hospital. The pediatric department contains 40 beds, 3 of which are for intensive care patients. The annual pediatric admission rate ranged from 9249 to 11,137 per annum during the 8-year period of the study. The incidence of DS is very high in Jerusalem, due to the high proportion of ultra-orthodox Jewish and of Arab population. These groups share their grand multiparity with advanced age at delivery, the avoidance of prenatal screening programs, and the avoidance of termination of pregnancy even if DS is identified prenatally.[4] Hence, our pediatric department has the unique opportunity to care for a high number of DS patients.

We reviewed the medical records of 222 children with DS, hospitalized in our medical center during a 9-year period (2000–2008). For all DS patients who were admitted due to RSV infection, we collected the following information: age, gender,

admission diagnosis, length of hospital stay, mechanical ventilation, need for pediatric intensive care unit, associated conditions, and outcome.

These results were compared with those of a previously published study from our pediatric department, describing the clinical and epidemiologic characteristics of RSV in the general population over a 5-year period 2002–2007. All children with positive RSV antigen in a nasal swab were included in this study and the number of admissions ranged from 127 to 165 per year.[5]

RESULTS

During the study period, there were 41 hospitalizations for RSV infection in 39 children with DS. Of the DS patients, 27 (66%) were males. Mean age was 1.3 years (range, 0 to 6.1 years). Four children were younger than 2 months, 17 (41%) were older than 1 year and 8 children (20%) were older than 2 years. This age distribution is in contrast to the findings in a previous study in our medical center in which only up to 4% of healthy children with hospitalization due to RSV infection were older than 2 years.[5] Mean duration of hospitalization was 10.9 ± 17 days, as compared with 4.9 ± 3 days in our cohort of healthy children ($P = 0.0007$).[5] Twenty-one children (51%) had an underlying congenital cardiac disease; 5 additional patients were born prematurely. One girl was admitted due to RSV infection 3 times. Two children required mechanical ventilation and 1 of them died.

DS infants with underlying conditions (congenital heart disease, n = 21; age <2 month, n = 3; prematurity, n = 5) had longer hospitalization stay than those without any other risk factors (12 vs. 7 days), but these differences did not reach statistical significance ($P = 0.23$).

DISCUSSION

We found that children with DS are at a high risk for a severe course of RSV infection, as was shown by Bloemers et al.[3]

Several pathophysiologic mechanisms could underlie the high risk of RSV-infection-associated hospitalization seen among children with DS. DS is associated with immunologic impairments partially explained by abnormal thymus function and reduced number of B cells and T cells,[7] decreased phagocytosis by neutrophils,[8] low serum immunoglobulin levels,[9] diminished lymphocyte numbers and responses to stimulations,[10] and possibly zinc deficiency in some instances.[6] All these could contribute to the predisposition to infections in this population, RSV included, to a longer and more complicated course of infection and to the occurrence of RSV infection beyond the first 2 years of life as found in this study.

Yet, our findings do not support the need for RSV prophylaxis in all infants born with DS, as Bloemers et al[3] suggested to consider. First, DS patients admitted with RSV in our study were significantly older than healthy children with RSV infection, and thus RSV prophylaxis for 1 or even 2 years would have not significantly reduced hospitalizations in this special population. Second, many of these patients deserve RSV prophylaxis even without DS, due to congenital heart disease or prematurity. Thus, we believe that giving palivizumab to all children with DS would not significantly reduce the burden of hospitalizations in these children.

REFERENCES

1. Garrison MM, Jeffries H, Christakis DA. Risk of death for children with Down syndrome and sepsis. *J Pediatr*. 2005;147:748–752.
2. Hill DA, Gridley G, Cnattingius S, et al. Mortality and cancer incidence among individuals with Down syndrome. *Arch Intern Med*. 2003;163:705–711.
3. Bloemers BL, van Furth AM, Weijerman ME, et al. Down syndrome: a novel risk factor for respiratory syncytial virus bronchiolitis—a prospective birth-cohort study. *Pediatrics*. 2007;120:e1076–e1081.
4. Zlotogora J, Haklai Z, Leventhal A. Utilization of prenatal diagnosis and termination of pregnancies for the prevention of Down syndrome in Israel. *Isr Med Assoc J*. 2007;9:600–602.
5. Arthur I, Eidelman MD, Orli Megged MD, et al. The burden of respiratory syncytial virus bronchiolitis on a pediatric inpatient service. *IMAJ*. 2009; 11:533–536.
6. Cossarizza A, Monti D, Montagnani G, et al. Precocious aging of the immune system in Down syndrome: alteration of B lymphocytes, T-lymphocyte subsets, and cells with natural killer markers. *Am J Med Genet Suppl*. 1990;7:213–218.
7. de Hingh YC, van der Vossen PW, Gemen EF, et al. Intrinsic abnormalities of lymphocyte counts in children with Down syndrome. *J Pediatr*. 2005; 147:744–747.
8. Licastro F, Melotti C, Parente R, et al. Derangement of non-specific immunity in Down syndrome subjects: low leukocyte chemiluminescence activity after phagocytic activation. *Am J Med Genet Suppl*. 1990;7:242–246.
9. Loh RK, Harth SC, Thong YH, et al. Immunoglobulin G subclass deficiency and predisposition to infection in Down's syndrome. *Pediatr Infect Dis J*. 1990;9:547–551.
10. Murphy M, Lempert MJ, Epstein LB. Decreased level of T cell receptor expression by Down syndrome (trisomy 21) thymocytes. *Am J Med Genet Suppl*. 1990;7:234–237.

TRENDS IN HOSPITALIZATIONS FROM ALL-CAUSE GASTROENTERITIS IN CHILDREN YOUNGER THAN 5 YEARS OF AGE IN BRAZIL BEFORE AND AFTER HUMAN ROTAVIRUS VACCINE INTRODUCTION, 1998–2007

Tatiana Miranda Lanzieri, MD, MSc, Iolanda Costa, MD,† Fakrudeen A. Shafi, MSc,‡ Maria Helena Cunha, MD, MPH,† Eduardo Ortega-Barria, MD,* Alexandre C. Linhares, MD, PhD,§ and Romulo E. Colindres, MD, MPH**

Abstract: Rotavirus vaccination was introduced in Brazil in March 2006. We describe trends in hospitalizations from all-cause gastroenteritis in children younger than 5 years during pre- and postvaccination periods using hospital discharge data from Brazil Hospital Information System (SIH-SUS). A reduction in gastroenteritis hospitalizations of 26% and 48% in 2006 and in 2007, respectively, was observed among children younger than 1 year compared with prevaccination period (1998–2005). The largest reduction rates among children younger than 1 year were noted in the South and Southeast regions, approximately 56% in 2007, where vaccine coverage was the highest.

Key Words: gastroenteritis, rotavirus, hospitalizations, human rotavirus vaccine, Brazil

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From the *GlaxoSmithKline Biologicals, Rio de Janeiro, Brazil; †Secretaria de Estado de Saúde do Estado do Pará, Belém, Brazil; ‡GlaxoSmithKline Biologicals, Bangalore, India; and §Instituto Evandro Chagas, Secretaria de Vigilância em Saúde, Ministério da Saúde, Belém, Pará, Brazil.

Address for correspondence: Tatiana M. Lanzieri, MD, MSc, GlaxoSmithKline Brazil, Estrada dos Bandeirantes, 8464-22783-110, Rio de Janeiro, Brazil. E-mail: tatiana.m.lanzieri@gsk.com.

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Rotavirus, the leading cause of severe dehydrating gastroenteritis (GE) in infants and young children, accounts for an estimated 2 million hospitalizations and 352,000 to 592,000 deaths

each year in children younger than 5 years worldwide.[1] A review of studies published during 1986–1999 indicated that rotavirus causes ~22% (range 17%–28%) of childhood diarrhea hospitalizations worldwide, increasing to 39% (range 29%–45%) during 2000–2004.[2] In Brazil, rotavirus infections were estimated to cause ~3.5 million diarrhea episodes, 655,853 outpatient visits, 92,453 hospitalizations, and 850 deaths of children ≤5 years of age each year before vaccine introduction.[3]

Brazil was one of the first countries worldwide to include the rotavirus vaccine in the official vaccination calendar targeting infants. The National Immunization Program of the Ministry of Health calculates vaccine coverage using the number of second doses of rotavirus vaccine administered to infants (recorded at local level) divided by the number of live births. National vaccine coverage was 46.5% in 2006 and 78.3% in 2007 and varied greatly by region.[4]

As recommended by the World Health Organization,[5] vaccine impact may be assessed by monitoring trends in hospitalizations from all-cause acute GE using secondary data sources. In Brazil, an estimated 76% to 81% of the population relies on public health care provided by the National Unified Health System (SUS),[6] which provides more than 1.3 million hospitalizations per month, through public, associated, and contracted hospitals throughout the country. The Hospital Information System (SIH-SUS), implemented in 1986, has been designed for the reimbursement of SUS hospitalizations. This countrywide public database contains information on medical procedures, diagnoses, type of admissions, selected demographic information, place and date of admission, length of stay, hospitalization costs, and other reimbursement data. Data are generated in the hospitals, analyzed by the municipal and state health departments, and transmitted to the national level. The SIH-SUS is being increasingly used for the analysis of relevant public health issues, including hospital morbidity and mortality, evaluation of the performance of medical care, epidemiologic surveillance, and in the validation of other health information systems.[7]

In this study, we describe the trends in hospitalizations from all-cause GE in children younger than 5 years before and after implementation of rotavirus universal mass vaccination in Brazil using data from the SIH-SUS.

METHODS

We extracted aggregated hospital discharge data from the SIH-SUS coded with all-cause GE among children <5 years of age from 1998 to 2007, using the International Classification of Diseases 10th Revision, which included codes A00 to A09 (intestinal infectious diseases). Mean, standard deviation (SD), and proportion of GE hospitalizations during prevaccination period (1998–2005) were calculated and compared with 2006 and 2007, by age group (younger than 1 year and 1–4 years) and by region.

RESULTS

During 1998–2007, a total of 1,071,755 hospitalizations among children younger than 1 year were caused by GE, representing 15% of all hospitalizations in this age group, which decreased from 16% during 1998–2005 to 14% in 2006 and 10% in 2007. The proportion of hospitalizations caused by GE among all hospitalizations among children younger than 1 year during 1998–2005 ranged from 9% (Southeast) to 26% (North) and decreased in 2006 (range 7%–23%) and 2007 (range 4%–18%).

The mean number of GE hospitalizations among children younger than 1 year during 1998–2005 was 115,748 (SD = 15,228) per year, with a downward annual trend averaging 5% (Fig. 1). In 2006 and 2007, GE hospitalizations decreased by

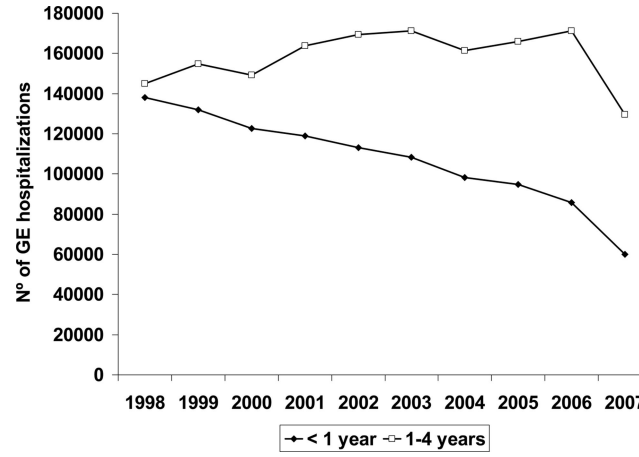

FIGURE 1. Trends in hospitalizations from all-cause gastroenteritis in children younger than 1 and 1 to 4 years, Brazil, 1998–2007.

25.8% (n = 85,835) and 48.2% (n = 59,939), respectively, compared with the prevaccination period of 1998–2005. In the Southeast and South regions, GE hospitalizations (1998–2005 means = 23,250 and 10,848, respectively) decreased by approximately 35% in 2006 (n = 15,136 and 7038) and 56% in 2007 (n = 10,168 and 4765). The North region (1998–2005 mean = 16,649), with the lowest vaccine coverage, had declines of 3.5% in 2006 (n = 16,060) and 28.8% in 2007 (n = 11,854). In the Midwest and the Northeast regions, respective decreases of 21.9% (from 1998–2005 mean of 8409 to 6570) and 27.5% (from 56,591 to 41,031) in 2006, and 47.7% (n = 4395) and 49.2% (n = 28,757) in 2007 were observed (Fig., Supplemental Digital Content 1, http://links.lww.com/INF/A435).

Among children aged 1 to 4 years, a total of 1,581,617 GE hospitalizations were recorded during 1998–2007, representing 18% of all hospitalizations in this age group. This proportion was 18% during 1998–2005 (range 12%–27%), increasing in all regions in 2006 (21%, range 13%–30%), and decreasing in 2007 (17%, range 10%–26%), except for the Northeast region.

The mean number of GE hospitalizations among children aged 1 to 4 years during 1998–2005 was 160,096 (SD = 9564) per year. A trend toward an increase by an annual average of 2% during 1998–2005 was followed by an increase by 7.0% (n = 171,368) in 2006 and a decrease by 19.1% in 2007 (n = 129,481) (Fig. 1). In the Midwest and the Northeast regions, respective increases by 10.9% and 11.7% were observed in 2006 (from 1998–2005 mean of 13,992 and 68,868 to 15,525 and 76,951), decreasing by 30.9% (n = 9661) and 12.9% (n = 59,964) in 2007. GE hospitalizations decreased in the Southeast and South regions by 8.4% and 6.7% in 2006 (from respective means of 33,785 and 19,121 during 1998–2005 to 30,956 and 17,848) and by approximately 35% in 2007 (n = 22,088 and 12,525). In the North region, an increase was observed both in 2006 and 2007, 23.7% and 3.8%, respectively (from 1998–2005 mean of 24,330 to 30,088 and 25,243) (Fig., Supplemental Digital Content 1, http://links.lww.com/INF/A435).

DISCUSSION

The introduction of rotavirus vaccination in Brazil in 2006 may have contributed to the marked decline in the number and proportion of all-cause GE hospitalizations among children younger than 1 year in 2007 compared with prevaccination period.

The proportion of GE hospitalizations among children younger than 1 year varied largely across regions; however, trends within each region show that the decrease in 2007 was much greater than the slight decrease observed annually during the prevaccination period.

The impact of vaccine is expected to be greater in children younger than 1 year in the first year of vaccination. After a few more years, impact in older children is also expected. Rotavirus vaccination began in March 2006; however, vaccine coverage was low and not homogeneous across all regions. Although vaccine coverage increased in 2007, it remained below the 95% level targeted by the Ministry of Health. We observed greater reduction rates in regions with higher vaccine coverage. It is worthy mentioning that a trend toward an increase in hospitalizations among children aged 1 to 4 years was reversed in 2007, which is possibly related to the partial vaccination of the 2006 cohort or indirect effect. In the United States, indirect benefits of vaccination seem to have occurred 1 year after the introduction of the human-bovine rotavirus vaccine.[8]

According to the World Health Organization rotavirus surveillance network, during 2001–2008, approximately 40% (range 10%–59%) of diarrhea hospitalizations among children younger than 5 years worldwide, and 34% (range 10%–51%) in the region of the Americas were attributed to rotavirus infection.[9] Results from the phase 3 clinical trials of human rotavirus vaccine (Rotarix, GlaxoSmithKline Biologicals, Rixensart, Belgium) in Latin America showed that hospitalization for GE of any cause was reduced by 39%.[10,11] These findings highlight that the burden of rotavirus disease on health systems would be substantially reduced with vaccination. In our study, we observed rates of reduction of ~25%–50% on both absolute numbers and proportion of all-cause GE hospitalizations among children younger than 1 year, which may represent a significant reduction of direct medical costs. In fact, a cost reduction of approximately US$ 10 million was observed in 2007 when costs of hospitalizations because of all-cause GE were compared with the average annual costs during 1998–2005.[12] Results from a cost-effectiveness study of a national rotavirus vaccination program for Brazilian children estimated that vaccination was likely to reduce the overall healthcare burden of rotavirus-associated GE (change in number of life-years saved) in the country by 75%.[13]

Nearly 80% of the total population in Brazil relies on health care provided by the SUS, varying from 68% in the Southeast to >90% in the North and Northeast.[6] One of the main limitations of this study was the inability to estimate rates of all-cause GE hospitalizations because of the lack of adequate denominators. Hospitalizations for diarrhea may be overrepresented in the North and Northeast, where a high percentage of the population is dependent on the SUS. Data on rotavirus-specific GE hospitalizations, stratification by other age groups (only 2-year olds, for instance), and month of hospitalization to describe seasonality patterns were not available. The effect of other interventions (sanitation, hygiene, breast-feeding, nutrition, water and food safety, and oral rehydration, increased access to care) known to influence diarrhea morbidity and mortality was not evaluated. However, the rate of increase in population coverage of sanitation, water supply or access to private health care remained constant during 1998–2007.[6]

Although this ecologic study analyzes all-cause GE hospitalizations, rotavirus vaccine introduction is likely to have been the major influence for the decreasing trends during 2006–2007. The impact was greater among children younger than 1 year and in regions with higher vaccine coverage. Trends in GE hospitalizations should continue to be assessed to monitor the impact of the vaccination program. After a few years, additional mortality data that become available should also be included in the analysis. Maximizing vaccine coverage should lead to the greatest impact.

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REFERENCES

1. Parashar UD, Hummelman EG, Bresee JS, et al. Global illness and deaths caused by rotavirus disease in children. *Emerg Infect Dis*. 2003;9:565–572.
2. Parashar UD, Gibson CJ, Bresee JS, et al. Rotavirus and severe childhood diarrhea. *Emerg Infect Dis*. 2006;12:304–306.
3. Sartori AMC, Valentim J, de Soárez PC, et al. Rotavirus morbidity and mortality in children in Brazil. *Rev Panam Salud Pública*. 2008;23:92–100.
4. DATASUS. Imunizações. Cobertura por ano e região [Immunizations. Coverage by year and region]. Available at: http://tabnet.datasus.gov.br/cgi/tabcgi.exe?pni/cnv/cpniuf.def. Accessed August 4, 2009.
5. WHO. Generic Protocol for Monitoring Impact of Rotavirus Vaccination on Gastroenteritis Disease Burden and Viral Strains. Version 6.2, 2008.
6. Rede Interagencial de Informações para a Saúde - Indicadores e Dados Básicos - Brasil – 2007 (IDB-2008) – Indicadores de Cobertura. [Interagency net of health information – Basic indicators and data – Brazil – 2007 – Coverage indicators]. Available at: http://tabnet.datasus.gov.br/cgi/idb2008/matriz.htm. Accessed January 25, 2009.
7. Bittencourt SA, Camacho LA, Leal MC. Hospital information systems and their application in public health. *Cad Saúde Pública*. 2006;22:19–30.
8. Delayed Onset and Diminished Magnitude of Rotavirus Activity—United States, November 2007–May 2008. *MMWR Morb Mortal Wkly Rep*. 2008; 57:697–700.
9. Centers for Disease Control and Prevention (CDC). Rotavirus surveillance—worldwide, 2001–2008. *MMWR Morb Mortal Wkly Rep*. 2008;57: 1255–1257.
10. Linhares AC, Velázquez FR, Pérez-Schael I, et al; Human Rotavirus Vaccine Study Group. Efficacy and safety of an oral live attenuated human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in Latin American infants: a randomised, double-blind, placebo-controlled phase III study. *Lancet*. 2008;371:1181–1189.
11. Ruiz-Palacios GM, Pérez-Schael I, Velázquez FR, et al; Human Rotavirus Vaccine Study Group. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. *N Engl J Med*. 2006;354:11–22.
12. DATASUS. Morbidade Hospitalar do SUS - por local de residência. Valor total por ano de competência e regiões. Available at: http://tabnet.datasus.gov.br/cgi/tabcgi.exe?sih/cnv/mruf.def. Accessed August 4, 2009.
13. de Soárez PC, Valentim J, Sartori AMC, et al. Cost-effectiveness analysis or routine rotavirus vaccination in Brazil. *Pan Am J Public Health*. 2008;23:221–230.

DISSEMINATED BARTONELLOSIS PRESENTING AS NEURORETINITIS IN A YOUNG ADULT WITH HUMAN IMMUNODEFICIENCY VIRUS INFECTION

Ankhi Dutta, MD, Heidi L. Schwarzwald, MD,† and Morven S. Edwards, MD**

Abstract: Rapidly declining visual acuity from neuroretinitis should prompt aggressive diagnostic intervention to preserve eyesight. We present a young adult with human immunodeficiency virus (HIV) infection in whom neuroretinitis was the presenting feature of disseminated bartonellosis. Tissue biopsy was required to establish the diagnosis and directed therapy was associated with restored vision.

Key Words: *Bartonella*, human immunodeficiency virus, infection

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From the Sections of *Infectious Diseases and †Retrovirology, Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston, TX.

Address for correspondence: Ankhi Dutta, MD, 1102 Bates Street, Suite 1120, Houston, TX 77030. E-mail: adutta@bcm.edu.

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Bartonella infections in immunocompromised individuals, including those with HIV, pose a diagnostic challenge. We present a young adult with HIV infection who developed neuroretinitis during immune reconstitution and was diagnosed with disseminated bartonellosis. His presentation illustrates the importance of maintaining a high index of suspicion for *Bartonella* infection in HIV patients with cat contact and the important role of tissue biopsy in confirming the diagnosis.

CASE REPORT

A 21-year-old man was admitted to Texas Children's Hospital with a 4-month long history of intermittent fever, 20-pound weight loss, nausea, and vomiting, and a 2-week history of blurred vision and headache. He had been diagnosed at 12 years of age with perinatally-acquired HIV infection and had been noncompliant with highly active antiretroviral therapy for the year before presentation. At evaluation 2 months before admission, his CD4 count was 34 cells/mm^3 and the viral load was 1,165,026 copies/mL. A chest radiograph was normal and a blood culture for *Mycobacterium avium-intracellulare* complex (MAC) was sterile. Therapy was initiated 3 weeks before admission with efavirenz/emtricitabine/tenofovir disoproxil fumarate (Atripla). One week later, he developed headaches and blurred vision. Ophthalmologic examination revealed bilateral papilledema.

The patient lived in Corpus Christi, Texas. He had no history of travel or known tuberculosis exposure. He smoked marijuana regularly but denied alcohol or other drug use. He was sexually active. He had 2 cats and a gerbil.

On admission, his temperature was 100.6°F and his weight was 57 kg. His general examination was notable for splenomegaly and ophthalmologic examination revealed bilateral papilledema with serous retinal detachment, cotton wool spots, subretinal fluid, and an infiltrative process involving the optic nerve. His visual acuity was 20/800 in the right and 20/400 in the left eye.

A white blood cell (WBC) count was 13,000/mm^3 with 53% neutrophils, 17% band forms, and 19% lymphocytes. Screening tests for hepatic and renal function were normal. Cerebrospinal fluid (CSF) had 12 WBC/mm^3 (90% monocytic), 5 red blood cells/mm^3, protein of 86 mg/dL, and glucose of 50 mg/dL. The opening CSF pressure was normal (12 cm of H_2O). Routine, acid-fast and fungal stains and cultures of CSF, cryptococcal antigen, and Venereal Disease Research Laboratory Test (VDRL) were negative or sterile. The CSF polymerase chain reaction (PCR) for enteroviruses, herpes simplex virus (HSV) 1 and 2, cytomegalovirus (CMV), *Toxoplasma gondii*, and *Bartonella* were negative. Serology and serum PCR for CMV and Epstein-Barr virus and serum cryptococcal antigen were negative. Fungal complement fixation testing and Toxocara titers were negative. Purified protein derivative testing and a QuantiFERON-TB gold test (Cellestis Ltd., Carnegie, Victoria, Australia) were negative.

Cranial magnetic resonance imaging (MRI) revealed bilateral papillitis and posterior choroidal surface enhancement and multiple parenchymal lesions most pronounced along the basal ganglia and hypothalamus. Abdominal ultrasound revealed splenomegaly with multiple microabscesses. A chest computed tomographic scan showed small peripheral pulmonary nodules with mediastinal lymphadenopathy.

Therapy was initiated with conventional amphotericin B and flucytosine for presumed cryptococcal meningitis and ganciclovir for treatment of possible CMV infection. Antiretroviral therapy was held. The patient's visual acuity progressively declined and when signs of increased intracranial pressure (vomiting and headache) had intensified by hospital day 8, doxycycline and rifampin were empirically initiated.

Open biopsy of a pulmonary nodule was performed on hospital day 10. Histopathologic examination revealed a discrete granuloma-like aggregate of epithelioid histiocytes, plasma cells, hemosiderin-laden macrophages, and a small central focus of necrosis with neutrophils. PCR for *T. gondii, M. tuberculosis*, HSV, and CMV as well as routine cultures, AFB stain and culture, fungal stain and culture were negative. The PCR for *Bartonella* species, performed on the lung tissue at the ARUP Laboratories (Salt Lake City, UT) was positive. Serum antibody titers to *Bartonella henselae* and *Bartonella quintana* were less than 1:64.

The patient became afebrile by hospital day 4. Amphotericin B, flucytosine, and ganciclovir were discontinued. Atripla was reinstituted on hospital day 14 when his vision had improved to 20/200. He was discharged home on hospital day 24 to continue doxycycline and rifampin. The CD4 cell count was 84 cells/mm^3 and the viral load was 250 copies/mL at 1 week after hospital discharge. At a 4-month follow-up visit, the patient's visual acuity had improved to 20/40. The CD4 cell count was 143 cells/mm^3 and the HIV viral load was $<$100 copies/mL. A 6-month course of doxycycline and rifampin is anticipated.

DISCUSSION

The seroprevalence of *Bartonella* in patients with HIV infection ranges from 16% to 40%, but seropositivity does not correlate directly with evidence of clinical infection.[1,2] The disease spectrum can include lymphadenopathy, fever of unknown origin, bacteremia, neuroretinitis, bacillary angiomatosis, bacillary peliosis, splenitis, endocarditis, and osseous involvement. The 2 common human pathogens are *B. henselae* and *B. quintana.*

Our patient illustrates the limitations of serologic testing for bartonellosis in an immunocompromised patient in the setting of disseminated infection. Antigen-based testing by PCR of lung tissue was required to establish the diagnosis. Intact cell-mediated immunity also is crucial to controlling proliferation of intracellular bacteria.[3] In HIV-infected individuals, a combination of weakened macrophage phagocytic function along with release of proinflammatory cytokines can facilitate progression of vascular proliferation resulting in angiogenesis and granuloma formation.[3]

Neuroretinitis in bartonellosis is well recognized as a manifestation of disseminated infection in immunocompetent hosts, but there is a paucity of data regarding this entity in patients with HIV infection. In the immunocompetent host, the hallmark of *Bartonella* neuroretinitis is a macular star lipid exudate.[4,5] Other eye findings include optic neuritis, macular hole, retinal white dot syndrome, retinal and chorioretinal inflammatory foci, peripapillary subretinal fluid, peripapillary angiomatosis, iridocyclitis, serous retinal detachment, arterial or venous occlusion or vasculitis.[4,5] Our patient did have serous retinal detachment with peripapillary subretinal fluid and optic neuritis but he lacked the distinctive macular star.

Some suggest that ocular bartonellosis is a hallmark of systemic disease. In HIV-positive patients, an abnormal vascular network with subretinal mass, unifocal choroiditis, and angiomatous lesions has been described.[6] A diagnostic challenge in these patients is that the findings can mimic eye involvement with CMV, mycobacterial infections including tuberculosis and atypical mycobacteria, HSV, varicella zoster, *Cryptococcus*, toxoplasmosis, toxocariasis, Kaposi sarcoma, extrapulmonary *Pneumocystis jirovecii*, and other fungal diseases. Neuroretinitis can occur with HIV infection itself or immune reconstitution syndrome in the absence of a secondary infection. We suspect that our patient's parenchymal brain lesions were a consequence of HIV immune

reconstitution. The improvement in his CD4 cell count and dramatic reduction in viral load several weeks into treatment for the *Bartonella* infection support this contention. Thus, the modest CSF pleocytosis with undetectable *Bartonella* antigen by PCR likely reflects a parameningeal process rather than invasion of the central nervous system.[7]

Hepatic or splenic microabscesses can occur in both immunocompetent and immunocompromised hosts but they are nonspecific and the finding of splenic microabscesses in our patient was not pathognomonic for *Bartonella* infection. In one report of 32 HIV-infected patients with unexplained fever, splenic microabscesses occurred in patients with tuberculosis (14), visceral leishmaniasis (7), MAC (5), *Candida* (1), *Rhodococcus* (1), *Salmonella* bacteremia (2), lymphoma (2), *P. jerovecii* (1 patient).[8] Documented pulmonary involvement with *Bartonella* is rare even in disseminated infection, but pulmonary infiltrates have been reported in both immunocompetent and immunocompromised patients with bartonellosis suggesting underdiagnosis because of a lack of respiratory symptoms.[9,10]

Coinfections of *Bartonella* with other organisms also can occur. Among 33 HIV-positive patients with *Bartonella* infection, one was coinfected with MAC and 2 with *Histoplasma capsulatum*.[11] Although disseminated MAC infections are common in these individuals, at least one-half of the patients infected with MAC have another identified infection.[12] Coinfection with CMV and tuberculosis also has occurred.[13,14]

Approaches to diagnosis of *Bartonella* in HIV-infected patients include serologic testing, culture of cutaneous lesions and evaluation by histopathology and PCR of material from tissue specimens. *Bartonella* is difficult to cultivate from blood or tissues. Plating onto chocolate or heart infusion agar with 5% rabbit blood with incubation in 5% CO_2 at 35 to 37°C for at least 21 days optimizes the yield. Indirect immunofluorescent antibody assay for antibodies to *Bartonella* and enzyme immunoassay for antibodies to *B. henselae* are available.[15] Serologic results can be difficult to interpret in HIV-infected patients because they do not reliably mount a response in the setting of a low CD4 cell count. Thus, tissue biopsy and *Bartonella* PCR should be undertaken, if feasible, as exemplified by our patient. Hematoxylin-eosin stain demonstrates a necrotizing granuloma, whereas Warthin-Starry stain can reveal small dark-staining bacteria. The PCR for *Bartonella* is commercially available and can be used in both blood and tissue specimens.

There are no controlled trials of treatment for bartonellosis in HIV-infected individuals. Erythromycin and doxycycline are first-line agents. A minimum of 3-months treatment duration is recommended.[15] If neuroretinitis or central nervous system disease is present, doxycycline and rifampin are indicated for treatment in children 8 years of age or older.[15,16] In younger children, trimethoprim-sulfamethoxazole and rifampin can be employed.[16] Since relapse can occur after a primary course, long-term suppressive therapy is recommended until the CD4 cell count exceeds 200 cells/mm^3.[15] Immune reconstitution syndrome has not been reported previously in HIV patients coinfected with *Bartonella.* We believe that this is a case of "unmasking" immune reconstitution disease provoked by *Bartonella* infection in an immunocompromised host because of the dramatic reduction of his viral load and increase in his CD4 cell counts within 3 weeks of initiating antiretroviral therapy.[17] Some experts recommend deferring antiretroviral therapy initiation for 2 to 4 weeks after initiating treatment for *Bartonella*.[15] Our patient's therapy was reinstituted after he had experienced initial improvement in visual acuity. Restoration of visual acuity in *Bartonella* neuroretinitis occurs in 1 to10 weeks after initiation of therapy in immunocompetent patients.[5] Our patient's vision improved within 2 weeks and was restored by 4 months after initiation of appropriate therapy.

In summary, disseminated bartonellosis should be considered in the differential diagnosis for fever and neuroretinitis and a history of cat contact should be sought. Splenic or liver microabscesses, cutaneous lesions, and lymphadenopathy are additional diagnostic clues but may not be consistently present. Our patient's recovery illustrates the gratifying outcome possible when treatment of neuroretinitis can be pathogen-directed.

REFERENCES

1. Pons I, Sanfeliu I, Nogueras MM, et al. Seroprevalence of *Bartonella spp*. infection in HIV patients in Catalonia, Spain. *BMC Infect Dis*. 2008;8:58.
2. Pape M, Kollaras P, Mandraveli K, et al. Occurrence of *Bartonella henselae* and *Bartonella quintana* among human immunodeficiency virus-infected patients. *Ann N Y Acad Sci*. 2005;1063:299–301.
3. Resto-Ruiz S, Burgess A, Anderson BE. The role of the host immune response in pathogenesis of *Bartonella henselae*. *DNA Cell Biol*. 2003;22: 431–440.
4. Ormerod LD, Dailey JP. Ocular manifestations of cat-scratch disease. *Curr Opin Ophthalmol*. 1999;10:209–216.
5. Reed JB, Scales DK, Wong MT, et al. *Bartonella henselae* neuroretinitis in cat scratch disease. Diagnosis, management, and sequelae. *Ophthalmology*. 1998;105:459–466.
6. Curi AL, Machado DO, Heringer G, et al. Ocular manifestation of cat-scratch disease in HIV-positive patients. *Am J Ophthalmol*. 2006;14:400–401.
7. Seah ABH, Azran MS, Rucker JC, et al. Magnetic resonance imaging abnormalities in cat-scratch disease encephalopathy. *J Neuro Ophthalmol*. 2003;23:16–21.
8. Bernabeu-Wittel M, Villanueva JL, Pachón J, et al. Etiology, clinical features and outcome of splenic microabscesses in HIV-infected patients with prolonged fever. *Eur J Clin Microbiol Infect Dis*. 1999;18:324–329.
9. Marseglia GL, Monafo V, Marone P, et al. Asymptomatic persistent pulmonary infiltrates in an immunocompetent boy with cat-scratch disease. *Eur J Pediatr*. 2001;160:260–265.
10. Caniza MA, Granger DL, Wilson KH, et al. Bartonella henselae: etiology of pulmonary nodules in a patient with depressed cell-mediated immunity. *Clin Infect Dis*. 1995;20:1505–1511.
11. Barat LM, Gunn JE, Steger KA, et al. Causes of fever in patients infected with human immunodeficiency virus who were admitted to Boston City Hospital. *Clin Infect Dis*. 1996;23:320–328.
12. Koehler JE, Sanchez MA, Tye S, et al. Prevalence of *Bartonella* infection among human immunodeficiency virus-infected patients with fever. *Clin Infect Dis*. 2003;37:559–566.
13. Rovery C, Rolain JM, Lepidi H, et al. *Bartonella quintana* coinfection with *Mycobacterium avium* complex and CMV in an AIDS patient: case presentation. *BMC Infect Dis*. 2006;6:89.
14. Núñez M, Miralles ES, Hilara Y, et al. Concurrent cytomegalovirus, M. tuberculosis and M. avium-intracellulare cutaneous infection in an HIV patient. *J Dermatol*. 1997;24:401–404.
15. Kaplan JE, Benson C, Holmes KH, et al. Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. Centers for Disease Control and Prevention (CDC); National Institutes of Health; HIV Medicine Association of the Infectious Diseases Society of America. *MMWR Recomm Rep*. 2009;58 (RR-4):1–207.
16. American Academy of Pediatrics. Cat Scratch Disease (*Bartonella henselae).* In: Pickering LK, Baker CJ, Kimberlin DW, et al, eds. *Red Book: 2009 Report of the Committee on Infectious Diseases.* 28th ed. Elk Grove Village, IL: American Academy of Pediatrics; 2009:249–250.
17. French MA. Immune reconstitution inflammatory syndrome: a reappraisal. *Clin Infect Dis*. 2009;48:101–107.

SYNDROME OF INAPPROPRIATE ANTIDIURETIC HORMONE ASSOCIATED WITH LOPINAVIR THERAPY

*Michael Mun-Kitt Yeong, MB BS,**
Pamela Palasanthiran, FRACP, MD, John B. Ziegler, FRACP, MD,* and Tony Walls, FRACP, MD*†*

Abstract: We report a case of the syndrome of inappropriate antidiuretic hormone associated with use of lopinavir in an HIV-infected child. This rare phenomenon has not previously been reported in children. Clinicians should be alert to the possibility of the syndrome of inappropriate antidiuretic hormone when prescribing lopinavir in children.

Key Words: HIV, hyponatremia, SIADH, lopinavir

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From the *Department of Immunology and Infectious Disease, Sydney Children's Hospital, Sydney, Australia; and †Department of Women's and Children's Health, University of New South Wales, Sydney, Australia.

Address for correspondence: Tony Walls, FRACP, MD, Department of Paediatrics, Christchurch School of Medicine, University of Otago, PO Box 4345 Christchurch 8140, New Zealand. E-mail: tony.walls@otago.ac.nz.

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CASE REPORT

A 13-year-old boy with perinatally acquired HIV infection presented with a 2 day history of fever, nausea, vomiting, diarrhea, and abdominal discomfort. There was no history of recent travel, no contacts with tuberculosis, animals, or anyone with a similar illness. He had been receiving trimethoprim-sulfamethoxazole prophylaxis for 2 months before this presentation and commenced HAART for the first time one week before his admission. Initiation of HAART had been delayed several weeks because of his reluctance to swallow tablets. There was no history of illicit drug usage and he was not taking any other medications. He was not symptomatic (CDC category N),[1] and HAART had been initiated for falling CD4 counts and rising HIV viral load. On the day he started treatment he weighed 36.7kg, his CD4 count was 70×10^9/L (CD4% = 10) with a viral load of 495,000 copies/mL. His initial daily regimen consisted of tenofovir-emtricitabine (Truvada 300/200 mg, 1 tablet daily) and lopinavir-ritonavir (Kaletra 200/50 mg, 2 tablets twice daily).[2]

On presentation he had a temperature of 39°C, weighed 36.2kg, and was clinically dehydrated. He had dry mucous membranes, his heart rate was 120 beats per minute and blood pressure was 118/72 mm Hg. There was no rash or lymphadenopathy and no abnormality was found on cardiac, respiratory, abdominal, or neurologic examination.

Initial investigations showed a hemoglobin of 97 g/L, white blood count of 2.97×10^9/L with 78% neutrophils, 15% lymphocytes, and 0.3% eosinophils. His erythrocyte sedimentation rate was 56 mm/h, C-reactive protein 14 mg/L (normal <3), and the blood film showed mild microcytosis and hypochromia. He was hyponatremic with serum sodium of 132 mmol/L, and hypokalemic with serum potassium of 3.1 mmol/L. Other serum electrolytes, amylase, and liver function tests were all normal.

A clinical diagnosis of gastroenteritis with mild dehydration was made and he was admitted for investigations and intravenous fluids. No antibiotics were given. Over the next 24 hours his serum sodium and serum potassium normalized and his gastrointestinal symptoms ceased. His intravenous fluids were stopped and he recommenced oral intake, but he remained intermittently febrile with temperatures ranging from 38.6°C to 40.2°C occurring once daily.

During the next 48 hours, hyponatremia recurred. He was clinically euvolemic and had no significant ongoing fluid losses. Plasma sodium was 128 mmol/L with an osmolality of 267 mmol/kg. A concurrent urine sample had sodium of 24 mmol/L and osmolality 495 mmol/kg. On the basis of his clinical status and electrolyte abnormalities a diagnosis of the syndrome of inappropriate antidiuretic hormone syndrome (SIADH) was made.[1] Fluid intake was restricted to 1000 mL per day.

Subsequent investigations demonstrated no bacterial or fungal growth on multiple blood, urine, and stool cultures. Stools were negative for ova, cysts, and parasites and enteric viruses. *Clostridium difficile* toxin was not identified. Serum PCR for cytomegalovirus, Epstein-Barr virus, herpes simplex virus, adenovirus and enterovirus were negative. There was no serologic evidence of syphilis, Q-fever, *Bartonella* or *Mycoplasma* infection. The initial chest radiograph was normal and cranial and thoracic computerized tomography did not show any abnormality. Endoscopy revealed mild oesophagitis, gastritis, and duodenitis only. A lumbar puncture was not felt to be indicated as he had no neurologic symptoms or signs.

SIADH persisted during his 2-week hospital admission, and despite fluid restriction and additional sodium and urea supplements his sodium remained below the normal range. An adverse drug reaction was considered in the differential diagnosis throughout his admission, but given his clinical presentation we elected to continue HAART until we had excluded an opportunistic infection. One week later, and 4 weeks after starting HAART, he developed a widespread, itchy maculopapular rash, highly suggestive of drug hypersensitivity. At this point his tenofovir-emtricitabine and lopinavir-ritonavir were discontinued, and he continued to receive trimethoprim-sulfamethoxazole prophylaxis only. The fevers and rash subsided within twenty-four hours and serum sodium remained normal as the fluid restriction was relaxed and he returned to a normal diet.

It was felt that the most likely cause of his SIADH was lopinavir. Thus, 2 weeks later his regimen was changed and he was given HAART with atazanavir boosted with ritonavir (ATV/r) and combination tenofovir/emtricitabine. His viral load became undetectable on this combination and no associated adverse effects or electrolyte disturbances developed. At follow-up he remains well, with an unchanged antiretroviral combination and an undetectable viral load.

DISCUSSION

Reports of SIADH associated with lopinavir are exceedingly rare and to our knowledge this is the first time it has been reported in a child. Roberts et al[3] described a 42-year-old Zimbabwean man who presented with confusion after commencing treatment with lamivudine, tenofovir, and lopinavir-ritonavir. SIADH began within a week of starting HAART and hyponatremia resolved once therapy was stopped. The substitution of lopinavir with atazanavir was tolerated without further electrolyte disturbances.

Similarly, in our case, there was a strong temporal association between the clinical features of SIADH and the introduction of lopinavir. We were able to substitute atazanavir for lopinavir without a recurrence of symptoms or electrolyte disturbance. These factors, and the lack of any identifiable infection or other possible cause, led us to conclude that our patient's SIADH was most likely related to the use of lopinavir.

Hyponatremia in HIV-infected patients is a well recognized phenomenon, and occurred in 35% to 50% of hospitalized patients before the introduction of highly active antiretroviral therapy.[4] This was most often related to associated opportunistic infections or an association with HIV encephalopathy. Hyponatremia associated with HAART is much less common, and has seldom been reported in children. Jugulete et al[5] described one patient with

transient hyponatremia from a cohort of 185 HIV-infected Romanian children treated with lopinavir-ritonavir. However, it is not clear from this report what the pathogenesis of the hyponatremia was, and in particular if it was related to SIADH.

The mechanism by which SIADH develops following exposure to antiviral drugs is unclear.[6] A wide range of drugs can cause SIADH either by stimulating the release of arginine vasopressin from the anterior pituitary or by enhancing its action. These include tricyclic antidepressants, SSRIs, carbamazepine, narcotics, antipsychotic drugs, nonsteroidal anti-inflammatory drugs and MDMA (ecstasy). We were unable to find reports of SIADH associated with other antiretroviral drugs. The immediate management of SIADH includes identifying and removing the underlying cause, while the optimal strategy for correcting serum sodium continues to be debated.

Commencing multiple drugs simultaneously in a HAART regimen makes identifying the cause of any adverse drug reaction difficult. We chose to reintroduce HAART without lopinavir as the limited literature on SIADH suggested this drug was the most likely cause. An alternative approach may have been to exclude all of the drugs used initially, but this would not have identified the cause of SIADH precisely and could potentially have limited future therapeutic options. Clinicians should be alert to the possibility of SIADH when prescribing lopinavir in children.

REFERENCES

1. CDC. 1994 Revised classification system for human immunodeficiency virus infection in children less than 13 years of age. *MMWR Recomm Rep.* 1994;43:1–10.
2. PENTA guidelines for the use of antiretroviral therapy. *HIV Med.* 2009;10: 591–613. Available at: www.hppmcs.org.
3. Roberts M, Aliyu S. Hyponatraemia associated with lopinavir-ritonavir? *Int J Infect Dis*. 2007;11:2064–2072.
4. Tolaymat A, Al-Mousily F, Sleasman J, et al. Hyponatremia in pediatric patients with HIV-1 infection. *South Med J*. 1995;88:1039.
5. Jugulete G, Mardarescu M, Petrea S, et al. Kaletra (lopinavir/ritonavir) on treatment of HIV-infected children. *Int Conf AIDS*. 2004;15:TuPeB4441.
6. Ellison D, Berl T. The syndrome of inappropriate antidiuresis. *N Eng J Med*. 2007;356:2064–2072.