Detection of Viruses in Young Children With Fever Without an Apparent Source

AUTHORS: Joshua M. Colvin, MD,^a Jared T. Muenzer, MD,^a David M. Jaffe, MD,^a Avraham Smason, BS,^a Elena Deych, MS,^b William D. Shannon, PhD,^b Max Q. Arens, PhD,^a Richard S. Buller, PhD,^a Wai-Ming Lee, PhD,^c Erica J. Sodergren Weinstock, PhD,^d George M. Weinstock, PhD,^d and Gregory A. Storch, MD^a

Departments of ^aPediatrics, and ^bMedicine, and ^cthe Genome Institute, Washington University School of Medicine, St Louis, Missouri; and ^dBiological Mimetics, Inc, Frederick, Maryland

KEY WORDS

fever, viral infection, polymerase chain reaction

ABBREVIATIONS

ED—emergency department

HHV-6—human herpesvirus 6

PCR—polymerase chain reaction

All of the authors except for Dr Lee participated in the design of the study; patient data were gathered by Drs Colvin, Muenzer, Jaffe, and Mr Smason; laboratory data were produced by Drs Colvin, Arens, Buller, Lee, and Storch; and all of the authors participated in the analysis of the data. The analysis of the data was led by Dr Storch and reviewed by Ms Deych and Dr Shannon, who also carried out all statistical analyses; and all of the authors take responsibility for the integrity of the data and the analysis. The manuscript was written by Dr Storch and all of the authors participated in the decision to publish the paper. Procedures for protecting the confidentiality of subjects were agreed to by the institutional review boards of Washington University and the National Institutes of Health. The study database was maintained by Dr Storch. It was used by the study statisticians, Ms Deych and Dr Shannon. All authors meet the criteria for authorship listed in the *Pediatrics* Author Guidelines and all authors have reviewed and approved the final manuscript.

Portions of this work was presented in part at the 2009 Pediatric Academic Societies annual meeting, April 30–May 5, 2009, Baltimore, MD; The First Annual Meeting of the Human Microbiome Project, January 19–21, 2010, Houston, TX; and the 2010 Pediatric Academic Societies annual meeting, May 1–4, 2010, Vancouver, Canada.

www.pediatrics.org/cgi/doi/10.1542/peds.2012-1391

doi:10.1542/peds.2012-1391

Accepted for publication Jul 30, 2012

Address correspondence to Gregory Storch, MD, Department of Pediatrics, Campus Box 8116, 660 S Euclid Ave, St Louis, MO 63110. E-mail: storch@wustl.edu

(Continued on last page)

WHAT'S KNOWN ON THIS SUBJECT: Fever without an apparent source is common in children. Currently in the United States, serious bacterial infection is uncommonly the cause. Most cases are assumed to be viral, but the specific viral causes have not been delineated. Antibiotics are frequently prescribed.

WHAT THIS STUDY ADDS: By using polymerase chain reaction, we detected pathogenic viruses frequently in children with fever without an apparent source. Adenovirus, human herpesvirus-6, enterovirus, and parechovirus were predominant. Testing of blood had high yield. Better recognition of viral etiologies may help reduce unnecessary antibiotic use.

abstract

OBJECTIVE: Fever without an apparent source is common in young children. Currently in the United States, serious bacterial infection is unusual. Our objective was to determine specific viruses that might be responsible.

METHODS: We enrolled children aged 2 to 36 months with temperature of 38°C or greater without an apparent source or with definite or probable bacterial infection being evaluated in the St Louis Children's Hospital Emergency Department and afebrile children having ambulatory surgery. Blood and nasopharyngeal swab samples were tested with an extensive battery of virus-specific polymerase chain reaction assays.

RESULTS: One or more viruses were detected in 76% of 75 children with fever without an apparent source, 40% of 15 children with fever and a definite or probable bacterial infection, and 35% of 116 afebrile children ($P < .001$). Four viruses (adenovirus, human herpesvirus 6, enterovirus, and parechovirus) were predominant, being detected in 57% of children with fever without a source, 13% of children with fever and definite or probable bacterial infection, and 7% of afebrile children ($P < .001$). Thirty-four percent of 146 viral infections were detected only by polymerase chain reaction performed on blood. Fifty-one percent of children with viral infections and no evidence of bacterial infection were treated with antibiotics.

CONCLUSIONS: Viral infections are frequent in children with fever without an apparent source. Testing of blood in addition to nasopharyngeal secretions expanded the range of viruses detected. Future studies should explore the utility of testing for the implicated viruses. Better recognition of viruses that cause undifferentiated fever in young children may help limit unnecessary antibiotic use. *Pediatrics* 2012;130:e1455–e1462

Fever without an apparent source is a common problem in children that may require medical evaluation.¹ Since implementation of immunization programs in the United States directed against *Haemophilus influenzae* type b and *Streptococcus pneumoniae*, systemic bacterial infection has become an uncommon cause, while localized bacterial infections, mostly urinary tract infections, account for ~5% to 10% of cases.^{2–5} Viral infections are believed to account for most of the remainder, but the specific viruses responsible have not been systematically delineated. The inability to accurately distinguish patients with occult bacteremia from those with viral infection can lead clinicians to prescribe antibiotic therapy for a substantial proportion of these patients.⁴ Several viruses including human herpesvirus 6 (HHV-6),⁶ enterovirus, and adenovirus can present as an undifferentiated febrile illness.^{7–10} In this study, we used broad panels of polymerase chain reaction (PCR) assays to detect those and other viruses in blood and nasopharyngeal secretions. Blood has not been used extensively as a specimen for detecting viruses in this setting, but we reasoned that fever without an apparent source may represent a systemic infection in which the causative agent might be present in the blood. Our broad aim is to expand knowledge of viral causes of fever to help curb use of broad-spectrum antibiotic therapy for febrile children, many of whom have viral infections.

METHODS

Subjects

Children aged 2 to 36 months were recruited from the emergency department (ED) or the ambulatory surgery department (afebrile children) at St Louis Children's Hospital during shifts when study personnel were available. The case group consisted of

children with temperature of 38°C or greater without an apparent source, who were having blood obtained for a blood count and/or a blood culture for clinical management. Children with clinical syndromes suggestive of viral respiratory infection, such as bronchiolitis, were not included. The decision to obtain blood for these studies was made by ED physicians as part of their standard care and was not a part of this study. No standard written clinical protocol was in place in the ED to govern these decisions. The elevated temperature was documented either in the ED or by a health care provider within 24 hours before the ED evaluation. Children were excluded if they had an underlying condition that predisposed them to infection, including cancer, immune deficiency, immunosuppressive therapy, cystic fibrosis, sickle-cell disease, or presence of an indwelling venous catheter. Children with a positive rapid test for influenza were also not included.

Two comparison groups were also included. The first was children aged 2 to 36 months evaluated in the ED who had temperature of 38°C or greater and definite or probable bacterial infection, including bacteremia, urinary tract infection, skin and soft tissue infection, bone or joint infection, and culture-positive bacterial gastroenteritis. The second group consisted of well children aged 2 to 36 months having outpatient surgery who had been afebrile for at least 7 days before surgery.

Children with fever were enrolled from mid-February 2007 through mid-February 2010. Afebrile control children were enrolled during a 12-month period starting in mid-February 2009. Children were enrolled by study personnel who obtained health information from each child's caregiver, including past history and recent symptoms that might indicate the presence of an acute infection.

The study was part of a Demonstration Project of the Human Microbiome Project and was approved by the Washington University Human Research Protection Office. Informed consent was obtained from parents or guardians of all subjects.

Specimens

Blood samples were obtained from a venipuncture performed to obtain blood for tests ordered by the physician caring for the patient. Up to 3 mL of blood for virus-specific PCR assays was collected into an EDTA tube that was stored at –80°C. Nasopharyngeal secretions were obtained by swabbing the posterior nasopharynx. Before 2008, a standard Dacron-tipped swab and viral transport media were used. Starting in 2008, these were replaced by a flocked swab and universal transport media (Copan Flexible Mini-Tipped Flocked Swab; Universal Transport Media, Murrieta, CA). Swab samples were stored at –80°C.

Virus Detection and Typing

Nucleic acid was extracted from 100-μL aliquots of plasma and whole blood by using the MagNa Pure automated processor with the LC Total Nucleic Acid Isolation kit (Roche Applied Science, Indianapolis, IN) and from 200-μL aliquots of nasopharyngeal samples by using a BioRobot M48 automated nucleic acid processor with the MagAttract Virus Mini kit (QIAGEN, Valencia, CA). All extracts were eluted in 100 μL. Blood samples were tested by using a battery of virus-specific PCR assays described in the Supplemental Table 5.^{11–20} This testing was performed on plasma for all viruses except cytomegalovirus and Epstein-Barr virus, for which testing was performed on whole blood. Because of limited sample volume, not all tests were performed on every sample. Testing of nasopharyngeal samples was performed by using commercial

multiplex assays supplemented by laboratory-developed assays. PCR results were not available for patient management. Because the multiplex respiratory panels do not distinguish rhinoviruses from enteroviruses, the identification of virus present in nasopharyngeal samples that were positive for rhinovirus/enterovirus was determined by nucleotide sequencing.²¹ Viruses detected in blood samples by the enterovirus reverse transcription–PCR assay were considered to be enteroviruses without additional testing. The serotype of adenoviruses from nasopharyngeal samples was determined by molecular methods at the Centers for Disease Control and Prevention (Atlanta, GA).²²

Statistical Analyses

We used Fisher's exact test to compare the frequency of viruses in different groups. For continuous variables, we used ANOVA and the Wilcoxon test to compare groups, depending on variable distribution. We used logistic regression for adjusted analyses.

RESULTS

Subjects

After children with fever were enrolled, their ED records were reviewed by study personnel. Only those children whose evaluation did not find a source for their fever were included in the fever without a source group. Specifically, children who were assigned diagnoses of upper respiratory infection, pneumonia, otitis media, or sinusitis as part of their ED evaluation were not included. This resulted in 75 children with fever without an apparent source, 15 with fever and definite or probable bacterial infection, and 116 afebrile children. Demographic characteristics of the groups are shown in Table 1, and diagnoses of children with definite or probable bacterial infection are shown in Table 2. Twenty-two (29%) of the children with

TABLE 1 Demographic Characteristics of Study Subjects, Maximum Temperatures, and Specimens Obtained

Characteristics	Subject Group, No. (% of total)			P Value
	Fever without a Source (n = 75)	Fever and Definite or Probable Bacterial Infection (n = 15)	Afebrile (n = 116)	
Age, mo				.005
1–12	44 (59)	3 (20)	69 (59)	
13–24	23 (31)	5 (33)	30 (26)	
25–35	8 (11)	7 (47)	17 (15)	
Mean (SD)	10.9 (8.6)	20.2 (9.5)	13.1 (8.6)	<.001
Median	8.0	20.0	11.0	.001
Race				<.001
African American	43 (57)	10 (67)	15 (13)	
Caucasian	29 (39)	4 (27)	98 (84)	
Other	3 (4)	1 (7)	3 (2.6)	
Gender				.539
Female	31 (41)	5 (33)	39 (34)	
Male	44 (59)	10 (67)	77 (66)	
Season				.09
Winter	12 (16)	3 (20)	31 (27)	
Spring	13 (17)	1 (7)	24 (21)	
Summer	30 (40)	4 (27)	43 (37)	
Autumn	20 (27)	7 (47)	18 (16)	
Maximum temperature in emergency department				
Mean (SD)	39.1 (0.64)	39.0 (0.63)	NA	
Median (IQR)	39.1 (38.6–39.5)	38.9 (38.3–39.4)	NA	.430
Admitted to hospital	22 (29)	15 (100)	NA	<.001
Median days of stay (IQR)	2.0 (2.0–3.0)	3.0 (2.0–4.0)	NA	.372
Specimens obtained				
Blood	71 (95)	15 (100)	108 (93)	
Nasopharyngeal ^a	73 (97)	11 (73)	110 (95)	
Both	69 (92)	11 (73)	102 (88)	

NA, not applicable; IQR, interquartile range.

^a If a nasopharyngeal swab was obtained for respiratory virus detection as part of the routine care of the child, residual material from that specimen was used for the study. For children whose routine care did not include a nasopharyngeal swab, a study specimen was obtained, consistent with the study protocol and informed consent.

fever without an apparent source were admitted to the hospital. Children in the groups with fever were more likely to be African American, reflecting different demographic characteristics of children seen in the ED and those having ambulatory surgery.

PCR Evidence of Viruses

In all, we detected 146 viruses in 206 study subjects, including 50 viruses detected only in blood, 79 detected only in nasopharyngeal secretions, and 17 detected in both samples. Children were classified as “positive” for a virus if the virus was detected in either blood

or nasopharyngeal secretions. The numbers of children positive for each virus are shown in Table 3. Seventy-six percent of the children with fever without a source were positive for one

TABLE 2 Serious Bacterial Infections

Infection	No. of Cases
Abscess	5 ^a
Bacteremia	3 ^b
Urinary tract infection	3 ^c
<i>Shigella</i> gastroenteritis	3
Mastoiditis	1

^a Four cutaneous abscesses caused by *Staphylococcus aureus* (3 methicillin-resistant), 1 retropharyngeal abscess.

^b Two *Streptococcus pneumoniae*, 1 methicillin-sensitive *S. aureus*.

^c All 3 caused by *Escherichia coli*, including 1 with positive blood culture.

TABLE 3 Viruses Detected^a

Virus	Total Subjects Positive (% of Total Tested) ^b			<i>P</i>
	Fever Without a Source	Fever and Definite or Probable Bacterial Infection	Afebrile	
Adenovirus	20 (32)	2 (18)	6 (6)	<.001
HHV-6	11 (17)	0 (0)	0 (0)	<.001
Enterovirus ^c	10 (16)	0 (0)	2 (2)	.003
Parechovirus	4 (7)	0 (0)	0 (0)	.018
Bocavirus	9 (14)	0 (0)	5 (5)	.114
Rhinovirus	7 (10)	2 (20)	17 (16)	.427
Picornavirus ^d	4 (6)	1 (11)	1 (1)	.080
KI virus	4 (5)	2 (13)	2 (1)	.062
WU virus	0 (0)	0 (0)	2 (2)	.581
Human metapneumovirus	3 (4)	1 (9)	1 (1)	.103
Cytomegalovirus	4 (6)	1 (8)	3 (3)	.398
Parainfluenza viruses	3 (4)	0 (0)	0 (0)	.085
Epstein-Barr virus	2 (3)	3 (23)	2 (2)	.008
Parvovirus B19	2 (3)	0 (0)	1 (1)	.660
Influenza A virus	1 (1)	0 (0)	0 (0)	.433
BK virus	0 (0)	0 (0)	1 (1)	1.000
Coronaviruses	0 (0)	0 (0)	2 (2)	1.000
Patients with ≥1 virus detected (% of patients)	57 (76%)	6 (40%)	41 (35%)	<.001
Total viruses detected	84	12	45	<.001
Children positive for adenovirus, HHV-6, enterovirus, or parechovirus (% of patients) ^e	43 (57%)	2 (13%)	8 (7%)	<.001

^a Subjects were considered positive if the indicated virus was detected in either the blood or the nasopharyngeal sample.

^b Because of limited sample volume, not all subjects were tested for each virus.

^c Confirmed as enterovirus by nucleotide sequencing for viruses detected in nasopharyngeal samples but not for those detected in plasma.

^d Detected by rhinovirus/enterovirus PCR performed on nasopharyngeal samples, but nucleotide sequence to allow discrimination between rhinovirus and enterovirus could not be performed for technical reasons.

^e The numbers in parentheses are the percent positive for 1 of the 4 viruses listed, calculated by using as the denominator the number of children in the study group. If the percentages are calculated by using as the denominator the number of children who were positive for 1 or more of the 4 viruses plus the number of children who were tested and were negative for each of the 4 viruses, the percentages of children positive for 1 of the 4 viruses are: children with fever without a source, 69%; children with fever and definite or probable bacterial infection, 18%; and afebrile children, 8%. The difference between these groups is statistically significant ($P < .001$).

or more viruses, compared with 40% of the children with fever and a definite or probable bacterial infection and 35% of afebrile children. Detection of multiple viruses in the same patient was common, with 84 viruses detected in 57 children with fever without a source, 12 viruses in 6 children with fever and definite or probable bacterial infection, and 45 viruses in 41 afebrile children. No patterns of specific viruses occurring together were apparent. Fifty-seven percent of children with fever without a source were positive for 1 of 4 viruses: adenovirus, HHV-6, enterovirus, or parechovirus, compared with 13% of children with fever and definite or probable bacterial infection and 7%

of afebrile children. Viruses detected in children with fever without a source tended to be viruses recognized as pathogenic, whereas viruses of low pathogenicity such as rhinoviruses or viruses of uncertain pathogenicity such as bocavirus and the KI and WU polyomaviruses made up a higher proportion of the viruses detected in the comparison groups.

Viruses Detected in Blood

Viruses detected in blood are shown in Fig 1. One or more viruses were detected in blood samples from 62% of 71 children with fever without a source who had blood samples available for testing, compared with 33% of 15 chil-

dren with fever and definite or probable bacterial infection and 9% of 108 afebrile children ($P < .001$ for differences among the 3 groups). Viruses detected most frequently in the blood of children with fever without a source were adenovirus (22%), HHV-6 (17%), and enterovirus (16%). Each of these viruses was detected significantly more often in the children with fever without a source compared with the other groups ($P < .001$ for each comparison). In addition to these viruses, parechovirus and bocavirus were also found significantly more often in blood samples from children with fever without a source ($P = .018$, $P = .048$). Other viruses that were found in the blood of children with fever without a source were cytomegalovirus (6%) and parvovirus B19 (3%). Epstein-Barr virus was detected significantly more often (23%) in children with a serious bacterial infection compared with the other groups ($P = .008$).

Viruses Detected in Nasopharyngeal Secretions

Viruses detected in nasopharyngeal secretions are shown in Fig 2. One or more viruses was detected in nasopharyngeal secretions of 49% of 73 children with fever without a source compared with 36% of 11 children with fever and definite or probable bacterial infection and 31% of 110 afebrile children ($P = .039$). The only individual virus found significantly more often in nasopharyngeal secretions of children with fever without a source was adenovirus, which was detected in 22% of 73 of those children who had samples available for analysis, compared with 18% of children with fever and definite or probable bacterial infection and 4% of afebrile children ($P < .001$). Of 16 adenoviruses that could be typed, 9 were type 1, 6 were type 2, and 1 was type 3. There was a trend toward greater detection of bocavirus in children with fever without a source, but

FIGURE 1

Viruses detected in blood. Subjects are represented by rows and viruses by columns. Detection of a virus is indicated by a horizontal line. A, Children with fever without a source. B, Children with fever and definite or probable bacterial infection. C, Afebrile children.

differences compared with the other patient groups were not significant. The virus detected most frequently in nasopharyngeal secretions was rhinovirus, which was detected in 16% of 107 afebrile children, 20% of 10 children with fever and definite or probable bacterial infection, and 10% of 68 children with fever without a source. These differences were not statistically significant ($P = .427$).

Possible Interactions Between Demographic Characteristics and Viruses Detected

Because of the higher proportion of afebrile children who were white compared with febrile children, we performed race-stratified analysis and examined whether there was an interaction between viruses detected and race that could affect the comparison of viruses detected in the different study groups. The same trends that were detected in the entire study population were present in both whites and African Americans, indicating the absence of a racial effect. In addition, after adjusting for group, neither race (nor race-by-group interaction), gender, nor age was a significant predictor of virus presence in a logistic regression model. Because afebrile controls were enrolled only during the 12-month period from mid-February 2009 to mid-February 2010, we also confirmed that there were no differences in viruses detected in children with fever without a source enrolled during this period (60% of the total) compared with those enrolled before this period (40% of the total).

Demographic and Clinical Characteristics of Children With Specific Viruses

Using separate multivariate logistic models, we compared demographic and clinical characteristics of children with no virus detected to those in children in whom we detected only adenovirus, HHV-6, or enterovirus, as well as those with any other virus detected. The only statistically significant difference was that white blood cell counts were higher in children with adenovirus ($P = .003$). Of the 11 children with only adenovirus detected who had a complete blood count performed, all but one had a white blood cell count greater than 15 000 cells/mm³. In contrast, only 1 of 8 children with only HHV-

FIGURE 2

Viruses detected in nasopharyngeal secretions. Subjects are represented by rows and viruses by columns. Detection of a virus is indicated by a horizontal line. A, Children with fever without a source. B, Children with fever and definite or probable bacterial infection. C, Afebrile children.

6 detected had a white blood cell count greater than 15 000 per mm³.

Antibiotic Utilization

Of the 75 children with fever without a source, 34 (45%) received antibiotics including 24 (55%) of those 2 to 12 months of age, 9 (39%) of those 13 to 24 months of age, and 1 (13%) of those older than 24 months ($P = .08$).

Antibiotic utilization according to virus detected is shown in Table 4. Fifty-one percent of children with one or more viruses detected and 63% of children with adenovirus, HHV-6, enterovirus, or parechovirus received antibiotics, including 10 (91%) of 11 children with adenovirus alone.

DISCUSSION

Fever without an apparent source in children under 3 years of age is common and often prompts medical evaluation.¹ Currently in the United States, systemic bacterial infection accounts for <1% of cases.^{2–4,23} In contrast, we detected viruses in 76% of children with fever without an apparent source and identified adenovirus, HHV-6, enterovirus, and parechovirus as the predominant viruses detected in this patient group. Notably, we also detected viruses in a substantial minority of children with fever and bacterial infection and in afebrile children, but the viruses were different and often of lower pathogenicity than the viruses detected in children with fever without an apparent source. While it is not clear that the viruses detected were always clinically significant, viruses that are well recognized as pathogens were detected much more frequently in children with fever without a source compared with afebrile children. The presence of viral nucleic acid in blood samples also provides additional support for the likely clinical significance

of the viruses detected. Testing of blood was important for maximizing the detection of viral agents, as was the use of molecular methods, since viruses such as HHV-6 and bocavirus are not well detected by conventional culture-based diagnostic testing.^{24,25} Consistent with this idea, detection of the known pathogenic viruses was infrequent in blood samples from the afebrile study participants.

The finding of adenovirus as the most common virus in children with fever without a source was unanticipated.²⁶ Some adenovirus serotypes may maintain latency in tonsillar tissue,²⁷ and we did detect adenovirus in some children who were afebrile and in some with definite or probable bacterial infection. However, the rate of detection was much higher in children with fever without a source, suggesting a causal association. Almost all of the febrile children with adenovirus had elevated leukocyte counts, possibly accounting for the finding that more than 90% of subjects with adenovirus and no other virus detected were treated with antibiotics. HHV-6, enteroviruses, and parechoviruses have each been detected previously in children with fever without a source.^{7,28} We tested for HHV-6 in plasma rather than whole blood, to avoid detecting latent virus in circulating mononuclear cells where it can be latent.²⁹ As in other recent studies,^{28,30} we detected most parechoviruses in children younger than 6 months.

This study has several limitations. First, the group of children with fever without a source was limited to children in whom blood was obtained for diagnostic studies. In the era of effective vaccines against invasive bacterial infections, the proportion of febrile children having blood drawn as part of evaluations for fever is decreasing. Indeed, in a study carried out in our ED that overlapped temporally with the first year of the study reported here, only 22% of children with temperatures of 39°C or greater had blood cultures obtained.³¹ Thus, it is likely that children with mild illness or illness very suggestive of viral infection were not included, and these populations may have been infected with different microbial agents. Second, because of funding limitations, staffing was not available in the ED to enroll potential subjects at all times, and thus the study does not include all patients meeting the eligibility criteria. We do not have data regarding possible differences between those enrolled and those not enrolled, and we cannot exclude unknown biases in subject enrollment. This may limit the generalizability of our results, and it will be important for this study to be replicated in other locations at other times. Third, detection of a virus in a febrile child, especially using highly sensitive molecular methods, does not prove that the virus is responsible for the child's illness. Indeed, we detected one or more viruses in 35% of well afebrile children, including some of the same viruses that were detected in febrile children. However, many of these were viruses of low pathogenicity such as rhinoviruses and the KI and WU polyomaviruses. Finally, our analysis was limited to blood and nasopharyngeal samples. Testing of other specimens, especially stool might have revealed the presence of additional viruses.

TABLE 4 Antibiotic Use According to Virus(es) Detected in Children With Fever Without a Source

Virus ^a	Subjects	No. (%) Receiving Antibiotics
Adenovirus	11	10 (91)
Enterovirus/parechovirus	8	5 (63)
HHV-6	8	2 (25)
Other single virus ^b	10	6 (60)
Multiple viruses	20	6 (30)
None ^c	18	5 (28)

^a No virus other than the specified virus was detected in plasma or nasopharyngeal samples.
^b Includes bocavirus (*n* = 3), rhinovirus (*n* = 2), metapneumovirus (*n* = 2), unclassified picornavirus (*n* = 1), CMV (*n* = 1), and KI virus (*n* = 1).
^c If this analysis is limited to children who were tested for all viruses, 2 (25%) of 8 children who had no virus detected received antibiotics.

More than half of children in this study with evidence of viral infection and no evidence for bacterial infection received antibiotics. It is our hope that better awareness of viral infection could lead to a significant decrease in antibiotic utilization in children with fever without a source. Previously the use of rapid tests for influenza has been associated with decreased antibiotic use,³² and development of rapid tests for the agents implicated in this study might have a similar effect. New technology allows PCR test results to be available with a turnaround time of ~1 hour.³³ However, it is also important to be aware that patients may have serious bacterial infection concurrent with viral infection. For this reason, the decision to withhold antibiotics based on presence of a virus, even one recognized as a pathogen, must be made carefully, with consideration of all clinical findings to avoid withholding antibiotics from a patient with serious

bacterial infection. A biomarker that accurately identifies patients with active bacterial infection would be an important adjunct to viral diagnostic testing and development of such a biomarker should be a priority for future research.

CONCLUSIONS

Known pathogenic viruses were detected much more frequently in children with fever without an apparent source, compared with children with fever and definite or probable bacterial infection or afebrile children, suggesting a causal relationship. The finding that 4 viruses were predominant is an important consideration for the design of future diagnostic tests. Testing of blood is required to maximize yield in children with fever without an apparent source. Additional study is required to determine whether specific viral diagnosis would be useful in clinical

management. These studies should be pursued, because better recognition of viral etiologies may help avoid unnecessary use of antibiotics.

ACKNOWLEDGMENTS

We thank the staff of the ED, the Ambulatory Surgery Department, and the Virology Laboratory at St Louis Children's Hospital for their assistance in carrying out this study. We thank Jennifer Loughman, PhD, and David Hunstad, MD, for assistance with figures (both from the Department of Pediatrics, Washington University); Carey-Ann Burnham, PhD (from the Department of Pathology, Washington University), and David Hunstad, MD, for their critical reading of the manuscript; Dean Erdman, PhD, and Xiaoyan Lu, MS, of the CDC for adenovirus typing; and Barbara Hartman (from the Department of Pediatrics, Washington University) for assistance with manuscript preparation.

REFERENCES

- Krauss BS, Harakal T, Fleisher GR. The spectrum and frequency of illness presenting to a pediatric emergency department. *Pediatr Emerg Care*. 1991;7(2):67–71
- Watt K, Waddle E, Jhaveri R. Changing epidemiology of serious bacterial infections in febrile infants without localizing signs. *PLoS ONE*. 2010;5(8):e12448
- Rudinsky SL, Carstairs KL, Reardon JM, Simon LV, Riffenburgh RH, Tanen DA. Serious bacterial infections in febrile infants in the post-pneumococcal conjugate vaccine era. *Acad Emerg Med*. 2009;16(7):585–590
- Waddle E, Jhaveri R. Outcomes of febrile children without localizing signs after pneumococcal conjugate vaccine. *Arch Dis Child*. 2009;94(2):144–147
- Hsiao AL, Chen L, Baker MD. Incidence and predictors of serious bacterial infections among 57- to 180-day-old infants. *Pediatrics*. 2006;117(5):1695–1701
- Pruksananonda P, Hall CB, Insel RA, et al. Primary human herpesvirus 6 infection in young children. *N Engl J Med*. 1992;326(22):1445–1450
- Dagan R, Hall CB, Powell KR, Menegus MA. Epidemiology and laboratory diagnosis of infection with viral and bacterial pathogens in infants hospitalized for suspected sepsis. *J Pediatr*. 1989;115(3):351–356
- Rotbart HA, McCracken GH Jr, Whitley RJ, et al. Clinical significance of enteroviruses in serious summer febrile illnesses of children. *Pediatr Infect Dis J*. 1999;18(10):869–874
- Ruuskanen O, Meurman O, Sarkkinen H. Adenoviral diseases in children: a study of 105 hospital cases. *Pediatrics*. 1985;76(1):79–83
- Edwards KM, Thompson J, Paolini J, Wright PF. Adenovirus infections in young children. *Pediatrics*. 1985;76(3):420–424
- Sanchez JL, Storch GA. Multiplex, quantitative, real-time PCR assay for cytomegalovirus and human DNA. *J Clin Microbiol*. 2002;40(7):2381–2386
- Wandinger K, Jabs W, Siekhaus A, et al. Association between clinical disease activity and Epstein-Barr virus reactivation in MS. *Neurology*. 2000;55(2):178–184
- Heim A, Ebnet C, Harste G, Pring-Akerblom P. Rapid and quantitative detection of human adenovirus DNA by real-time PCR. *J Med Virol*. 2003;70(2):228–239
- Cone RW, Huang ML, Hackman RC, Corey L. Coinfection with human herpesvirus 6 variants A and B in lung tissue. *J Clin Microbiol*. 1996;34(4):877–881
- Kidd IM, Clark DA, Ait-Khaled M, Griffiths PD, Emery VC. Measurement of human herpesvirus 7 load in peripheral blood and saliva of healthy subjects by quantitative polymerase chain reaction. *J Infect Dis*. 1996;174(2):396–401
- Sumino KC, Walter MJ, Mikols CL, et al. Detection of respiratory viruses and the associated chemokine responses in serious acute respiratory illness. *Thorax*. 2010;65(7):639–644
- Arthur RR, Dagostin S, Shah KV. Detection of BK virus and JC virus in urine and brain tissue by the polymerase chain reaction. *J Clin Microbiol*. 1989;27(6):1174–1179
- Le BM, Demertzis LM, Wu G, et al. Clinical and epidemiologic characterization of WU

- polyomavirus infection, St. Louis, Missouri. *Emerg Infect Dis*. 2007;13(12):1936–1938
19. Hormozdi DJ, Arens MQ, Le BM, Buller RS, Agapov E, Storch GA. KI polyomavirus detected in respiratory tract specimens from patients in St. Louis, Missouri. *Pediatr Infect Dis J*. 2010;29(4):329–333
 20. Nix WA, Maher K, Johansson ES, et al. Detection of all known parechoviruses by real-time PCR. *J Clin Microbiol*. 2008;46(8):2519–2524
 21. Lee WM, Kiesner C, Pappas T, et al. A diverse group of previously unrecognized human rhinoviruses are common causes of respiratory illnesses in infants. *PLoS ONE*. 2007;2(10):e966
 22. Lu X, Erdman DD. Molecular typing of human adenoviruses by PCR and sequencing of a partial region of the hexon gene. *Arch Virol*. 2006;151(8):1587–1602
 23. Herz AM, Greenhow TL, Alcantara J, et al. Changing epidemiology of outpatient bacteremia in 3- to 36-month-old children after the introduction of the heptavalent-conjugated pneumococcal vaccine. *Pediatr Infect Dis J*. 2006;25(4):293–300
 24. Krueger GR, Ablashi DV, Josephs SF, et al. Clinical indications and diagnostic techniques of human herpesvirus-6 (HHV-6) infection. *In Vivo*. 1991;5(3):287–295
 25. Allander T. Human bocavirus. *J Clin Virol*. 2008;41(1):29–33
 26. Gray GC, McCarthy T, Lebeck MG, et al. Genotype prevalence and risk factors for severe clinical adenovirus infection, United States 2004–2006. *Clin Infect Dis*. 2007;45(9):1120–1131
 27. Wold WSM, Horowitz MS. Adenoviruses. In: Knipe DM, Howley PM, eds. *Fields virology*. Vol 2. 5th ed. Philadelphia: Wolters Kluwer/Lippincott Williams & Wilkins; 2007:2395–2436
 28. Harvala H, Robertson I, Chieochansin T, McWilliam Leitch EC, Templeton K, Simmonds P. Specific association of human parechovirus type 3 with sepsis and fever in young infants, as identified by direct typing of cerebrospinal fluid samples. *J Infect Dis*. 2009;199(12):1753–1760
 29. Huang LM, Kuo PF, Lee CY, Chen JY, Liu MY, Yang CS. Detection of human herpesvirus-6 DNA by polymerase chain reaction in serum or plasma. *J Med Virol*. 1992;38(1):7–10
 30. Selvarangan R, Nzabi M, Selvaraju SB, Ketter P, Carpenter C, Harrison CJ. Human parechovirus 3 causing sepsis-like illness in children from midwestern United States. *Pediatr Infect Dis J*. 2011;30(3):238–242
 31. Colvin JM, Jaffe DM, Muenzer JT. Evaluation of the precision of emergency department diagnoses in young children with fever. *Clin Pediatr (Phila)*. 2012;51(1):51–57
 32. Bonner AB, Monroe KW, Talley LI, Klasner AE, Kimberlin DW. Impact of the rapid diagnosis of influenza on physician decision-making and patient management in the pediatric emergency department: results of a randomized, prospective, controlled trial. *Pediatrics*. 2003;112(2):363–367
 33. Poritz MA, Blaschke AJ, Byington CL, et al. FilmArray, an automated nested multiplex PCR system for multi-pathogen detection: development and application to respiratory tract infection. *PLoS ONE*. 2011;6(10):e26047

(Continued from first page)

PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275).

Copyright © 2012 by the American Academy of Pediatrics

FINANCIAL DISCLOSURE: The authors have indicated they have no financial relationships relevant to this article to disclose.

FUNDING: Funding for this study was provided by grant 1UAH2AI083266-01 from the National Institute of Allergy and Infectious Diseases, a Demonstration Project of the Human Microbiome Project; grant UL1 RR024992 from the National Institutes of Health National Center for Research Resources; and Washington University Institute for Clinical and Translational Sciences, and Training grant T32HD049338 from the National Institute of Child Health and Human Development. The funding agencies did not have any role in the performance of the study, the interpretation of study data, or the preparation of the article. Funded by the National Institutes of Health (NIH).