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Subclinical VZV reactivation in immunocompetent children hospitalized in the ICU associated with prolonged fever duration*

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Abstract

A prospective observational study was conducted to examine whether asymptomatic VZV reactivation occurs in immunocompetent children hospitalized in an ICU and its impact on clinical outcome. A secondary aim was to test the hypothesis that vaccinated children have a lower risk of reactivation than naturally infected children. Forty immunocompetent paediatric ICU patients and healthy controls were enrolled. Patients were prospectively followed for 28 days. Clinical data were collected and varicella exposure was recorded. Admission serum levels of TNF-a, cortisol and VZV-IgG were measured. Blood and saliva samples were collected for VZV-DNA detection via real-time PCR. As a comparison, the detection of HSV-DNA was also examined. Healthy children matched for age and varicella exposure type (infection or vaccination) were also included. VZV reactivation was observed in 17% (7/39) of children. Children with VZV reactivation had extended duration of fever (OR = 1.17; 95% CI, 1.02–1.34). None of the varicella-vaccinated children or healthy controls had detectable VZV-DNA in any blood or saliva samples examined. HSV-DNA was detected in saliva from 33% of ICU children and 2.6% of healthy controls. Among children with viral reactivation, typing revealed wild-type VZV and HSV-1. In conclusion, VZV reactivation occurs in immunocompetent children under severe stress and is associated with prolonged duration of fever.

Keywords: Herpes-virus, reactivation, saliva, stress, varicella, varicella vaccine, viraemia
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*Correction added on 21st January 2013, after first online publication: in the left hand column of Table 3, "Anti-TNF levels" was corrected to "TNF-α levels"

Introduction

Varicella zoster virus (VZV) is a member of the alpha herpes-virus subfamily. In primary infection, varicella, has low morbidity and mortality rates in immunocompetent children and induces long-lasting immunity [1]. Higher rates are observed in infants, adults and immunocompromised patients [1]. During primary infection the virus establishes latency in sensory ganglia. Viral reactivation results in herpes zoster (HZ), characterized by a painful dermatomal rash, the frequency of which increases with age or reduced cellular immunity [2]. While VZV-DNA is readily detectable in blood and saliva of patients with recent HZ, it is less often detected in asymptomatic immunocompromised patients and healthy elderly individuals [3–5]. Both VZV-DNA and viable virus have been detected in the saliva of healthy astronauts during and after space flights, supporting the notion that asymptomatic VZV reactivation may occur in response to stress [6,7].

It is not known whether asymptomatic VZV reactivation occurs under stress among intensive care unit (ICU) patients. A prospective study was conducted to evaluate whether

children requiring intensive care have a greater likelihood of presenting with asymptomatic VZV reactivation.

Materials and Methods

Study design

A prospective study was conducted in the paediatric ICU of a tertiary children's hospital during 2008 and 2009 and in healthy paediatric outpatients. The study was approved by the hospital ethics committee and written informed consent for study participation was obtained from the legal guardians upon study enrollment.

Study population

Children aged >12 months, admitted to the ICU, were eligible for inclusion. Exclusion criteria included expected survival <72 h, expected ICU stay <48 h, and known immunodeficiency or long-term therapy with steroids or other immunosuppressive agents. All children enrolled were screened for the presence of VZV-IgG antibodies. All children's immunization cards were reviewed. Past natural infection or varicella vaccination were noted. Unvaccinated children without documentation of previous varicella infection and with no detectable VZV serum antibodies were considered unexposed to VZV and therefore excluded from further analyses.

Study participants were prospectively evaluated for 28 days following ICU admission. Children discharged prior to day 28 of hospitalization were contacted by phone to record patient outcome. Standardized data collection forms, including information on interventions, medications and complications, were used during their hospitalization (ICU and ward). A brief questionnaire and patients' immunization cards were used to obtain demographic characteristics, as well as prior history of varicella disease or vaccination, respectively. To preserve anonymity all patient data and samples were coded. Upon hospital admission the PRISM III scale was used to assess clinical severity [8]. Serum samples were also obtained to measure TNF-α and cortisol levels, as well as to assess pre-existing VZV and herpes simplex virus (HSV) immunity. Peripheral whole blood samples were obtained on a weekly basis until the date of hospital discharge and stored at -35°C. Saliva samples were obtained twice weekly during the same time period using special synthetic swabs (Salivetta–Salivette, Sarsted®). Enrolled children in the ICU were also examined for the presence of HSV-1-DNA, because HSV-1 is known to reactivate under stress [9].

Control group

Healthy children were examined for the presence of VZV and/or HSV-1-DNA in blood and saliva samples. Parents of controls were asked to provide written informed consent. Children matched (1:1) for age and varicella exposure (natural infection, vaccination, neither) with the ICU study population were sampled once upon presentation at the outpatient department for routine examination.

Serology testing

Prior immunity to VZV (either natural or vaccine related) and HSV was investigated using the commercially available enzyme-linked immunosorbent assays (ELISA), including Enzygnost Anti-VZV/IgG (Siemens, Marburg, Germany) and Herpes Simplex 1 ELISA IgG/IgM (gG1 recombnant) (Vircell SL, Santa Fe, Spain) according to the manufacturer's instructions. TNF-a serum levels were quantified by the Elisa technique (Bender MedSystems, GmbH, Vienna, Austria) in accordance with the manufacturer's instructions. Serum cortisol concentration was measured in samples collected in the morning after fasting and stored at -85°C, using an electrochemiluminescence immunoassay (Roche Co., Basel, Switzerland) according to the manufacturer's protocol.

DNA extraction and PCRs

DNA was extracted from 200 μL of either whole blood or saliva using a QIAamp® DNA Blood Mini Kit (Qiagen Ltd, Crawley, West Sussex, UK) according to the manufacturer's instructions. Amplification of VZV-DNA was performed using a quantitative real-time PCR assay with a sensitivity of $\log_{10}$ 0.4 genome copies (gc) per μL (10 copies/μL) as described previously [10]. The presence of VZV-DNA was further confirmed using an in-house biallelic real-time PCR assay targeting SNP 108111 in ORF 62, which distinguishes wild-type and vaccine strains [10,11]. Amplification of HSV-1 and HSV-2-DNA was achieved using in-house real-time PCR assays. To ensure sample integrity, PCR amplification of the human β-actin gene was performed. The laboratory was blinded to the origin of the samples. In both ICU patients and controls, the detection of viral DNA in either a blood or saliva sample was defined as viral reactivation.

Statistical analysis

Categorical variables were compared between groups using the chi-squared test. Fisher's exact test was used where cell sizes did not exceed five patients. The Mann–Whitney *U*-test was used to compare mean values of continuous variables between groups. Spearman and Pearson correlations were carried out to assess the correlation between categorical and continuous variables, respectively, and the outcomes of interest. Univariate logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (95% CIs) for evaluating the association between all independent variables

examined (i.e. age, fever duration, anti-tnf levels, cortisol levels, intubation, and days in ICU) and the occurrence of VZV reactivation (yes vs. no) and HSV reactivation (yes vs. no). Stepwise multivariate regression analysis was applied to evaluate the association between the aforementioned factors and VZV reactivation. A p-value of 0.05 was used as the criterion of significance. All statistical analyses were performed using SAS v9.2 (SAS Institute, USA).

Results

Patient demographics and serological status

The study population consisted of 40 children (23 boys) with a mean age (±SD) of 9.04 ± 4.28 years (range, 1.1–16.3 years). Expected short ICU stay was the most common exclusion criterion. Most participants (n = 27) were admitted due to trauma or following surgery, while 13 children were admitted for medical reasons. Admission PRISM III score was lower in children admitted for medical reasons (6.5 ± 4.4 vs. 9.9 ± 5, p 0.027). Most children (27/40, median age 11 years) had a past history of varicella 0.3–12 years before admission (median time 7 years). Of those, only 11 (40.7%) had the diagnosis of chickenpox noted in their immunization cards. Six children (median age, 3.8 years) had documented immunization against varicella (median 2 years before admission) with a single varicella vaccine dose, while parents of seven unvaccinated children (median age 4.3 years) did not recall varicella infection in their child. The majority of children (35/40) had detectable VZV-IgG levels. Of the remaining five VZV-negative children, two had documented vaccination and another two had had chickenpox diagnosed by a paediatrician. A 3-year-old unvaccinated child with negative history of varicella was considered unexposed to VZV and therefore excluded from the analysis. Thus, 6/7 unvaccinated children with a negative history of chickenpox had detectable VZV antibodies and were assumed to have had subclinical natural infection. Hence, 39 children admitted to the ICU were evaluated. Overall, 23/39 children included in the analysis had detectable IgG antibodies against HSV.

Detection of viral DNA in ICU children and healthy controls

Detection of VZV-DNA in ICU children. Varicella zoster virus DNA was detected in the saliva and/or blood of 7/39 (17.9%) children (four boys) hospitalized in the ICU (Table 1). Three children were admitted for medical reasons. In five cases where typing was possible, the viral DNA was confirmed as wild type. Two children who had VZV-DNA detected in the blood and had subsequent blood samples available for testing, were then negative for viral DNA. None of the participants with detectable VZV-DNA had clinical signs of VZV infection at the time of sampling or during the follow-up period of 28 days. None of six vaccinated children were VZV-DNA positive, as compared with 4/27 (14.8%) seropositive children with a history of varicella and 3/6 (50%) seropositive children with no history of chickenpox. There were no differences in overall frequency of sampling in the three groups (Table 2). VZV-DNA was detected in 3/11 and 3/51 whole blood samples obtained from children with past subclinical varicella and past symptomatic varicella infection, respectively (p 0.06) (Table 2). There was no difference in VZV-DNA detection between children with past varicella (both symptomatic and subclinical) and vaccinated children (7/33 vs. 0/6, p 0.278), nor in the frequency of VZV-DNA detection in blood and saliva in these groups.

Neither of the children with VZV present in saliva had been intubated. Although mean time to first detection of VZV was 6.6 days (range 1–15 day), three children had VZV-DNA present in the initial sample collected within 24 h of admission to the ICU; two in blood and one in saliva. Two were after scheduled surgery (lumbar body fusion and benign cerebral tumour) and the third after trauma.

Detection of HSV-DNA in ICU children. Overall, HSV-DNA was detected in saliva from 13 children (33.3%), with a mean time to detection of 3.4 days (1–9 days) following admission to the ICU. HSV-DNA was detected significantly more frequently than VZV-DNA (13/39 children vs. 7/39, p 0.039). HSV-1-DNA was not detected in any blood samples but was significantly more frequently detected in saliva samples obtained from children admitted for medical reasons (11/38 vs. 9/93, p 0.005). Detection of both VZV and HSV in the same patient was never observed. In six children HSV-1-DNA was recovered in saliva obtained within 24–36 h of ICU admission, with viral loads ranging from $\log_{10}$ 5.0 gc/mL to $\log_{10}$ 12.3 gc/mL. No further HSV was detected in one or more salivary samples taken subsequently from five of the six children. One child continued to have detectable HSV in three further samples and overall HSV-DNA was detected in multiple samples, obtained over 7–9 days after ICU admission, in 3/13 children. Detection of HSV-1 antibodies was poorly predictive of reactivation (PPV = 34.8%).

Detection of VZV or HSV-DNA among controls. As with the study population, 6/39 healthy children were VZV IgG-positive despite a negative/unknown history of exposure. HSV serology was not evaluated in control children. No control child had VZV-DNA in blood or saliva (0/39 vs. 7/39 children in the study population, p 0.012). HSV-DNA was not detected in any blood sample obtained from healthy

TABLE 1. VZV-DNA viral load in children hospitalized in the ICU presenting with VZV-DNA shedding

No.	Age (years)	Sex	VZV exposure	Admission reason	Day of detection[a]	Blood or saliva	Viral load (log_{10} gc/mL)
1	11	F	Varicella	Medical	15	Blood	5.5
2	13	F	Varicella	Surgical	1	Blood	5.49
3	15	M	Varicella	Surgical	1	Saliva	5.3
4	10	M	Varicella	Surgical	12	Blood	5.78
5	6	F	Unknown	Trauma	1	Blood	5.54
6	3.4	M	Unknown	Medical	7	Blood	5
7	1.5	M	Unknown	Trauma	9	Blood	5.3
					11	Saliva	5.6

[a]Days after ICU admission.

TABLE 2. Patients' VZV exposure and detection of VZV-DNA in blood and saliva samples

	N	Varicella infection (*N* = 27)	Varicella vaccine (*N* = 6)	Negative history (*N* = 6)	p-value[a]
VZV-DNA shedding in ICU children	39	4/27 (14.8%)	0/6	3/6 (50%)	0.09
VZV-DNA(+) blood samples	74	3/51 (5.9%)	0/12	3/11 (27.3%)	0.06
VZV-DNA(+) saliva samples	131	1/93 (0.01%)	0/18	1/20 (5%)	0.32

[a]Fisher's exact test p-value for the comparison between children with primary varicella infection and seropositive children with negative history of exposure to VZV.

TABLE 3. Clinical characteristics and laboratory findings of study population according to VZV reactivation

	VZV reactivation *n* = 7	No reactivation *n* = 32	p-value
Age (mean years ± SD)	9.94 ± 4.06	11.97 ± 18.59	0.579[a]
Male gender, *n* (%)	4 (57.1)	19 (59.4)	0.617[b]
PRISM score (mean score ± SD)	7.00 ± 2.71	9.19 ± 5.35	0.134[a]
VZV history			
Past varicella infection (*N* = 27)	4 (57.1)	23 (71.9)	
Vaccinated (*N* = 6)	0 (0.0)	6 (18.8)	
Negative/unknown history (*N* = 6)	3 (42.8)	3 (9.4)	
VZV IgG	6 (85.7)	29 (90.6)	0.698[b]
Intubation, *n* (%)	2 (28.6)	10 (31.2)	0.635[c]
Total days in ICU (mean days ± SD)	8.71 ± 9.72	4.13 ± 6.34	0.270[a]
Total days fever (mean days ± SD)	11.7 ± 10.4	3.5 ± 4.9	*0.030*[a]
Total days on antibiotic treatment (mean days ± SD)	13.00 ± 9.98	10.28 ± 6.76	0.514[a]
TNF-α levels	11.0 ± 13.9	13.7 ± 15.0	0.744[a]
Cortisol levels	950.2 ± 550.4	501.9 ± 365.2	0.059[a]

[a]Mann–Whitney *U*-test p-value.
[b]Chi-square p-value.
[c]Fisher's exact test p-value.

controls but was present in saliva from one (2.6%) control child.

Risk factors for viral reactivation in ICU children

Demographic, clinical and laboratory data upon admission were similar between children with and without VZV reactivation (Table 3). Children admitted to the ICU for management of medical conditions (mainly severe infections, sepsis) were more likely to be positive for any viral DNA in either blood or saliva samples than children admitted for surgical interventions (p 0.08).

HSV-1-DNA was significantly more often detected in saliva samples collected from children admitted to the ICU for medical management (11/38 saliva samples) compared with those collected from children admitted after surgery or trauma (9/93 saliva samples, p 0.005). VZV-DNA was also more often detected in blood samples in medical patients (3/25 vs. 3/49) but this difference did not reach statistical significance (p 0.39).

By univariate regression analysis, VZV reactivation, but not viral load, was associated with a prolonged course of fever (>38°C axillary) during hospitalization (11.7 vs. 3.5 days; OR, 1.17; 95% CI, 1.02–1.34) and this association remained significant in multivariate analysis (AOR, 1.17; 95% CI, 1.01–1.37). Although VZV-DNA detection was significantly associated with elevated cortisol levels upon admission (Wald p-value, 0.04), this association was not significant in multivariate analysis (AOR, 1.00; 95% CI, 1.00–1.01).

Discussion

The results from this study showed that half of the children (20/39) admitted to the ICU had reactivated alpha herpesviruses over a 28-day period. HSV-1 reactivation is well recognized [12]. However, this is the first time that VZV-DNA detection, consistent with subclinical VZV reactivation, is described among immunocompetent children under severe stress (7/39, 17.9%). Of note, VZV-DNA was not detected in any of the healthy controls (p 0.016).

Varicella zoster virus DNA was mainly detected in blood samples. While VZV-DNA in the blood may not necessarily represent circulating virus, the data supports the likelihood

that viral reactivation is occurring. VZV-DNA has been detected in peripheral blood of patients with varicella and HZ and in up to 9% of asymptomatic blood donors, 17% of cancer patients and 27.3% of elderly subjects [3–5,13,14]. VZV-DNA was less often detected in saliva than in blood samples (2/131 vs. 6/74, p 0.026) (Table 2). VZV-DNA and viable virus have also been detected in saliva from asymptomatic immunocompetent astronauts, as well as up to 5% of adults with HIV [6,7,15]. VZV remains latent in multiple sensory ganglia neurons, cranial nerves and enteric ganglia [16,17]. Most HZ cases involve the thorax, with only 20% arising from the trigeminal ganglion [18]. Whether VZV-DNA in saliva is the result of activation in the trigeminal ganglion or viraemia needs further exploration [19].

Varicella zoster virus DNA has been detected in the saliva of 100%, 52% and 59% of patients with acute HZ, Ramsey Hunt syndrome and zoster sine herpete, respectively [20,21].

Varicella zoster virus DNA was detected less often (17.9% vs. 33% of children) and later than HSV (6.6 vs. 3.4 days post-admission, p 0.009). The latter finding fits with the more prolonged time to reactivation seen in other scenarios such as following transplantation [22]. HSV-1 was only detected in saliva. This is not surprising given that disseminated HSV infection and viraemia have only been described in neonates and immunocompromised patients while normal healthy individuals rarely shed HSV-1 asymptomatically [12,23,24]. Accordingly, we detected HSV-DNA in saliva obtained from one (2.6%) of our healthy controls. The frequency of HSV-DNA detection in saliva in our study (33%) was similar to previous studies [12]. HSV-DNA was detected significantly more often in saliva samples obtained from children admitted for medical reasons, although they had lower PRISM III scores. Unlike others we did not find an association of intubation with HSV-DNA detection or HSV viral load in our cohort (data not shown). As previously reported, HSV antibodies were a poor predictor of HSV reactivation, suggesting low sensitivity of the Elisa test used [24].

For the majority of patients, VZV or HSV-1-DNA was detected only once, as previously described [7]. Three children shed HSV-1-DNA for 7–9 days, while shedding of virus for more than 3 days has been reported in up to 10% of healthy adult patients [24].

The main limitations of this study include the size of the study population and infrequent sampling. The small sample size limits interpretation of the observations made. Importantly, more frequent blood sampling might have provided valuable additional information regarding our understanding of VZV reactivation under severe stress. The detection of HSV-1 for a prolonged period (7–9 days) may reflect biases in the study design, because VZV-DNA was mainly detected in blood samples, which were only collected once a week, whereas saliva was collected bi-weekly.

However, three potential important new findings of relevance to clinical management were identified.

Interestingly, detection of VZV-DNA was independently associated with longer duration of fever among ICU children. Whether VZV viraemia caused prolonged fever or vice versa remains unclear. The clinical implications of endogenous VZV reactivation need further evaluation. Alternatively, children with prolonged fever might represent sicker patients in whom VZV reactivation occurred due to their underlying disease. Our cohort was comprised of relatively healthy children, only 30% (12/40) of whom were intubated and most of whom had a short ICU stay (median 2.5 days) (Table 1). In contrast, asymptomatic reactivation of other herpes-viruses has been described in immunocompetent adult ICU patients [9,12,25] and has been associated with more severe morbidity and mortality [25]. We did not find an association of VZV or HSV-1 viral DNA load with clinical outcome. Herpes-virus reactivation was not associated with an increased severity score (PRISM III) on admission; however, detection of VZV-DNA was weakly associated with higher admission cortisol levels. While this finding needs to be verified with a larger cohort, it supports the hypothesis that stress is a trigger for VZV reactivation in immunocompetent children.

Secondly, the mode of primary VZV exposure may have influenced whether VZV reactivation occurred. None of six children immunized with the Oka vaccine had reactivated VZV. Although vaccinated children were not found to present a significantly reduced risk of VZV reactivation compared with those after natural infection (symptomatic and/or subclinical), this is possibly due to our main study limitation, the small number of children examined. Decreased incidence of HZ among vaccinees has been reported, possibly because skin infection is reduced and hence the ability to establish latency is diminished [26,27]. However, the Oka strain can establish a latent infection, as evidenced by the detection of Oka VZV-DNA in the ganglia of vaccinated children on autopsy [17]. Therefore, larger studies are needed to examine whether vaccinees are less prone to 'asymptomatic' VZV reactivation. Interestingly, wild-type VZV-DNA was more commonly detected in whole blood (3/6 children, 3/11 blood samples) from children with subclinical infection than that from those with a history of chickenpox (4/27 children, 3/51 blood samples). While only weakly associated, it is possible that subclinical chickenpox was associated with lower levels of viraemia and associated cellular immunity, predisposing these children to greater risk of reactivation under stress (Table 2).

Finally, reactivation of both HSV-1 and VZV in a single individual was not documented. HSV and VZV have been

simultaneously detected in skin lesions from immunocompetent and immunocompromised subjects, as well as in autopsied cranial and cervical ganglia [28–30]. However, a study of asymptomatic salivary viral shedding also failed to detect both viruses in the same sample [31]. This observation needs further investigation with a larger study.

In conclusion, this study provides the first evidence that VZV reactivation occurs among ICU hospitalized immunocompetent children under severe stress and that reactivation is associated with a prolonged course of fever during hospitalization. VZV-DNA was not detected in children vaccinated against varicella but was more common in those with subclinical primary infection. Although the clinical significance of these findings needs further investigation, they may have important implications for susceptible patients and staff, especially if one considers that in temperate regions most children are exposed to VZV during early childhood and have VZV-DNA detected in saliva [1,19]. These findings may also have implications for the role of endogenous boosting in maintaining VZV-specific immunity and long-term protection against varicella in the era of varicella vaccination.

Authorship/Contribution

VP and JB are responsible for the conception of the study. All authors made a substantial contribution to the design of the study and interpretation of data. EC performed the data analysis. VP prepared the first draft of the manuscript. All authors critically revised the manuscript and have approved the final version to be published.

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