Viral DNAemia and Immune Suppression in Pediatric Sepsis

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Objectives: Demonstrate that DNA viremia is common in pediatric sepsis and quantitate its associations with host immune function and secondary infection risk.

Design: Retrospective analysis of a prospective cohort study.

Patients: Seventy-three children admitted with sepsis-induced organ failure.

Interventions: None.

Measurements and Main results: This study was performed as an ancillary investigation to a single-center prospective study of children with severe sepsis. Longitudinally collected, batched, frozen plasma was examined using real time–polymerase chain reaction for the presence of cytomegalovirus, Epstein-Barr virus, herpes simplex virus, human herpes virus-6, torque teno virus, and adenovirus DNA. Innate immune function was also measured longitudinally via quantification of ex vivo lipopolysaccharide -induced tumor necrosis factor-α production capacity. Viral DNAemia with a virus other than torque teno virus was detected in 28 of 73 subjects (38%) and included cytomegalovirus 5%, Epstein-Barr virus 11%, herpes simplex virus 4%, human herpes virus-6 8%, and adenovirus 26%. In addition, torque teno virus was detected in 89%. Epstein-Barr virus DNAemia was associated with preexisting immune suppression ($p = 0.007$) Viral DNAemia was associated with preexisting immune suppression and high risk for the subsequent development of secondary infection ($p < 0.05$ for both). Subjects with viral DNAemia had lower innate immune function over time compared with those who were virus negative ($p < 0.05$).

Conclusions: DNAemia from multiple viruses can be detected in septic children and is strongly associated with preexisting immune suppression and secondary infection risk. The role of DNA viruses in the perpetuation of impaired host defense in this setting should be the subject of prospective study. (*Pediatr Crit Care Med* 2018; 19:e14–e22)

Key Words: immunology; immunosuppression; inflammation; pediatrics; sepsis; viruses

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Sepsis is associated with mortality rates as high as 25% in pediatric patients (1). For decades, evidence-based treatment of sepsis has been limited to antimicrobials, supportive care, and source control. Failure of novel therapeutics may reflect a limited understanding of the host response to sepsis. Improved understanding of this response is necessary in order to develop new approaches to pediatric sepsis management in hopes of improving outcomes.

Recent adult data suggest that reactivation of latent viruses such as cytomegalovirus occurs frequently in the setting of severe sepsis and that the degree of reactivation is associated with the risk for adverse outcomes (2–5). This phenomenon has not been demonstrated in septic children. In addition, failure of the host immune system is increasingly recognized as an important feature of severe sepsis in adults and children (6–9). This secondary immune suppression can affect both the adaptive (lymphocyte) and innate (monocyte, neutrophil) arms of the immune system. Severe lymphopenia in the context of sepsis has been associated with adverse outcomes in critically ill children and adults (10, 11). In addition, we have previously demonstrated that innate immune suppression, characterized by a reduced ability of patients' whole blood to produce the proinflammatory cytokine tumor necrosis factor (TNF)–α upon ex vivo stimulation with lipopolysaccharide, is associated with increased risks of secondary infection and/or death across a variety of critical diagnoses including sepsis (9, 12, 13).

The relationships between sepsis-associated immune suppression and viral reactivation are unknown in any patient population. We therefore designed this observational study to test the hypotheses that viral DNA can be detected in the plasma of critically ill children with severe sepsis and that this viral DNAemia is associated with biomarkers of immune suppression in this population.

MATERIALS AND METHODS

This study represents an ancillary investigation to a single-center prospective study of children with severe sepsis in the PICU at the University of Pittsburgh Medical Center. The parent study, carried out as a pilot study for the Collaborative Pediatric Critical Care Research Network (CPCCRN), included longitudinal measurement of immune function in enrolled subjects. Stored plasma from these subjects was used for viral DNA analysis in this study. The University of Pittsburgh Institutional Review Board approved this study. Patients meeting the consensus definition of severe sepsis (Systemic Inflammatory Response Syndrome + suspicion of infection + one or more organ failures) (14) and having an indwelling arterial or central venous line for blood draws were eligible for participation. Those whose families provided informed consent within 24 hours of the diagnosis of sepsis were entered into the study. Non-English speaking patients and patients in whom aggressive therapy was not sought were excluded. Patients were included in the present study if they had one or more plasma samples with at least 100 µL of plasma remaining from the parent study. Immune function measurements and plasma samples were obtained upon study enrollment and then twice weekly until ICU discharge and stored at –80°C.

Demographic features, results of routine laboratory studies, and selected clinical findings were recorded. Variables of interest included age, sex, preexisting immune suppression, use of extracorporeal therapies, lymphopenia, neutropenia, and presence of viral DNAemia. Preexisting immune suppression was defined as the use of immunosuppressive therapy, a history of cancer, or a history of transplantation. Lymphopenia was defined as an absolute lymphocyte count less than 1,000 cells/mm^3 for greater than 48 hours. Neutropenia was defined as an absolute neutrophil count less than 1,000 cells/mm^3 for greater than 48 hours. Viral DNAemia was defined as a positive polymerase chain reaction (PCR) assay for a virus other than torque teno virus (TTV), as performed in our laboratory, on at least two of three replicates for any sample during an episode. TTV DNAemia was excluded from this definition due to its high prevalence in the healthy population.

Total nucleic acid was extracted from the thawed plasma using the NuclieSENS easyMag automated nucleic acid extractor (Biomerieux, Durham, NC). Laboratory-developed PCR assays for the following viruses were performed on nucleic acid extracted from frozen banked plasma samples: cytomegalovirus (15), Epstein-Barr virus (EBV) (5), herpes simplex virus (5), human herpes virus-6 (5), TTV (16), and adenovirus (17, 18). Presence of TTV and level of TTV viral load were included as separate variables. Patients with preexisting diagnoses of viral DNAemia were excluded from analysis to avoid including subjects with primary infections.

Innate immune function was quantified at the time of the parent study performance by measurement of whole blood ex vivo lipopolysaccharide-induced TNFα production capacity as previously described (12). Briefly, within 60 minutes of sample collection, 50 µL of whole blood was added to 500 µL of highly standardized stimulation solution containing 500 pg/mL of lipopolysaccharide (kits produced by the Immune Surveillance Laboratory at The Research Institute at Nationwide Children's Hospital, Columbus, OH) and incubated for 4 hours at 37°C. The supernatants were then collected and stored at –80°C for batch analysis of TNFα. Stimulation assays were performed in duplicate for each blood sample, and TNFα values were averaged from each set of duplicates. Stimulation solution, which was shipped monthly, was manufactured and quality controlled such that the intrabatch coefficient of variation for TNFα production from healthy donor replicates was determined to be less than 10% prior to each shipment. Immune paralysis was defined, for the purposes of these analyses, as ex vivo lipopolysaccharide-induced TNFα production capacity less than 200 pg/mL beyond day 3 of sepsis as previously described (9). TNFα production capacity values below this threshold have been associated with adverse outcomes from pediatric critical illness in prior single- and multicenter studies (9, 12, 19). TNFα production capacity was also evaluated over time as a continuous variable.

Abstracted clinical and laboratory data were sent to the CPCCRN Data Coordinating Center for analysis. In addition to viral DNAemia, variables of interest included the development of immune paralysis and secondary infection. Secondary infection was defined as a clinically indicated positive microbiological test, culture, or PCR, that identified a new bacterial, fungal, or viral infection after 48 hours following hospital admission.

Univariate analysis of risk factors was performed using the Fisher exact test. Those risk factors with a *p* value of less than 0.1

were included in multiple logistic regression models which were fit in order to adjust for the effects of covariates associated with outcomes of interest. The presence of viral DNAemia was forced into the models, and the remaining candidate variables were entered in stepwise fashion. Adjusted odds ratios (aORs) with 95% CI are shown. A multiple linear regression model was used to evaluate the relationship between viral DNAemia and duration of stay in the PICU. Covariates included age, gender, and initial severity of illness at the time of sepsis onset as measured by Pediatric Risk of Mortality (PRISM) III score (20). Probability curves were also generated to examine time to viral DNAemia for subgroups. Episodes were censored by the date of last measurement. The Wilcoxon rank-sum test was used to compare maximum TTV measurements against preexisting immune suppression. TNFα production capacity (innate immune function) was also analyzed as a continuous variable, with relationships between TNFα response and viral DNAemia over time evaluated by generalized estimating equations to account for repeating measures within subjects. Data are shown as median and interquartile range throughout. SAS statistical software, version 9.4 (Cary, NC) was used for analysis.

RESULTS

One-hundred consecutive patient episodes meeting the inclusion criteria were enrolled in the original study, of which 75 had sufficient plasma available to participate in the current study. For subjects with multiple septic episodes ($n = 2$), only the first episode was used for these analyses, leaving a final sample size of 73 subjects. Of these subjects, 69 (95%) had TNFα production capacity data available that were concurrent with their viral PCR data. We compared the demographics of subjects in the primary study who did ($n = 73$) and did not ($n = 25$) have samples available for this secondary analysis. We found no significant differences in age, gender, cancer or transplant status, initial severity of illness (PRISM III score), frequency of immunoparalysis, or lymphopenia between groups ($p > 0.05$ for all, data not shown). Serious underlying diseases were common in the cohort; 21 episodes occurred in subjects who had received a transplant (including three with cancer who had received a hematopoietic stem cell transplant), and nine occurred in children who were diagnosed with cancer but had not undergone a transplant. A variety of other underlying diagnoses were noted including chronic lung disease, prematurity, mitochondrial disorders, epilepsy, hydrocephalus, static encephalopathy, autoimmune hemolytic anemia, short gut syndrome, nephrotic syndrome, Hirshprung disease, chronic liver failure, and trisomy 21. Viral PCR testing was performed on 191 samples (1–9 per subject), and viral DNAemia was documented in 28 of the 73 subjects (38%). Demographic and clinical features illustrated in **Table 1** did not differ significantly between subjects with and without viral DNAemia

TABLE 1. The Characteristics of Septic Subjects With and Without Viral DNAemia (Excluding Torque Teno Virus)

Characteristics	Viral DNAemia ($n = 28$), *n* (%)	No Viral DNAemia ($n = 45$), *n* (%)	OR (Exact 95% CI)	[a]*p*
Age group (yr)				0.896
0–5	16 (57)	26 (58)	0.97 (0.34–2.83)	
5–12	6 (21)	8 (18)	1.26 (0.31–4.78)	
≥ 12	6 (21)	11 (24)	0.84 (0.22–2.94)	
Female gender	13 (46)	20 (44)	1.08 (0.38–3.09)	1.000
Underlying disease				
Cancer	4 (14)	5 (11)	1.08 (0.20–5.12)	1.000
Transplant	10 (36)	9 (20)	1.94 (0.60–6.27)	0.282
No cancer or transplant	14 (50)	31 (69)	0.45 (0.15–1.34)	0.139
Preexisting immune suppression[b]	21 (75)	19 (42)	4.11 (1.31–13.64)	0.008
Plasma exchange	6 (21)	4 (9)	2.80 (0.58–14.76)	0.168
Immune paralysis[c]	11 (39)	10 (22)	2.26 (0.71–7.22)	0.183
Laboratory findings				
Lymphopenia[d]	10 (36)	17 (38)	0.92 (0.30–2.70)	1.000
Neutropenia[e]	7 (25)	6 (13)	2.17 (0.54–8.83)	0.225

OR = odds ratio.

[a]Fisher exact test.

[b]Based on underlying condition and/or receipt of immune suppressant therapy.

[c]Immune paralysis is defined as an ex vivo lipopolysaccharide-induced tumor necrosis factor-α production capacity < 200 pg/mL beyond 3 d of sepsis.

[d]Lymphopenia is defined as an absolute lymphocyte count < 1,000 cells/mm^3 for > 48 hr.

[e]Neutropenia is defined as an absolute lymphocyte count < 1,000 cells/mm^3 for > 48 hr.

except for preexisting immune suppression. Preexisting immune suppression was present in 40 subjects, of whom 21 (53%) had viral DNAemia. In comparison, of the 33 subjects without preexisting immune suppression, only seven (21%) had viral DNAemia ($p = 0.008$). The time to viral DNAemia in septic patients with preexisting immune suppression was significantly shorter compared with those without preexisting immune suppression (**Fig. 1*A***) ($p = 0.0052$). Subjects that left the ICU without viral DNAemia were censored and no longer considered at risk; the number at risk is shown. In 19 subjects, viral DNAemia was present in samples collected on study day 1, whereas nine subjects became virus positive later in their sepsis course (median of 4 d).

The detection of individual viruses in subjects with and without preexisting immune suppression is shown in **Table 2**. The presence of preexisting immune suppression was significantly associated with EBV DNAemia ($p = 0.007$) and a trend toward being positive for more than one virus, excluding TTV ($p = 0.0722$). The most commonly detected virus, excluding TTV, was adenovirus which was present in 13 (33%) of subjects with preexisting immune suppression and six (18%) of subjects without preexisting immune suppression. There were no significant differences in age, gender, rates of preexisting immune suppression, rates of lymphopenia, or neutropenia between episodes with and without adenovirus. In only one of the 13 subjects with preexisting immune suppression (and none of the six without) was the adenovirus diagnosed by the care team. More than one virus (excluding TTV) was identified in eight subjects, including seven with preexisting immune suppression. TTV was detected in 89% of subjects with no difference in frequency between those with and without preexisting immune suppression. The median maximum TTV level, however, was higher in those with preexisting immune suppression compared with those without preexisting immune suppression (532,331 [1–2.5 × 10^7] vs 5,767 [0–7.1 × 10^5]; $p = 0.04$).

Factors associated with the development of immune paralysis are

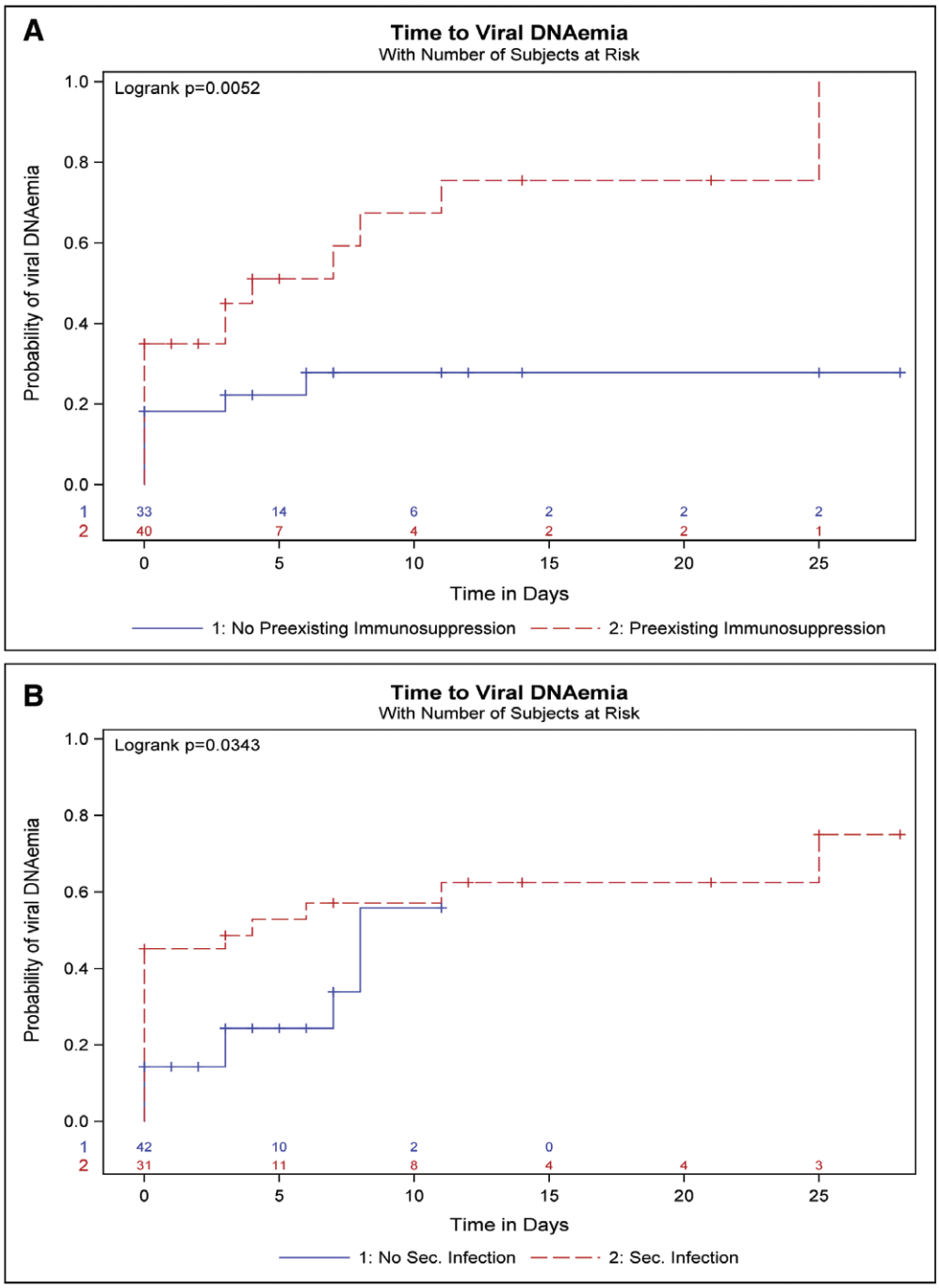

Figure 1. The time to viral DNAemia was shorter in those with preexisting immune suppression (**A**). The time to viral DNAemia was shorter in those who developed secondary infection (**B**). In these Kaplan-Meier plots, subjects are censored at the time of death or ICU discharge. The lines represent the probability of viral DNAemia in the population of subjects who remain at risk at a given time point. The number of patients at risk is shown. Sec. = secondary.

TABLE 2. Detection of Viral DNAemia in Septic Subjects With and Without Preexisting Immune Suppression

Virus	Preexisting Immune Suppression		p	Overall
	Not Present (*n* = 33), *n* (%)	Present (*n* = 40), *n* (%)		(*n* = 73), *n* (%)
Cytomegalovirus	0 (0)	4 (10)	0.122	4 (5)
Epstein-Barr virus	0 (0)	8 (20)	0.007	8 (11)
Human herpes virus-6	1 (3)	5 (13)	0.212	6 (8)
Herpes simplex virus	1 (3)	2 (5)	1	3 (4)
Adenovirus	6 (18)	13 (33)	0.191	19 (26)
TTV positive	30 (91)	35 (88)	0.722	65 (89)
TTV viral load upper quartile	4 (12)	11 (28)	0.148	15 (21)
Two or more viruses detected (excluding TTV)	1 (3)	7 (18)	0.065	8 (11)

TTV = torque teno virus.

TABLE 3. Univariate and Multivariate Analyses of Factors Associated With Immune Paralysis[a]

	Immune Paralysis (*n* = 21), *n* (%)	No Immune Paralysis (*n* = 52), *n* (%)	OR (Exact 95% CI)	[b]p	Adjusted OR (95% CI)	χ^2 p
Viral DNAemia (excluding TTV)	11 (52)	17 (33)	2.26 (0.71–7.22)	0.183	3.08 (0.91–10.46)	0.071
Age group (yr)				0.236		
0–5	9 (43)	33 (63)	0.43 (0.13–1.37)			
5–12	6 (29)	8 (15)	2.20 (0.53–8.57)			
≥ 12	6 (29)	11 (21)	1.49 (0.38–5.37)			
Female gender	9 (43)	24 (46)	0.88 (0.27–2.73)	1.000		
Primary infection						
Viral	4 (19)	11 (21)	0.88 (0.18–3.53)	1.000		
Bacterial	14 (67)	32 (62)	1.25 (0.39–4.31)	0.792		
Fungal	1 (5)	4 (8)	0.60 (0.01–6.62)	1.000		
Preexisting immune suppression	15 (71)	25 (48)	2.70 (0.82–9.76)	0.118		
Plasma exchange	4 (19)	6 (12)	1.80 (0.33–8.66)	0.459		
TTV positive	18 (86)	47 (90)	0.64 (0.11–4.56)	0.682		
TTV viral load upper quartile	8 (38)	7 (13)	3.96 (1.02–15.31)	0.027		
Extracorporeal membrane oxygenation or dialysis	6 (29)	9 (17)	1.91 (0.47–7.20)	0.341		
Laboratory findings						
Lymphopenia	15 (71)	12 (23)	8.33 (2.34–31.43)	< 0.001	9.79 (2.89–33.17)	< 0.001
Neutropenia	7 (33)	6 (12)	3.83 (0.91–16.07)	0.042		

OR = odds ratio, TTV = torque teno virus.

[a]Immune paralysis is defined as an ex vivo lipopolysaccharide-induced tumor necrosis factor-α production capacity < 200 pg/mL beyond 3 day of sepsis.

[b]Fisher exact test.

depicted in **Table 3**. In univariate analysis, significant factors included upper quartile TTV viral load ($p = 0.027$), lymphopenia ($p < 0.001$), and neutropenia ($p = 0.042$). The final multivariable logistic regression model for immune paralysis was significant only for lymphopenia (aOR, 9.8; 95% CI, 2.9–3; $p < 0.001$) although there was a trend toward an association

with viral DNAemia (aOR, 3.1; 95% CI, 0.9–10.5; $p = 0.07$). The time to viral DNAemia did not differ significantly in those with and without immune paralysis ($p = 0.27$).

When TNFα production capacity was analyzed as a continuous variable, innate immune function was significantly lower over time in children who demonstrated viral DNAemia (excluding TTV) compared with those who were never virus positive ($p = 0.026$) (**Fig. 2**). There was no difference in TNFα production capacity between these groups on day 1 of illness (498 [50–1,492] vs 661 [333–1,283] pg/mL; $p = 0.37$) despite the fact that 75% of virus-positive subjects with available TNFα response data were already virus positive in day 1 samples.

Factors associated with secondary infections are summarized in **Table 4**. In univariate analysis, statistically significant factors included viral DNAemia ($p = 0.004$), female gender ($p = 0.02$), and use of an extracorporeal device (extracorporeal membrane oxygenation or renal replacement therapy) ($p = 0.043$). The final multivariable logistic regression model for secondary infections included viral DNAemia (odds ratio [OR], 5.5; 95% CI, 1. 8–16.4; $p = 0.003$) and female gender (OR, 0.24; 95% CI, 0.08–0.73; $p = 0.012$). Thus, while female gender was protective, the presence of viral DNAemia was associated with development of secondary infection even when adjusting for gender. The time to viral DNAemia, excluding TTV, was shorter in patients who developed secondary infection ($p = 0.034$) (**Fig. 1*B***). Subjects that left the ICU without viral DNAemia were censored and no longer considered at risk; the number at risk is shown.

The mortality for the cohort as a whole was 11% (8/73) with five deaths occurring in subjects with viral DNAemia and three deaths occurring in subjects without ($p = 0.25$). Duration of ICU stay, however, was longer in children with viral DNAemia (15 [7–22] vs 8 [4–14] d; $p = 0.028$). This relationship remained significant after adjusting for age, gender, and initial PRISM III score ($p = 0.038$ for viral DNAemia).

DISCUSSION

This study demonstrates that viral DNAemia is common in critically ill children with severe sepsis. Further, the data suggest an association between viral DNAemia, immune suppression, and susceptibility to secondary infection in this population. Children with preexisting immune suppression were at the greatest risk for viral DNAemia in our cohort, and children with viral DNAemia demonstrated lower innate immune function over time, as measured by TNFα production capacity, compared with children without viral DNAemia. Although lymphopenia was associated with immune paralysis as has been previously reported (10, 11), lymphopenia was not associated with either viral DNAemia or secondary infection risk in this cohort. Viral DNAemia, however, was strongly associated with secondary infection risk in the multivariate logistic regression model. Our findings raise the possibility that viral DNAemia, acquired either through new infection or reactivation of latent infection, may perpetuate host immune suppression and contribute to secondary infection risk.

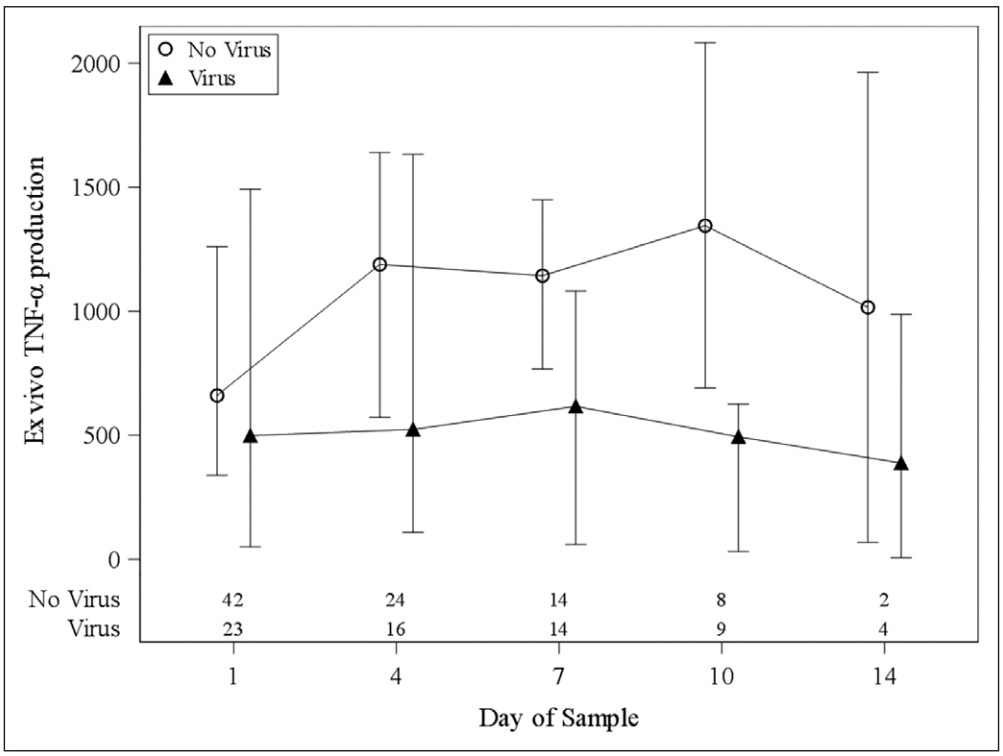

Figure 2. Children who demonstrated viral DNAemia at any time (excluding torque teno virus) had lower innate immune function over time as evidenced by persistently reduced tumor necrosis factor (TNF)–α production capacity ($p = 0.026$). Previous studies using this method have demonstrated TNFα production capacities of approximately 1,000 pg/mL in healthy children (12, 13). Symbols represent medians, error bars represent interquartile ranges. The numbers of samples available at each time point are shown.

The phenomenon of sepsis-induced immune suppression is characterized by dysfunction of both the innate and adaptive arms of the immune system (21). Apoptosis of peripheral and splenic lymphocytes (10, 11, 22) often occurs alongside decreased monocyte human-leukocyte antigen-antigen D related expression and reduced whole blood ex vivo lipopolysaccharide-induced TNFα production capacity (9, 21, 23). These features, if severe and persistent, have been found in multiple adult and pediatric studies of severe sepsis to be associated with the subsequent development of secondary infection and death (6, 7, 9, 10, 12, 24). Neither immune paralysis, as defined by a TNFα production capacity below a threshold of 200 pg/mL beyond day 3 of sepsis, nor lymphopenia were associated with

TABLE 4. Univariate and Multivariate Analyses of Factors Associated With Secondary Infection

	Secondary Infection ($n = 31$), n (%)	No Secondary Infection ($n = 42$), n (%)	OR (Exact 95% CI)	[a]p	Adjusted OR (95% CI)	χ^2 p
Viral DNAemia (excluding TTV)	18 (58)	10 (24)	4.43 (1.45–13.74)	0.004	5.46 (1.81–16.45)	0.003
Age group (yr)				0.398		
0–5	18 (58)	24 (57)	1.04 (0.37–2.96)			
5–12	4 (13)	10 (24)	0.47 (0.10–1.90)			
≥ 12	9 (29)	8 (19)	1.74 (0.50–6.02)			
Female gender	9 (29)	24 (57)	0.31 (0.10–0.91)	0.020	0.24 (0.08–0.73)	0.012
Primary infection						
Viral	9 (29)	6 (14)	2.45 (0.66–9.51)	0.150		
Bacterial	20 (65)	26 (62)	1.12 (0.39–3.30)	1.000		
Fungal	3 (10)	2 (5)	2.14 (0.23–26.93)	0.645		
Preexisting immune suppression	18 (58)	22 (52)	1.26 (0.45–3.57)	0.644		
Plasma exchange	7 (23)	3 (7)	3.79 (0.76–24.44)	0.086		
TTV positive	29 (94)	36 (86)	2.42 (0.39–25.94)	0.454		
TTV viral load upper quartile	9 (29)	6 (14)	2.45 (0.66–9.51)	0.150		
Extracorporeal membrane oxygenation or dialysis	10 (32)	5 (12)	3.52 (0.93–14.74)	0.043		
Laboratory findings						
Lymphopenia	11 (35)	16 (38)	0.89 (0.30–2.59)	1.000		
Neutropenia	6 (19)	7 (17)	1.20 (0.29, – 4.74)	0.768		

OR = odds ratio, TTV = torque teno virus.
[a]Fisher exact test.

viral DNAemia or secondary infection risk in this cohort. When the TNFα response was evaluated as a continuous variable, however, children with viral DNAemia demonstrated failure to recover innate immune function over time compared with children without viral DNAemia. It is possible, therefore, that viral DNAemia and/or factors that promote its presence may perpetuate innate immune suppression in a way that places the host at risk for nosocomial infection despite a TNFα response greater than 200 pg/mL. The concept of viral DNAemia as a predictor of immune system failure and infection risk should be the subject of further study in a larger, multicenter cohort.

Walton recently demonstrated that reactivation of latent viruses was linked to high rates of secondary infections and mortality in adult septic patients (5). Our data are the first to demonstrate this phenomenon in septic children. Of the viruses that were detected in our study, cytomegalovirus and EBV were detected only in subjects with preexisting immune suppression; however, the other four viruses tested were detected in patients with and without known immune suppression. EBV and cytomegalovirus DNAemia have been well described in the immune suppressed population and our findings are consistent with this literature. Interestingly, adenovirus was very commonly detected. Adenovirus is recognized as a cause of severe infection in immunosuppressed children (25) and was recently shown to be a common cause of fever of unknown source in nonimmunosuppressed children 2–36 months old (26). It is also known that adenovirus can exist as a latent infection with the potential for subsequent reactivation, particularly in the setting of infection with species C (27). The care team only diagnosed one episode of adenovirus, underlining the potential need for enhanced surveillance to diagnose the burden of adenovirus infection in critical illness. Because there are possible therapies for serious adenovirus infections (28), it will be important to follow up on this observation in future studies.

TTV is an anellovirus that is not currently linked to any human disease, although its genome can be detected in the blood of as many as 60% of healthy people (5). Interestingly, while TTV was present in 89% of our subjects, high levels of the virus were associated with preexisting immune suppression. Though not significant in multivariable analyses, the strong association of high TTV viral load with immune paralysis in univariate analyses is intriguing and is deserving of future study in a larger cohort, as evidence in adults suggests that TTV may serve as a biomarker of immune suppression after transplantation (29, 30).

There are several limitations to this study. First, despite being the largest study of its kind in children, the sample size is small and this cohort may not be representative of the larger population of critically ill children. The subjects in the current study may have different rates of viral DNAemia or immune dysfunction compared with a broader population of critically ill children. No statistically significant conclusions could be drawn as to whether a specific type of primary infection (bacterial, viral, or fungal) was associated with viral DNAemia. Further, this study does not define a mechanism by which viral DNAemia occurs. The laboratory methods employed in this study do not establish whether episodes of DNAemia represented new, primary viral infections or reactivation of latent virus. Because nucleic acids were extracted from plasma rather than from whole blood, viruses latent within WBCs were not evaluated. The relationships between viral DNAemia, nosocomial infection, and immune function, however, strongly suggest a potentially important relationship between viral DNAemia and impairment of the host immune response. Finally, this study does not establish cause and effect relationships between viral DNAemia, immune function, and outcomes. Rather, it highlights novel associations that should be the subject of prospective study.

CONCLUSIONS

In conclusion, viral DNAemia was common in children with severe sepsis and was most strongly associated with preexisting immune suppression. There were also strong associations of viral DNAemia with secondary infection risk and lower innate immune function over time. Causal relationships between viral DNAemia and immune dysfunction in the setting of pediatric severe sepsis, along with the measurement of viral DNAemia as a potential marker of immune suppression, should be investigated in larger multicenter trials. An understanding of this biology may help inform the identification of therapeutic targets and determine whether treatment of these DNA viruses can improve outcomes in septic children.

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