Application of Transcriptional Signatures for Diagnosis of Febrile Infants within the Pediatric Emergency Care Applied Research Network (PECARN)

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Abstract

Fever is one of the most important reasons for childhood visits to emergency departments (EDs) and accounts for approximately 5.4 million ED visits annually in the United States.[1–3] Infants 60 days of age or younger are of greatest concern among febrile children as their risk of serious bacterial infection is greatest (the current estimates of prevalence are between 6 - 10%).[4,5] Missed bacterial infections in young febrile infants can result in meningitis, sepsis and death; therefore early and accurate identification of bacterial infections is critical.[5] Although most febrile children have non-bacterial infections (mostly viral or presumed viral), some of these infants have serious bacterial infections (defined here as, bacteremia, urinary tract infection (UTI), bacterial meningitis, lobar pneumonia, or bacterial enteritis).

Although many pathogens are difficult to culture in the laboratory, the diagnosis of bacterial and non-bacterial infections in young febrile infants is largely dependent on culture and/or detection (for example by rapid diagnostic tests) of the microbial pathogen in the appropriate clinical specimens (for example cerebrospinal fluid, blood, urine, or respiratory secretions).[6] Dependence on cultures to discriminate between bacterial and non-bacterial infections can be inaccurate, costly, inconvenient and is often impractical due to the time needed for the growth of cultures (a minimum of 48 hours).

Advances in techniques for conducting high through-put ribonucleic acid (RNA) analysis have led to a better understanding of the host-pathogen response during infections. Thus, a novel approach to distinguish febrile infants infected with bacterial pathogens from those infected with non-bacterial pathogens is to examine the host response to infection. Recent data indicate that different pathogens induce distinct transcriptional "biosignatures" in the RNA of blood leukocytes that can be reliably measured by microarray analysis. In children with confirmed bacterial and viral infections, microarray assays in a single blood sample can discriminate between bacterial and viral infections with 95% accuracy.[7]

The Pediatric Emergency Care Applied Research Network (PECARN)[8] evaluates more than 800,000 children annually in its 18 participating EDs, including approximately 144,000 children with fever and at least 4,000 febrile infants 60 days of age or younger. The network offers the ideal setting to prospectively collect clinical data and blood samples in a large cohort of febrile infants to identify transcriptional diagnostic signatures that may discriminate bacterial from non-bacterial infections.

The ultimate goal of this project is to incorporate transcriptional biosignatures into the evaluation of febrile infants in the ED to facilitate the diagnosis of infants with bacterial and non-bacterial infections. The objectives of the current proposal are to introduce mi-

croarray technology in PECARN, to demonstrate the ability of this network to consistently and reliably collect high-quality small volume blood samples for RNA analysis, to establish diagnostic bacterial and non-bacterial biosignatures in young febrile infants, and assess the accuracy of procalcitonin in identifying young febrile infants with serious bacterial infections in the context of a multi-center ED setting.

1 Study Summary

1.1 Specific Aims

Specific Aim 1. To educate and train a core group of PECARN investigators to conduct research in RNA microarray transcriptional technologies in febrile infants.

Specific Aim 2. To demonstrate the ability of the PECARN network to consistently obtain small-volume, high-quality, RNA samples that can be used for microarray analysis from febrile infants 60 days of age or younger across multiple participating PECARN EDs.

Specific Aim 3. To define the initial diagnostic "bacterial" and "non-bacterial" biosignatures using RNA microarray transcriptional technologies in young febrile infants presenting to the ED.

Specific Aim 4. To conduct limited validation testing of the initial diagnostic biosignatures on an independent group of febrile infants presenting to the participating EDs.

Specific Aim 5. To determine the accuracy of procalcitonin (PCT) for identifying young febrile infants with serious bacterial infections.

1.2 Patient Eligibility

1.2.1 Inclusion Criteria

Patients will be eligible for enrollment if they:

- are 60 days of age or younger; AND
- are febrile with documented rectal temperature $\geq$ 38 °C in the ED OR have a history of fever (temperature, measured by any route, $\geq$ 38 °C at home / outside clinic) within 24 hours of ED presentation; AND

- are being evaluated for serious bacterial infections with screening tests, as per clinical site current practice, including but not limited to blood culture.

1.2.2 Exclusion Criteria

Patients will be ineligible for enrollment if ANY of the following is present or anticipated:

- prematurity (born less than 37 weeks gestational age); OR
- administration of antibiotics within 4 days of ED presentation, OR
- overwhelming clinical sepsis (i.e. requiring emergent interventions such as endotracheal intubation, use of vasoactive medications or cardiopulmonary resuscitation) OR
- major congenital abnormality; OR
- inborn errors of metabolism; OR
- congenital heart disease, OR
- chronic lung disease, OR
- disease or medication that would affect the immune system, OR
- indwelling catheters or shunts, OR
- evidence of focal infections such as abscesses, cellulitis, or other focal infections (otitis media is *not* an exclusion criterion), OR
- lack of permission from the parent or legally authorized representative (LAR).

1.3 Summary of Study

Enrolled infants will have up to 2 mls of whole blood obtained during the same venipuncture necessary for obtaining the screening blood tests needed to evaluate the febrile infant in the ED. This sample will be split into a 1.0 ml RNA sample, which will be stored and shipped to the Bioinformatics Core at the Baylor Institute for Immunology Research (Dallas, TX) for further processing; and the remaining available 1.0 ml PCT sample, which will be stored and shipped to the Hemostasis Reference Laboratory, Inc. (Ontario,

CA). The samples will be identified only with a study ID number and the RNA sample may also be labeled with a study tube barcode. Some sites may be unable to collect the PCT sample in which case only 1 ml of blood will be collected and will be used for the RNA sample.

Clinical data will be sent to the CDMCC at the University of Utah, and the data will be de-identified to produce an analytical database. This database will be used to provide clinical correlation with the microarray and PCT results.

1.4 Anticipated Recruitment and Study Duration

In the first year of the study, each clinical site will collect 1 to 3 blood samples. In years two and three, each clinical site will screen and enroll eligible infants until they obtain approximately 25-150 blood samples per year. It is anticipated that 20 to 22 sites will participate in the study, so the anticipated total number of samples that will be collected is 2,250 over the initial three year accrual period. In years 4 and 5, the target sample collection is an additional 1,000 samples per year. In year 6, additional patients will be enrolled in order to reach the targeted rate of serious bacterial infection within the sample size. Therefore, a total target sample collection of approximately 4,800 samples will be obtained over the entire study period.

2 Background and Significance

Fever without a source is an important reason for childhood visits to the ED, and accounts for 15% of all visits in infants 60 days of age or younger.[1–3] Most of these febrile infants (with fever defined as rectal temperatures $\geq$ 38 °C) will have a non-bacterial cause of fever. Most of these infants are clinically indistinguishable from those with serious bacterial infections when they first present to the ED.

2.1 Current Approach to the Febrile Infant

Traditional risk stratification strategies (including those which involve clinical and laboratory evaluation) have been largely derived from three prospective studies, commonly referred to as the Rochester, Philadelphia and Boston criteria.[5] These strategies have been evaluated extensively and components of each have been endorsed for incorporation into guidelines for the evaluation of febrile infants by a consensus panel of experts in the early 1990's.[9] These guidelines recommend laboratory evaluation (blood tests, urine tests,

chest radiographs and lumbar puncture for cerebrospinal fluid analysis), hospitalization, and empiric treatment with antibiotics for a subset of the youngest infants at highest risk for serious bacterial infections, until bacterial cultures are reliably negative (minimum of 48 hours).

The introduction of two protein conjugate bacterial vaccines (the *Hemophilus influenzae* type b and pneumococcal vaccines [PCV7]) has dramatically altered the evaluation of febrile children *older* than 3 months of age.[10,11] The impact of these vaccines is substantially less in young infants due to the timing of vaccine administration. Because the risk of serious bacterial infections in the youngest febrile infants remains high (estimated as 8.8% (95% CI 7.2,10.6) in infants younger than 30 days and 7.3% (95%CI 6.2,8.5%) in those 30-60 days old,[4,5] experts continue to recommend laboratory screening for serious bacterial infections in this age group.[12]

In the meantime, the use of rapid viral detection technologies is changing management strategies.[13–18] Documentation of specific viral infections has been shown to decrease, but not eliminate the risk of serious bacterial infections in young febrile infants. For example, the prevalence of bacteremia and meningitis is lower in febrile infants with documented respiratory syncytial virus (RSV) infections than in those without such infections (1.1% vs. 2.3%); unfortunately, the risk of serious bacterial infections was not negligible.[19,20] The addition of biomarkers such as C-reactive protein, procalcitonin and interleukins to routine screening tests for detecting serious bacterial infections has been unsuccessful in improving the clinician's ability to evaluate and manage febrile infants.[21–28] However, these biomarkers have not been studied in large infant populations, and recent studies suggest that PCT may be useful in identifying serious bacterial infections in other populations.[22,23,25–27]

2.2 Assessing the Host Response to Infection

Recent evidence indicates that different microbial pathogens induce specific host responses that can be identified using microarray assays in leukocytes.[7] These host response patterns are known as "transcriptional signatures" or "molecular biosignatures." The use of microarray techniques for measuring the host response to the infecting microbe is a novel diagnostic strategy that should be prospectively evaluated. This strategy shifts the paradigm in the diagnosis of infectious diseases from pursuing the direct identification of the pathogen to examining the host response as the source of a unique disease biosignature, and accurately identifying the pathogen from the biosignature.[6,7,29,30]

2.3 Transcriptional Patterns in Respiratory Infections

Blood leukocytes carry biosignatures that can be used to differentiate infections caused by various pathogens. Gene expression profiles were obtained for 95 pediatric patients admitted to the hospital with infections caused by a variety of pathogens including: Influenza A virus (n=18), Gram-negative (Escherichia coli n=32) or Gram-positive (Staphylococcus aureus n=29 and Streptococcus pneumoniae n=16) bacteria. Data were acquired using Affymetrix U133A GeneChips (22,283 probe sets). This analysis identified 137 genes, known as classifier genes, that allow discrimination between patients with infections caused by different pathogens. When these classifier genes were used in a different group of 27 pediatric patients with pneumonia and 7 healthy children, the pathogens causing the infection in these patients were correctly identified. In Figure 1 on the facing page, there are four prototypical expression signatures: healthy controls were clearly distinguishable from those children with acute infections. A second signature was associated with samples from patients with Influenza A infection (including interferon-inducible genes) and was clearly different from a third signature, which characterized bacterial infections caused by S. aureus and S. pneumoniae (including neutrophil-associated genes). Three samples belonging to the influenza A group and one from the S. aureus group were characterized by a fourth signature (marked with asterisk), which combined elements of the previous ones, suggesting the possibility of a co-infection caused by both a viral and a bacterial pathogens. These results clearly demonstrate that blood leukocyte transcriptional patterns can distinguish the different microbial pathogens in patients presenting with similar symptoms.

2.4 Class Prediction Analysis to Discriminate Bacterial Infection

Class prediction analysis has been used to identify tumor markers for cancer prognosis.[31,32] We have implemented this strategy to select blood transcriptional signatures to differentiate patients with acute infections caused by viral vs. bacterial infections. We identified 35 genes with the best ability to discriminate patients with influenza A virus infection from patients with bacterial infection caused by either E. coli or S. pneumoniae (Figure 2 on the next page, left panel).[7] These 35 classifier genes were then tested in a second group of 37 patients. The 37 children were classified with 95% accuracy: one patient was misclassified and one patient was indeterminate in class prediction (Figure 2 on the facing page, right panel).

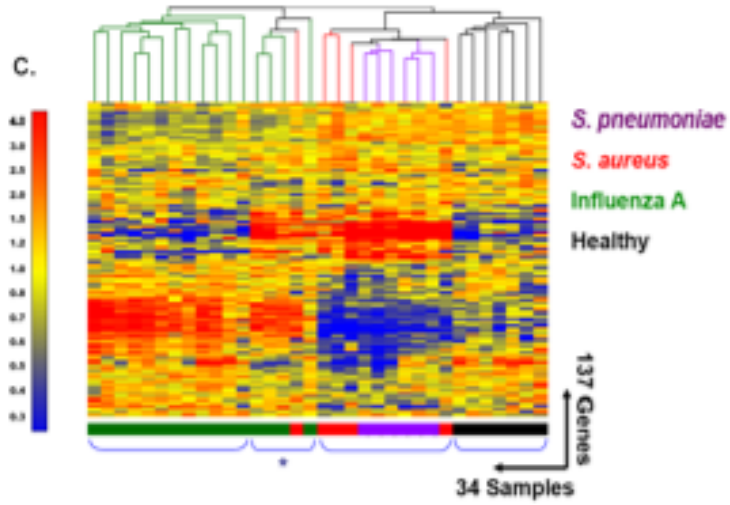

Figure 1: Hierarchical clustering of 137 discriminative genes in 34 individuals (27 patients with pneumonia and 7 age-matched healthy controls).

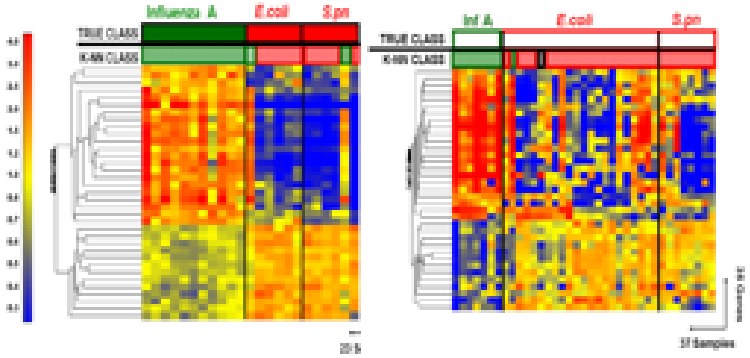

Figure 2: Gene expression on U133A GeneChips. Left: Set of 35 genes that discriminates patients with acute Influenza A infection (Green) and bacterial infections (Red). Right: the classifier genes were tested in an independent set of patients.

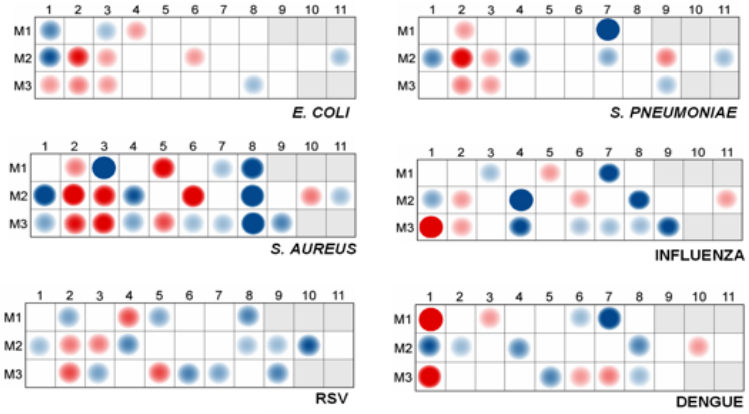

Figure 3: Disease specific fingerprints in patients with infectious diseases.

2.5 Disease Specific Biosignatures

To simplify interpretation of the large amount of data from microarray analyses, we have developed a novel strategy based on transcriptional modules (i.e. sets of co-expressed genes). The modules were extracted from an extensive patient transcriptional database (239 samples x 44,000 transcripts) using a custom unsupervised mining algorithm. The samples were obtained from 8 cohorts of patients with a wide range of disorders. Therefore modules contain little noise (because the probability for multiple transcripts to follow a defined expression pattern across hundreds of samples only by chance is very small), and are not disease-specific. When this analytical strategy is applied to patients presenting with different febrile illnesses, it allows a very accurate discrimination of patients with a number of different bacterial and viral infections. The disease fingerprints that are generated using this approach permit a much easier readout to distinguish between viral and bacterial pathogens, and will facilitate implementation of this technology in the ED. (Figure 3).

2.6 Significance of Present Study

The application of transcriptional microarray techniques to a large-scale multi-center network poses a number of theoretical and technical challenges.[33] The more obvious challenges include training PECARN investigators and staff to properly handle samples and maintain them in a cold environment, obtaining small volume blood samples in the "chaotic" ED environment,

verifying the quality of the RNA in these samples after shipping to a central laboratory, and confirming that we can identify diagnostic "bacterial" and "non-bacterial" biosignatures in young febrile infants presenting to the ED. The relative value of biomarker measurements, such as procalcitonin, compared with microarray signatures and clinical findings needs to be rigorously assessed. This study addresses each of these specific challenges, and will continue to position PECARN for future molecular studies that will contribute to improving pediatric emergency care.

3 Specific Aims

Specific Aim 1. To educate and train a core group of PECARN investigators to conduct research in RNA microarray transcriptional technologies in febrile infants.

Specific Aim 2. To demonstrate the ability of the PECARN network to consistently obtain small-volume, high-quality, RNA samples that can be used for microarray analysis from febrile infants 60 days of age or younger across multiple participating PECARN EDs.

Specific Aim 3. To define the initial diagnostic "bacterial" and "non-bacterial" biosignatures using RNA microarray transcriptional technologies in young febrile infants presenting to the ED.

Specific Aim 4. To conduct limited validation testing of the initial diagnostic biosignatures on an independent group of febrile infants presenting to the participating EDs.

Specific Aim 5. To determine the accuracy of procalcitonin (PCT) for identifying young febrile infants with serious bacterial infections.

4 Patient Eligibility

4.1 Inclusion Criteria

Patients will be eligible for enrollment if they:

- are 60 days of age or younger; AND
- are febrile with documented rectal temperature $\geq$ 38 °C in the ED OR have a history of fever (temperature, measured by any route, $\geq$ 38 °C at home / outside clinic) within 24 hours of ED presentation; AND

- are being evaluated for serious bacterial infections with screening tests, as per clinical site current practice, including but not limited to blood culture.

4.2 Exclusion Criteria

Patients will be ineligible for enrollment if ANY of the following is present or anticipated:

- prematurity (born less than 37 weeks gestational age); OR
- administration of antibiotics within 4 days of ED presentation, OR
- overwhelming clinical sepsis (i.e. requiring emergent interventions such as endotracheal intubation, use of vasoactive medications or cardiopulmonary resuscitation) OR
- major congenital abnormality; OR
- inborn errors of metabolism; OR
- congenital heart disease, OR
- chronic lung disease, OR
- disease or medication that would affect the immune system, OR
- indwelling catheters or shunts, OR
- evidence of focal infections such as abscesses, cellulitis, or other focal infections (otitis media is *not* an exclusion criterion), OR
- lack of permission from the parent or legally authorized representative (LAR).

4.3 Inclusion of Women and Minorities

Study subjects in this project are all infants. The gender, ethnic and racial composition of patients enrolled in all PECARN studies is a function of the underlying referral population at the clinical centers selected by the Maternal and Child Health, Emergency Medical Services for Children (EMSC) Program to participate in the network. During this study, the Central Data Management and Coordinating Center (CDMCC) will monitor patient accrual by race, ethnicity, and gender. If necessary, additional recruitment efforts will be made at specific centers to ensure that the aggregate patient sample contains appropriate gender and minority subsets.

4.4 Anticipated Recruitment and Study Duration

In the first year of the study, each clinical site will collect 1 to 3 blood samples. In years two and three, each clinical site will screen and enroll eligible infants until they obtain approximately 25-150 blood samples per year. It is anticipated that 20 to 22 sites will participate in the study, so the anticipated total number of samples that will be collected is 2,250 over the initial three year accrual period. In years 4 and 5, the target sample collection is an additional 1,000 samples per year. In year 6, additional patients will be enrolled in order to reach the targeted rate of serious bacterial infection within the sample size. Therefore, a total target sample collection of approximately 4,800 samples will be obtained over the entire study period.

5 Study Methods

Specific Aim 1. To educate and train a core group of PECARN investigators to conduct research in RNA microarray transcriptional technologies in febrile infants.

PECARN investigators and study coordinators will receive training in conjunction with an already scheduled Steering Committee meeting. This training will include a review of molecular biology as it pertains to this study, explanation of the importance of maintaining a continuous cold temperature for samples, and thorough discussion of the ethical issues that are raised by microarray studies. The training will enable the successful implementation of the remaining objectives of this study, as well as preparing PECARN for future studies involving genomics and proteomics.

Specific Aim 2. To demonstrate the ability of the PECARN network to consistently obtain small-volume, high-quality, RNA samples that can be used for microarray analysis from febrile infants 60 days of age or younger across multiple participating PECARN EDs.

After the initial training, the Bioinformatics Core will ship the special tubes for RNA storage (Baby Tempus, Applied Biosystems, Foster City, CA) to each participating PECARN site. These tubes will be used to collect and store blood samples from eligible participants. Although only 2-3 samples will be obtained from each site for this specific aim (i.e. a total of 50 samples to establish quality of RNA), the Bioinformatics Core will ship 5 tubes to each site, so that replacements are available in case of sample loss due to spillage or tube breaks.

Blood samples will be collected during routine venipuncture for standard evaluation of febrile infants at the participating centers of this study (which may vary from site to site). After informed consent, one extra ml of blood will be withdrawn and stored (between – 20 to – 80 °C) in special tubes (for high-quality RNA storage) at each site until at least 2-3 samples are available for transport to the Bioinformatics Core. All participating sites must have access to appropriate storage facilities (including freezers that can store blood samples between – 20 to – 80 °C).

Shipped samples will be processed and evaluated for quality at the Bioinformatics Core. The RNA will be extracted following the established protocols in the Bioinformatics Core and the quality of the RNA will be assessed. Only blood samples with satisfactory RNA will be included in the analysis. Approximately 50 samples will be run on the gene chips to determine whether they yield consistent results. On the basis of the experience of several ongoing multi-center and international studies conducted by the Bioinformatics Core using the newly adapted protocol for RNA extraction, our goal is that $> 90\%$ of samples will produce high-quality RNA that is adequate for microarray analysis.

Specific Aim 3. To define the initial diagnostic "bacterial" and "non-bacterial" biosignatures using RNA microarray transcriptional technologies in young febrile infants presenting to the ED.

Each participating site will screen and enroll eligible infants using the same method as described earlier. The blood samples will be stored at the appropriate temperature, and will be shipped to the Bioinformatics Core in 2 separate batches per year from each participating ED. It is anticipated that $\approx$ 1,050 samples per year will be collected. From samples collected during Years 2 and 3, the CDMCC will identify, from clinical laboratory results, all infants with proven bacterial infections, and all infants with proven viral infections. In addition, a similar number of infants with negative laboratory studies will be identified. The CDMCC will randomly select between 20 and 50 infants in each group, and will provide a list of samples to the Bioinformatics Core for analysis with gene chips. Additional random lists may be provided by the CDMCC upon request for further signature development.

For the purposes of this specific aim we will classify febrile infants into two groups. Those with:

Bacterial infections: As defined by a positive bacterial culture of the blood, CSF or urine.

Non bacterial infections: As defined by negative bacterial cultures of the blood, CSF and urine.

On the basis of our previous studies,[7] a minimum 20 samples of proven bacterial infections and 20 with non-bacterial infections (see Section 5.2 on page 20 for discussion of study power) will be required to define the diagnostic biosignatures. We will process as many samples as are available, up to 50 in each group. It is important to clarify that the goal of this specific aim is not to define signatures for the different bacterial infections such as bacteremia, meningitis or UTI, or for specific pathogens causing these infections. Rather, the objective is to define the "common bacterial infection" signature, which will be different than the "non-bacterial" signature. As we increase our accrual of enrolled infants and biologic samples in subsequent years and are able to analyze a larger number of samples, we will define more specific signatures such as those for UTI, bacteremia and meningitis, as well as pathogen-specific (bacterial and viral) signatures.

Specific Aim 4. To conduct limited validation testing of the initial diagnostic biosignatures on an independent group of febrile infants presenting to the participating EDs.

The CDMCC will identify, from samples that were not analyzed for the previous specific aim, and from samples collected in Years 4 through 6, all infants with proven bacterial and viral infections, and will provide a list of samples to the Bioinformatics Core for processing. The Bioinformatics Core will test the diagnostic biosignatures obtained in the previous specific aim. The Bioinformatics Core will not know the clinical information for these specimens; this will validate the accuracy with which the initial biosignatures are able to classify these unknown "test" samples.

In contrast to the "training set" of samples used to identify the diagnostic biosignatures, the "test set" does not need to include an equivalent number of patients in each group. Accordingly, we plan to perform two different validation analyses. In the initial validation, we will analyze equal numbers of samples between the two groups, for example 20 bacterial and 20 non-bacterial infections. In the second validation, we will simulate a more realistic situation in which frequencies of bacterial and non-bacterial infections in the sampled groups are similar to the frequencies among all samples; accordingly we plan to run samples from at least 20 bacterial and 100 non-bacterial infections. This limited validation will provide a very early assessment of the potential diagnostic value of this methodology for evaluation of young febrile infants in the EDs across the network.

As we conduct this study, we expect to identify a small number of patients with "mixed signatures" (as shown in Figure 1 on page 11), reflecting bacterial and other non-bacterial components. This may represent co-infections. We plan to better define these cases as we advance the project in subsequent years and obtain the necessary funding to analyze a larger number of samples. However, the goal of the present project is to identify microarray-based diagnostic signatures that do not miss bacterial infections that require antibiotic therapy. Thus, in this limited validation, the Bioinformatics Core will classify the mixed signature group with the bacterial group.

Samples that have been stored, but have not been analyzed during this project, will be kept for a maximum period of 10 years, to be analyzed at a later time as additional funding becomes available for the project. In order to refine the biosignatures it will be necessary to analyze as many of these samples as possible. Samples will not be used for any purposes unrelated to the long term goals of this study. If the initial results indicate that the study should not be continued, all samples from this study will be destroyed as per standard laboratory practices.

Specific Aim 5. To determine the accuracy of procalcitonin (PCT) for identifying young febrile infants with serious bacterial infections.

We will evaluate the test characteristics of PCT for identifying febrile infants with serious bacterial infections and compare these test characteristics to those of traditional screening tests for SBI. In these analyses, we will use both the currently used reference standard for SBI (positive cultures) as well as the RNA diagnostic biosignatures for SBI. We will also evaluate the laboratory data from standard screening tests accumulated during routine evaluation of the study patients.

We will calculate the sensitivity, specificity, predictive values, and likelihood ratios for white cell count (CBC), absolute neutrophil count (ANC), band count (BC), band to neutrophil ratio, and procalcitonin (PCT) for identifying SBI in young febrile infants, defined by both positive cultures and bacterial biosignature.

Bacteremia Rate Review in Population not Enrolled into the Study: The intent of this review is to get a better estimate of the rate of bacteremia over the study period in infants $\leq$ 60 days of age who are not enrolled in the study. Personnel at each study site will screen emergency department (ED) patient visit logs or laboratory logs to identify infants who presented

to the ED during the study period and who had blood cultures obtained in the ED. Patients previously enrolled with a positive blood culture will not be included in this review. Sites will then upload all de-identified positive blood culture reports into an electronic data collection repository and these reports will have a unique study identification number (ID).

The de-identified, uploaded reports will be reviewed by designated study investigators to determine bacteremia rates. This review will be conducted a minimum of 2 times during the course of the study.

Serious Bacterial Infection (SBI) Review: A few select sites will perform a more detailed retrospective review of the overall Serious Bacterial Infection (SBI) rates at their sites. SBI is defined here as bacteremia, urinary tract infection (UTI), or bacterial meningitis. These sites will perform a comprehensive review of patient records to ensure eligibility. Patients previously enrolled with positive culture reports will not be included in this review.

These sites will review blood, urine and CSF culture reports, both positive and negative, and upload the de-identified positive reports into an electronic data collection repository. Sites may also be requested to upload urinalysis results in cases where the urine culture results are unclear and additional relevant clinical data is needed. Patient records previously uploaded during the bacteremia review will be eligible as part of this review, but will not have blood culture reports uploaded a second time. All reports will be de-identified and have a unique study identification number (ID).

The reports and other relevant clinical data provided will be reviewed by designated study investigators to determine the rate of SBI at these select sites. This review will be conducted a minimum of 2 times during the course of the study.

5.1 Analytical Strategy

The overall principle for the analyses is to use an initial group of patients to identify the diagnostic biosignatures by comparing 20 to 50 positive cases (in this study bacterial infections) to a similar number of matched-controls (in this study the non-bacterial infections). This initial group of cases and controls is known as the "training set" and will be used to generate the initial diagnostic biosignature. The accuracy of this diagnostic biosignature will then be evaluated in a blinded fashion using an independent group of patients, known as "test set" in which the accuracy of transcriptional

biosignatures will be compared to the reference standard bacterial cultures and viral testing.

Three different strategies of biomarker discovery will be used for the identification of the diagnostic signatures:

1. Class prediction algorithms (such as K-Nearest Neighbors) will be used to identify sets of genes discriminating bacterial infections vs. non-bacterial infections by direct comparison between the groups as illustrated in Figure 2 on page 11.[7]

2. Class prediction will also be carried out on a module-by-module basis. Profiles obtained from patients with bacterial infections will be compared with patients with nonbacterial infections, as shown in Figure 3 on page 12.[30]

3. Alternatively, markers will be identified through the analysis of patterns of significance across datasets. The main advantage of this approach is that it can be used to compare diseases across multiple datasets, each being analyzed in relation to an existing healthy reference. This has allowed us to identify blood transcriptional markers that are exquisitely disease specific, differentiating for instance patients with a non-infectious disease such as systemic arthritis from a number of confounding infectious diseases that cause similar symptoms.[34]

5.2 Sample Size and Power Analysis

Traditional hypothesis testing approaches will not be used in this pilot study and therefore specific power calculations are not included. Our proposed sample size assumes that "effect sizes" (differences in biosignature patterns between subjects with "bacterial" and "non-bacterial infections") are of the magnitudes encountered in our previous microarray studies.[7] In those studies, a minimum of 20 infants per group ("bacterial" and "non-bacterial infections") were required to identify the diagnostic biosignatures.[7] Because the scope of this study is to analyze a limited number of samples and although we aim at a 90% sensitivity and specificity, we will be unable to precisely define the test characteristics (i.e. sensitivity, specificity, with narrow CI) of the signatures with the anticipated numbers of bacterial infections. This further underscores the need for collecting approximately 2,250 samples in the initial three year pilot project and expanding the available samples by

accruing up to 2,800 additional samples in years 4, 5, and 6 in order to better define pathogen-specific biosignatures as we have done in our previous studies in hospitalized children.[7]

6 Data Management

Clinical data will be collected at the time of enrollment, including the Yale Observation Scale[35] and final results of laboratory tests that were obtained as part of the evaluation of the febrile infant will be obtained from the hospital laboratory and/or the infant's medical record. We will also collect the disposition information from the infant's medical record. For patients who did not have a lumbar puncture and CSF culture done in the ED and are discharged home we will follow up by telephone to collect information on any return visit made within the 7 days following their initial ED discharge. If the patient made a return visit we will collect the results of any laboratory test performed during that visit from the patient's medical record. The information needed for the bacteremia rate review will be obtained from emergency department (ED) patient visit logs or laboratory logs. Information needed for the more comprehensive SBI review will be obtained from the infant's medical record.

Data will be entered into an electronic data capturing system (EDCS) used by the PECARN CDMCC. The Study Coordinator may choose to print hard copy forms to use as worksheets. If used, the paper worksheets should be retained at the clinical center in a locked file cabinet within a locked office until the study is complete and all PECARN publications have been accomplished. These paper worksheets will be subject to site monitoring reviews (see Section 7 on page 23).

The following data elements will be obtained and recorded when a patient is enrolled into the study, and when the laboratory results are available:

1. Study ID Number
2. Date of Enrollment
3. Date of Birth
4. Gender
5. Race

   American Indian or Alaska Native A person having origins in any of the original peoples of North and South America, including

Central America, and who maintains tribal affiliation or community attachment.

Asian A person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent, including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam.

Black or African American A person having origins in any of the black racial groups of Africa.

Native Hawaiian or Other Pacific Islander A person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands.

White A person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

Other (provide text) Should provide text description.

Stated as Unknown Explicitly stated as unknown.

6. Ethnicity
   - Hispanic or Latino
   - Not Hispanic or Latino
   - Stated as Unknown
7. Duration of fever
8. Blood culture
9. Yale Observation Scale
10. CBC and differential (if obtained)
11. Urinalysis (if obtained)
12. Urine culture (if obtained)
13. Cerebrospinal fluid cell count, glucose, protein, Gram stain, and culture (if obtained)
14. Other bacterial cultures (if obtained)
15. Viral studies (if obtained)
16. C-reactive protein (if obtained)

17. Procalcitonin (if obtained)
18. Interleukins (if obtained)
19. Chest radiograph (if obtained)
    - Positive for pneumonia
    - Negative for pneumonia
20. Disposition
21. Telephone Follow-up (if applicable)

When the subject is enrolled in the EDCS, a study number will be automatically provided to the clinical site. Blood samples will be labeled with the study ID number and the study tube barcode. No clinical data will accompany the blood samples.

The CDMCC will recode the dates of enrollment and birth to calculate the subject's age, in days, and the resulting analytical database fulfills the definition of a de-identified database according to HIPAA definitions. This activity (recoding the dates to calculate age) is done under Business Associate Agreements with each clinical site (see Section 8.5 on page 27).

7 Training and Site Monitoring

PECARN investigators and study coordinators will receive training in conjunction with an already scheduled Steering Committee meeting. This training will include a review of molecular biology pertaining to this study, explanation of the importance of maintaining a continuous cold temperature for samples, and thorough discussion of the ethical issues that are raised by microarray studies. The training will enable the successful implementation of the remaining objectives of this study, as well as preparing PECARN for future studies involving genomics and proteomics.

Training sessions were conducted at the beginning of years 2 and 3 enrollment to review lessons learned and to reemphasize study procedures. Additional training will be provided in years 4, 5, and 6, as needed.

PECARN investigators and study coordinators will be trained to use the EDCS for entry of clinical data for this study. The EDCS is already in use in PECARN studies, and training will focus on the specific data screens developed for this study.

Site monitoring visits will be performed by staff from the CDMCC, PECARN Nodal Administrators or designee, to ensure that all regulatory

requirements are being met and to monitor the quality of the data collected. The first site monitoring visit is anticipated toward the end of Year 1, and site monitoring will occur in subsequent years. During site monitoring visits, patient forms and original source documents will also be inspected. The primary criterion for data element verification is identification in the source document, which is the medical record. Thus, the medical record, either hard copy file or access to electronic medical record system, must be available for review by the site monitor. In addition, the site monitor will inspect the laboratory facilities that are used for processing, storing, and shipping the specimens in this study.

Remote site monitoring will also be performed by CDMCC staff to verify selected data elements. This will require the clinical site staff to locate the relevant source document, make a copy, de-identify the material with a black marker, and send the copy to the CDMCC via FAX server, email or secured mail where the information will be compared with data stored in the EDCS. Remote monitoring reports will be distributed to the study investigators and coordinators. The documents will be retained in accordance with Federal requirements (see Section 9 on page 27).

8 Human Subjects

This study requires approval of the Institutional Review Board (IRB) at every participating clinical site and the CDMCC at the University of Utah. The CDMCC will track IRB approval status at every clinical site, and will not permit enrollment of infants without documentation of initial and ongoing approval from each site's IRB.

8.1 Obtaining Permission for Participation

The parents or legally authorized representative (LAR) of infants who are eligible for this study will be provided with information about the study, including the potential risks and benefits, and written permission will be obtained for participation of the infant in the study.

Waiver of Consent/HIPAA for Bacteremia/SBI Retrospective Review: We request a waiver of consent and HIPAA authorization for the two (bacteremia and serious bacterial infection) retrospective reviews as these procedures are observational in nature, with minimal risk to the patient.

8.2 Potential Risks and Benefits

There are no major risks associated with participating in this study. Blood sampling will be carried out during a venipuncture that is already required as part of the evaluation of the febrile infant, and no separate venipuncture will be performed to enable participation in this study. A potential risk is disclosure of confidential clinical information, or perceived association of the gene expression data with an identifiable individual.

Measurement of RNA expression with microarray technology does not permit identification of an individual, in contrast to DNA sequencing. *In fact, processing samples for this study destroys the DNA present in the blood.* Thus, the analysis, and storage of RNA samples in this study poses no risk to individuals participating in the study.

There are no potential benefits for subjects who participate in this study. The results of microarray analyses will not be available during the management of the infant, and will not be available to the clinical site. The results of the study are intended to lead to improved methods for accurate diagnosis of febrile infants in the future.

8.3 Protection Against Risks

The risk of disclosure of confidential clinical data is minimized by keeping identifiable information at the clinical site in locked offices, and by minimizing identifying information stored in the EDCS at the CDMCC. Patient names and medical record numbers will not be sent to the CDMCC at the University of Utah, and will not be entered into the EDCS. The only linkage between the final analytical clinical data and the gene expression data will be the study ID number. The analytical clinical database will contain no patient identifiers and will fulfill the definition of a de-identified dataset as defined by the Health Insurance Portability and Accountability Act (HIPAA). This analytical database will be the only one available for the analysis of the current and future derivative studies.

8.4 Data Security

The CDMCC at the University of Utah has a dedicated, locked server room within its offices, and the building has 24 hour on-site security guards. The data coordinating center has a state-of-the-art computer infrastructure and coordinates its network infrastructure and security with the Health Sciences Campus (HSC) information systems at the University of Utah. This provides the data coordinating center with effective firewall hardware, auto-

matic network intrusion detection, and the expertise of dedicated security experts working at the University. Network equipment includes three high-speed switches and two hubs. User authentication is centralized with two Windows 2003 domain servers. Communication over public networks is encrypted with virtual point-to-point sessions using secure socket layer (SSL) or virtual private network (VPN) technologies, both of which provide at least 128 bit encryption. The EDCS, eRoom^{TM} (used for communication about the study) and other web applications use the SSL protocol to transmit data securely over the Internet.

Direct access to CDMCC computers is only available while physically located inside the data coordinating center offices, or via a VPN client. All network traffic is monitored for intrusion attempts, security scans are regularly run against our servers, and our IT staff are notified of intrusion alerts. Security is maintained with Windows 2003 user/group domain-level security. Users are required to change their passwords every 90 days, and workstations time out after 10 minutes of inactivity. All files are protected at group and user levels; database security is handled in a similar manner with group level access to databases, tables, and views in Microsoft SQL Server.

The investigators and staff of the data coordinating center are fully committed to the security and confidentiality of all data collected for PECARN studies. All personnel at the data coordinating center at the University of Utah have signed confidentiality agreements concerning all data encountered in the center. Violation of these agreements may result in termination from employment at the University of Utah. In addition, all personnel involved with data coordinating center data systems have received Human Subjects Protection and HIPAA education.

The Bioinformatics Core at Baylor Institute for Immunology Research (BIIR) is equipped with a data center which has been designed to provide a secure, high availability, computing environment. It is equipped with 5 brand new IBM servers and an 11 Terabyte IBM storage area network (SAN) server, expandable to 60 Terabytes (2 x 64 bit Intel Xeon Dual Core 2.8GHz/800MHz servers with fiber optic connection to the SAN). Data storage is fully redundant and mirrored to off-site servers. This setup insures data safety while minimizing downtime in the event a hardware failure should occur. In this study, no identifiable clinical information about study subjects will be sent to or stored at the Bioinformatics Core.

8.5 Health Insurance Portability and Accountability Act

Registration of research subjects in the EDCS used by the CDMCC at the University of Utah requires a date of birth, race, ethnicity, and gender. These demographic data are held in database tables that are separate from coded research data (including clinical data). These demographic data are required for Federal reporting purposes to delineate subject accrual by race, ethnicity, and gender.

Additional potential identifying information includes the date of admission, date of discharge and birth date. Staff at the CDMCC will use these dates to calculate patient age. The analytical data sets (used for study analyses and archived at the end of the study) will be de-identified, and will exclude these specific dates.

The data coordinating center produces the de-identified research data sets that will be used for all analyses in this project. Since the raw data includes potential identifiers, such as dates of birth and admission, all sites have been offered a Business Associate Agreement (BAA) with the University of Utah. The BAA explains that the data coordinating center is producing the de-identified data using the data submitted by the site, and the University of Utah assumes responsibility to preserve the confidentiality of the original data. Copies of executed Business Associate Agreements are maintained at the data coordinating center in Utah.

Patient identification at the HEDA site is present in subject study files, in order to enable auditing of data quality, and contact information is recorded in order to accomplish telephone follow-up. These data will not be sent to the data coordinating center, but will be retained in locked filing cabinets in locked offices in the HEDA itself. These records should be retained until the study data have been completely cleaned, data lock has occurred, and all primary and secondary publications have been completed. In accordance with Section 9, these records will be retained for at least 3 years after completion of the research. At that time, all records with identifying information will be destroyed.

9 Record Retention

For federally funded studies subject to the Common Rule, records relating to the research conducted shall be retained for at least 3 years after completion of the research. Completion of the research for this protocol should be anticipated to include planned primary and secondary analyses, as well as subsequent derivative analyses. Completion of the research also includes

additional enrollment of subjects and processing of additional samples, if funding becomes available for these activities. Finally, completion of the research also entails completion of all publications relating to the research. All records shall be accessible for inspection and copying by authorized representatives of the regulatory authorities at reasonable times and in a reasonable manner [45 CFR §46.115(b)].

References

[1] J. Chamberlain, K. Patel, and M. Pollack. Association of emergency department care factors with admission and discharge decisions for pediatric patients. *J Pediatr*, 149(5):644–649, Nov 2006. 0022-3476 (Print) Journal Article Multicenter Study Research Support, U.S. Gov't, P.H.S.

[2] P. Mahajan, M. Hamre, S. Knazik, and X. Chen. Evaluation of febrile infants < 60 days of age: Review of NHAMCS data. *PAS: Pediatric Academic Societies Annual Meeting*, Toronto, Canada, 2007.

[3] L. McCaig and E. Nawar. National Hospital Ambulatory Medical Care Survey: 2004 emergency department summary. *Adv Data*, 372:1–29, Jun 2006. 0147-3956 (Print) Journal Article.

[4] R. Bachur and M. Harper. Predictive model for serious bacterial infections among infants younger than 3 months of age. *Pediatrics*, 108(2):311–6, Aug 2001. 1098-4275 (Electronic) Journal Article.

[5] MB Harper. Update on the management of the febrile infant. *Clin Ped Emerg Med*, 5 (1):5–12, 2004.

[6] D. Relman. The human body as microbial observatory. *Nat Genet*, 30(2):131–3, Feb 2002. 1061-4036 (Print) Comment News.

[7] O Ramilo, W Allman, W Chung, A Mejias, M Ardura, C Glaser, K. Wittkowski, B Piqueras, J Banchereau, A. Palucka, and D Chaussabel. Gene expression patterns in blood leukocytes discriminate patients with acute infections. *Blood*, 109(5):2066–77, Mar 2007. 0006-4971 (Print) Journal Article Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't.

[8] PECARN. The Pediatric Emergency Care Applied Research Network (PECARN): rationale, development, and first steps. *Acad Emerg Med*, 10(6):661–8, Jun 2003. 1069-6563 (Print) Journal Article.

[9] L. Baraff, J. Bass, G. Fleisher, J. Klein, G. McCracken, K. Powell, and D. Schriger. Practice guideline for the management of infants and children 0 to 36 months of age with fever without source. Agency for Health Care Policy and Research. *Ann Emerg Med*, 22(7):1198–210, Jul 1993. 0196-0644 (Print) Guideline Journal Article Meta-Analysis Practice Guideline Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.

[10] K. Bisgard, A Kao, J Leake, P. Strebel, B. Perkins, and M Wharton. Haemophilus influenzae invasive disease in the united states, 1994-1995: near disappearance of a vaccine-preventable childhood disease. *Emerg Infect Dis*, 4(2):229–37, Apr 1998. 1080-6040 (Print) Journal Article.

[11] S Black, H Shinefield, R Baxter, R Austrian, L Bracken, J Hansen, E Lewis, and B Fireman. Postlicensure surveillance for pneumococcal invasive disease after use of heptavalent pneumococcal conjugate vaccine in Northern California Kaiser Permanente. *Pediatr Infect Dis J*, 23(6):485–9, Jun 2004. 0891-3668 (Print) Comparative Study Journal Article.

[12] J. Klein. Management of the febrile child without a focus of infection in the era of universal pneumococcal immunization. *Pediatr Infect Dis J*, 21(6):584–8; discussion 613–4, Jun 2002. 0891-3668 (Print) Journal Article Review.

[13] A. Bonner, K. Monroe, L. Talley, A. Klasner, and D. Kimberlin. 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*, 112(2):363–7, Aug 2003. 1098-4275 (Electronic) Clinical Trial Journal Article Randomized Controlled Trial.

[14] C. Byington, H Castillo, K Gerber, J. Daly, L. Brimley, S Adams, J. Christenson, and A. Pavia. The effect of rapid respiratory viral diagnostic testing on antibiotic use in a children's hospital. *Arch Pediatr Adolesc Med*, 156(12):1230–4, Dec 2002. 1072-4710 (Print) Journal Article Research Support, Non-U.S. Gov't.

[15] C. Byington, F. Enriquez, C Hoff, R Tuohy, E. Taggart, D. Hillyard, K. Carroll, and J. Christenson. Serious bacterial infections in febrile infants 1 to 90 days old with and without viral infections. *Pediatrics*,

113(6):1662–6, Jun 2004. 1098-4275 (Electronic) Journal Article Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.

[16] C Rocholl, K Gerber, J Daly, A. Pavia, and C. Byington. Adenoviral infections in children: the impact of rapid diagnosis. *Pediatrics*, 113(1 Pt 1):e51–6, Jan 2004. 1098-4275 (Electronic) Journal Article Research Support, Non-U.S. Gov't.

[17] V Sharma, M. Dowd, A. Slaughter, and S. Simon. Effect of rapid diagnosis of influenza virus type A on the emergency department management of febrile infants and toddlers. *Arch Pediatr Adolesc Med*, 156(1):41–3, Jan 2002. 1072-4710 (Print) Journal Article.

[18] R Vega. Rapid viral testing in the evaluation of the febrile infant and child. *Curr Opin Pediatr*, 17(3):363–7, Jun 2005. 1040-8703 (Print) Journal Article Review.

[19] D. Levine, S. Platt, P. Dayan, C. Macias, J. Zorc, W Krief, J Schor, D Bank, N Fefferman, K. Shaw, and N Kuppermann. Risk of serious bacterial infection in young febrile infants with respiratory syncytial virus infections. *Pediatrics*, 113(6):1728–34, Jun 2004. 1098-4275 (Electronic) Comparative Study Journal Article Multicenter Study Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.

[20] W. Krief, D. Levine, S. Platt, C. Macias, P. Dayan, J. Zorc, N. Fefferman, and N. Kuppermann. Influenza virus infection and the risk of serious bacterial infections in young febrile infants. *Pediatrics*, 124(1):30–39, July 2009.

[21] B. Bonsu, M Chb, and M. Harper. Identifying febrile young infants with bacteremia: is the peripheral white blood cell count an accurate screen? *Ann Emerg Med*, 42(2):216–25, Aug 2003. 0196-0644 (Print) Journal Article Validation Studies.

[22] E. Carrol, P Newland, F. Riordan, A. Thomson, N Curtis, and C. Hart. Procalcitonin as a diagnostic marker of meningococcal disease in children presenting with fever and a rash. *Arch Dis Child*, 86(4):282–5, Apr 2002. 1468-2044 (Electronic) Journal Article.

[23] A Fernandez Lopez, C Luaces Cubells, J. Garcia Garcia, and J Fernandez Pou. Procalcitonin in pediatric emergency departments for the early diagnosis of invasive bacterial infections in febrile infants: results of a multicenter study and utility of a rapid qualitative test for this

marker. *Pediatr Infect Dis J*, 22(10):895–903, Oct 2003. 0891-3668 (Print) Comparative Study Journal Article Multicenter Study.

[24] A. Hsiao and M. Baker. Fever in the new millennium: a review of recent studies of markers of serious bacterial infection in febrile children. *Curr Opin Pediatr*, 17(1):56–61, Feb 2005. 1040-8703 (Print) Journal Article Review.

[25] A. Jones, J. Fiechtl, M. Brown, J. Ballew, and J. Kline. Procalcitonin test in the diagnosis of bacteremia: A meta-analysis. *Ann Emerg Med*, Dec 2006. 1097-6760 (Electronic) Journal article.

[26] A. Lacour, A Gervaix, S. Zamora, L Vadas, P. Lombard, J. Dayer, and S Suter. Procalcitonin, IL-6, IL-8, IL-1 receptor antagonist and C-reactive protein as identificators of serious bacterial infections in children with fever without localising signs. *Eur J Pediatr*, 160(2):95–100, Feb 2001. 0340-6199 (Print) Journal Article.

[27] Kannikeswaran. Mahajan. P. Procalcitonin as a marker of serious bacterial infections (SBI). *Society for Pediatric Research Annual Meeting*, San Francisco, CA., 2006.

[28] P. Pulliam, M. Attia, and K. Cronan. C-reactive protein in febrile children 1 to 36 months of age with clinically undetectable serious bacterial infection. *Pediatrics*, 108(6):1275–9, Dec 2001. 1098-4275 (Electronic) Evaluation Studies Journal Article Research Support, Non-U.S. Gov't.

[29] C. Cummings and D. Relman. Genomics and microbiology. microbial forensics–"cross-examining pathogens". *Science*, 296(5575):1976–9, Jun 2002. 1095-9203 (Electronic) Comment Journal Article.

[30] A Mejias. Differential gene expression profiles in peripheral blood mononuclear cells of children with respiratory syncytial virus (RSV) and influenza virus infections. *45th Interscience Conference on Antimicrobial Agents and Chemotherapy*, Washington DC, 2005.

[31] A. Alizadeh, M. Eisen, R. Davis, C Ma, I. Lossos, A Rosenwald, J. Boldrick, H Sabet, T Tran, X Yu, J. Powell, L Yang, G. Marti, T Moore, J Hudson, L Lu, D. Lewis, R Tibshirani, G Sherlock, W. Chan, T. Greiner, D. Weisenburger, J. Armitage, R Warnke, R Levy, W Wilson, M. Grever, J. Byrd, D Botstein, P. Brown, and L. Staudt. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature*, 403(6769):503–11, Feb 2000. 0028-0836

(Print) Journal Article Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.

[32] T. Golub, D. Slonim, P Tamayo, C Huard, M Gaasenbeek, J. Mesirov, H Coller, M. Loh, J. Downing, M. Caligiuri, C. Bloomfield, and E. Lander. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. *Science*, 286(5439):531–7, Oct 1999. 0036-8075 (Print) Journal Article Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.

[33] E Zerhouni. Medicine. the NIH Roadmap. *Science*, 302(5642):63–72, Oct 2003. 1095-9203 (Electronic) Journal Article.

[34] D Chaussabel, W Allman, A Mejias, W Chung, L Bennett, O Ramilo, V Pascual, A. Palucka, and J Banchereau. Analysis of significance patterns identifies ubiquitous and disease-specific gene-expression signatures in patient peripheral blood leukocytes. *Ann N Y Acad Sci*, 1062:146–54, Dec 2005. 0077-8923 (Print) Comparative Study Journal Article Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't.

[35] P McCarthy, M Sharpe, S Spiesel, T Solan, B Forsyth, T DeWitt, H Fink, M Baron, and D Cicchetti. Observation scales to identify illness in febrile children. *Pediatrics*, 70:802–09, 1982.