Open Forum Infectious Diseases

MAJOR ARTICLE

Detection of Viral Infection and Bacterial Coinfection and Superinfection in Coronavirus Disease 2019 Patients Presenting to the Emergency Department Using the 29-mRNA Host Response Classifier IMX-BVN-3: A Multicenter Study

Wolfgang Bauer,[1,©] Sven Gläser,[2,3] Dorina Thiemig,[3] Katrin Wanner,[2,a] Alexander Peric,[4] Steffen Behrens,[5] Johanna Bialas,[6] Angelika Behrens,[7] Noa Galtung,[1] Oliver Liesenfeld,[8] Lisa Sun,[8] Larissa May,[9] Sharron Mace,[10] Sebastian Ott,[11,12] and Silvan Vesenbeckh[11,13]

[1]Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Department of Emergency Medicine, Berlin, Germany, [2]Klinik für Innere Medizin–Pneumologie, Vivantes Klinikum Spandau und Klinik für Innere Medizin–Pneumologie und Infektiologie, Vivantes Klinikum Neukölln, Berlin, Germany, [3]Klinik für Innere Medizin–Pneumologie und Infektiologie, Vivantes Klinikum Neukölln, Berlin, Germany, [4]Klinik für Innere Medizin–Pneumologie und Infektiologie, Vivantes Klinikum im Friedrichshain, Berlin, Germany, [5]Klinik für Innere Medizin–Kardiologie, Vivantes–Netzwerk für Gesundheit/Vivantes Humboldt-Klinikum and Klinik für Innere Medizin–Kardiologie und konservative Intensivmedizin, Vivantes–Netzwerk für Gesundheit/Vivantes Klinikum Spandau, Berlin, Germany, [6]Labor Berlin–Charité Vivantes Services GmbH, Berlin, Germany, [7]Klinik für Innere Medizin, Gastroenterologie und Pneumologie, Evangelische Elisabeth Klinik Krankenhausbetriebs gGmbH, Berlin, Germany, [8]Inflammatix Inc, Burlingame, California, USA, [9]Department of Emergency Medicine, University of California, Davis School of Medicine, Sacramento, California, USA, [10]Department of Emergency Medicine, Cleveland Clinic, Cleveland, Ohio, USA, [11]Department of Pulmonary Medicine, St Claraspital AG, Basel, Switzerland, [12]University of Bern, Bern, Switzerland, and [13]Department of Pulmonology, University Hospital Zürich, Zürich, Switzerland

Background. Identification of bacterial coinfection in patients with coronavirus disease 2019 (COVID-19) facilitates appropriate initiation or withholding of antibiotics. The Inflammatix Bacterial Viral Noninfected (IMX-BVN) classifier determines the likelihood of bacterial and viral infections. In a multicenter study, we investigated whether IMX-BVN version 3 (IMX-BVN-3) identifies patients with COVID-19 and bacterial coinfections or superinfections.

Methods. Patients with polymerase chain reaction–confirmed COVID-19 were enrolled in Berlin, Germany; Basel, Switzerland; and Cleveland, Ohio upon emergency department or hospital admission. PAXgene Blood RNA was extracted and 29 host mRNAs were quantified. IMX-BVN-3 categorized patients into very unlikely, unlikely, possible, and very likely bacterial and viral interpretation bands. IMX-BVN-3 results were compared with clinically adjudicated infection status.

Results. IMX-BVN-3 categorized 102 of 111 (91.9%) COVID-19 patients into very likely or possible, 7 (6.3%) into unlikely, and 2 (1.8%) into very unlikely viral bands. Approximately 94% of patients had IMX-BVN-3 unlikely or very unlikely bacterial results. Among 7 (6.3%) patients with possible (n = 4) or very likely (n = 3) bacterial results, 6 (85.7%) had clinically adjudicated bacterial coinfection or superinfection. Overall, 19 of 111 subjects for whom adjudication was performed had a bacterial infection; 7 of these showed a very likely or likely bacterial result in IMX-BVN-3.

Conclusions. IMX-BVN-3 identified COVID-19 patients as virally infected and identified bacterial coinfections and superinfections. Future studies will determine whether a point-of-care version of the classifier may improve the management of COVID-19 patients, including appropriate antibiotic use.

Keywords. bacterial infection; coinfection; COVID-19; PCR; SARS-CoV-2; superinfection; viral infection.

Bacterial coinfections and superinfections (hereafter referred to simply as "coinfections") have been described in viral respiratory tract infections including coronavirus disease 2019 (COVID-19) and influenza [1, 2]. Because bacterial coinfections contribute to morbidity and mortality of the condition, early and accurate diagnosis is of the utmost importance to facilitate appropriate antibacterial therapy [3]. Bacterial coinfections in patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have not yet been investigated in detail. In a recent meta-analysis, Langford et al [4] reported that bacterial coinfections are relatively infrequent in hospitalized patients with COVID-19; across 24 studies including 3338 patients, 3.5% of

Received 29 May 2022; editorial decision 22 August 2022; accepted 24 August 2022; published online 25 August 2022

[a]Present affiliation: Universitätsspital Zürich, Klinik für Infektionskrankheiten und Spitalhygiene, Zürich, Switzerland.

Correspondence: Wolfgang Bauer, MD, Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Department of Emergency Medicine, Hindenburgdamm 30, 12203 Berlin, Germany (wolfgang.bauer@charite.de).

Open Forum Infectious Diseases®

https://doi.org/10.1093/ofid/ofac437

patients were found to have bacterial coinfections (estimated on presentation), whereas 14.3% of patients developed secondary bacterial infections. The overall proportion of COVID-19 patients with bacterial infection was 6.9%, more common in critically ill patients (8.1%). Although antibiotics are ineffective for the treatment of COVID-19, they were prescribed in 71.9% of patients with suspected or documented COVID-19, most likely based on the difficulty in ruling out bacterial coinfection on presentation and the possibility of bacterial secondary infection [4]. Furthermore, during the first year of the COVID-19 pandemic, 30% of outpatient visits for COVID-19 among Medicare beneficiaries in the United States (US) were linked to an antibiotic prescription, and the highest rates of antibiotic prescribing were observed in the emergency department [5].

Thus, in the absence of rapid and accurate diagnostic solutions, clinicians need to balance the need for rapid antimicrobial therapy to prevent mortality in sepsis patients with bacterial coinfections versus their role as antimicrobial stewards against the overuse of antibiotics and subsequent harm associated with bacterial resistance [6].

Inflammatix Bacterial Viral Noninfected version 3 (IMX-BVN-3) is a host messenger RNA (mRNA)–based classifier to determine the likelihood of a bacterial infection and the likelihood of a viral infection [7, 8]. Several clinical studies have demonstrated its high accuracy for the detection of bacterial and/or viral infections [9–12].

In the present prospective multicenter study, we investigated the performance of the IMX-BVN-3 classifier to (1) confirm viral infection in patients with COVID-19 and to (2) identify patients with bacterial coinfection in a cohort of COVID-19 patients admitted to the emergency departments of 5 sites in Europe and the US.

METHODS

Patient Enrollment

We enrolled 127 patients presenting to the following healthcare institutions after receiving a positive nucleic acid test for SARS-CoV-2 between 20 March 2020 and 29 April 2021: Emergency Department, Charité Universitätsmedizin Berlin, Germany (Campus Benjamin Franklin, n = 19); Emergency Department, Cleveland Clinic (Cleveland, Ohio, US, n = 1); Emergency Department and Department of Pulmonary Medicine, St Claraspital (Basel, Switzerland, n = 45); Departments of Internal Medicine and Cardiology, Vivantes Humboldt-Klinikum and Vivantes Klinikum Spandau (Berlin, Germany, n = 20); Department for Internal Medicine, Gastroenterology and Pulmonology, Evangelische Elisabeth Klinik (Berlin, Germany, n = 7); and Departments of Internal Medicine–Pulmonology and Infectious Diseases, Vivantes Klinikum Neukölln and Vivantes Klinikum Friedrichshain (both Berlin, Germany, n = 19).

All sites enrolled patients under the same protocol, Inflammatix (INF-10). Inclusion criteria were age ≥18 years, presenting with COVID-19 (symptomatic with either nonsevere or severe symptoms of COVID-19) and testing positive for SARS-CoV-2 prior to or at presentation, being able to provide informed consent or consent by a legally authorized representative, and willing to participate in a 30-day follow-up interview. Exclusion criteria were patient-reported treatment with systemic antibiotics, systemic antiviral agents, or systemic antifungal agents (use of topical antibiotics, topical antivirals, topical antifungals, or use of perioperative [prophylactic] antibiotics did not result in exclusion) within the past 7 days prior to enrollment; prisoners or mentally disabled persons were not eligible. Patients were otherwise managed at the enrolling sites as per standard of care. Inflammatory biomarker (procalcitonin [PCT], C-reactive protein [CRP]) results were obtained from the electronic health records using samples collected at the time of patient enrollment. Sixteen patients were excluded from the analysis due to incomplete data. The study design is shown in Figure 1. A standardized case report form was used to collect patient data including visit date, informed consent, demographics, medical history, inclusion and exclusion criteria, sample collection, discharge, and follow-up including subject disposition.

Ethical Approvals

The study protocol and informed consent forms were approved by the relevant ethics committees (central US institutional review board Pro00042993, EKNZ Basel 2020-0759, Vivantes/Charité EA2/071/20, and Charité EA4/167/18). Patients' written informed consent was obtained; those unable to consent due to altered mental state or the severity of their condition were enrolled on an interim basis until they, or their legal guardian, were able to retroactively consent.

Specimen Collection and Collection of Patient Data

Whole blood (2.5 mL) was collected in PAXgene Blood RNA tubes (PreAnalytiX, Hombrechtikon, Switzerland) and frozen locally following the instructions of the manufacturer. Samples were shipped to Inflammatix for processing. RNA was extracted and 29 host mRNAs were quantified using the nCounter FLEX platform (NanoString, Seattle, Washington) as described previously [12]. Following RNA amplification and hybridization, results were processed blinded using the previously locked IMX-BVN-3 classifier. The IMX-BVN-3 classifier determines both the likelihood of bacterial and viral infection (each very unlikely, unlikely, possible, or very likely) [8, 9]. Patient data including clinical evolution and outcome, laboratory results (microbiology, virology, biomarkers), and patient disposition data were obtained from the respective electronic health records and entered into a secure electronic database.

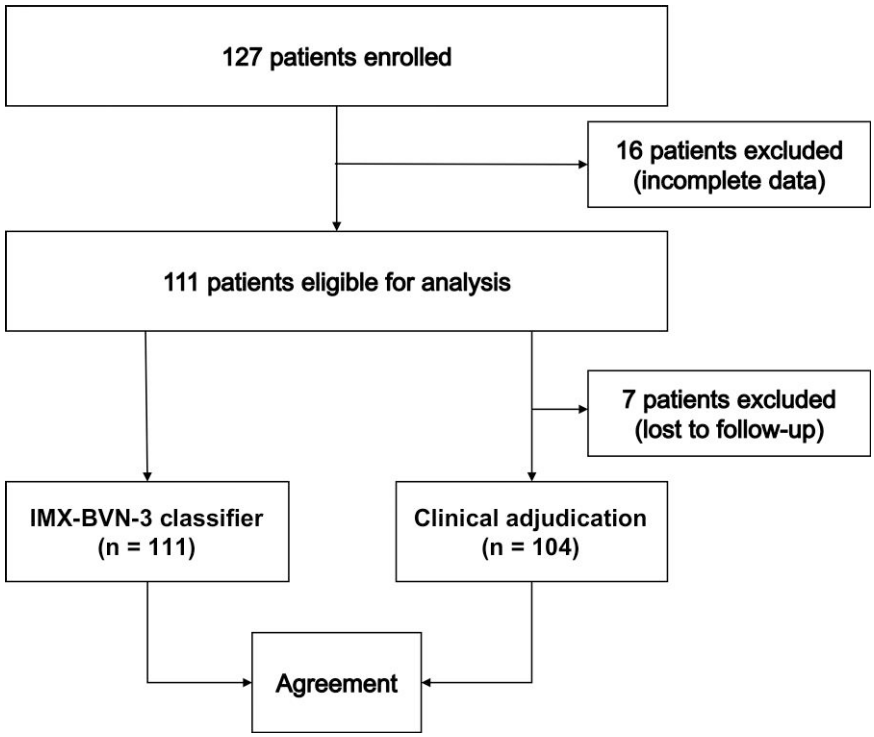

Figure 1. Study design overview. Abbreviation: IBX-BVN-3, Inflammatix Bacterial Viral Noninfected version 3.

Polymerase Chain Reaction for Detection of SARS-CoV-2

Detection of SARS-CoV-2 was performed using polymerase chain reaction (PCR) technologies following local procedures. Tests used included the cobas SARS-CoV-2 Duo on the cobas 6800 system, SARS reverse-transcription PCR (TibMolBiol) on the cobas LightCycler 480 system, Xpert Xpress SARS-CoV-2 (Cepheid), and BD SARS-CoV-2 for BD MAX system (Becton Dickinson); tests varied per site and changed over the time of enrollment. All PCR technologies used were commercially available solutions and had been cleared or authorized by the respective regulatory agencies (CE mark, license from the Swiss Agency for Therapeutic Products, Swiss Medic, or US Food and Drug Administration emergency use authorization).

Clinical Adjudication of Infection Status

To define the infection status, clinical adjudication was performed on all patients for whom access to electronic health records was available (n = 104). Principal investigators at the clinical sites chosen for clinical adjudication were board-certified physicians trained in emergency medicine, infectious diseases, and/or internal medicine. Adjudicators reviewed available records of all patients to determine whether a bacterial coinfection was present (guidance was provided to standardize how microbiology and/or supporting information, including clinical presentation, patient history, laboratory data including biomarkers such as PCT, and imaging to distinguish infection from colonization or contamination, should be used). Results were used to determine the rate of bacterial coinfections overall, as well as to determine the rate of true-positive and true-negative bacterial results for the IMX-BVN-3 classifier.

Statistical Analysis

Annotated data in a secure electronic database were compared with results obtained in IMX-BVN-3. The continuous variables were summarized by mean (standard deviation [SD]) or median (interquartile range) depending on the normality of the data. Categorical variables were summarized by number of patients and the percentages. The χ^2 test was used to test the differences between bacterial and viral bands in patients with and without clinical outcomes, and Wilcoxon rank-sum test was used to compare laboratory values between patients with or without coinfections. Sensitivity, specificity, and likelihood ratio were used for evaluating the diagnostic performance of IMX-BVN-3 for the identification of viral infections and bacterial coinfections. Logistic regression was used for evaluating the associations between coinfection and patients' demographics and clinical outcomes.

RESULTS

Patient Characteristics

The median age of the cohort of 111 patients enrolled with PCR-confirmed SARS-CoV-2 infection was 61.8 years (SD, 17

Table 1. Demographic and Other Characteristics in Patients Enrolled With Polymerase Chain Reaction–Confirmed Severe Acute Respiratory Syndrome Coronavirus 2 Infection

Patient Characteristics	Overall (N = 111)	Clinically Adjudicated[a] (n = 104)	Bacterial Coinfection Absent (n = 86)	Bacterial Coinfection Present (n = 18)
Age, y, mean (SD)	60.81 (16.99)	61.55 (16.82)	60.22 (17.55)	67.89 (11.01)
Male sex	54 (48.6)	49 (47.1)	41 (47.7)	8 (44.4)
Race/ethnicity				
Asian	6 (5.4)	3 (2.9)	3 (3.5)	0 (0.0)
Black or African American	2 (1.8)	1 (1.0)	1 (1.2)	0 (0.0)
White	102 (91.9)	99 (95.2)	81 (94.2)	18 (100.0)
Latin American	1 (0.9)	1 (1.0)	1 (1.2)	0 (0.0)
Hospitalization	108 (97.3)	101 (97.1)	83 (96.5)	18 (100.0)
Length of hospital stay, d, mean (SD)	11.78 (10.65)	11.15 (9.10)	10.23 (8.81)	15.39 (9.44)
Patients receiving ICU-level care[b]	14 (12.6)	14 (13.5)	6 (7.0)	8 (44.4)
Deaths	5 (4.5)	5 (4.8)	2 (2.3)	3 (16.7)
ICU care and/or deaths	16 (14.4)	16 (15.4)	8 (9.3)	8 (44.4)
ICU admission, ICU-level care, and/or deaths	21 (18.9)	20 (19.2)	12 (14.0)	8 (44.4)
Underlying conditions				
Diabetes	33 (29.7)	32 (30.8)	24 (27.9)	8 (44.4)
Cardiovascular conditions	55 (49.5)	54 (51.9)	43 (50.0)	11 (61.1)
Pulmonary conditions	15 (13.5)	15 (14.4)	13 (15.1)	2 (11.1)
Renal insufficiency	16 (14.5)	16 (15.5)	12 (14.1)	4 (22.2)
Immunosuppression	19 (17.1)	18 (17.3)	16 (18.6)	2 (11.1)
Cancer	10 (9.1)	10 (9.7)	10 (10.6)	1 (5.6)

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: ICU, intensive care unit; SD, standard deviation.

[a]Clinical adjudication was performed in 104 patients (7 patients lost to follow-up).

[b]ICU-level care: receipt of vasopressors, mechanical ventilation, or renal replacement therapy.

years) and 54 (48.6%) were male; most patients were White (91.9%) and enrolled at the European sites (99%). The most frequent underlying conditions were cardiovascular disease (49.5%) and diabetes (29.7%) (Table 1).

Laboratory Values at Enrollment

Laboratory values obtained at enrollment for the overall cohort and separated by coinfection status are presented in Table 2. Laboratory values were characteristic of a SARS-CoV-2–infected cohort. Patients with coinfection had significantly higher PCT and CRP compared to those patients without coinfections ($P < .05$, Wilcoxon rank-sum test). Coinfected patients also showed higher leukocyte and neutrophil counts associated with lower lymphocyte counts compared to patients without bacterial infections ($P < .05$, Wilcoxon rank-sum test).

Identification of Viral Infection in COVID-19 Patients Using IMX-BVN-3

One hundred two of 111 (91.9%) of patients with a positive SARS-CoV-2 PCR result were classified as possible or very likely viral in the IMX-BVN-3 classifier ("true positives"), whereas 9 were classified as unlikely or very unlikely (Table 3). IMX-BVN-3 showed a positive agreement for ruling in viral infections (patients in the very likely or possible viral interpretation bands) of 91.9%; for the 9 patients (8.1%) with unlikely or very unlikely viral results ("false negatives"), we investigated clinical and virological findings as potential causes for false-negative results in the IMX-BVN-3 classifier (Supplementary Table 1). We found that 3 patients had high cycle threshold values in SARS-CoV-2 PCR tests, potentially indicating low viral load, and 3 had very likely or possible bacterial infections in IMX-BVN-3; in 2 patients, the IMX-BVN-3 viral scores were close to the cutoff value (0.2548) between the unlikely and possible viral interpretation bands. Median PCT and CRP concentrations were 0.07 ng/mL and 31 mg/L, respectively (Table 2).

Detection of Bacterial Coinfections Using IMX-BVN-3

Among 104 patients for whom clinical adjudication was performed, IMX-BVN-3 classified 7 (6.7%) into the possible (n = 4) or very likely (n = 3) bacterial interpretation bands, suggesting bacterial coinfections (Table 4). Clinical adjudication confirmed coinfections in all 3 patients with very likely IMX-BVN-3 results, and 3 of the 4 patients with possible IMX-BVN-3 results were also confirmed to have coinfections. One patient did not have clinical or microbiological evidence of bacterial infection and was not treated with antibiotics (Supplementary Table 2). Of interest, 1 of 7 patients had a PCT concentration of 1.66 ng/mL, whereas the other 6 had PCT concentrations of <0.25 ng/mL.

Table 2. Laboratory Values in Patients Enrolled With Polymerase Chain Reaction–Confirmed Severe Acute Respiratory Syndrome Coronavirus 2 Infections and Separated by Coinfection Status

Laboratory Value	Overall (N = 111)	Coinfection Status[a]		*P* Value
		Absent (n = 86)	Present (n = 18)	
Procalcitonin, ng/mL	0.07 (0.05–0.14)	0.06 (0.05–0.11)	0.23 (0.13–0.64)	<.001
CRP, mg/L	31.0 (12.6–80.5)	21.00 (11.00–62.38)	99.55 (41.20–151.27)	<.001
D-dimer, mg/L	0.66 (0.40–1.14)	0.61 (0.40–1.10)	0.69 (0.30–1.67)	.446
LDH, U/L	262.00 (204.75–342.00)	258.00 (186.00–339.00)	315.00 (238.00–369.00)	.103
Leukocytes, cells/nL	5.33 (4.00–7.40)	5.11 (3.90–6.62)	7.65 (4.98–9.77)	.024
Lymphocytes, %	18.85 (12.30–28.82)	21.60 (15.50–30.40)	12.10 (8.05–15.42)	.001
Neutrophils, %	69.80 (60.38–77.82)	67.30 (59.90–74.20)	78.85 (73.88–82.82)	.001
Thrombocytes, mg/dL	200.00 (149.50–266.00)	202.50 (151.50–262.25)	194.00 (147.00–302.50)	.723
Ferritin, µg/L	264.00 (108.00–722.50)	282.50 (93.50–737.75)	248.50 (184.80–613.25)	.837
Lactate, mmol/L	0.90 (0.70–1.20)	0.90 (0.70–1.17)	0.90 (0.66–1.21)	.901

Data are presented as median (interquartile range) unless otherwise indicated.

Abbreviation: CRP, C-reactive protein; LDH, lactate dehydrogenase.

[a]Clinical adjudication was performed in 104 patients (7 patients lost to follow-up).

Ninety-seven of 104 patients (93.3%; 7 lost to follow-up) were classified into the unlikely or very unlikely bacterial interpretation bands. Clinical adjudication revealed an additional 12 (12.4%) bacterial infections that were not identified by IMX-BVN-3 (Table 4); 6 of these had unlikely bacterial and 6 had very unlikely bacterial score (9 had very likely viral scores; 3 had likely viral scores).

Performance characteristics for PCT and CRP are shown in Supplementary Table 3*A* and 3*B*. Of note, results for both PCT and CRP were available to the clinical experts performing clinical adjudication of bacterial infection.

DISCUSSION

In the present multicenter study, we investigated the performance of the host response IMX-BVN-3 classifier for the identification of viral infection (COVID-19) and bacterial coinfection in patients with positive SARS-CoV-2 PCR tests upon presentation. First, we observed high agreement for the identification of viral infection (ie, COVID-19) using IMX-BVN-3. One hundred two of 111 patients were correctly identified as virally infected ("true positive") with a very likely or possible viral result in IMX-BVN-3, resulting in an agreement of 91.9%. Interestingly, among the 9 patients who had unlikely or very unlikely results, we found several potential causes for the "false-negative" IMX-BVN-3 result, including high SARS-CoV-2 PCR cycle threshold values potentially indicating low viral load, greater than a week's delay between the positive SARS-CoV-2 PCR test and the IMX-BVN-3 blood draw, and viral scores close to the cutoff value for the possible viral interpretation band (Supplementary Table 1). Furthermore, some patients also had positive IMX-BVN-3 bacterial scores; as the IMX-BVN-3 classifier determines viral and bacterial infections concomitantly, the viral and bacterial scores are partly interdependent and a high bacterial score may lower the viral score. This study is the second demonstration of the performance of host response IMX-BVN-3 for the detection of viral infections. Previously, the performance had only been demonstrated in viral infections other than SARS-CoV-2 [9]. This is of importance, as the IMX-BVN-3 classifier was not trained using COVID-19 patient data but was still able to identify the vast majority of these. In a separate study in the US, we observed a similar agreement of 93.8% for the identification of COVID-19 [11]. The potential causes for "false-negative" IMX-BVN-3 viral results were similar in these independent studies.

The host response classifier does not specify the exact virus but provides the likelihood of any acute viral infection. Viral infections therefore appear to show common pathophysiological signals captured in the target gene set used by IMX-BVN-3. Thus, IMX-BVN-3 may support rapid decision making, ranging from initiation of antiviral therapy to contact precautions and further diagnostic testing. IMX-BVN-3 may also be used to identify novel viral infections early on in future epidemics, when virus-specific tests are not yet available. Existing biomarkers used for the identification of infections (eg, PCT and CRP) fail to identify viral infections. The overall agreement

Table 3. Inflammatix Bacterial Viral Noninfected Version 3 Viral Interpretation Bands in 111 Patients With Polymerase Chain Reaction–Confirmed Severe Acute Respiratory Syndrome Coronavirus 2 Infection

IMX-BVN-3 Viral Band	No. (%)
Very likely	91 (82)
Possible	11 (9.9)
Unlikely	7 (6.3)
Very unlikely	2 (1.8)

Abbreviation: IMX-BVN-3, Inflammatix Bacterial Viral Noninfected version 3.

Table 4. Inflammatix Bacterial Viral Noninfected Version 3 Bacterial Interpretation Bands Compared to Clinically Adjudicated Bacterial Infection Status

IMX-BVN-3 Bacterial Band	Bacterial Infection Status by Clinical Adjudication, No. (%)[a]		% in Band	Sensitivity, %	Specificity, %	Likelihood Ratio
	Not Infected (n = 86)	Infected (n = 18)				
Very likely	0 (0.0)	3 (16.7)	2.9	NR	100	Infinite
Possible	1 (1.2)	3 (16.7)	3.8	NR	98.9	14.3
Unlikely	38 (44.2)	6 (33.3)	42.3	66.7	NR	0.75
Very unlikely	47 (54.7)	6 (33.3)	51	66.7	NR	0.61

Abbreviations: IMX-BVN-3, Inflammatix Bacterial Viral Noninfected version 3; NR, not reported.

[a]Clinical adjudication was performed in 104 of 111 patients (7 patients lost to follow-up).

of IMX-BVN-3 classifier results for the detection of SARS-CoV-2 appears to be similar to the sensitivity of PCR tests for the detection of SARS-CoV-2; a recent systematic review and meta-analysis of 32 studies comprised of 18 000 patients revealed heterologous false-negative rates in quantitative PCR ranging from 2% [13] to 58% [14] with an overall summary estimate of 12% [15–17]. In addition to the occurrence of false-negative results, SARS-CoV-2 PCR tests can also generate "false-positive" results at repeat testing; persistently or intermittently PCR-positive results raise the question of the true risk of disease transmission and the safe duration of self-isolation [16–19].

The second important finding of our study is the fact that the IMX-BVN-3 classifier identified bacterial coinfections, a critical need to allow clinicians to either initiate appropriate antimicrobial therapy or withhold such therapy in COVID-19 patients, thus acting as antimicrobial stewards. All of the 3 patients with a very likely bacterial IMX-BVN-3 score were confirmed by clinical adjudication to have a coinfection; similarly, 3 of 4 patients with likely bacterial IMX-BVN-3 score had a coinfection. Among those patients with unlikely and very unlikely bacterial infections, clinical adjudication revealed an additional 12 bacterial infections. Unfortunately, we were unable to determine in all cases whether bacterial infections were coinfections or superinfections, that is, when exactly during the clinical course these bacterial infections occurred. Furthermore, the IMX-BVN algorithm distributes probability across noninfected, viral, and bacterial classes, so to some degree the presence of a viral infection may lead to a lower bacterial score, and vice versa (though both should drop the noninfected probability to very low).

Clinical actions to limit harm during the current COVID-19 pandemic may have marked effects on antimicrobial resistance, which is less acute but not less crucial [20]. Our finding is of interest as PCT and CRP did not appear to be reliable biomarkers of bacterial coinfection in COVID-19 patients and do not support clinicians in identifying or ruling out bacterial (co-)infections. In this regard, Relph et al [21] recently reported that among 1040 hospitalized adults with COVID-19 in the United Kingdom, admission PCT measurement was neither clinically significant nor diagnostically useful, with an area under the curve of 0.56. Importantly, ongoing and future studies in larger cohorts will determine the performance of IMX-BVN-3 and the prevalence of bacterial infections in COVID-19 patients.

In this regard, it is of importance that the classifier version IMX-BVN-3 investigated here will be revised to the classifier IMX-BVN-4, incorporating additional patient cohorts. IMX-BVN-4 will be integrated into a point-of-care test (in development) that will generate results in approximately 30 minutes to potentially become a valuable part of the clinical toolset in diagnosing and treating patients with suspected infections. Updating classifier versions using real-world data is a key tool in increasing overall performance and utility of tests; rather than updating target genes by changing the assay chemistry, such updates can be performed more easily via software updates following regulatory guidelines. However, further prospective validation of any classifier will be needed prior to their application in clinical practice including randomized interventional trials to assess the effect of the host response diagnostic on patient outcomes.

Of interest, older patients tended to have a higher risk of developing coinfection; although age was not a significant factor in our logistic model (point estimate >1), this trend is consistent with results from a recent meta-analysis [4]. Also, mechanical ventilation was positively associated with bacterial coinfection, as previously reported [4].

This study has several limitations. First, we enrolled patients at emergency departments and on wards if they had a positive SARS-CoV-2 PCR test. While enrolling at multiple sites in the US and Europe under the same protocol, our patient cohort remains relatively small. Also, while we performed clinical adjudication to ensure the appropriate diagnosis of bacterial coinfections (including microbiology, biomarkers, and clinical information) at clinical sites, the exact time of bacterial infection could not be determined in all cases and infections may have happened days after enrollment and blood collection for the IMX-BVN-3 classifier. Last, retrospective records review to allow clinical adjudication of infection status may have

introduced bias. However, clinical adjudication to determine infection status was performed by board-certified clinical experts adhering to study-specific guidance for use of clinical data, biomarkers, and imaging.

In conclusion, the IMX-BVN-3 classifier holds promise as a tool to concurrently identify viral infections and bacterial coinfections in patients with COVID-19. Future studies will need to demonstrate whether the classifier can assist physicians in more informed decision making on initiation or withholding of antimicrobial therapy, thereby allowing for antimicrobial stewardship.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. We thank the staff at the clinical sites and all patients for their support.

Potential conflicts of interest. L. S. and O. L. are employees of and stock option holders of Inflammatix. The institution of N. G. and W. B. received funding from Inflammatix for the conduct of clinical studies. N. G. and W. B. received a travel grant from Inflammatix to attend conferences. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Klein EY, Monteforte B, Gupta A, et al. The frequency of influenza and bacterial coinfection: a systematic review and meta-analysis. Influenza Other Respir Viruses **2016**; 10:394–403.
2. Wu CP, Adhi F, Highland K. Recognition and management of respiratory coinfection and secondary bacterial pneumonia in patients with COVID-19. Cleve Clin J Med **2020**; 87:659–63.
3. Martin-Loeches I, Sanchez-Corral A, Diaz E, et al. Community-acquired respiratory coinfection in critically ill patients with pandemic 2009 influenza A(H1N1) virus. Chest **2011**; 139:555–62.
4. Langford BJ, So M, Raybardhan S, et al. Bacterial co-infection and secondary infection in patients with COVID-19: a living rapid review and meta-analysis. Clin Microbiol Infect **2020**; 26:1622–9.
5. Tsay SV, Bartoces M, Gouin K, Kabbani S, Hicks LA. Antibiotic prescriptions associated with COVID-19 outpatient visits among Medicare beneficiaries, April 2020 to April 2021. JAMA **2022**; 24:2018–9.
6. Huttner BD, Catho G, Pano-Pardo JR, Pulcini C, Schouten J. COVID-19: don't neglect antimicrobial stewardship principles! Clin Microbiol Infect **2020**; 26: 808–10.
7. He YD, Wohlford EM, Uhle F, Buturovic L, Liesenfeld O, Sweeney TE. The optimization and biological significance of a 29-host-immune-mRNA panel for the diagnosis of acute infections and sepsis. J Pers Med **2021**; 11:735.
8. Ducharme J, Self WH, Osborn TM, et al. A multi-mRNA host-response molecular blood test for the diagnosis and prognosis of acute infections and sepsis: proceedings from a clinical advisory panel. J Pers Med **2020**; 10:266.
9. Bauer W, Kappert K, Galtung N, et al. A novel 29-messenger RNA host-response assay from whole blood accurately identifies bacterial and viral infections in patients presenting to the emergency department with suspected infections: a prospective observational study. Crit Care Med **2021**; 49:1664–73.
10. Safarika A, Wacker JW, Katsaros K, et al. A 29-mRNA host response test from blood accurately distinguishes bacterial and viral infections among emergency department patients. Intensive Care Med Exp **2021**; 9:31.
11. Ram-Mohan N, Rogers AJ, Blish CA, et al. Detection of bacterial coinfections and prediction of fatal outcomes in COVID-19 patients presenting to the emergency department using a 29 mRNA host response classifier. medRxiv [Preprint]. Posted online 17 March **2022**. doi:10.1101/2022.03.14.22272394
12. Mayhew MB, Buturovic L, Luethy R, et al. A generalizable 29-mRNA neural-network classifier for acute bacterial and viral infections. Nature Comm **2020**; 11:1177.
13. Lu Y, Li L, Ren S, et al. Comparison of the diagnostic efficacy between two PCR test kits for SARS-CoV-2 nucleic acid detection. J Clin Lab Anal **2020**; 34: e23554.
14. Shen N, Zhu Y, Wang X, et al. Characteristics and diagnosis rate of 5630 subjects receiving SARS-CoV-2 nucleic acid tests from Wuhan, China. JCI Insight **2020**; 5: e137662.
15. Pecoraro V, Negro A, Pirotti T, Trenti T. Estimate false-negative RT-PCR rates for SARS-CoV-2. A systematic review and meta-analysis. Eur J Clin Invest **2022**; 52: e13706.
16. Watson J, Whiting PF, Brush JE. Interpreting a covid-19 test result. BMJ **2020**; 369:m1808.
17. Surkova E, Nikolayevskyy V, Drobniewski F. False-positive COVID-19 results: hidden problems and costs. Lancet Respir Med **2020**; 8:1167–8.
18. Plebani M. Persistent viral RNA shedding in COVID-19: caution, not fear. EBioMedicine **2021**; 64:103234.
19. Vibholm LK, Nielsen SSF, Pahus MH, et al. SARS-CoV-2 persistence is associated with antigen-specific CD8 T-cell responses. EBioMedicine **2021**; 64: 103230.
20. Nieuwlaat R, Mbuagbaw L, Mertz D, et al. Coronavirus disease 2019 and antimicrobial resistance: parallel and interacting health emergencies. Clin Infect Dis **2021**; 72:1657–9.
21. Relph KA, Russell CD, Fairfield CJ, et al. Procalcitonin is not a reliable biomarker of bacterial coinfection in people with coronavirus disease 2019 undergoing microbiological investigation at the time of hospital admission. Open Forum Infect Dis **2022**; 9:ofac179.