BRIEF REPORT

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An Infant with Human Parechovirus Type 3 Infection with a Distinctive Rash on the Extremities

Abstract: **Human parechovirus type 3 (HPeV3) is known to cause sepsis-like syndrome and meningoencephalitis in neonates and young infants. We herein report a neonatal case of sepsis-like syndrome due to HPeV3 infection, diagnosed using polymerase chain reaction (PCR), with a distinctive erythematous rash present mainly on the soles and palms that helped in the diagnosis of the disease. Combining the unique characteristics of rash and confirmation by PCR at the early stage of the disease led to the diagnosis of HPeV3, distinguishing it from sepsis and other critical disease conditions, and allowing for appropriate, rapid management.**

Human parechoviruses (HPeVs) are part of newly recognized genus *Parechovirus* in the family *Picornaviridae* (1). Similar to enteroviruses, HPeV type 3 (HPeV3) has been known to be a major cause of sepsis-like illness and meningoencephalitis in neonates and young infants with various grades of neurologic morbidity and mortality (1).

The signs and symptoms of HPeVs infection are nonspecific. The common findings of neonatal sepsis-like syndrome due to HPeV3 are fever, tachycardia, tachypnea, and rash. Although maculopapular rash has been reported in patients with HPeV3 infections (2–4), detailed descriptions of the rash have been limited.

CASE REPORT

A 19-day-old, full-term, male neonate was admitted to our hospital for a 1-day history of high fever with rhinorrhea. His family history was significant for upper respiratory infection symptoms, with a fever in all family members. Physical examination revealed a toxic general appearance with a temperature of 39°C, a heart rate of 202/minute, a respiratory rate of 68/minute, a saturation of peripheral oxygen of 96% (on room air), and blood pressure of 78/50 mmHg. Physical examination was unremarkable, without any obvious signs of the source of the fever. A sepsis examination was initiated. His cerebrospinal fluid (CSF) showed no pleocytosis, with normal glucose and protein levels. A chest radiograph revealed no abnormality. Neonatal sepsis was suspected, and ampicillin and cefotaxime were initiated, but the fever, tachycardia, and tachypnea continued despite antibiotic therapy. The blood culture taken on admission was negative at 48 hours. On day 2 of hospitalization his physical examination revealed a rash on his extremities, most notably on the lower legs and the soles of his feet (Figs. 1 and 2). RNA extracted from the serum, CSF, and nasopharyngeal swab were tested for HPeVs using polymerase chain reaction (PCR) (5), and all were positive. The genetic sequences of the viral protein (VP) regions according to PCR were consistent with HPeV3. We discontinued antibiotic therapy based on the positive results of PCR on day 3 of hospitalization. The infant's rash and other signs resolved spontaneously on day 5 of hospitalization, and he was discharged from the hospital on day 8 of hospitalization without any sequelae.

DISCUSSION

Our patient demonstrated a distinctive distribution of the rash—erythema on his soles and a maculopapular rash, predominantly on his lower legs—that were not described in the previous literature (2–4). A rash limited to the distal part of the extremities and erythema on the palms and soles are rare physical findings in the neonatal and infantile period, which could be specific for this HPeV3 strain and a clue for diagnosing HPeV3 infection. Our recent experience in neonates and infants with PCR-confirmed HPeV3 infection also supports these findings; 80% (12/15) demonstrated a rash on the palms and soles (6). HPeV3 infection should be considered in the differential diagnosis of neonates and young infants with sepsis-like syndrome and distinctive rash on the palms

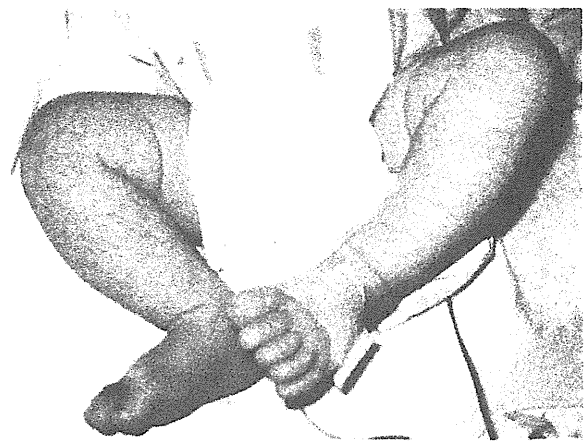

Figure 1. Maculopapular rash on legs and erythema on soles.

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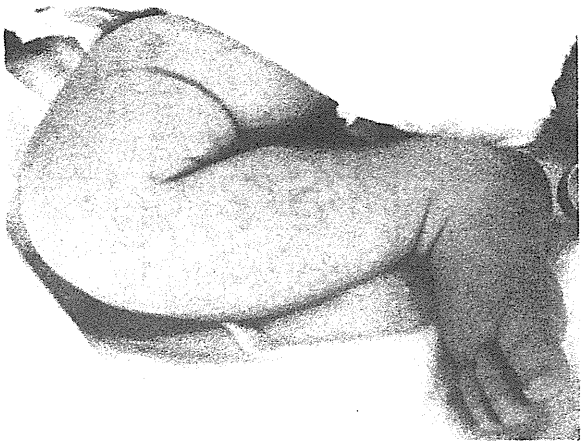

Figure 2. Erythema on the right sole and maculopapular rash on the right lower leg.

and soles. The PCR analysis for HPeVs is the most reliable diagnostic method, leading to appropriate diagnosis and a decrease in the inappropriate use of antimicrobials.

REFERENCES

1. Harvala H, Wolthers KC, Simmonds P. Parechoviruses in children: understanding a new infection. Curr Opin Infect Dis 2010;23:224–230.
2. Verboon-Maciolek MA, Krediet TG, Gerards LJ et al. Severe neonatal parechovirus infection and similarity with enterovirus infection. Pediatr Infect Dis J 2008;27:241–245.
3. Boivin G, Abed Y, Boucher FD. Human parechovirus 3 and neonatal infections. Emerg Infect Dis 2005;11:103–105.
4. Bangalore H, Ahmed J, Bible J et al. Abdominal distension: an important feature in human parechovirus infection. Pediatr Infect Dis J 2011;30:260–262.
5. Nix WA, Maher K, Johansson ES et al. Detection of all known parechoviruses by real-time PCR. J Clin Microbiol 2008;46:2519–2524.
6. Komuro H, Kobayashi Y, Funaki T, Miyata I, Miyairi I, Takayama JI, Saitoh A. Dermatologic manifestation of human parechovirus type 3 infections in neonates and infants. Presented at the 2012 Pediatric Academic Societies, Boston, MA, April 28-May 1.

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Review

Current issues with the immunization program in Japan: Can we fill the "vaccine gap"?

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ABSTRACT

The "vaccine gap" is a term which has been used in Japan to indicate that the current immunization program is behind compared to the programs in other developed countries. The current national immunization program (NIP) which was established under the Japanese Immunization Law includes only six vaccines (eight targeted diseases), and the rest of available vaccines have been categorized as voluntary vaccines, which require out-of-pocket expense in order for the patients to receive them. This has led the vaccination rates for the voluntary vaccines remaining low, and the incidence of the target diseases remaining high. In addition, there are a few domestic rules that exist for immunizations including (1) subcutaneous injection is the standard method of vaccination, (2) the thigh is not considered to be the common site of vaccination in infants, and (3) the intervals of administration of inactivated and live vaccines are strictly determined by law. Along with the "vaccine gap" and the domestic rules, some movements to improve our current NIP are underway; including increased calls to change the NIP from civilians and professionals, the establishment of a group by the representatives from 13 medical professional societies asking the government to consider the immunization policy a "national policy" and seeking the establishment of a new and reorganized national immunization technical advisory group (NITAG). In addition, the Vaccination Subcommittee of Health Sciences Council was formed in the government to reform the current Immunization Law and NIP, which established a new national program for three voluntary vaccines funded by a temporary budget. We hope these new movements will fill the "vaccine gap" and that the NITAG will help ensure that vaccine policy becomes a national policy, and will provide necessary vaccinations without out-of-pocket expense to protect children in Japan from vaccine preventable diseases.

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Abbreviations: Hib, *Haemophilus influenzae* type b; VZV, varicella zoster virus; NIP, national immunization program; HPV, human papillomavirus vaccine; PCV7, seven-valent conjugate pneumococcal vaccine; HBV, hepatitis B virus; VPD, vaccine-preventable diseases; NITAG, National immunization technical advisory group; DTaP, diphtheria, tetanus-toxoid, and acellular pertussis; DTwP, diphtheria, tetanus-toxoid, and whole cell pertussis; MMR, mumps, measles, rubella; JPS, Japan Pediatric Society; IPV, inactivated polio vaccine; BCG, Bacille de Calmette et Guérin; OPV, oral polio vaccine; ACIP, Advisory Committee on Immunization Practices; VAPP, vaccine-associated poliomyelitis paralysis.

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1. Introduction

The "vaccine gap" is a term which has been used for the last decade to indicate that the immunization program in Japan has been behind compared to the programs in other developed countries [1]. The best example is that the *Haemophilus influenzae* type b (Hib) vaccine, which has been known to be safe and the most effective vaccine for preventing invasive Hib infections [2], was introduced in Japan in 2008, which was more than 20 years later than other countries. In addition, some important vaccines, including the mumps vaccine, and varicella zoster virus (VZV) vaccine, have been available in Japan for the last two decades; however, they have not been in the national immunization program (NIP). Furthermore, the Hib vaccine, human papillomavirus vaccine (HPV), seven-valent conjugate pneumococcal vaccine (PCV7), and rotavirus vaccine have only recently been introduced in Japan since 2008; however, none of them is part of the NIP. Lastly, the hepatitis B virus (HBV) vaccine has so far only been used as a selective vaccination; and the universal HBV vaccination has not yet been included in the NIP. All these vaccines not in the NIP have been categorized as voluntary vaccines, as opposed to vaccines under the Japanese Immunization Law. To receive the voluntary vaccines, individuals must pay out-of-pocket expense, which has been a major obstacle to increasing the vaccination rates for each voluntary vaccine and decreasing the incidence of vaccine-preventable diseases (VPD).

The reasons why the "vaccine gap" exists are multi-factorial; a long history of fear about vaccinations, the existence of the Immunization Law imposing strict rules for immunization practice, the ineffectiveness of a systematic national surveillance system for VPD, insufficient resources of vaccine education for both medical personnel and civilians, and the lack of an effective national immunization technical advisory group (NITAG). In this review, we summarize these factors contributing to the "vaccine gap" and discuss a few current issues related to immunization in Japan.

2. The history of vaccine fears in Japan

The initial Immunization Law was launched in 1948, with the goal of decreasing the incidence of endemic diseases, such as smallpox, diphtheria, polio, tetanus, pertussis, tuberculosis, etc. After the significant improvement of the sanitary status and the distribution of available vaccines under the revised immunization law, the incidence of the endemic diseases in Japan decreased significantly. During the subsequent period, the vaccination rates for the targeted diseases were high, because receiving vaccines was considered a duty, and there was a penalty if citizens did not receive the required vaccines. Furthermore, Japanese scientists contributed to develop some novel vaccines to the world, including the VZV vaccine in 1974 [3] and the DTaP (diphtheria, tetanus-toxoid, and acellular pertussis) vaccine in 1981 [4].

There were two major events that impacted the immunization program in Japan. First, two fatalities after DTwP (diphtheria, tetanus-toxoid, and whole cell pertussis) vaccination were reported in 1975, and the vaccine was withheld for six years until a new acellular pertussis combination vaccine was available [4]. After that event, civilians started to have doubts about receiving the immunization because the risks of vaccination were emphasized by the mass media. As expected, the temporary discontinuation of the DTwP vaccine led to the resurgence of 13,000 pertussis cases and 20 deaths reported in 1979 [4]. The second event was in 1989 when the mumps, measles, rubella (MMR) vaccine caused vaccine-related aseptic meningitis due to the mumps component of the vaccine. The incidence of meningitis was estimated to be one case in every 500–900 vaccinations [5]. The vaccine was withdrawn from the market in 1993 based on the Japanese government's decision, and monovalent measles and rubella vaccines were recommended for children >1 year of age. Twelve years were required for the marketing of a new combination vaccine of measles and rubella, without the mumps component, and the lack of a combination vaccine with the mumps component is the reason why the disease is still endemic in Japan and many children have been suffering from its complications [6]. The delay of introducing the mumps strain causing less aseptic meningitis, which was carried out in order to protect domestic Japanese vaccine manufacturers, was criticized by the vaccine authorities [7]. The Japanese government was also sued several times for being responsible for vaccine adverse effects in the 1980s and 90s, including severe adverse events after small pox immunization. In addition, there were negative campaigns against the influenza vaccine by both citizens and medical professionals doubting its effectiveness and believing it to cause serious adverse effects. After these multiple events, the government has had difficulty in defending their vaccination policy, because they hesitate to be responsible for their decisions related to immunization. In 1994, the Immunization Law was revised, and the immunization was changed from a civic "duty" to an "effort duty", and mass immunization in schools was thus discontinued and changed to immunization on an individual basis. These movements decreased the vaccination rates in general and led to a failure to introduce new vaccines between 1991 and 2007, which led to the creation of the "vaccine gap". Only two new vaccines were licensed in Japan between 1990 and 2007 (hepatitis A virus, and measles and rubella combination vaccine) compared to 17 vaccines (including combination vaccines) introduced in the US during the same period.

3. Factors contributing to the "vaccine gap"

3.1. Vaccines under the law and voluntary vaccines

There is a unique classification of vaccines in Japan; vaccines defined by the immunization law, and voluntary vaccine not regulated by Japanese law (Table 1). Several important vaccines, including the mumps vaccine, VZV vaccine, and HBV vaccine have remained categorized as voluntary vaccines which require individuals to pay out-of-pocket, and considers them to be less important vaccines compared to the vaccines under the law. This led to low vaccination rates for these voluntary vaccines, and the incidence of the target diseases has remained high [8]. In contrast, the use of the HBV vaccine has been limited to children whose mothers are positive for the HBV surface antigen and individuals at high risk for HBV. Although the HBV carrier rate used to be high in Japan and the rate has decreased significantly due to selective immunization, we nevertheless hope to reduce the rate even further

Table 1
A comparison of vaccines under the immunization law and voluntary vaccines for children in Japan.

	Vaccines under the immunization law	Voluntary vaccines
Regulated by the immunization law	Yes	No
Vaccination fee	Almost free of charge (provided by the government and the local sector)	Out-of-pocket expense
Compensation for adverse effects	By the immunization law	By the PMDA law
Vaccines	Diphtheria, pertussis, tetanus vaccine (DTaP, DT) BCG Oral polio vaccine Measles rubella vaccine (MR, M, R) Japanese encephalitis vaccine Diphtheria tetanus vaccine	*Haemophilus influenzae* type b vaccine[a] 7 valent pneumococcal conjugate vaccine[a] Hepatitis B virus vaccine[b] Mumps vaccine Varicella zoster virus vaccine Human papillomavirus vaccine[a] Influenza vaccine Hepatitis A virus vaccine Rotavirus vaccine

PMDA: Pharmaceutical and medical devices agency.
[a] Supported by the temporary budget for fiscal years 2010–2011 and 2011–2012.
[b] Selective immunization is covered by the national health insurance system.

in order to reduce the drop out of selective immunization and to prevent horizontal transmission through intra-familial, intra-institutional, or sexual routes. Japan is surrounded by countries with a high incidence of HBV infection [9]. In addition, genotype A, which tends to shift to chronic hepatitis, is the predominant genotype accounting for up to 60% of acute HBV infections in Japan [10]. Furthermore, it is estimated that one third of pediatric HBV carriers were transmitted the disease by non-vertical transmission [11]. These facts strongly emphasize the importance of universal HBV vaccination. The new vaccines introduced after 2008, including the Hib vaccine, HPV vaccine, PCV7, and rotavirus vaccine, have also been categorized as voluntary vaccines as of March, 2012. The fee for receiving these vaccines for parents has been high, and the economic burden associated with these VPD has been also high.

3.2. Subcutaneous vs. intramuscular vaccination

Currently, subcutaneous vaccination is the standard method of vaccination in Japan. Intramuscular injection is limited to specific vaccines, including the HPV vaccine, the adjuvanted 2009 A/H1N1 vaccine, and the HBV vaccine for subjects older than 10 years of age. The reason why intramuscular injection has been restricted is that there was a report with an accumulation of approximately 3700 cases with contracture of the gluteal quadriceps muscle in the 1970s due to the frequent intramuscular injection of antibiotics or antipyretics for the treatment of common respiratory infections [12]. Although no case of muscle contracture has been reported due to the injection of vaccines, the Japanese Pediatric Society (JPS) made a statement that there is no safe place for intramuscular injection in children in 1972 to reduce unnecessary injections for common respiratory infections [12]. Since then, intramuscular injections to children have remarkably decreased, but the majority of vaccines have been administered subcutaneously.

Intramuscular injection is known to have benefits compared subcutaneous injection [13]; it causes fewer local reactions such as pain, redness, and swelling, and results in equal or greater immunogenicity in children immunized with the diphtheria, tetanus-toxoid vaccine [14]. In infants that received a tetravalent combination vaccine (diphtheria, pertussis, Hib and IPV), intramuscular injection also showed fewer local reactions and equal immunogenicity compared to subcutaneous injection [15]. Because intramuscular injection is the standard method of vaccination for the majority of vaccines (except for some live vaccines) in other countries, intramuscular injection should be reconsidered as a method of vaccination for Japanese children.

3.3. Anatomical site of vaccinations

The most common location used for the vaccination of children in Japan is the lateral side of the upper arms. The anterior frontal aspects of the thighs have not been used as the site of injection due to the fear of muscle contracture. When simultaneous vaccination is required to provide appropriate vaccines for children, especially in early infancy, Japanese physicians have started to raise questions about where to inject multiple vaccines in the small area of the upper arms in infants. In addition, it has been reported that it is best to separate the injection sites by at least one inch if the same anatomical site is used for intramuscular injection [16], but there have been no such studies regarding subcutaneous injection at the same anatomical site. To provide a sufficient location for vaccination, the anterior frontal aspects of the thighs should be included as a location of vaccination for Japanese children.

3.4. Obstacles impacting simultaneous vaccination

Simultaneous vaccination is a common and safe practice used to vaccinate children [17], and it is known to be efficacious to provide vaccines in a timely manner in order to appropriately protect children from VPD, to save time for caregivers and medical care personnel, and also to decrease the medical costs [18]. However, this practice has not been well distributed and understood in Japan, because there had been no need to perform simultaneous vaccination due to the lack of necessary vaccines, especially during early infancy. Following the introduction of the Hib vaccine and PCV7, there has been a need for simultaneous vaccination. Currently, there are doubts about the safety and efficacy of simultaneous vaccination voiced by both medical professionals and civilians.

To reduce the number of shots for young infants and children, it is necessary to develop and introduce combination vaccines. The usefulness of combinations vaccines has been confirmed by several studies to decrease the numbers of vaccinations and increase the vaccination rates [19–21]; however, there are currently only three combination vaccines available in Japan; the DTaP, DT, and MR vaccines produced by the domestic vaccine companies. Moreover, there is currently no combination vaccine containing components of HBV, Hib, or an inactivated polio vaccine (IPV). At this moment, the numbers of shots that should be completed with the current JPS recommended immunization program during early infancy is high. To date, due to the limited use of combination vaccines in Japan, simultaneous vaccination is a necessary practice to protect children from VPD. This message was clearly noted by the JPS in 2011. However, there has been a gap between the statement and actual practice. To widely distribute simultaneous

vaccination to protect children from VPD beginning from early infancy in Japan, both medical professionals and civilians need to understand the importance and safety of simultaneous vaccination. Furthermore, the introduction of combination vaccines to reduce the number of shots and increase the vaccination rates is urgently needed.

3.5. Rules about the vaccination intervals

Under the immunization law, the intervals at which different inactivated vaccines and live vaccines are given are strictly set to be greater than six days and greater than 27 days, respectively. These numbers were established by the Immunization Law to ensure that the responsible vaccine could be identified if an adverse event occurred after vaccination. These intervals prevent the general public from getting their vaccinations in a timely manner, especially after receiving lives vaccines [Bacille de Calmette et Guérin (BCG) and oral polio vaccine (OPV)] during early infancy. In general, the intervals of different vaccines are only set when parenteral live vaccines are given (28 days) [22]; therefore, these rules should be reconsidered to increase the vaccination rates and increase the opportunities for the vaccination of Japanese children.

3.6. The lack of an effective national immunization technical advisory group (NITAG)

The NITAG is important because it makes decisions that determine the national policy of vaccination; however, such a group does not exist in the current Japanese system. There have been a few committees organized by separate departments of the Ministry of Labor, Welfare, and Health to discuss issues related to immunization; however, there was little discussion regarding the long-term vision of national vaccination strategies and such committees have not been held either regularly or continuously. Because current infectious disease epidemiology clearly indicates that several VPD are still endemic in Japan and affect Japanese children, it is necessary to consider developing a vaccine policy setting system in Japan [23].

4. Current issues

4.1. Refusal of the oral polio vaccine due to fear of vaccine-associated poliomyelitis paralysis (VAPP)

Although the development of IPV using Sabin strain-derived vaccine was initiated in the 1990s in Japan, there has been delay of in the process for its production and authorization, which has therefore led to the current problematic situation, namely that Japan is the only developed country still routinely using the OPV as of March, 2012. There have been many programs on television and articles in newspapers describing the fears of vaccine-associated poliomyelitis paralysis (VAPP), which estimated the incidence of VAPP as 1.4 cases per one million vaccinations in Japan for the last 15 years based on the number of cases that have been reported as VAPP [24]. This led to a decrease in the OPV vaccination. Although a combination vaccine including Sabin strain-derived, inactivated polio and DTaP is expected to be licensed in Japan by the end of 2012 at the latest and the Director of the Ministry of Health, Labor, and Welfare has been trying to facilitate the process, there are caregivers who have had their children receive an imported and unlicensed IPV in Japan by paying for the vaccine out-of-pocket, and more than 20,000 children have been vaccinated this way. If adverse effects occur due to the vaccine, the government cannot be held responsible; the patients will have to be compensated by the insurance system of the importing company, with strict limitations for compensation. Some parents have been waiting for the IPV + DTaP combination vaccine, and have not had their children receive the OPV, which leads to the risk of developing a wild polio infection if the disease moves into Japan. The JPS warned the public about this situation and that everyone should avoid an unvaccinated status, because there are still some outbreak cases of wild polio that have occurred in various countries, including cases in China in August 2011 [25]. This issue will continue until the new Sabin strain-derived IPV + DTaP vaccine is licensed.

4.2. Temporary withholding of the Hib and PCV7 vaccines after a report of seven fatalities

On March 8, 2011, the Hib vaccine and PCV7 were temporarily withheld due to a report of accumulation of seven fatalities that occurred one to seven days after simultaneous vaccinations with the Hib vaccine and/or PCV7 and/or the DTaP vaccine or BCG. Detailed case analyses demonstrated that there was no causative relationship between these deaths and the vaccines according to an expert committee organized by the Ministry of Health, Labor, and Welfare. Two of the cases had severe congenital heart diseases; three patients had risk factors for sudden infant death syndrome; and one case was reported to have a human metapneumovirus infection. The accumulated fatality rates (including adverse events) were 0.13 and 0.15 per one million vaccinations for Hib and PCV7 in Japan between 2005 and 2010, respectively, which have been reported to range between 0.04–1.0 and 0.1–0.6, respectively, in other countries. No specific lots were identified to be responsible for causing the events. Additionally, no deviation in the process of vaccine certification was found. Therefore, the Scientific Committee assembled by the government concluded that there was no relationship between the vaccines and the fatal events, and vaccinations with both vaccines were resumed on April 1, 2011, 22 days after the interruption. Although no causal relationship between the vaccines and fatalities were identified, the following sentences were added to the package inserts for Hib and PCV7; Physicians need to notify their patients or the patients' guardians that there is an option for single vaccination, and single vaccination should thus be considered, especially for children with underlying diseases. These specific notes in the package inserts, which were added without the authorization of vaccine specialists, led to physician confusion regarding whether simultaneous vaccination is safe for Japanese children.

5. New movements to improve the immunization system in Japan

Although these critical issues have been discussed for decades [26], some important movements to improve our current NIP are underway. First, both the general public and medical professionals have voiced a desire to change the NIP, and these voices have become stronger every year. Approximately 2.7 million signatures from civilians and medical professionals led by the Japanese Medical Association were collected and presented to the government asking them to improve the NIP [27]. Second, representatives from 13 medical professional societies gathered to ask the government to consider immunization policy as a "national policy" and seeking the establishment of a new NITAG system to provide expert opinions for the government [23]. Finally, along with these movements, the government launched the Vaccination Subcommittee of Health Sciences Council to discuss the reform of the current Immunization Law and NIP in Japan [28]. All of these new movements led to the government's decision to establish a new national program for the Hib vaccine, PCV7, and HPV vaccines funded by a temporary budget. The government is further considering continuing this budget and

including these vaccines into the NIP, along with other important vaccines currently categorized as voluntary vaccines, such as the universal HBV vaccine, VZV vaccine, and mumps vaccine. Furthermore, the JPS launched a new immunization schedule which put the vaccines in an order of requirement and does not distinguish between the vaccines under the law and voluntary vaccines [29]. It is hoped that these new movements will reform the immunization law and improve the NIP in Japan, and that this will lead to the government providing necessary vaccines for all children without out-of-pocket expenses for the guardians in order to make sure that all children are protected from VPD.

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We dedicated this manuscript to Dr. Hitoshi Kamiya (1943–2011) who devoted his life to improving the immunization systems in Japan.

References

[1] Murashige N, Matsumura T, Masahiro K. Disseminating Japan's immunisation policy to the world. Lancet 2011;377(January (9762)):299.
[2] Swingler G, Fransman D, Hussey G. Conjugate vaccines for preventing Haemophilus influenzae type B infections. Cochrane Database of Systematic Reviews 2007;(2):CD001729.
[3] Takahashi M, Otsuka T, Okuno Y, Asano Y, Yazaki T. Live vaccine used to prevent the spread of varicella in children in hospital. Lancet 1974;2(November (7892)):1288–90.
[4] Noble GR, Bernier RH, Esber EC, Hardegree MC, Hinman AR, Klein D, et al. Acellular and whole-cell pertussis vaccines in Japan. Report of a visit by US scientists. JAMA (The Journal of The American Medical Association) 1987 Mar 13;257(10):1351–6.
[5] Ueda K, Miyazaki C, Hidaka Y, Okada K, Kusuhara K, Kadoya R. Aseptic meningitis caused by measles-mumps-rubella vaccine in Japan. Lancet 1995;346(September (8976)):701–2.
[6] Hashimoto H, Fujioka M, Kinumaki H. An office-based prospective study of deafness in mumps. The Pediatric Infectious Disease Journal 2009;28(March (3)):173–5.
[7] Plotkin SA. Commentary: Is Japan deaf to mumps vaccination. The Pediatric Infectious Disease Journal 2009;28(March (3)):176.
[8] Sadzot-Delvaux C, Rentier B, Wutzler P, Asano Y, Suga S, Yoshikawa T, et al. Varicella vaccination in Japan, South Korea, and Europe. Journal of Infectious Diseases 2008;197(March (Suppl. 2)):S185–90.
[9] Clements CJ, Baoping Y, Crouch A, Hipgrave D, Mansoor O, Nelson CB, et al. Progress in the control of hepatitis B infection in the Western Pacific Region. Vaccine 2006;24(March (12)):1975–82.
[10] Matsuura K, Tanaka Y, Hige S, Yamada G, Murawaki Y, Komatsu M, et al. Distribution of hepatitis B virus genotypes among patients with chronic infection in Japan shifting toward an increase of genotype A. Journal of Clinical Microbiology 2009;47(May (5)):1476–83.
[11] Komatsu H, Inui A, Sogo T, Hiejima E, Kudo N, Fujisawa T. Source of transmission in children with chronic hepatitis B infection after the implementation of a strategy for prevention in those at high risk. Hepatology Research 2009;39(June (6)):569–76.
[12] Japan Pediatric Society. Committee on muscular contracture. A report of muscular contracture. Japan Pediatric Society 1987:1067–105 [in Japanese].
[13] Petousis-Harris H. Vaccine injection technique and reactogenicity–evidence for practice. Vaccine 2008;26(November (50)):6299–304.
[14] Mark A, Carlsson RM, Granstrom M. Subcutaneous versus intramuscular injection for booster DT vaccination of adolescents. Vaccine 1999;17(April (15–16)):2067–72.
[15] Carlsson RM, Claesson BA, Kayhty H, Selstam U, Iwarson S. Studies on a Hib-tetanus toxoid conjugate vaccine: effects of co-administered tetanus toxoid vaccine, of administration route and of combined administration with an inactivated polio vaccine. Vaccine 1999;18(October (5–6)):468–78.
[16] Centers for Disease Control and Prevention (U.S.), National Immunization Program (Centers for Disease Control and Prevention). Epidemiology and prevention of vaccine-preventable diseases. [Atlanta, Ga]: Dept. of Health & Human Services, Public Health Service: v.
[17] Plotkin SA, Orenstein WA, Offit PA. Vaccines. 5th ed. Philadelphia, Pa: Saunders/Elsevier; 2008.
[18] King GE, Hadler SC. Simultaneous administration of childhood vaccines: an important public health policy that is safe and efficacious. The Pediatric Infectious Disease Journal 1994;13(May (5)):394–407.
[19] Bedford H, Lansley M. More vaccines for children? Parents' views. Vaccine 2007;25(November (45)):7818–23.
[20] Marshall GS, Happe LE, Lunacsek OE, Szymanski MD, Woods CR, Zahn M, et al. Use of combination vaccines is associated with improved coverage rates. The Pediatric Infectious Disease Journal 2007;26(June (6)):496–500.
[21] Kalies H, Grote V, Verstraeten T, Hessel L, Schmitt HJ, von Kries R. The use of combination vaccines has improved timeliness of vaccination in children. The Pediatric Infectious Disease Journal 2006;25(June (6)):507–12.
[22] General recommendations on immunization — recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 2011 Jan 28, 60(2):1–64.
[23] Kamiya H, Okabe N. Leadership in Immunization: the relevance to Japan of the U.S.A. experience of the centers for disease control and prevention (CDC) and the advisory committee on immunization practices (ACIP). Vaccine 2009;27(March (11)):1724–8.
[24] Ministry of health, labor, and welfare [in Japanese] http://www.mhlw.go.jp/stf/shingi/2r9852000001e323-att/2r9852000001e3gx.pdf.
[25] Zarocostas J. Imports of polio to China and western Africa threaten global eradication. BMJ 2011;343:d6186.
[26] Gomi H, Takahashi H. Why is measles still endemic in Japan. Lancet 2004;364(July (9431)):328–9.
[27] Japan Medical Association [in Japanese] http://www.med.or.jp/nichinews/n230105b.html.
[28] Ministry of health, labor, and welfare [in Japanese] http://www.mhlw.go.jp/stf/shingi/2r98520000008f2q.html.
[29] The Japan Pediatric Society [in Japanese] http://www.jpeds.or.jp/saisin/saisin_110427.pdf.

Utility of gram stain of endotracheal aspirates on empiric therapy in children with hospital-acquired pneumonia☆

Dear Editor,

We read "Gram stain/aolc screening for detection of catheter related bloodstream infection"[1] with great interest which demonstrated that Gram/AOLC screening has modest sensitivity and high specificity for the diagnosis of catheter-related bloodstream infection. Early and prompt diagnosis of healthcare-associated infections (HAI) is important because it leads to appropriate antimicrobial selections and decrease the morbidity and mortality of patients.

To evaluate the other common HAI, hospital-acquired pneumonia (HAP), we investigated the usefulness of Gram stain of endotracheal aspirates (ETA) for the early diagnosis of HAP in children to select the appropriate initial antibiotic therapy. Guidelines for the management of HAP in adults emphasize that broad-spectrum combination antibiotic therapy (two different types of antipseudomonal agents, and vancomycin or linezolid) should be empirically initiated in patients with risk factors for multidrug-resistant organisms including *Pseudomonas aeruginosa*, *Acinetobacter* species, Extended-spectrum β-lactamase producing *Klebsiella pneumoniae* and methicillin-resistant *Staphylococcus aureus*.[2] Whereas the overuse of broad-spectrum antibiotics can lead to increased hospital expenditures, while also potentially promoting antibiotic resistance.[3] Gram stain of sputum has been used for the early diagnosis of pneumonia and the selection of initial antibiotic therapy in adults[4]; however, the usefulness of Gram stain in children has not been reported except for one report in extremely preterm neonates.[5]

The study subjects included 53 children diagnosed with HAP at the largest pediatric hospital in Japan, between April 2009 and February 2011. Pneumonia was defined by the CDC criteria, including radiologic, clinical, and laboratory findings.[6] HAP is defined as pneumonia that occurs ≥48 h after admission. We selected patients who were qualified to receive broad-spectrum combination antibiotics based on the risk factors for multidrug-resistant organisms according to the adult guidelines[2]; the risk factors are antimicrobial therapy in preceding 90 days, current hospitalization of ≥5 days, hospitalization for ≥2 days in the preceding 90 days, chronic dialysis within 30 days, and immunosuppressive disorders and/or therapies.

All patients with suspected HAP on mechanical ventilation (MV) or tracheostomy were evaluated by collecting ETA. All Gram stain specimens were examined for the number of polymorphonuclear neutrophils and squamous epithelial cells with Geckler classifications. Nurses, who submitted the sputum specimens other than Geckler 5 classification, were suggested to resubmit the other sputum specimens. Data was collected from only children whose Gram stain findings were evaluated as Geckler 5. There were six samples classified as Geckler 4 or 6 which were excluded from the study. Bacterial counts were recorded as: +1, +2, +3 and +4 were <1 bacterium, 1, 2–10 and >10 noted per high-powered field, respectively. The criteria of dominant organism were utilized when one or two types of morphology are seen on Gram stain. Bacterial growth from sputum culture was semiquantified as few, 1+, 2+ and 3+. By comparing with the quantitative culture method, bacteria semiquantified as 1+, 2+ and 3+ corresponded to at least 10^4, 10^6 and 10^8 cfu/ml, respectively. The causative organisms were considered to be "definitive" when one or more pathogenic organisms were isolated from ETA at concentrations of ≥+2 and those organisms were consistent with the clinical courses of the patients.

Empiric antibiotic therapies for HAP were initiated based on the results of Gram stain. The use of vancomycin was considered when Gram-positive cocci were dominantly observed (Group 1). One or two antipseudomonal agents were initiated when Gram-negative bacilli (GNB) were observed with consideration of the patients' background information (Group 3). Gram-negative cocci were mainly observed in other cases, and a narrow-spectrum antibiotic was selected (Group 2).

The majority of HAP (77%) cases occurred while the patients were on MV, and half of these required MV for ≥10 days (Table 1). Among the causative organisms (n = 68), multidrug-resistant organisms were responsible for 21% of HAP cases (Fig. 1). *Moraxella catarrhalis* (37%) was the most common organism, followed by *Haemophilus influenzae* (19%), *P. aeruginosa* (16%). *S. aureus* was isolated (9%), and six percent was methicillin-susceptible *S. aureus* and three percent was methicillin-resistant *S. aureus*.

Gram stain of ETA was highly sensitive (92%) and specific (81%) to identify causative organisms, and the specificity (97%) was enhanced when dominant organisms were observed.

The current study results showed that Gram stain of ETA was useful for predicting the causative organisms of HAP in children if stringent criteria were fulfilled using Geckler classifications. The present findings of Gram stain are superior to other studies in adults (sensitivity 62–91% and specificity 64–74%).[7–9] The most probable reason of the difference was the use of stringent criteria for the Gram stain in the current study. We only selected patients with HAP, who were qualified to receive broad-spectrum combination antibiotics according to the risk factors for multi-drug resistant bacteria based on the adult guideline.[2] All antibiotic selections were determined based on the results of Gram stain findings. As a result, vancomycin or anti-pseudomonal agents were selected initially in only 40% of patients and no patient received both antipseudomonal agents and vancomycin or linezolid; whereas, the selection of antibiotics was appropriate in 92% of the patients on the basis of drug sensitivity results. These favorable results are consistent with previous reports in adults[7] and in extremely preterm neonates.[5]

In conclusion, this study showed that Gram stain of ETA based on stringent criteria is useful for predicting

Table 1 Baseline characteristics.

Characteristics	All patients (n = 53)	Group 1 (n = 7)	Group 2 (n = 11)	Group3 (n = 35)
Gender, male	26 (49%)	2 (29%)	4 (36%)	20 (57%)
Age, year (median)	2.80	0.81	2.63	3.24
<1	14 (26%)	4 (57%)	4 (36%)	6 (17%)
1–12	29 (55%)	3 (43%)	7 (64%)	19 (54%)
≥13	10 (19%)	0	0	10 (29%)
Length of hospital stay, ≥10 days	35 (66%)	6 (86%)	5 (45%)	24 (69%)
Ventilator-associated pneumonia	41 (77%)	7 (100%)	9 (82%)	25 (71%)
Duration of mechanical ventilation, ≥10 days	22/41 (54%)	5/7 (71%)	2/9 (22%)	15/25 (60%)
Tracheostomy	24 (45%)	0	5 (45%)	19 (54%)
Admission to Intensive Care Unit	35 (66%)	7 (100%)	8 (73%)	20 (57%)
Underlying diseases				
Neuromuscular diseases	25 (47%)	3 (43%)	5 (45%)	17 (49%)
Gastrointestinal diseases	17 (32%)	0	4 (36%)	13 (37%)
Anatomical abnormalities in respiratory system	11 (21%)	1 (14%)	2 (18%)	8 (23%)
Patients with immunosuppressive agents	11 (21%)	0	2 (18%)	9 (26%)
Chromosomal abnormalities	10 (19%)	1 (14%)	3 (27%)	6 (17%)
Heart diseases	8 (15%)	1 (14%)	3 (27%)	4 (11%)
Metabolic diseases	7 (13%)	2 (29%)	0	5 (14%)
Others	12 (23%)	1 (14%)	3 (27%)	7(20%)
Recent antibiotic changes within 72 h	13 (25%)	1 (14%)	5 (45%)	7 (20%)

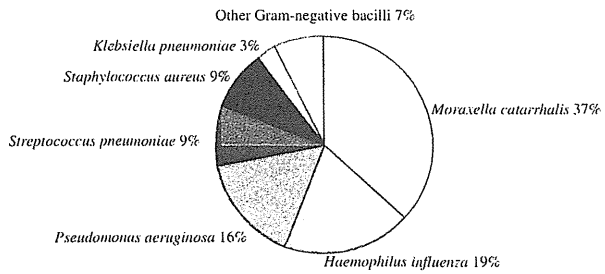

Figure 1 Causative organisms of Hospital-acquired pneumonia based on the semiquantitative culture results (n = 68). Culture-identified organisms were recognized as causative organisms when organisms were isolated from ETA at concentrations of ≥+2 and those organisms were consistent with the clinical courses of patients.

the causative organisms of HAP and selecting appropriate narrow-spectrum antibiotics in children. Further prospective, randomized studies are necessary to evaluate the usefulness of Gram stain of ETA in children with HAP.

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Contributor's statement

Chikara Ogimi: Dr. Ogimi conceptualized and designed the study, drafted the initial manuscript, and approved the final manuscript as submitted.

Kensuke Shoji: Dr. Shoji conceptualized and designed the study, acquired data, revised the article critically for important intellectual content, and approved the final manuscript as submitted.

Tomohiro Katsuta: Dr. Katsuta conceptualized and designed the study, acquired data, revised the article critically for important intellectual content, and approved the final manuscript as submitted.

Yasushi Watanabe: Mr. Watanabe designed the study, coordinated and supervised data collection, revised the article critically for important intellectual content, and approved the final manuscript as submitted.

Akihiko Saitoh: Dr. Saitoh conceptualized and designed the study, coordinated and supervised analysis and interpretation of data, reviewed and revised the manuscript totally, and approved the final manuscript as submitted.

References

1. Zach J, Zollner-Schwetz I, Krause R, Zebisch A, Rohn A, Sill H. Gram stain/aolc screening for detection of catheter related bloodstream infection. *J Infect* 2010;**60**(3):254–5.
2. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. *Am J Respir Crit Care Med* 2005;**171**(4):388–416.
3. Trouillet JL, Chastre J, Vuagnat A, Joly-Guillou ML, Combaux D, Dombret MC, et al. Ventilator-associated pneumonia caused by potentially drug-resistant bacteria. *Am J Respir Crit Care Med* 1998;**157**(2):531–9.
4. Rea-Neto A, Youssef NC, Tuche F, Brunkhorst F, Ranieri VM, Reinhart K, et al. Diagnosis of ventilator-associated pneumonia: a systematic review of the literature. *Crit Care* 2008;**12**(2):R56.
5. Katayama Y, Minami H, Enomoto M, Takano T, Hayashi S, Lee YK. Usefulness of gram staining of tracheal aspirates in initial therapy for ventilator-associated pneumonia in extremely preterm neonates. *J Perinatol* 2010;**30**(4):270–4.
6. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific

types of infections in the acute care setting. *Am J Infect Control* 2008;**36**(5):309–32.
7. Veinstein A, Brun-Buisson C, Derrode N, Alvarez A, Pinsard M, Robert R, et al. Validation of an algorithm based on direct examination of specimens in suspected ventilator-associated pneumonia. *Intensive Care Med* 2006;**32**(5):676–83.
8. Papazian L, Autillo-Touati A, Thomas P, Bregeon F, Garbe L, Saux P, et al. Diagnosis of ventilator-associated pneumonia: an evaluation of direct examination and presence of intracellular organisms. *Anesthesiology* 1997;**87**(2):268–76.
9. Blot F, Raynard B, Chachaty E, Tancrede C, Antoun S, Nitenberg G. Value of gram stain examination of lower respiratory tract secretions for early diagnosis of nosocomial pneumonia. *Am J Respir Crit Care Med* 2000;**162**(5):1731–7.

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CD4+ T-lymphocytopenia in HIV-negative tuberculosis patients in Sudan

As Wang already stated population frequencies of CD4+ T cells in tuberculosis (TB) patients vary significantly in different studies.[1] CD4+ T lymphocytes are important in the protective immunity to TB, since they prevent re-activation of the infection through cell-mediated responses. In humans, the importance of CD4+ T lymphocytes in the control of TB can be demonstrated by the high increased susceptibility to both acute and reactivated TB in HIV-infected individuals.[2] In the past it has been demonstrated that CD4+ T-lymphocytopenia is also found in patients with severe pulmonary tuberculosis without evidence of HIV infection in patients originating from Argentina, Saudi Arabia and Botswana,[3–5] but no data is available from the Sudan, another country with a high TB prevalence. Tuberculosis patients with CD4+ T-lymphocytopenia usually have a more severe disease with extra pulmonary involvement (pleural effusion, adenopathy or military disease) and co-infections associated with low CD4+ counts.[2] In Sudan because of the lack of normal reference ranges for hematological parameters in healthy subjects, many investigators interpret their data using normal values derived from populations in Europe and the United States.[6] This range is 450–1350 cell/μl.[6] Due to differences in the genetic, environmental, nutritional, gender, age and attitude backgrounds, it has been shown that CD4+ counts are different for each population tested both in the absolute and the percent quantities.[6] Therefore in order to determine if CD4+ T-lymphocytopenia is also common among HIV-negative tuberculosis patients in Sudan, local CD4+ reference counts need to be established first. We performed a prospective, cross sectional, observational, descriptive case study in 2008–2010 in Abu-Anjaa Chest Hospital at Omdurman, Omdurman Teaching Hospital, Elshab Hospital, Academy Hospital, Baharri, Ebrahim Malik, Mayoo Hospital, Sudan. A population comprised of 55 healthy unrelated people from the Sudan (Table 1) was used to determine the normal CD4+ T-cells in the Sudanese population by performing CD4 differential count using flow-cytometry method. Flow-cytometry was performed at Voluntary Counseling and Testing Centre (VCT) (Omdurman Teaching Hospital) using Partec Flow-Cytometry instrument (Cyflow®Counter) and the Partec CD4 easy count kit according to the instructions. As seen in the Tables 1 and 2, our healthy controls had lower CD4+ cell counts 319–1075 cell/μl (student's t test, $p = 0.0001$) than this reference range. Our finding of low CD4+ count among controls in Sudan adds to emerging data supporting the importance of establishing CD4+ count reference ranges for local populations. Differences in CD4+ count among healthy persons has been shown in previous studies and has been related to a variety of factors including genetics factors, and to differences in the methodologies used for CD4+-cell count.[5] Published reference ranges for CD4+ count in HIV-negative populations from Africa and Asia vary widely.[6–13] When we compare the CD4+ counts found for the healthy controls in our study with a mean CD4+ cell counts of 458.8 cell/μl to the values reported for healthy adult Dutch population (mean = 993),[10] Swiss (mean = 691),[7]Tanzanians (mean = 843),[13] Kuwaitis (mean = 1050),[8] Indians (mean = 865)[12] and Chinese (mean = 785)[9] a significant lower CD4+ count is found in the Sudanese population. The CD4+ count of the Sudanese population was even lower than that of populations from neighboring countries such as the Ethiopian population (mean = 1235)[10] and the Ugandan population (mean = 1256).[11]

In order to determine if Sudanese HIV-seronegative tuberculosis patients also suffered from CD4+ T-lymphocytopenia, CD4+ counts were determined in a population of 100 HIV-negative tuberculosis patients (based on the

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Letter to the Editor

Detection of enteroviral RNA from preserved umbilical cord

Keywords:
Umbilical cord
Infant
Enterovirus
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Reverse transcriptase polymerase chain reaction

1. Introduction/background

Enterovirus (EV) is known to cause serious neonatal infections, both pre- and post-natally.[1]

We experienced a neonate who developed hepatic encephalopathy by fulminant hepatitis. The onset of symptoms was on the 3rd day of life, when the illness progressed rapidly and required intensive care. The patient was born in July, which is known to be the season for EV infection.[2] The serum, cerebrospinal fluid of the patient proved to be positive for EV by real-time RT-PCR. More importantly, the mother had suffered fever and diarrhea for one day, three days prior to her labor, which was suggestive of peripartum EV infections.[2–10] Furthermore, maternal feces obtained 19 days after the diarrhea was positive for EV.

Therefore, we sought to assess preserved (dried) umbilical cord containing clots of cord blood to determine the infection route of this case.

2. Materials and methods

2.1. Specimen

A small portion (160 mg) of preserved umbilical cord was provided from the patient's family.

2.2. RNA extraction from preserved umbilical cord

RNA was extracted according to the following provisional protocol: Poly[A] (Roche Applied Science, IN, USA) was dissolved in nuclease free water, to a final concentration of 1 μg/μL for use as carrier RNA; A2001HRS tubes containing zirconia beads (Sarstedt K.K., Tokyo, Japan) were pre-filled with 1000 μL of ISOGEN (Nippon Gene Co., Ltd., Tokyo, Japan); a 62.2 mg fragment of the specimen was soaked into the tubes and continuously vortexed for 110 min; 800 μL of the aqueous phase was transferred to another fresh tube and divided into two aliquots after adding 5 μg of carrier RNA and 400 μL fresh ISOGEN; each aliquot (approximately 600 μL) was mixed vigorously with 120 μL of chloroform for 15 s, incubated at room temperature for 3 min, centrifuged at 12,000 × g, 4 °C for 10 min; the inorganic phase was collected and combined into one (approximately 700 μL), which was further purified on PureLink Mini columns (Invitrogen, CA, USA) according to the manufacturer's instructions. Finally, RNA was eluted with 80 μL of nuclease free water.

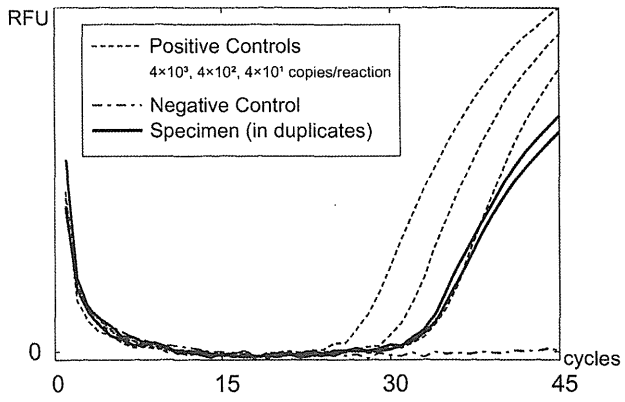

Fig. 1. Amplification result of RNA extracted from preserved umbilical cord specimen. RNA specimen was amplified in duplicate (solid lines). Both reactions were positive for enterovirus, with estimated concentration of 68.6 and 48.8 copies/reaction.

2.3. Realtime RT-PCR detection of EV

EV was detected using the methods described previously,[11] with the alteration of PCR reagents to One Step PrimeScript RT-PCR Kit (Takara Bio Inc., Shiga, Japan). Briefly, 4 μL of template was amplified in a 20 μL reaction under the following conditions: 42 °C 5 min; 95 °C 3 min; 45 cycles of 95 °C 5 s and 60 °C 20 s.

3. Results

3.1. Real-time RT-PCR detection of EV

Extracted RNA was amplified in duplicates with a negative control (nuclease free water) and 3 positive controls (4×10^3, 4×10^2, 4×10^1 copy/reaction). EV was successfully detected from the patient's sample (Fig. 1).

4. Discussion

We have succeeded in detecting EV RNA from preserved umbilical cord.

Although the most common infection route of EV is post-natal infection,[1] sufficient proof to determine the infection route is difficult.

Abbreviation: RT-PCR, reverse transcriptase polymerase chain reaction.

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We were not able to confirm whether the mother had been viremic prepartum, which is the limitation of retrospective investigations. However, the following circumstances were suggestive: maternal febrile illness 3 days prior to delivery, viral RNA detected from maternal feces, and the season being the "enteroviral season". Taking these facts as circumstantial evidence that the mother had been viremic, positive results from preserved umbilical cord provided crucial information to presume pre-natal infection. To our knowledge, contrary to the case with DNA viruses,[1,2] reports proving RNA viral genome from preserved umbilical cord are scarce, if not none. Umbilical cords are preserved as a custom in Japan and some Southeast Asian countries.

However, umbilical cord is very stiff when dried (preserved), and it turns resilient and slippery after soaking, neither form can be easily cut or divided at the lab bench nor can be lysed in ISOGEN. Our protocol requires improvements to overcome the troublesome physical natures of the specimen.

This is the first report of detecting EV RNA from preserved umbilical cord. Our experience adds to proving the potency of preserved umbilical cords as a resource to determine the causative agent of neonatal infections and/or their infection routes.

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Competing interests

None declared.

Ethical approval

Approved by the Internal Review Board at the National Center for Child Health and Development in Tokyo.

References

1. White DO, Fenner F. *Medical virology*. 4th ed. San Diego: Academic Press; 1994. p. 381–406 [Chapter 23, Picornavirinae].
2. Lake AM, Lauer BA, Clark JC, Wesenberg RL, McIntosh K. Enterovirus infections in neonates. *J Pediatr* 1976;**89**:787–91.
3. Modlin JF, Polk BF, Horton P, Etkind P, Crane E, Spiliotes A. Perinatal echovirus infection: risk of transmission during a community outbreak. *N Engl J Med* 1981;**305**:368–71.
4. Krajden S, Middleton PJ. Enterovirus infections in the neonate. *Clin Pediatr (Phila)* 1983;**22**:87–92.
5. Kaplan MH, Klein SW, McPhee J, Harper RG. Group B coxsackievirus infections in infants younger than three months of age: a serious childhood illness. *Rev Infect Dis* 1983;**5**:1019–32.
6. Modlin JF. Perinatal echovirus infection: insights from a literature review of 61 cases of serious infection and 16 outbreaks in nurseries. *Rev Infect Dis* 1986;**8**:918–26.
7. Abzug MJ, Levin MJ, Rotbart HA. Profile of enterovirus disease in the first two weeks of life. *Pediatr Infect Dis J* 1993;**12**:820–4.
8. Lin TY, Kao HT, Hsieh SH, Huang YC, Chiu CH, Chou YH, et al. Neonatal enterovirus infections: emphasis on risk factors of severe and fatal infections. *Pediatr Infect Dis J* 2003;**22**:889–94.
9. Tebruegge M, Curtis N. Enterovirus infections in neonates. *Semin Fetal Neonatal Med* 2009;**14**:222–7.
10. Abzug MJ. Nonpolio enteroviruses. In: Kliegman RM, Stanton BF, St Geme 3rd JW, Schor NF, Behrman RE, editors. *Nelson textbook of pediatrics*. 19th ed. Philadelphia: Saunders; 2011. p. 1088–94.
11. Mohamed N, Elfaitouri A, Fohlman J, Friman G, Blomberg J. A sensitive and quantitative single-tube real-time reverse transcriptase-PCR for detection of enteroviral RNA. *J Clin Virol* 2004;**30**:150–6.
12. Ogawa H, Baba Y, Suzutani T, Inoue N, Fukushima E, Omori K. Congenital cytomegalovirus infection diagnosed by polymerase chain reaction with the use of preserved umbilical cord in sensorineural hearing loss children. *Laryngoscope* 2006;**116**:1991–4.

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Letters to the Editor

Bacteraemia associated with intravascular catheter colonisation with *Staphylococcus aureus* in children☆

Madam,

A recent study in adults showed that patients with *Staphylococcus aureus* colonisation of an intravascular catheter but without bacteraemia, less than 24 h after the catheter removal, had a 14% chance of developing subsequent *S. aureus* bacteraemia (SAB).[1] Without administering effective therapy, the rate of subsequent SAB increases to 24%. Based on this study, the Infectious Disease Society of America guidelines recommended that patients whose catheter tip grows *S. aureus* but whose simultaneous blood cultures have negative results should receive a course of anti-staphylococcal antibiotics for five to seven days.[2] Because *S. aureus* infection is known to be a leading cause of bacteraemia and is associated with severe morbidity and mortality in hospitalised children[3] and no previous study had examined the incidence of subsequent SAB in paediatric populations, we investigated the incidence of subsequent SAB, and examined the clinical outcomes of positive catheter tip cultures for *S. aureus* without documented bacteraemia in children.

Children with *S. aureus* cultured from a catheter tip during the period March 2002 to February 2009 were identified in the database at the National Center for Child Health and Development, Tokyo. From the database, it was determined whether blood cultures had been performed for the patients in the 24 h before or after intravenous catheter removal and whether the patients had any blood cultures positive for *S. aureus* during the six months after the catheter removal. Patients with blood cultures that were positive for *S. aureus* during the period from 48 h before to 24 h after the intravenous catheter removal were excluded from the analysis. The patient's clinical characteristics were retrospectively extracted from the medical records. Subsequent SAB was defined when the following conditions were met: (i) the catheter tip culture was positive for *S. aureus*; (ii) simultaneous blood culture was negative; (iii) blood culture was positive for *S. aureus* >24 h after catheter removal; (iv) anti-staphylococcal antibiotic sensitivities between the catheter tip culture and blood culture were the same.[1] The appropriate treatment was defined as any therapy to which the cultured *S. aureus* strain was susceptible and treatment was started ≤24 h after removal of the catheter and continued for a minimum of three days.[1] The Mann–Whitney test and the Fisher's exact test were used for statistical analyses.

During the 84-month period, 167/5784 (3%) patients had catheter tip cultures that were positive for *S. aureus*. Among them, 92/167 (55%) had simultaneous blood cultures examined. A total of 38/92 (41%) of these patients with positive catheter tip cultures for *S. aureus* had negative simultaneous blood cultures for *S. aureus*, including 30 with venous catheters (central venous catheter; CVC or peripherally inserted central catheter; PICC) and eight with arterial catheters. We found that 4/38 (11%) patients met the definition of subsequent SAB, and all four of these patients were neonates and infants. The baseline clinical characteristics of two groups (patients with or without subsequent SAB) were summarised in Table I. The patients who had positive catheter tip cultures for *S. aureus* who developed subsequent SAB developed the bacteraemia within 34 days of the catheter removal. Patients who developed subsequent SAB were younger and had a higher rate of extremely low birth weight (ELBW) than those without subsequent SAB, but they did not differ significantly. Of the four patients with subsequent SAB, all patients were neonates and infants; two patients were ELBW infants aged less than a week, other patients were an eight-month-old female who underwent live-donor liver transplantation for biliary atresia (postoperative day 61) and the other was a seven-month-old male with methylmalonic academia. Except in the last case, meticillin-resistant *S. aureus* (MRSA) was identified from cultures and they eventually died despite appropriate antibiotic therapy. The relationship between subsequent SAB and the high mortality rate in patients with MRSA was inconclusive because the number of cases was small and they had significant underlying medical conditions.

Intravenous catheter tip colonisation with *S. aureus* has been considered as a risk factor for bacteraemia. In a previous study, a catheter tip culture positive for *S. aureus* was strongly associated with bacteraemia.[4] Other studies reported that appropriate antibiotic therapy for patients with a catheter tip culture positive for *S. aureus* can reduce the incidence of subsequent SAB.[1,5,6] In the current study, colonisation of the intravenous catheter with *S. aureus* was associated with an 11% incidence of subsequent bacteraemic complications, and these complications occurred more frequently in patients in the younger age group (0–11 months)

Table I
Patients' characteristics

Variables	Patients without subsequent SAB ($N = 34$)	Patients with subsequent SAB ($N = 4$)	*P*-value
Age, median months (range)	10 (0–119)	3.5 (0–8)	NS
Male (%)	22 (65%)	2 (50%)	NS
Comorbid conditions			
Gastrointestinal diseases	4 (12%)	0 (0%)	
Post-surgical status	13 (38%)	1 (25%)	NS
ELBW infants	5 (15%)	2 (50%)	
Others	12 (35%)	1 (25%)	
Duration of i.v. catheter, median days (range)	7 (3–212)	6 (5–18)	NS
Purpose of i.v. catheter			
Total parenteral nutrition	15 (44%)	2 (50%)	
Inotropic therapy	11 (32%)	2 (50%)	NS
Arterial line	8 (24%)	0 (0%)	
Immunosuppressive therapy	4 (12%)	1 (25%)	NS
Systemic corticosteroid use	4 (12%)	1 (25%)	NS
Appropriate antibiotic treatment	19 (56%)	3 (75%)	NS
Death	5 (15%)	3 (75%)	0.02

SAB, *Staphylococcus aureus* bacteraemia; ELBW, extremely low birth weight; i.v., intravenous; NS, not significant.

☆ This study was presented in part at the 47th Infectious Disease Society of America Annual Meeting, Philadelphia, PA, October, 2009.

than the older age group (12–119 months) (17% vs 0%). The incidence of subsequent SAB in children was similar to that reported in a study of adults (11% vs 14%);[1] however, our study showed that subsequent SAB was more common in neonates and infants. Larger prospective studies are needed to determine the optimal treatment strategy for children who demonstrate *S. aureus* colonisation in the intravascular catheter without bacteraemia.

Conflict of interest statement
None declared.

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References

1. Ekkelenkamp MB, van der Bruggen T, van de Vijver DA, Wolfs TF, Bonten MJ. Bacteremic complications of intravascular catheters colonized with *Staphylococcus aureus*. *Clin Infect Dis* 2008;**46**:114–118.
2. Mermel LA, Allon M, Bouza E, *et al.* Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2009; **49**:1–45.
3. Frederiksen MS, Espersen F, Frimodt-Moller N, *et al.* Changing epidemiology of pediatric *Staphylococcus aureus* bacteremia in Denmark from 1971 through 2000. *Pediatr Infect Dis J* 2007;**26**:398–405.
4. Peacock SJ, Eddleston M, Emptage A, King A, Crook DW. Positive intravenous line tip cultures as predictors of bacteraemia. *J Hosp Infect* 1998; **40**:35–38.
5. Ruhe JJ, Menon A. Clinical significance of isolated *Staphylococcus aureus* central venous catheter tip cultures. *Clin Microbiol Infect* 2006;**12**: 933–936.
6. Park KH, Kim SH, Song EH, *et al.* Development of bacteraemia or fungaemia after removal of colonized central venous catheters in patients with negative concomitant blood cultures. *Clin Microbiol Infect* 2010;**16**:742–746.

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Single dose antibiotic prophylaxis for patients who undergo hip fracture surgery

Madam,

We read with interest the recent audit by Bigsby *et al.*, which highlights the importance of communication of guidelines within a department.[1] In our own practice we report similar findings, but achieved higher compliance with our single dose regimen. In keeping with national guidelines, we use single dose prophylactic antibiotics for hip fracture surgery in our department.[2] Specifically, we use intravenous cefuroxime 1.5 g and teicoplanin 400 mg on induction of anaesthesia. In penicillin-allergic patients and patients with a previous history of *Clostridium difficile*-associated diarrhoea (CDAD) we omit cefuroxime.

The clinical notes and medication charts were audited prospectively for 50 consecutive patients admitted with hip fracture to the University Hospital of Wales from 4 August 2010. For each patient we recorded the type of antibiotic used, route of administration and number of doses administered against our local hospital guideline. Twelve patients were male and 38 were female of mean age 84 years (range: 60–100 years). The majority of patients (78%) were treated with hemiarthroplasty or sliding hip screw. The remainder had cannulated screws or pertrochanteric nailing. In all, 96% of patients received induction antibiotics in accordance with hospital guidance (Figure 1). Two patients received a reduced dose of cefuroxime and one patient received an additional dose of teicoplanin 400 mg for undocumented reasons.

In addition to increased patient morbidity, surgical site infections increase hospital stay by 6.5 days and cost in excess of £3000 extra per patient, so must be avoided.[3] Antibiotic prophylaxis for patients who undergo hip fracture surgery reduces the incidence of deep and superficial wound infection, from 10.40% in the no-antibiotic group to 5.39% (NNT 20) with antibiotic.[4] Evidence suggests that there is no difference in the rate of deep or superficial wound infection when using single dose prophylaxis compared to multiple doses.[4] We administer antibiotics on induction because the lowest risk of surgical wound infection is achieved when the antibiotic is administered within 30 min prior to incision.[2] This allows time for blood and bone levels to reach minimum inhibitory concentration.[5] Teicoplanin is used to target meticillin-resistant *Staphylococcus aureus*. Compliance with this regime in our department was higher than other regimes described in previous studies (96% vs 75.8%).[1] As suggested by previous authors, we expect that this is due to good communication of the guideline within the department, as it is printed on our hip fracture proforma.[1] We also suggest that it is due to the simplicity of the guideline. Apart from high compliance, our regime has several benefits when compared to multiple dose prophylaxis. Single dose antibiotic prophylaxis reduces the risk of adverse reactions and economic costs of antibiotic and nursing time compared with multiple doses.[4] It has also been shown that CDAD rates can be reduced by changing prophylactic antibiotic regimens from three doses to one dose of cefuroxime (4.2% to 1.6%).[6] Patients who sustain hip fracture are at particular risk of this because the majority are aged >65 years, which is a risk factor for the development of CDAD itself.[7] The outcome of this infection is a one-year mortality as high as 17%.[8] None of the patients in our audit had treatment with total hip

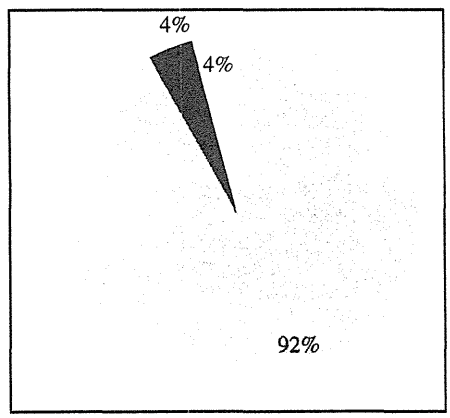

Figure 1. Induction antibiotic. Light grey: cefuroxime 1.5 g i.v./teicoplanin 400 mg i.v.; dark grey: cefuroxime 750 mg i.v.; white: teicoplanin 400 mg i.v. (penicillin allergy).

A Universal Preemptive Therapy for Cytomegalovirus Infections in Children After Live-Donor Liver Transplantation

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Background. Cytomegalovirus (CMV) infection remains the most common and critical viral infection that occurs after liver transplantation (LT). The current set of guidelines recommends prophylaxis over a preemptive therapy for pediatric LT; however, the data regarding the optimal approach after LT in children are limited.

Methods. We conducted a universal preemptive therapy for CMV infection in 113 children (median: 16 months) after live-donor LT at the largest pediatric LT center in Japan between November 2005 and August 2009. CMV-pp65 antigenemia was monitored weekly regardless of the subjects' CMV serostatus after LT, and ganciclovir therapy was initiated when CMV-pp65 antigenemia was positive.

Results. The overall success rate of LT was 91.7%. CMV-pp65 antigenemia became positive in 37 (33%) recipients, and the positivity with their CMV serostatus was as follows: donor (D)+/recipient (R)−: 62%, D+/R+: 36%, D−/R+: 11%, and D−/R−: 8%. Among the D+/R− (n=29) and D+/R+ (n=44) recipients, 38% (n=11) and 64% (n=28) recipients were able to avoid the use of ganciclovir, respectively. Human CMV disease was documented in six (5%) recipients, and they were successfully treated with ganciclovir without any sequelae.

Conclusions. A universal preemptive therapy for CMV infection after live-donor LT was successful for reducing the use of antiviral agents and for controlling CMV infection and disease in children.

Keywords: Cytomegalovirus, Liver transplantation, Children, Preemptive therapy, Ganciclovir.

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Cytomegalovirus (CMV) remains the most common infection that occurs after liver transplantation (LT) in children (*1*). Human CMV (HCMV) can cause various diseases affecting different organs, and it may contribute to rejection (*2*), fungal infections (*3*), and the risk for the Epstein-Barr virus-related posttransplant lymphoproliferative disorders (*4*). However, recent advances in preventive strategies for CMV have led to a significant decrease in the incidence of HCMV disease (*5*).

There have been two major approaches to control CMV infection after LT: universal prophylaxis and preemptive therapy. Universal prophylaxis provides antiviral therapy for subjects with a high risk of developing HCMV disease, such as seronegative recipients receiving seropositive grafts, for certain period of time (e.g., 2–14 weeks) (*6*, *7*). In contrast, a preemptive approach provides antiviral therapy for subjects who have positive results for routine monitoring of CMV antigenemia or polymerase chain reaction (PCR). Previous studies have shown the effectiveness of preemptive therapy for solid organ transplant recipients, primarily in adults (*7–10*), but little data are available for children after LT (*11*, *12*). Furthermore, no study has evaluated a preemptive therapy for children with the highest risk of developing a CMV infection (e.g., donor (D)+/recipient (R)−). Children are at a higher risk of developing a CMV infection because pediatric organ recipients have a higher chance of being seronegative for CMV compared with adult recipients. Thus, more information is necessary to elucidate the importance of preemptive therapy to prevent and treat HCMV infections and disease.

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The authors declare no conflicts of interest.

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A.S., S.S., A.F., T.S., and M.K. participated in the research design; A.S., S.S., C.O., and M.K. participated in the writing of the manuscript; A.F., T.S., Tos.K., S.K., Tom.K., K.S., M.K. participated in the performance of the research; and A.S., S.S., and M.K. participated in data analysis.

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TABLE 1. The clinical outcome in recipients based on the donor's and recipient's cytomegalovirus serologic status

D/R CMV serologic status						
Donor	Recipient	N (%)	Age median month (IQR)	Number of recipients with CMV-pp65 Ag positive (%)	Number of recipients with CMV diseases (%)	Number of recipients with acute rejection (%)
+	−	35 (31%)	19 (9–52)	22 (63%)	4 (11%)	22 (42%)
+	+	53 (47%)	8 (14–103)	20 (38%)	1 (2%)	10 (29%)
−	+	9 (8%)	8 (5–10)	1 (11%)	0 (0%)	2 (9%)
−	−	16 (14%)	30 (10–60)	1 (6%)	1 (6%)	5 (31%)
Total		113 (100%)	16 (8–66)	44 (39%)	6 (5%)	39 (5%)

D, donor; R, recipient; CMV, cytomegalovirus; Ag, antigen; IQR, interquartile range.

The pediatric LT program at the National Center for Child Health and Development, Tokyo, is the largest pediatric LT program in Japan. The program has performed a universal preemptive therapy monitoring CMV-pp65 antigenemia weekly for CMV prevention for all recipients regardless of their CMV status since November 2005. We herein report the impact of preemptive therapy on the incidence and clinical outcome of HCMV infection and disease in children after live-donor LT.

RESULTS

Patient Characteristics

We performed 113 live-donor LT at our institution between November 2005 and December 2009, and the clinical course and outcome of these patients were monitored for at least 6 months after LT. The baseline information of the 113 children who received live-donor LT is summarized in Table 1. The median age of the recipients was 16 months (range: 1 month–21 years). The most common indication for live-donor LT was biliary atresia (n=49, 43%), followed by metabolic diseases (n=26, 23%), acute liver failure (n=20, 18%), liver cirrhosis (n=10, 9%), vascular abnormalities (n=5, 4%), and hepatic tumors (n=3, 3%). Eleven patients (9.7%) died after live-donor LT during the first year after LT, and the reasons for their deaths were not directly related to HCMV disease.

CMV Serostatus and CMV-pp65 Antigenemia Positivity

The CMV serostatus of the donors and recipients is presented in Table 1. The median time to become positive for CMV-pp65 antigenemia was 33 days postoperatively (interquartile range [IQR]: 17.5 days, range: −8 to 115 days). Of note, two cases were CMV-pp65 antigenemia positive before LT, and both patients were treated with ganciclovir (GCV) pre and postoperatively. The median CMV-positive cells were 3 of 50,000 cells (IQR: 8/50,000 cells, range: 1–201/50,000 cells). The highest CMV-pp65 antigenemia positivity was observed in 63% (22/35) of patients in the D+/R− group, followed by 38% (20/53) in the D+/R+ group. In the D−/R+ and D−/R− groups, only one recipient in each group was positive for CMV-pp65 antigenemia (Table 1). As expected, the proportion of recipients who remained negative for CMV-pp65 antigenemia for the 6 months after LT was significantly affected by the CMV serostatus of the donors and recipients (Figure 1).

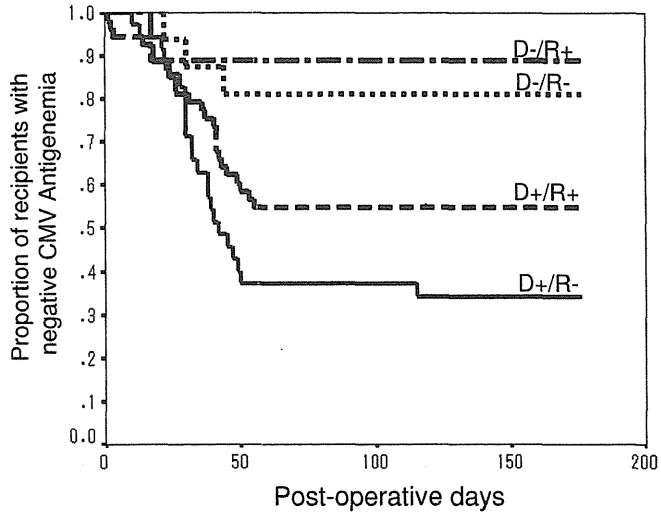

FIGURE 1. A proportion of patients were negative for cytomegalovirus antigenemia based on cytomegalovirus serostatus for the first 180 days after live-donor liver transplantation. D, donor; R, recipient.

Treatment for CMV-pp65 Antigenemia

During the observational period, 44 (39%) recipients had positive tests for CMV-pp65 antigenemia. Among the 44 patients, 38 (86%) patients received intravenous (IV) GCV as an initial therapy. The median duration of GCV therapy was 14 days (range: 6–24 days). IV foscarnet was given to four (9%) recipients when infection by GCV-resistant strains was suspected after GCV induction therapy; however, no GCV resistant strain was identified by the sequence analyses. GCV was switched to valganciclovir for three (8%) recipients after successful GCV induction therapy when valganciclovir became available in 2009. Neutropenia (absolute neutrophile counts $<500/\mu L$) was observed in three (7%) recipients, but their neutropenia resolved after discontinuation of therapy. Retreatment with GCV was required for four (9%) recipients. Of note, six (14%) recipients who had positive CMV-pp65 antigenemia after LT did not require antiviral treatment because repeated CMV-pp65 antigenemia in the same week became indeterminate or negative when the patients' immunosuppressive therapy was decreased. Four patients were treated with IV immunoglobulin to control HCMV disease.

Treatment of Recipients at High Risks for CMV

Among the highest risk group for CMV (D+/R−), 20 of 35 (57%) recipients required GCV due to positivity for CMV-pp65 antigenemia, with a median duration of 14 days (IQR: 3.8 days). Two (6%) patients did not receive GCV because the repeated CMV-pp65 antigenemia evaluation in the same week dropped to within an indeterminate range. In contrast, 15 of 35 (43%) recipients were able to avoid the use of GCV. Similarly, among the patients in the D+/R+ group, 16 of 53 (30%) recipients required GCV therapy for positive CMV-pp65 antigenemia, with a median duration of 14 days (IQR: 3.0 days). Four patients (8%) did not receive GCV because the repeated CMV-pp65 antigenemia evaluation in the same week was negative or within an indeterminate range. In contrast, 37 of 53 (70%) recipients were able to avoid the use of GCV that they would have received if they were on universal prophylaxis.

Comparison Between Recipients Positive for CMV-pp65 Antigenemia and Those Negative for CMV-pp65 Antigenemia

We compared the baseline characteristics of recipients who were positive for CMV-pp65 antigenemia and those who were negative for CMV-pp65 antigenemia (Table 2). No differences were found between the two groups in age or sex. Recipients who received LT for acute liver failure tended to have a higher rate of positive CMV-pp65 antigenemia (70%, 14/20), in contrast, recipients with cholestatic liver diseases tended to have a lower rate of positive CMV-pp65 antigenemia (20%, 10/49). Among patients with positive CMV-pp65 antigenemia, 95% of recipients (42/44) were in either D+/R− or D+/R+ groups.

TABLE 2. The baseline characteristics of recipients determined by CMV-pp65 antigenemia status

Variables	CMV-pp65antigenemia negative (N = 69)	CMV-pp65 antigenemia positive (N = 44)	*P*
Median age, mo (IQR)	16 (8–81)	17 (7–56)	0.48
Gender, male, N (%)	28 (41)	24 (55)	0.15
Baseline diseases, N (%)			
Biliary atresia	39 (57)	10 (23)	<0.001
Metabolic diseases	14 (20)	12 (27)	0.39
Acute liver failure	6 (9)	14 (32)	0.002
Cirrhosis	5 (7)	5 (11)	0.45
Tumor	3 (4)	0 (0)	0.28
Vascular	2 (3)	3 (7)	0.38
CMV serologic status, N (%)			
D+/R−	13 (19)	22 (50)	<0.001
D+/R+	33 (48)	20 (45)	0.80
D−/R+	8 (12)	1 (2)	0.09
D−/R−	15 (22)	1 (2)	0.004

D, donor; R, recipient; CMV, cytomegalovirus.

Clinical Outcome

All patients were successfully treated with GCV for 2 to 4 weeks, and their symptoms and signs resolved and CMV-pp65 antigenemia became negative when the treatment was completed. Overall, only 6 of 113 (5%) recipients developed HCMV disease including CMV syndrome (n=3), hepatitis (n=1), enterocolitis (n=1), and pneumonitis (n=1). No complications or sequelae were noted in these four recipients with HCMV disease. Seven (6%) recipients were retreated with GCV when CMV-pp65 antigenemia became positive after the completion of the initial GCV therapy.

Acute rejection was observed in 39 (35%) recipients after LT. The median duration from LT to the time when the diagnosis of acute rejection was made was 12 days (range: 6–50 days) postoperatively. No significant difference was found between the time for acute rejection and the CMV serostatus; 11.5 days (range: 7–50 days) in the D+/R− group, 12 days (range: 6–37 days) in the D+/R+ group, 22.5 days (range: 13–32 days) in the D−/R+ group, and 16 days (range: 8–30 days) in the D−/R− group (P=0.39). In addition, no association was found between the incidence of acute rejection and CMV-pp65 antigenemia; among 39 patients positive for CMV-pp65 antigenemia, 17 patients (44%) developed acute rejection, whereas 27 of 74 (36%) of the patients negative for CMV-pp65 antigenemia after LT developed acute rejection (P=0.46). Only two patients (both were D+/R+) developed acute rejection subsequent to positive CMV-pp65 antigenemia.

Overall, 11 of 113 (9.7%) patients died after the live-donor LT due to sepsis (n=5, 45%), graft failure (n=5, 45%), and tumor recurrence (n=1, 10%). Among children with graft failure, no recipients had positive CMV-pp65 antigenemia after LT. Therefore, no recipients died due to HCMV infection or disease.

DISCUSSION

This is the first study to demonstrate that a universal preemptive therapy was effective to prevent the development of HCMV disease in children after LT, notably in children with the highest risk for HCMV disease (e.g., D+/R−). This universal approach was successful to target therapy to the recipients who developed early evidence of CMV reactivation and thereby decrease drug costs and toxicity for patients who do not need the treatments.

Reviewing the current guidelines proposed by the Transplantation Society International CMV Consensus Group (*13*), the administration of GCV for 12 weeks is the recommended regimen for any D+/R− transplants and R+ liver transplants, and no preemptive approach is recommended. The only recommendation by some experts for a preemptive approach is 2 to 4 weeks of prophylaxis, followed by preemptive therapy as an alternative therapy (*14*). No current recommendation is available with respect to a universal preemptive approach for pediatric LT recipients (*15*).

The major advantage of preemptive therapy is to limit the periods of antiviral treatment in children in whom antivirals are necessary for preventing HCMV disease. Gerna et al. (*12*) demonstrated equal efficacy of universal prophylaxis and preemptive therapy in 21 pediatric liver transplant recipients for preventing HCMV diseases, although the duration of

antiviral therapy was significantly shorter in the preemptive group compared with the universal prophylaxis group (18 vs. 30 days). Madan et al. (*11*) demonstrated the usefulness of a combination of a short course of antiviral prophylaxis (≥14 days) for high-risk patients (D+/R−) and preemptive monthly CMV PCR monitoring in children after LT. With this approach, 12 subjects (9.8%) developed HCMV disease, and there were no mortalities secondary to CMV. This approach spared a total of 39% of the patients from the use of antiviral medications beyond their initial postoperative prophylactic period. In this study, we were able to avoid the use of GCV in 13 of 35 (37%) recipients in the D+/R− group and 37 of 53 (70%) recipients in the D+/R+ group. In addition, the long-term use of GCV or valganciclovir for the treatment or prevention of CMV in children is a concern. The major toxicities in patients receiving GCV are bone marrow suppression and renal toxicity (*16*). Additionally, animal studies have suggested that GCV may cause gonadal toxicity (*17*) or carcinogenicity (*18*). Finally, the long-term use of antivirals is costly and may increase the chance of developing GCV-resistant virus strains after prolonged exposure (*19*). The major advantages of universal prophylaxis are that there is no need for virologic monitoring for CMV infection after LT. Regular monitoring of CMV-pp65 antigenemia or CMV-DNA PCR is costly and labor intensive; however, a few studies suggested that the cost of the preemptive approach is actually lower than the cost of universal prophylaxis in adults (*20*, *21*). In preemptive approach, a delayed response to the test results may delay the treatment for patients, thus resulting in increased diseases and higher costs of treatment.

Given the challenge of characterizing the recipient serostatus in those less than 18 months of age, any CMV seropositive recipient who is less than 18 months of age should be considered to be seronegative, as maternal antibodies may account for this finding. Therefore, CMV urine culture or nucleic acid amplification testing for all seropositive recipients is recommended (*13*). However, negative tests do not exclude the exposure to CMV, because negative tests may result from the intermittent shedding of the virus. In this study, not all recipients less than 18 months of age with positive CMV antibodies were tested for CMV-pp65 antigenemia at baseline; therefore, we analyzed the data solely using their CMV serostatus. In this study, 28 (53%) patients among the 53 D+/R+ patients were less than 18 months of age, and we assumed that the majority of patients were seronegative (D+/R−). This assumption favors the use of a preemptive approach because it allows for the inclusion of more recipients with the highest risk (D+/R−) of developing CMV reactivation.

Oral valganciclovir was used for three patients after successful GCV induction therapy. Valganciclovir has excellent oral bioavailability, resulting in systemic drug levels that are comparable with IV GCV in patients after LT (*22*). The oral bioavailability of valganciclovir in neonates and infants has also been confirmed by other studies (*23*). However, the current guideline does not recommend preemptive therapy using oral valganciclovir for LT recipients (*15*) due to the absence of adequate pharmacokinetics and efficacy data. In addition, one study indicated that the incidence of HCMV disease was higher in the patients receiving valganciclovir compared with GCV in the subgroup analysis of CMV D+/R− solid organ transplant recipients (*24*). Because CMV infection usually becomes apparent approximately 3 weeks after LT, the majority of recipients can tolerate oral valganciclovir by that time, and the option of using oral medication is practical and reasonable. Further studies to compare these two medications are needed to demonstrate the effectiveness of valganciclovir in children after LT.

There are a few limitations to this study. First, because of the study design, we had a limited number of recipients after LT and were unable to directly compare universal prophylaxis and a universal preemptive therapy for HCMV disease in children. Second, we have used the cutoff for a positive CMV-pp65 antigenemia as ≥5/50,000 cells, but this is not the standardized value. Further studies are necessary to determine the optimal cutoff value of CMV-pp65 antigenemia and to compare it with the results of CMV-DNA PCR. Third, this study included only subjects who received live-donor LT, which requires less immunosuppression compared with cadaveric LT; therefore, caution is required when this approach is used for the patients who receive a cadaver liver for the LT. Finally, judicious use of IV sodium was not performed in four recipients. Sodium was initiated when CMV-pp65 antigenemia persistently increased after more than 2-week course of GCV induction therapy; however, the event of delayed antigenemia decrease after appropriate treatment with GCV without GCV resistant virus has been clearly documented (*25*, *26*).

In conclusion, a universal preemptive therapy in children after live-donor LT was safe and successful at the largest pediatric LT center in Japan. A further prospective study is necessary to identify the best approach to preventing and treating HCMV disease in children after LT.

MATERIALS AND METHODS

Subjects

The study subjects comprised 113 children who received live-donor LT at the National Center for Child Health and Development in Tokyo, the largest pediatric LT center in Japan, between November 2005 and December 2009. This study was a retrospective evaluation of the standard protocol, which has been performed at our institution. The following information was extracted from the medical record database; age, gender, and HCMV serostatus of the donors and recipients, baseline diseases, reasons for LT, the first postoperative date positive for HCMV-pp65 antigenemia, antiviral treatment and its duration, HCMV disease, clinical outcome, and mortality.

Monitoring of CMV-pp65 Antigenemia

CMV-pp65 antigenemia was measured by a direct immunoperoxidase technique using a horseradish peroxydase-conjugated F(ab')2 fragment of human monoclonal (humab C7), designated horseradish peroxydase-C7 (*27*). The measurements were performed weekly for the first 3 months postoperatively, while the recipients were hospitalized, then monthly in an outpatient setting until 6 months after LT. We determined that the presence of more than 5 CMV antigen-positive cells/50,000 cells indicated that the patient was positive, and 1 to 5 positive cells/50,000 cells were considered to be an indeterminate result. Once the result was positive, a repeated CMV-pp65 antigenemia was performed within the same week in some of the patients, particularly when the value was close to the cutoff. The test results were returned to the primary care physicians less than 48 hr after the sample was taken and then were used to select the patients' treatment strategies.

Immunosuppressive and Antiviral Therapies

After LT, standard immunosuppression consisted of corticosteroids and tacrolimus. The corticosteroid was started intraoperatively (10 mg/kg/dose) and continued with tapering for the first 3 months after LT (1.0 mg/kg/day IV [days 1–3], 0.5 mg/kg/day IV [days 4–6], 0.3 mg/kg/day IV [day 7], 0.3 mg/kg/day orally (PO) [days 8–28], 0.1 mg/kg/day PO [days 29–90]). Tacrolimus was also started 1 day before surgery, and the dose was adjusted to maintain a trough level of 10 to 15 mg/L for the first 2 weeks, followed by 8 to 10 mg/L (days 15–28 after LT), 6 to 8 mg/L (days 29 to 90), and 4 to 6 mg/L (after day 91). If CMV-pp65 antigenemia was noted to be positive, then a dosage of tacrolimus was reduced down to 75% of the regular dosage, and IV GCV (5 mg/kg/dose, every 12 hr) was initiated for the first 2 weeks, followed by a maintenance dose of IV GCV (5 mg/kg/dose, every 24 hr), and the treatment continued until CMV-pp65 antigenemia became negative. If CMV-pp65 antigenemia was indeterminate, immunosuppressive therapy was reduced as far as possible, and CMV-pp65 antigenemia was reevaluated twice a week. We also switched from GCV to valganciclovir (16 mg/kg/dose PO every 12 hr, available from August 2008), which is an oral tablet that was crushed and dissolved in syrup and used as a maintenance therapy when recipients (1) were able to tolerate to oral medication and (2) demonstrated a trend of decrease in CMV-pp65 antigenemia after GCV induction therapy. The dosing of valganciclovir in maintenance was based on the study performed for controlling HCMV disease in infants (*23, 28*). Sodium (100 mg/kg/dose, every 24 hr) was empirically used when CMV-pp65 antigenemia did not improve after decreasing the dose of immunosuppressants and administering a 2- to 3-week course of GCV induction therapy. When sodium was empirically started for possible GCV-resistant CMV, the UL97 genetic sequencing or by an analysis of other known genetic sequences related to resistance was examined (*29*). IV immunoglobulin was used when HCMV disease was not able to be controlled by GCV or sodium.

CMV Serostatus of the Donor and Recipient

The CMV serostatus of donors and recipients was determined by the CMV IgG determined by preoperative enzyme immunoassay. Patients were defined as seropositive if the antibody titer was ≥1:4 and negative if the antibody titer was less than 1:4.

Diagnosis of Acute Rejection and Treatment

Acute rejection was clinically diagnosed on the bases of an increase in liver enzymes (more than three times the upper limit of normal range or an increase of more than 50% over the previous record) with or without histological evidence. Histological diagnosis and grading of acute rejection were performed according to the standardized criteria (*30*). Graft biopsies were considered when other etiologies other than rejection were suspected in clinical course. All the rejection episodes were treated with a corticosteroid bolus injection. Steroid-resistant rejection was treated with additional immunosuppressants, such as mycophenolate mofetil.

Outcome Measures

The primary endpoint of this study was the proportion of recipients with event-free survival 6 months after LT. Events were defined as the occurrence of CMV infection based on the CMV-pp65 antigenemia, active HCMV disease, or death from any cause. HCMV disease was defined when recipients developed the following diseases with positive CMV-pp65 antigenemia; CMV syndrome without any evidence of acute rejection, enterocolitis, pneumonitis, and meningitis.

Statistical Analysis

We used the SPSS 15.0 software package (SPSS, Inc., Chicago, IL) for all analyses. The χ^2 test and Fisher's exact test were used to compare categorical variables between the groups. The Mann-Whitney U test and the Kruskal-Wallis test were used to compare continuous variables between two groups and more than three groups, respectively. P values were calculated as two trailed and were considered to be significant if P less than 0.05.

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REFERENCES

1. Fridell JA, Jain A, Reyes J, et al. Causes of mortality beyond 1 year after primary pediatric liver transplant under tacrolimus. *Transplantation* 2002; 74: 1721.
2. Gupta P, Hart J, Cronin D, et al. Risk factors for chronic rejection after pediatric liver transplantation. *Transplantation* 2001; 72: 1098.
3. George MJ, Snydman DR, Werner BG, et al. The independent role of cytomegalovirus as a risk factor for invasive fungal disease in orthotopic liver transplant recipients. Boston Center for Liver Transplantation CMVIG-Study Group. Cytogam, MedImmune, Inc. Gaithersburg, Maryland. *Am J Med* 1997; 103: 106.
4. Kim JM, Lee SK, Kim SJ, et al. Risk factors for posttransplant lymphoproliferative disorder in pediatric liver transplant recipients with cytomegalovirus antigenemia. *Transplant Proc* 2010; 42: 895.
5. Hodson EM, Jones CA, Webster AC, et al. Antiviral medications to prevent cytomegalovirus disease and early death in recipients of solid-organ transplants: A systematic review of randomised controlled trials. *Lancet* 2005; 365: 2105.
6. Small LN, Lau J, Snydman DR. Preventing post-organ transplantation cytomegalovirus disease with ganciclovir: A meta-analysis comparing prophylactic and preemptive therapies. *Clin Infect Dis* 2006; 43: 869.
7. Kalil AC, Levitsky J, Lyden E, et al. Meta-analysis: The efficacy of strategies to prevent organ disease by cytomegalovirus in solid organ transplant recipients. *Ann Intern Med* 2005; 143: 870.
8. Singh N, Wannstedt C, Keyes L, et al. Efficacy of valganciclovir administered as preemptive therapy for cytomegalovirus disease in liver transplant recipients: Impact on viral load and late-onset cytomegalovirus disease. *Transplantation* 2005; 79: 85.
9. Singh N, Wannstedt C, Keyes L, et al. Valganciclovir as preemptive therapy for cytomegalovirus in cytomegalovirus-seronegative liver transplant recipients of cytomegalovirus-seropositive donor allografts. *Liver Transpl* 2008; 14: 240.
10. Diaz-Pedroche C, Lumbreras C, San Juan R, et al. Valganciclovir preemptive therapy for the prevention of cytomegalovirus disease in high-risk seropositive solid-organ transplant recipients. *Transplantation* 2006; 82: 30.
11. Madan RP, Campbell AL, Shust GF, et al. A hybrid strategy for the prevention of cytomegalovirus-related complications in pediatric liver transplantation recipients. *Transplantation* 2009; 87: 1318.
12. Gerna G, Lilleri D, Callegaro A, et al. Prophylaxis followed by preemptive therapy versus preemptive therapy for prevention of human cytomegalovirus disease in pediatric patients undergoing liver transplantation. *Transplantation* 2008; 86: 163.
13. Kotton CN, Kumar D, Caliendo AM, et al. International consensus guidelines on the management of cytomegalovirus in solid organ transplantation. *Transplantation* 2010; 89: 779.
14. Martin JM, Danziger-Isakov LA. Cytomegalovirus risk, prevention, and management in pediatric solid organ transplantation. *Pediatr Transplant* 2011;15:229–236.
15. Cytomegalovirus. *Am J Transplant* 2004; 4(suppl 10): 51.
16. Biron KK. Antiviral drugs for cytomegalovirus diseases. *Antiviral Res* 2006; 71: 154.
17. Faqi AS, Klug A, Merker HJ, et al. Ganciclovir induces reproductive hazards in male rats after short-term exposure. *Hum Exp Toxicol* 1997; 16: 505.
18. Marshall BC, Koch WC. Antivirals for cytomegalovirus infection in neonates and infants: Focus on pharmacokinetics, formulations, dosing, and adverse events. *Paediatr Drugs* 2009; 11: 309.
19. Limaye AP, Corey L, Koelle DM, et al. Emergence of ganciclovir-resistant cytomegalovirus disease among recipients of solid-organ transplants. *Lancet* 2000; 356: 645.
20. Kusne S, Grossi P, Irish W, et al. Cytomegalovirus PP65 antigenemia monitoring as a guide for preemptive therapy: A cost effective strategy

for prevention of cytomegalovirus disease in adult liver transplant recipients. *Transplantation* 1999; 68: 1125.
21. Seehofer D, Meisel H, Rayes N, et al. Prospective evaluation of the clinical utility of different methods for the detection of human cytomegalovirus disease after liver transplantation. *Am J Transplant* 2004; 4: 1331.
22. Pescovitz MD, Rabkin J, Merion RM, et al. Valganciclovir results in improved oral absorption of ganciclovir in liver transplant recipients. *Antimicrob Agents Chemother* 2000; 44: 2811.
23. Kimberlin DW, Acosta EP, Sanchez PJ, et al. Pharmacokinetic and pharmacodynamic assessment of oral valganciclovir in the treatment of symptomatic congenital cytomegalovirus disease. *J Infect Dis* 2008; 197: 836.
24. Paya C, Humar A, Dominguez E, et al. Efficacy and safety of valganciclovir vs. oral ganciclovir for prevention of cytomegalovirus disease in solid organ transplant recipients. *Am J Transplant* 2004; 4: 611.
25. Gerna G, Lilleri D, Zecca M, et al. Rising antigenemia levels may be misleading in pre-emptive therapy of human cytomegalovirus infection in allogeneic hematopoietic stem cell transplant recipients. *Haematologica* 2005; 90: 526.
26. Gerna G, Sarasini A, Lilleri D, et al. In vitro model for the study of the dissociation of increasing antigenemia and decreasing DNAemia and viremia during treatment of human cytomegalovirus infection with ganciclovir in transplant recipients. *J Infect Dis* 2003; 188: 1639.
27. Sano K, Tanaka K, Uemoto S, et al. Cytomegalovirus infection in living related liver transplantation: Rapid diagnosis by human monoclonal antibody staining of blood leucocytes. *Transplant Sci* 1994; 4: 105.
28. Shoji K, Ito N, Ito Y, et al. Is a 6-week course of ganciclovir therapy effective for chorioretinitis in infants with congenital cytomegalovirus infection? *J Pediatr* 2010; 157: 331.
29. Scott GM, Isaacs MA, Zeng F, et al. Cytomegalovirus antiviral resistance associated with treatment induced UL97 (protein kinase) and UL54 (DNA polymerase) mutations. *J Med Virol* 2004; 74: 85.
30. Banff schema for grading liver allograft rejection: An international consensus document. *Hepatology* 1997; 25: 658.