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Association of RNA Biosignatures With Bacterial Infections in Febrile Infants Aged 60 Days or Younger

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Abstract

IMPORTANCE—Young febrile infants are at substantial risk of serious bacterial infections; however, the current culture-based diagnosis has limitations. Analysis of host expression patterns ("RNA biosignatures") in response to infections may provide an alternative diagnostic approach.

OBJECTIVE—To assess whether RNA biosignatures can distinguish febrile infants aged 60 days or younger with and without serious bacterial infections.

DESIGN, SETTING, AND PARTICIPANTS—Prospective observational study involving a convenience sample of febrile infants 60 days or younger evaluated for fever (temperature >38° C) in 22 emergency departments from December 2008 to December 2010 who underwent laboratory evaluations including blood cultures. A random sample of infants with and without bacterial infections was selected for RNA biosignature analysis. Afebrile healthy infants served as controls. Blood samples were collected for cultures and RNA biosignatures. Bioinformatics tools were applied to define RNA biosignatures to classify febrile infants by infection type.

EXPOSURE—RNA biosignatures compared with cultures for discriminating febrile infants with and without bacterial infections and infants with bacteremia from those without bacterial infections.

MAIN OUTCOMES AND MEASURES—Bacterial infection confirmed by culture. Performance of RNA biosignatures was compared with routine laboratory screening tests and Yale Observation Scale (YOS) scores.

RESULTS—Of 1883 febrile infants (median age, 37 days; 55.7%boys), RNA biosignatures were measured in 279 randomly selected infants (89 with bacterial infections—including 32 with bacteremia and 15 with urinary tract infections—and 190 without bacterial infections), and 19 afebrile healthy infants. Sixty-six classifier genes were identified that distinguished infants with and without bacterial infections in the test set with 87%(95%CI, 73%-95%) sensitivity and 89% (95%CI, 81%-93%) specificity. Ten classifier genes distinguished infants with bacteremia from those without bacterial infections in the test set with 94%(95%CI, 70%-100%) sensitivity and 95%(95%CI, 88%-98%) specificity. The incremental *C* statistic for the RNA biosignatures over the YOS score was 0.37 (95%CI, 0.30–0.43).

CONCLUSIONS AND RELEVANCE—In this preliminary study, RNA biosignatures were defined to distinguish febrile infants aged 60 days or younger with vs without bacterial infections. Further research with larger populations is needed to refine and validate the estimates of test accuracy and to assess the clinical utility of RNA biosignatures in practice.

Febrile infants aged 60 days or younger are at substantial risk of serious bacterial infections (defined as bacteremia, urinary tract infections [UTIs], and bacterial meningitis), estimated at 7% to 9% in this population.[1] Current guidelines recommend obtaining blood, urine, and cerebrospinal fluid (CSF) cultures, with consideration of antibiotic treatment and hospitalization until culture results are negative. Although these strategies aim to identify and treat all young febrile infants with serious bacterial infections, the costs and risks are high.[2] The lack of an optimal management strategy has led to substantial variation in the care for this vulnerable population, unnecessarily exposing many infants to potential harm.

Culture of bacteria remains the reference standard for the diagnosis of serious bacterial infections, but cultures are limited by time to bacterial growth and false-positive and false-negative results.[3–6] A genomic approach based on analysis of the host response to infection has been investigated as an alternative.[3,7–10] Microbial pathogens induce specific host responses or “RNA biosignatures” that can be identified using microarray analyses of blood leukocytes. Studies involving older children and adults have demonstrated the value of this approach to distinguish bacterial from viral infections.[10–15] It is unknown, however, whether measuring host responses can discriminate febrile infants aged 60 days or younger with and without bacterial infections because their immature immune systems may not generate a sufficiently robust host response and because the duration of illness when they present for medical attention is short.

The objective of this preliminary study was to determine whether RNA biosignatures could distinguish febrile infants aged 60 days or younger with and without bacterial infections. We determined the performance characteristics of RNA biosignatures compared with standard bacterial cultures.

Methods

Study Design, Setting, and Population

This was a prospective observational study that included 22 emergency departments (EDs) in the Pediatric Emergency Care Applied Research Network (PECARN). A convenience sample of infants aged 60 days or younger were evaluated for fever (rectal temperatures >38° C documented in the ED, home, or clinic). Those who underwent laboratory evaluations that included at least 1 blood culture were enrolled. Febrile infants presenting to any of the 22 EDs when study research staff members were available were screened for study eligibility. The parents or guardians of eligible infants were approached, and their infants were enrolled upon receiving written informed consent. The study was approved by the institutional review boards at all sites. For this preliminary analysis, a random sample of enrolled infants was selected (described below).

The goal was to focus on infants who posed diagnostic quandaries; therefore, infants with clinical sepsis, prematurity, significant comorbidities, focal bacterial infections (except otitis media), and those already receiving antibiotics were excluded. Laboratory evaluations, aside from blood cultures and blood draws for RNA biosignatures, were performed at the treating physician’s discretion and typically included white blood cell counts, urinalyses and urine cultures, and CSF analyses and cultures. Based on culture results, febrile infants were

assigned either to the bacterial infection group (bacteremia, UTI, bacterial meningitis) or to the without-bacterial-infection group.

Key Points

Question

Can the host response measured by RNA biosignatures distinguish young febrile infants with and without bacterial infections?

Findings

In this prospective observational study of 279 febrile infants 60 days or younger, 66 classifier genes distinguished infants with and without bacterial infections with 87%sensitivity and 89% specificity. Ten classifier genes distinguished infants with bacteremia from those without bacterial infections with 94% sensitivity and 95%specificity.

Meaning

In this preliminary study, host RNA biosignatures accurately distinguished febrile infants 60 days or younger with and without bacterial infections.

Study Definitions

Bacteremia and bacterial meningitis were defined as growth of a single pathogen in the blood or CSF, respectively.[16] Growth of multiple bacteria or those not commonly considered pathogens (eg, coagulase-negative *Staphylococcus*, diphtheroids, bacillus species) were categorized as contaminants. Urinary tract infection was defined as the growth of a single pathogen in the urine with colony counts (colony-forming units [CFUs]) meeting 1 of 3 criteria: (1) 1000 CFUs/mL by suprapubic aspiration; (2) 50 000 CFUs/mL from a catheterized specimen; or (3) 10 000 CFUs/mL from a catheterized specimen in association with an abnormal urinalysis (positive for leukocyte esterase or nitrites, or >5 white blood cell counts per high-power field on microscopic examination).[17] Patients were classified as having bacterial infections or not in a blinded fashion following study definitions, before the RNA biosignature results were available.

Training and Test Sets and Sample Size Determination for Microarray Analysis

Best practices for microarray analyses include 2 independent patient sets: one to define (training set) and the other to validate (test set) candidate RNA biosignatures. The initial goal was to define the bacterial infection biosignature, which would be different from the biosignature for infants without bacterial infections. Because there was no information regarding RNA biosignatures in infants aged 60 days or younger, the proposed sample size implicitly assumed that effect sizes (differences in RNA biosignatures between patients with and without bacterial infections) would be of the magnitude encountered in previous microarray studies in other age groups.[7,18] It was estimated that 30 to 40 infants with bacterial infections and 30 to 40 without bacterial infections (15–20 for the training set and 15–20 for the test set in each group) would be required to define the diagnostic biosignatures.[19] To ensure that the analysis was not underpowered, additional cases in both

groups of febrile infants were included. Because patients were recruited from 22 different EDs over 2 years, the samples analyzed constitute a diverse study population.

Infants with and without bacterial infections included in the analyses were randomly selected from the study population. To have a balanced representation of bacterial infections, the PECARN data coordinating center selected infants at random from the groups with bacteremia and UTIs. Febrile infants whose bacterial cultures tested negative were heterogeneous because not every patient was tested for viral infections. To represent the clinical setting, we divided patients without bacterial infections into 4 groups: those with proven viral infections, those tested for at least 3 respiratory viruses but with negative results, others tested for at least 1 virus and with negative results, and those not tested for viruses. Patients were selected from each group by the data coordinating center.

Each group (with and without bacterial infections) was divided into training and test sets at random, stratified by age, sex, and infection type to attain matched training and test sets for analysis. Following this approach, the RNA biosignatures were first identified in the training sets and then validated in the test sets.[7,18] For comparison, healthy control afebrile infants with neither signs of acute infections nor antibiotic therapy in the preceding 2 weeks were also included. These infants were enrolled during primary care visits or at the time of elective surgery not involving the respiratory tract.

Study Procedures

For each patient, investigators prospectively recorded demographics, physical examination findings including Yale Observation Scale (YOS) scores, and laboratory results. The YOS is a clinical score that provides a quantitative assessment of risk for serious bacterial infections in febrile infants aged 60 days or younger based on simple clinical and observational parameters. It includes 6 items, with each item scored on a 1-to-3-to-5 scale, yielding a total YOS score ranging from 6 (perfect score) to 30 (most ill-appearing infant). A YOS score of 10 or less is considered normal.[20] Blood samples for microarray analyses were placed into tempus tubes (Applied Biosystems), frozen, and batch-shipped to a central laboratory where they were hybridized on Illumina HT12 V4 beadchips.[18] The data were deposited in the NCBI Gene Expression Omnibus (GEO accession number: GSE64456).

Statistical and Microarray Data Analysis

Illumina chips were analyzed with GenomeStudio and GeneSpring GX 7.3 (Agilent Technologies) software.[7,12,21] Analyses of RNA transcripts included *supervised analyses* (for class comparisons between predefined sample groups) and *unsupervised analyses* (unbiased grouping of samples based on their molecular profiles without prior knowledge of sample classification). To identify differentially expressed genes, Mann-Whitney testing (2 sided) for comparisons between 2 groups (P values <.01 were considered significant) with Benjamini-Hochberg corrections for multiple testing, and a 1.25-fold or higher change in expression level were used.[12,21] Functional analyses of differentially expressed genes were performed using modular analyses per infection group and per individual patient. Modules are groups of genes with similar biological functions.[13,14,21–23] For these analyses, modular maps were derived independently for the training and test sets in both groups of patients

(infants with and without bacterial infections) using the healthy control group as a reference. Modules related to interferon and inflammation genes are particularly relevant to identify differences in gene expression between infants with and without bacterial infections because these modules represent the major immune pathways activated in response to infections. In addition to the group analyses, a modular analysis for each individual patient was also performed.[12,13,21–23] Next class prediction using the κ–nearest neighbors (KNN) algorithm[7] was performed to identify genes that best discriminated between infants with and without bacterial infections in the training set and then validated in the test set. This was followed by a subgroup analysis, again using the KNN algorithm, to identify classifier genes that best discriminated infants with bacteremia from those without bacterial infections. Sensitivity and specificity of these classifier genes were calculated to assess discrimination first in the training set using the leave-one-out cross-validation method and then were validated in the test set.[7]

Sample proportions and exact (Clopper-Pearson) confidence intervals were used to estimate the test characteristics.[24] The incremental *C* statistic for the RNA biosignature over the YOS score was estimated using logistic regression, once using the YOS as a continuous variable and again as a dichotomous variable (normal vs abnormal, that is, YOS ≤10 vs YOS >10).[25] Other continuous and categorical variables such as demographic, clinical and laboratory findings of infants with and without bacterial infections were summarized using SAS statistical software version 9.4 (SAS Institute Inc).

Results

To accrue a sufficient sample size of patients with bacteremia to define the bacterial biosignatures, parents of 2820 febrile infants were approached from December 2008 to December 2010, and 1883 infants (67%; median age, 37 days, 55.7%boys) were enrolled.

Patients With Bacterial Infections

The data coordinating center randomly selected 33 infants with bacteremia and 58 with UTIs. One blood sample in each group did not have adequate RNA for analysis. Thus, 89 infants with bacterial infections were included for microarray analyses (among patients with bacteremia, 1 also had meningitis, 15 had UTIs, and 1 had meningitis and UTI).

Patients Without Bacterial Infections

Of the 204 infants randomly selected, 190 had adequate RNA for analysis. Among the selected infants with proven viral infections, 94.4% (51 of 54) with enteroviral infections, 92.3% (48 of 52) with influenza infections, and 100% (12 of 12) with other viral infections had adequate RNA for analysis. Among the other groups selected for analysis, 90.7% (39 of 43) with negative results for at least 3 viruses had adequate RNA, as did 97.4% (38 of 39) with no viral testing performed and 50% (2 of 4) with other viral tests performed with negative results.

Healthy Controls

Twenty-five healthy afebrile infants were enrolled as controls, with a median age of 78 days (interquartile range, 44–185 days). Of those, 76%(19 of 25) had samples with adequate RNA for analysis.

Figure 1 summarizes the overall study design, patient enrollment, and the allocation of samples obtained from the different patient groups and healthy controls for the various analyses performed. eTable 1 (in the Supplement) provides a complete description of the patients and pathogens.

The demographics and clinical and laboratory parameters of the 279 patients analyzed (89 with and 190 without bacterial infections) are summarized in Table 1 and Table 2. Patient groups were comparable with regards to demographics.

Biosignatures of Infants With and Without Bacterial Infections

The first step was to define each of the specific RNA biosignatures of patients with and without bacterial infections compared with healthy controls. Class comparisons identified 3753 differentially expressed RNA transcripts between 44 infants with bacterial infections included in the training set and 19 healthy controls (Figure 2, A). Unsupervised hierarchical clustering of the 3753 RNA transcripts applied to a separate test set of infants (45 with bacterial infections) correctly grouped 44 of 45 (97%) infants with bacterial infections (Figure 2, B). A similar analysis performed with the group without bacterial infections compared with the 19 controls identified differential expression of 2486 transcripts in the training set (95 without bacterial infections) (Figure 2, C). Unsupervised hierarchical clustering of the 2486 transcripts applied to a separate test set (95 without bacterial infections) correctly grouped 72 of 95 (76%) infants without bacterial infections (Figure 2, D).

Modular Analysis of Biosignatures of Infants With and Without Bacterial Infections

To characterize the biological differences between infants with and without bacterial infections, we applied a modular analytical strategy.[12,18,21,23] For these analyses, modular maps were derived independently for the training and test sets for each of the infection groups and were compared with the healthy controls. This analytical method confirmed the reproducibility of the RNA biosignatures. As shown in Figure 3 and Figure 4, the correlations between the training and test sets were statistically significant for both the group with bacterial infection biosignatures ($r = 0.98$; $P < .001$; Figure 4, A, and Figure 3, A and B) and the group without bacterial infection biosignatures ($r = 0.99$, $P < .001$; Figure 4, B and Figure 3, C and D). This analysis also demonstrated differences in expression of the inflammation (M4.6, M5.1, M5.7) and interferon modules (M1.2, M3.4, M5.12) between infants with bacterial infections (Figure 3, A and B) and without bacterial infections (Figure 3, C and D).

Modular maps for each individual patient further illustrate the biological differences between the groups.[13,22] The group with bacterial infections (eFigure 1A in the Supplement) showed marked overexpression of inflammation modules and almost no overexpression of

interferon modules. Among infants without bacterial infections, patients with influenza and enterovirus infections (eFigure 1B in the Supplement) had marked overexpression of interferon modules and mild overexpression of inflammation modules. Patients infected with other viruses, those who were virus-negative, and those not tested for viruses (eFigure 1C in the Supplement) had mild overexpression of inflammation modules and overexpression of interferon modules in certain patients only.

Classifier Genes to Discriminate Febrile Infants With and Without Bacterial Infections

The KNN algorithm identified 66 classifier genes (eTable 3 in the Supplement) that best discriminated infants with and without bacterial infections. Leave-one-out cross-validation in the training set showed 82% (95% CI, 67%-92%) sensitivity and 88%(95%CI, 80%-94%) specificity. Validation in the test set demonstrated 87% (95% CI, 73%-95%) sensitivity and 89% (95%CI, 81%-93%) specificity (Table 3 and Figure 5, A and B).

Because bacteremia is one of the most challenging diagnoses of the invasive infections in this population, we conducted a similar analysis to identify genes that best discriminated infants with bacteremia from those without bacterial infections. The KNN algorithm identified 10 classifier genes (eTable 4 in the Supplement) that best classified these 2 groups of patients. Leave-one-out cross-validation in the training set showed 75% (95% CI, 48%–93%) sensitivity and 93% (95%CI, 85%–97%) specificity, while the test set demonstrated 94%(95%CI, 70%–100%) sensitivity and 95%(95%CI, 88%–98%) specificity (Table 3 and Figure 5, C and D).

RNA Biosignatures vs Microbiological Cultures

Table 3 and eTable 2 (in the Supplement) demonstrate the results of the RNA biosignature analysis in categorizing patients with and without bacterial infections, compared with standard bacterial cultures. Bacterial culture-positive patients who had RNA biosignatures consistent with nonbacterial infections had mean white blood cell and absolute neutrophil counts similar to infants who had negative cultures. Furthermore, patients with negative bacterial cultures whose RNA biosignatures suggested bacterial infections had mean white blood cell and absolute neutrophil counts similar to infants with positive bacterial cultures. Three patients with positive blood cultures for viridans streptococci were classified a priori as having bacteremia; however, their profiles (using the 10 classifier genes) appeared different from those of patients with bacteremia caused by other pathogens (Figure 3C and D, arrows). In addition, the individual modular profiles of these 3 infants with suspected viridans streptococcus bacteremia lacked the overexpression of inflammation genes observed in other cases of bacteremia (eFigure 2 in the Supplement).

RNA Biosignatures vs YOS Scores

The incremental diagnostic value of the RNA biosignatures compared with clinical observation, the YOS score, was calculated, and *C* statistics are summarized in eTable 5 (in the Supplement). The bacterial RNA biosignature was notably more predictive of bacterial infection than clinical examination (YOS) (*C* statistic, 0.89 vs 0.53) and added significantly to prediction beyond the YOS alone (incremental *C* statistic, 0.37; 95%CI, 0.30–0.43).

Potential Clinical Utility of RNA Biosignatures

To assess the potential clinical utility of the RNA biosignatures, we calculated posttest positive and negative probabilities. The prevalence of serious bacterial infections in the parent study cohort was 9.0%, bacteremia was 2.1%, and bacterial meningitis was 0.38%, similar to the recent literature.[26] Using the sensitivity and specificity of RNA biosignatures for the training and test sets (Table 3) and focusing on the most important serious bacterial infections (bacteremia and bacterial meningitis), the posttest negative probabilities were 0.4% and 0%, respectively, in the training set and 0.2%and 0%, respectively, in the test set (eTable 6 in the Supplement).

Discussion

In this preliminary study, we evaluated and demonstrated the accuracy of RNA biosignatures in febrile infants aged 60 days or younger at the time of their initial evaluation in the ED. We identified unique RNA biosignatures that discriminated between infants with and without bacterial infections.

Microbiologic cultures of sterile fluids remain the standard for diagnosing bacterial infections in young febrile infants but have limitations and require invasive procedures to obtain samples (urinary catheterization, lumbar puncture).[27] The false-positive rate of blood cultures in infants may be as high as 10%, and the false-negative rate may be even higher.[6,28,29] Microarray-based assays to measure host responses to pathogens have been suggested as an alternative approach to diagnosis of infectious diseases.[8,9,30,31] Studies in older children and adults have documented the potential value of this approach for diagnosis of viral and bacterial infections, and tuberculosis.[9–11,13–15,18,31] Despite the young age of the febrile infants evaluated, they carried robust RNA biosignatures and demonstrated that regardless of the etiology of the infections, their immune systems are programmed to respond not only with shared elements induced by common microbes but also with specific patterns that allow discrimination by class of pathogen.

Among patients with discordance between microbiological cultures and RNA biosignatures, traditional screening tests for bacterial infections (white blood cell count, absolute neutrophil count) suggested agreement with the diagnostic categorization of the RNA biosignatures. There were 3 infants classified as having viridans streptococcus bacteremia by culture (and considered to have bacteremia a priori for analysis), but whose RNA biosignatures, modular analyses, and screening tests suggested a lack of bacterial infection. There is controversy surrounding growth of viridans streptococcus from the blood of febrile infants and whether growth represents contaminated cultures or potential pathogens.[16,32–34] The RNA biosignatures and the traditional screening tests of these 3 infants suggest that growth likely represented contaminated cultures. Although this observation is limited to 3 patients, it suggests a potential additional benefit of microarray analysis in clarifying the true infection status of patients that canaid in solving the conundrum of unclear culture results.

The clinical utility of the RNA biosignatures in the evaluation of febrile infants will ultimately be determined by their ability to exclude the diagnosis of serious bacterial infections rapidly and reliably. The biosignatures contributed substantially more than the

YOS in identifying those with bacterial infections. Most importantly, the posttest negative probabilities for bacteremia and bacterial meningitis were very low, suggesting future clinical application of RNA biosignatures for excluding serious bacterial infections in febrile infants aged 60 days or younger.

The study has limitations. RNA biosignatures were evaluated on a convenience sample; therefore, the results are not necessarily generalizable to febrile infants whose guardians declined enrollment. However, the febrile infants analyzed were enrolled in many centers during a 2-year period, and the rate of serious bacterial infections observed in the parent cohort was similar to that described in the current literature.[26] Patient diagnoses were adjudicated before the analysis, and bacterial cultures were considered the reference standard for purposes of analysis, despite a known substantial rate of false- positive and false-negative results. In addition, viral testing was not consistently performed for all study participants. Therefore, the ability to interpret the biosignatures of infants with potential bacterial-viral coinfections was limited; however, the ultimate goal of the study was to identify the RNA biosignatures of febrile infants with bacterial infections. The analysis was based on sampling blood just once in the course of the illness. It appears that the biosignatures were stable, as patients presented to the ED at variable times during their illnesses. The microarray analyses were performed on frozen samples batched in a central laboratory. It is unlikely, although possible, that the RNA biosignatures would be different if analyzed at the time blood cultures were obtained. However, novel polymerase chain reaction–based assays with rapid turnaround times should allow a practical implementation in the future.[31] In addition, the analytic sample was relatively small; therefore, the study will require external validation in a larger patient population.

As technology advances, RNA biosignatures may prove to be an alternative and accurate method to identify infants with bacterial infections. This would help clinicians target evaluation and therapy when they are needed and avoid invasive procedures, antibiotics, and hospitalizations when they are not.

Conclusions

In this preliminary study, RNA biosignatures were defined to distinguish febrile infants aged 60 days or younger with and without bacterial infections. Further research with larger populations is needed to refine and validate the estimates of test accuracy and to assess the clinical utility of RNA biosignatures in practice.

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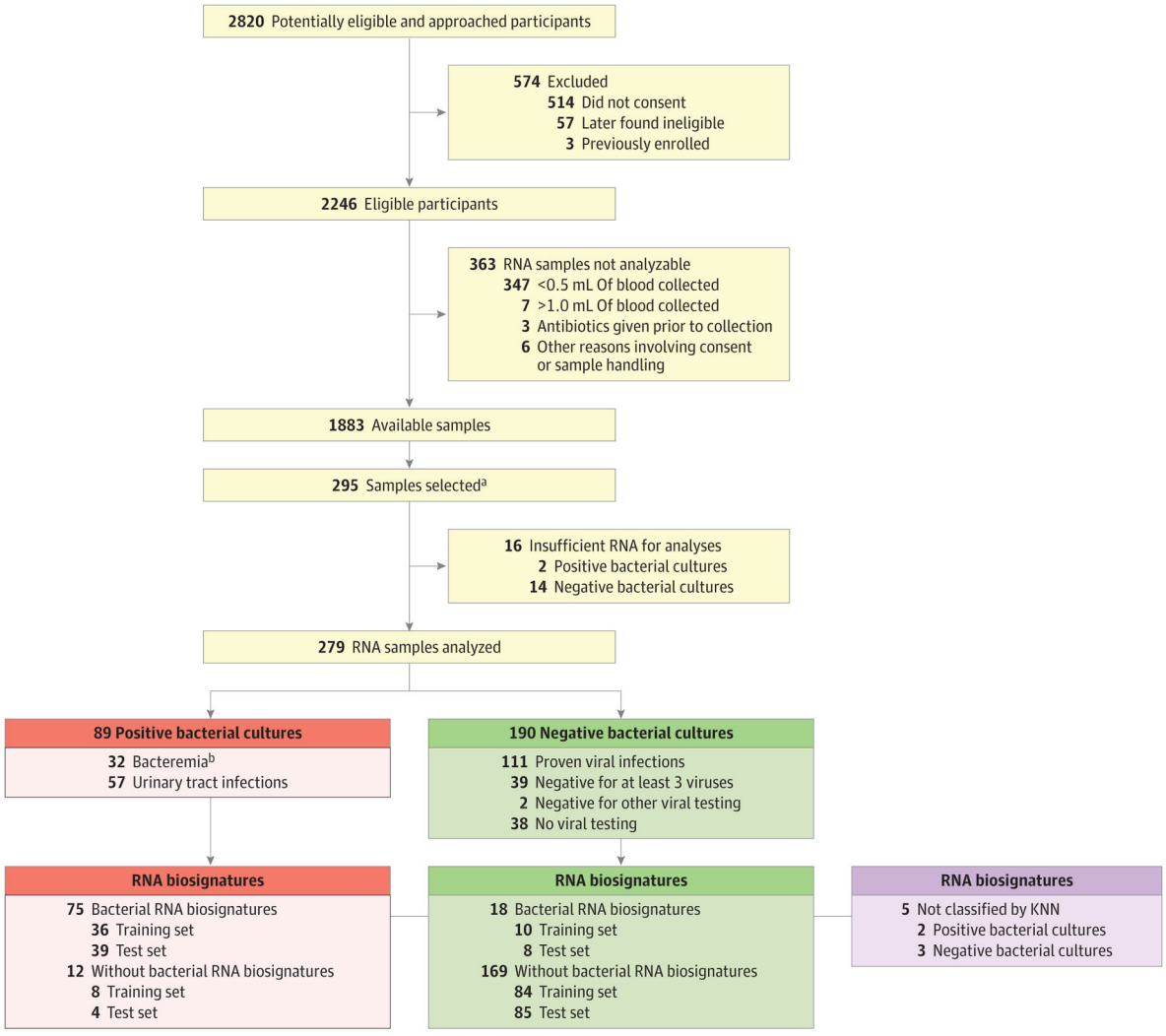

Types and Numbers of Samples Allocated to Each Type of Analysis

	Healthy Controls[c] (n = 19)		Bacterial Infections (n = 89)		Without Bacterial Infections (n = 190)	
Analyses	Training Set	Test Set	Training Set	Test Set	Training Set	Test Set
Bacterial biosignatures	19	19	44	45		
Without bacterial biosignatures	19	19			95	95
KNN bacterial infection vs without bacterial infection			44	45	95	95
KNN bacteremia vs without bacterial infection			16	16	95	95

Figure 1. FlowDiagram of Enrollment and Allocation to Different Groups for Microarray Analyses

The same 19 healthy controls were used for both the bacterial and without bacteria biosignatures analyses. KNN indicates κ–nearest neighbors; UTI, urinary tract infection.

[a]RNA samples selection strategy is described in detail in the Methods section.

[b]Two patients with bacteremia also had bacterial meningitis, one each in the training and test sets.

[c]Healthy afebrile control infants enrolled during routine primary care visits or at the time of elective surgery were also included as comparators for the analyses.

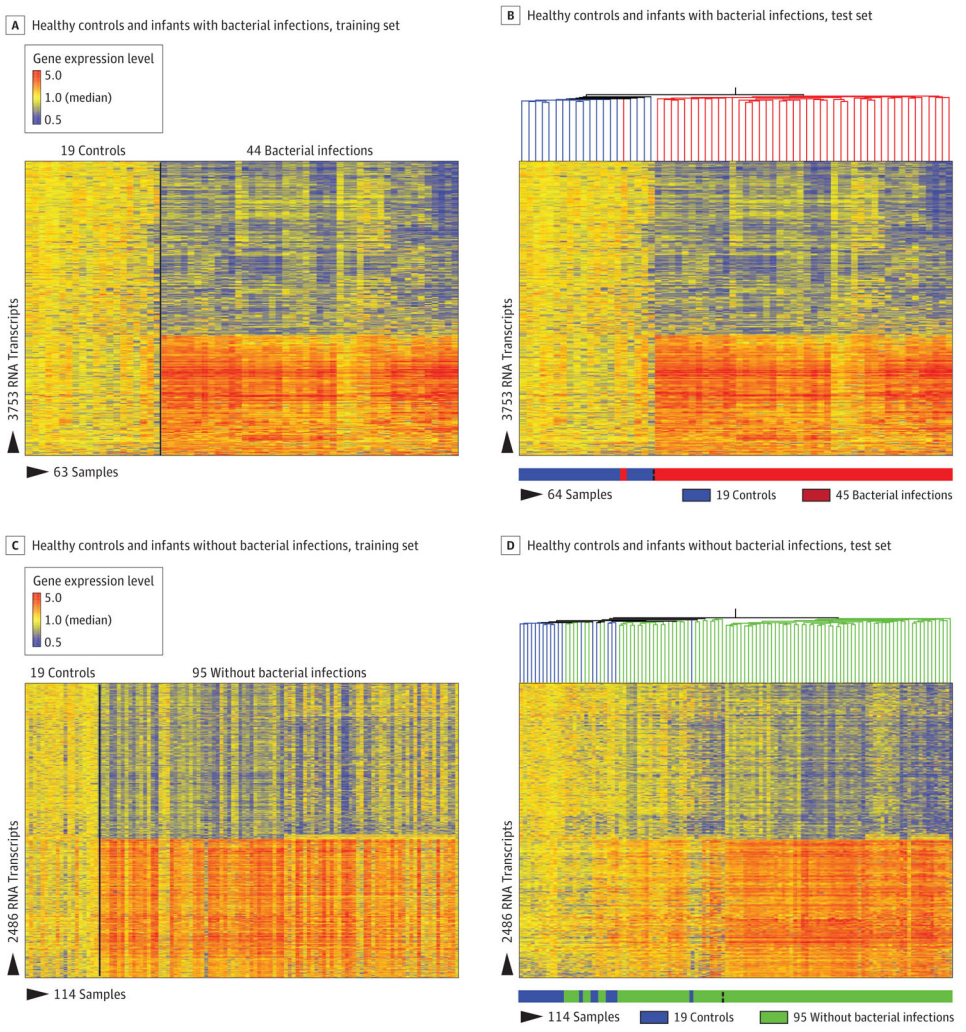

Figure 2. RNA Biosignatures of Young Febrile Infants With and Without Bacterial Infections

For panels A and C, the heat map representing RNA biosignatures are identified by class comparisons by nonparametric test (Mann-Whitney test, $P < .01$), with the Benjamini-Hochberg multiple test correction and 1.25-fold change. Transcripts are represented in rows and infants are represented in columns. Color bars indicate the normalized gene expression level (red, overexpressed; blue, underexpressed) relative to the healthy control infants (yellow, median gene expression of the healthy controls). The scale indicates the relative-fold differences in gene expression levels. For panels B and D, the dendrogram branches of the gene tree structure are colored according to condition (blue, healthy controls; red, bacterial infection; green, without bacterial infection). Unsupervised hierarchical clustering (euclidean distance, average linkage) of the transcripts applied to a separate test set of infants. For these analyses, the computer algorithm groups the samples of patients and healthy controls according to the similarities in gene expression patterns; this is performed in a blinded fashion because the samples are not preassigned to the infection or control group.

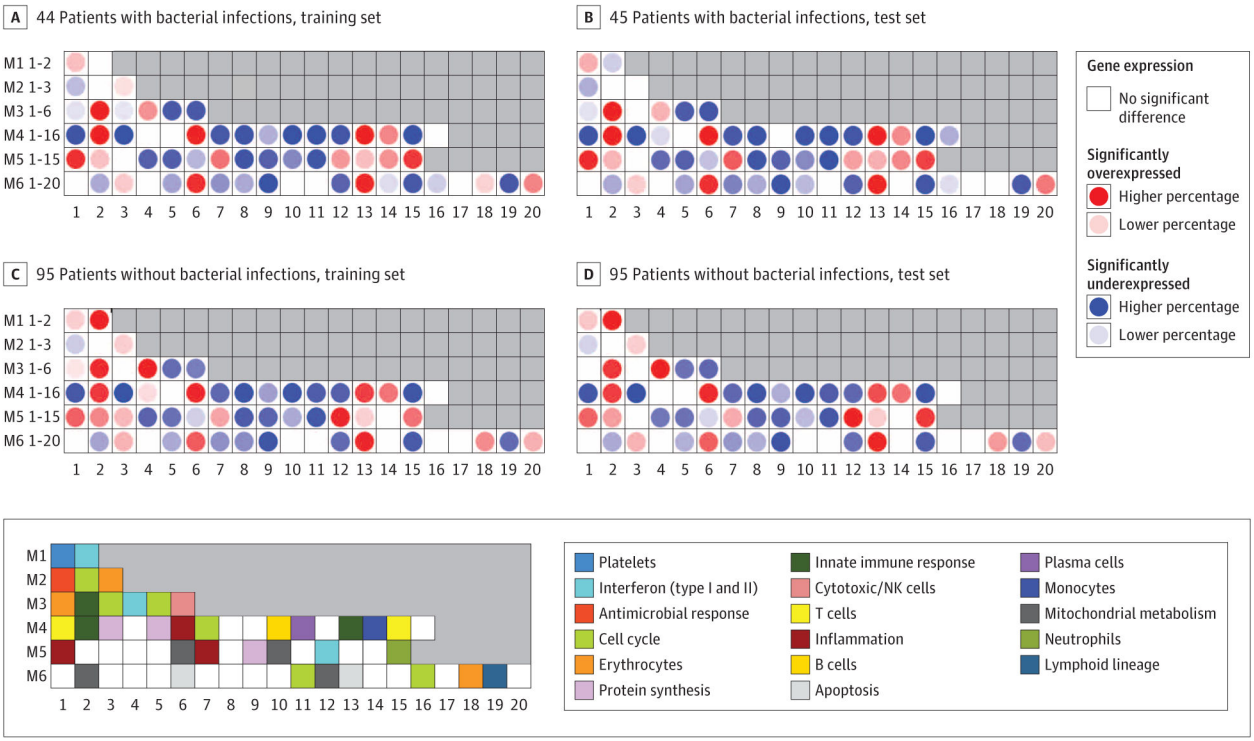

Figure 3. Transcriptional Modular Analysis of Young Febrile Infants With and Without Bacterial Infections

Modular maps were derived independently for the training and test sets. Modules (groups of coordinately expressed genes with similar biological function) are shown as the percentage of genes in that particular module that are expressed significantly differently from healthy controls. They are represented as dots on a grid. Module functional annotation is indicated by the color-code legend key. The first row on the grid includes modules that were identified in the first round of selection, when the modules were first described (M1; subnetwork constituted of genes coclustering; the letter Mis used to name the modules). Modules that were identified in subsequent rounds of selection make up the next rows (M2, M3, M4, M5, M6).[22] In this display only modules from the first 6 rounds of selection are shown because they are considered the most biologically relevant and have better functional characterization.[23]

A and B, the average modular transcriptional profile for patients with bacterial infections vs healthy controls is shown.

C and D, the average modular transcriptional profile for patients without bacterial infections vs healthy controls.

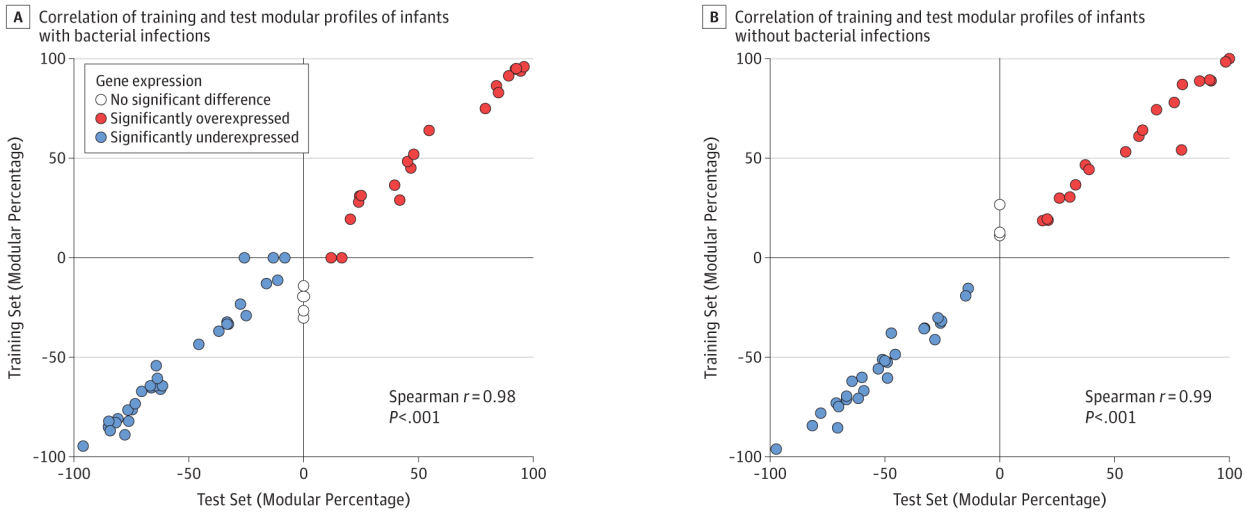

Figure 4. Correlation of Training and Test Modular Profiles of Infants

The scatterplots represent the correlation between the modular expression of the training and test sets of the patients with bacterial infections. Red dots represent modules with significantly overexpressed genes; blue dots, significantly underexpressed genes vs healthy controls. Dots with no color indicate no significant differences in the expression of the genes included in that module vs healthy controls.

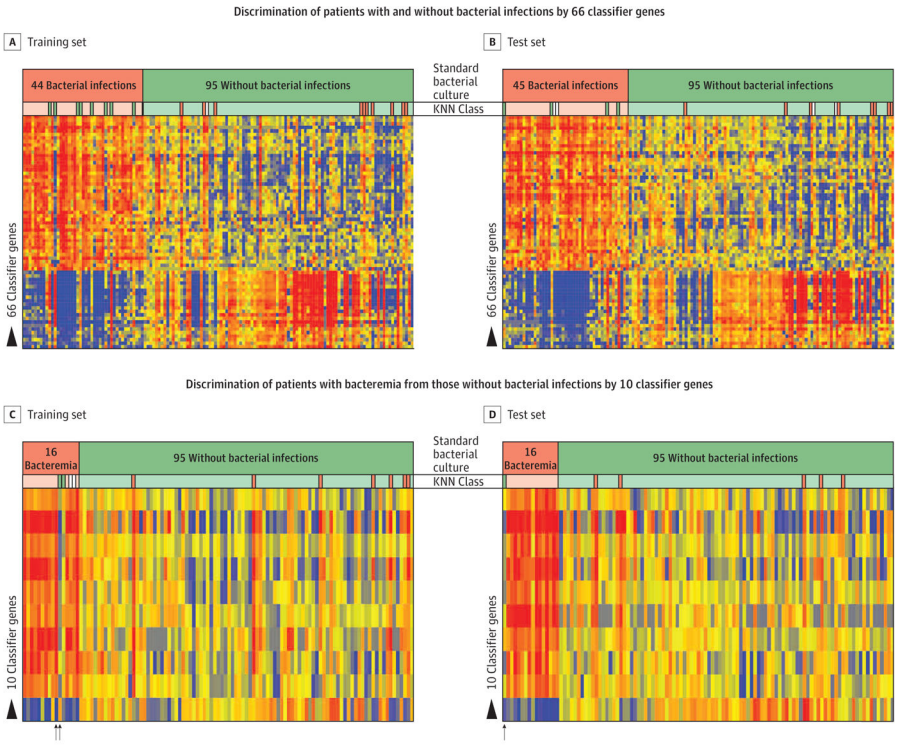

Figure 5. Discrimination of Febrile Infants With and Without Bacterial Infections by Classifier Genes

The rectangles located on the top of the heat maps represent the patient's classification according to standard bacterial cultures in dark colors (red for patients with bacterial infections and green for patients without bacterial infections) and below according to the κ–nearest neighbors (KNN) algorithm in light colors (white represents the patients not classified by the KNN algorithm). The arrows located at the bottom of the heat maps indicate the transcriptional profiles of 3 patients (2 in panel C and 1 panel D) with positive blood cultures for viridans streptococcus. The profiles of these 3 patients appear visually different from those of most other patients with bacteremia.

A, Application of the KNN algorithm to the training set composed of febrile infants with and without bacterial infections identified classifier genes (listed in eTable 3 in the Supplement) that best discriminated the 2 groups.

B. The accuracy of the classifier genes was confirmed in an independent test set of patients with and without bacterial infections. The heat maps represent the expression levels of the classifier genes in the training and test sets. Overexpressed transcripts are shown in red and underexpressed transcripts in blue. Genes are ordered in the heat map from top to bottom according to their ability to discriminate between the groups. A similar approach was followed to identify classifier genes to discriminate infants with bacteremia and those without bacterial infections.

C, The KNN algorithm identified the classifier genes (listed in eTable 4 in the Supplement) in the training set composed of infants with bacteremia and those without bacterial infections that best discriminated the 2 groups.

D, The accuracy of the classifier genes was confirmed in an independent test set of patients with bacteremia and without bacterial infections. The heat maps represent the expression levels of the classifier genes in the training and test sets.

Table 1

Demographics and Laboratory Parameters of Study Population by Bacterial Culture Status[a]

	Bacterial Infections			
	Bacteremia[b] (n = 32)	UTI (n = 57)	All (n = 89)	Without Bacterial Infections (n = 190)
Age, mean (SD), d	29 (18)	35 (15)	33 (16)	35 (14)
Age, 0–30 d, No. (%)	21 (66)	22 (39)	43 (48)	67 (35)
Girls, No. (%)	18 (56)	25 (44)	43 (48)	80 (42)
Temperature, mean (SD), °C	38.8 (0.5)	38.6 (0.5)	38.7 (0.5)	38.5 (0.5)
YOS score, median (IQR)[c]	8 (6–10)	6 (6–9)	6 (6–10)	6 (6–8)
Urine culture obtained, No. (%)	32 (100)	57 (100)	89 (100)	185 (97)
CSF culture obtained, No. (%)	31 (97)	49 (86)	80 (90)	154 (81)
Disposition, No. (%)				
Discharged	0 (0)	4 (7)	4 (4)	46 (24)
Admitted or transferred	32 (100)	53 (93)	85 (96)	144 (76)
WBC count × 1000/μL, mean (SD)	12.0 (5.6)	15.3 (7.2)	14.1 (6.8)	10.3 (4.4)
ANC × 1000/μL, mean (SD)	6.4 (4.3)	8.3 (5.1)	7.6 (4.9)	3.9 (2.8)

Abbreviations: ANC, absolute neutrophil count; CSF, cerebrospinal fluid; IQR, interquartile range; UTI, urinary tract infection; WBC, white blood cell; YOS, Yale Observation Scale.

[a] Patients were enrolled from December 2008 through December 2010.

[b] Two patients with bacteremia also had bacterial meningitis, 1 each in the training and test sets

[c] See the Methods section for the definition of the YOS score. The total YOS score ranges from 6 (a perfect score) to 30 (for the most ill-appearing infant). A YOS score of 10 or less is considered normal.

Table 2

Demographics and Laboratory Parameters of Study Population by Culture Status and Training-Test Assignment[a]

	Training Set				Test Set			
	Bacterial Infections				Bacterial Infections			
	Bacteremia[b] (n = 16)	UTI (n = 28)	All (n = 44)	Without Bacterial Infections (n = 95)	Bacteremia[b] (n = 16)	UTI (n = 29)	All (n = 45)	Without Bacterial Infections (n = 95)
Age, mean (SD), d	28 (17)	32 (16)	31 (16)	33 (15)	29 (19)	38 (13)	35 (16)	37 (13)
Age, 0–30 d, No. (%)	11 (69)	15 (54)	26 (59)	42 (44)	10 (63)	7 (24)	17 (38)	25 (26)
Girls, No. (%)	9 (56)	11 (39)	20 (45)	40 (42)	9 (56)	14 (48)	23 (51)	40 (42)
Temperature, mean (SD), °C	38.7 (0.5)	38.5 (0.4)	38.6 (0.5)	38.5 (0.5)	38.8 (0.5)	38.7 (0.5)	38.7 (0.5)	38.5 (0.5)
YOS score, median (IQR)[c]	8 (6–14)	6 (6–8)	8 (6–10)	6 (6–8)	6 (6–9)	6 (6–10)	6 (6–10)	6 (6–10)
Urine culture obtained, No. (%)	16 (100)	28 (100)	44 (100)	92 (97)	16 (100)	29 (100)	45 (100)	93 (98)
CSF culture obtained, No. (%)	16 (100)	23 (82)	39 (89)	80 (84)	15 (94)	26 (90)	41 (91)	74 (78)
Disposition, No. (%)								
Discharged	0 (0)	3 (11)	3 (7)	22 (23)	0 (0)	1 (3)	1 (2)	24 (25)
Admitted or transferred	16 (100)	25 (89)	41 (93)	73 (77)	16 (100)	28 (97)	44 (98)	71 (75)
WBC × 1000/μL, mean (SD)	12.1 (6.8)	16.6 (8.1)	15.0 (7.9)	10.0 (4.3)	12.0 (4.2)	14.0 (6.1)	13.3 (5.5)	10.6 (4.6)
ANC × 1000/μL, mean (SD)	7.2 (5.3)	8.7 (5.4)	8.1 (5.4)	3.7 (2.5)	5.6 (3.0)	8.0 (4.8)	7.1 (4.4)	4.2 (3.0)

Abbreviations: ANC, absolute neutrophil count; CSF, cerebrospinal fluid; IQR, interquartile range; UTI, urinary tract infection; WBC, white blood cell; YOS, Yale Observation Scale.

[a]Patients were enrolled from December 2008-December 2010.

[b]Two patients with bacteremia also had bacterial meningitis, 1 each in the training and test sets.

[c]See the Methods section for the definition of the YOS score. The total YOS score ranges from 6 (a perfect score) to 30 (for the most ill-appearing infant). A YOS score of 10 or less is considered normal.

Table 3

Performance Characteristics of RNA Biosignatures Compared With Bacterial Cultures

	Training Set (n = 139)			Test Set (n = 140)		
	Bacterial Infection	**Without Bacterial Infection**	**Total**	**Bacterial Infection**	**Without Bacterial Infection**	**Total**
Patients correctly classified, No. (%)	36	84	120 (86.33)	39	85	124 (88.57)
Patients incorrectly classified, No. (%)	8	10	18 (12.95)	4	8	12 (8.57)
Patients not classified, No. (%)	0	1	1 (0.72)	2	2	4 (2.86)
Sensitivity (95% CI), %	82 (67–92)			87 (73–95)		
Specificity (95% CI), %	88 (80–94)			89 (81–93)		
	Training Set (n = 111)			**Test Set (n = 111)**		
	Bacteremia	**Without Bacteremia**	**Total**	**Bacteremia**	**Without Bacteremia**	**Total**
Patients correctly classified, No. (%)	12	88	100 (90.09)	15	90	105 (94.59)
Patients incorrectly classified, No. (%)	4	7	11 (9.91)	1	5	6 (5.41)
Patients not classified, No. (%)	0	0	0	0	0	0
Sensitivity (95% CI), %	75 (48–93)			94 (70–100)		
Specificity (95% CI), %	93 (85–97)			95 (88–98)		