Supplementary Online Content

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eTable 1. List of Genes Used in the IMX Transcriptomic Metric

eFigure 1. AUROC Figures of Discrimination of Bacterial Infection for IMX-BVN-3 and Procalcitonin and IL-6 Levels

eFigure 2. AUROC Figures of Discrimination of 30-Day Mortality for IMX-BVN-3 and Procalcitonin and IL-6 Levels

eTable 2. Sensitivity, Specificity, and Odds Ratios for the Various Tests and Models

eFigure 3. Linear Mixed-Effects Modeling of IMX-BVN-3 Scores Over Time in the Patients With Sepsis (Cohort A [Blue]) and Patients at Risk of Sepsis (Cohort B [Red])

eFigure 4. Linear Mixed-Effects Modeling of IMX-SEV-3 Scores in Patients With Those Dead (Blue Lines) and Alive (Red Lines) at 30 Days

eFigure 5. A Framework for Using IMX-SEV-3 Severity Scores to Further Stratify Risk for 30-day Mortality

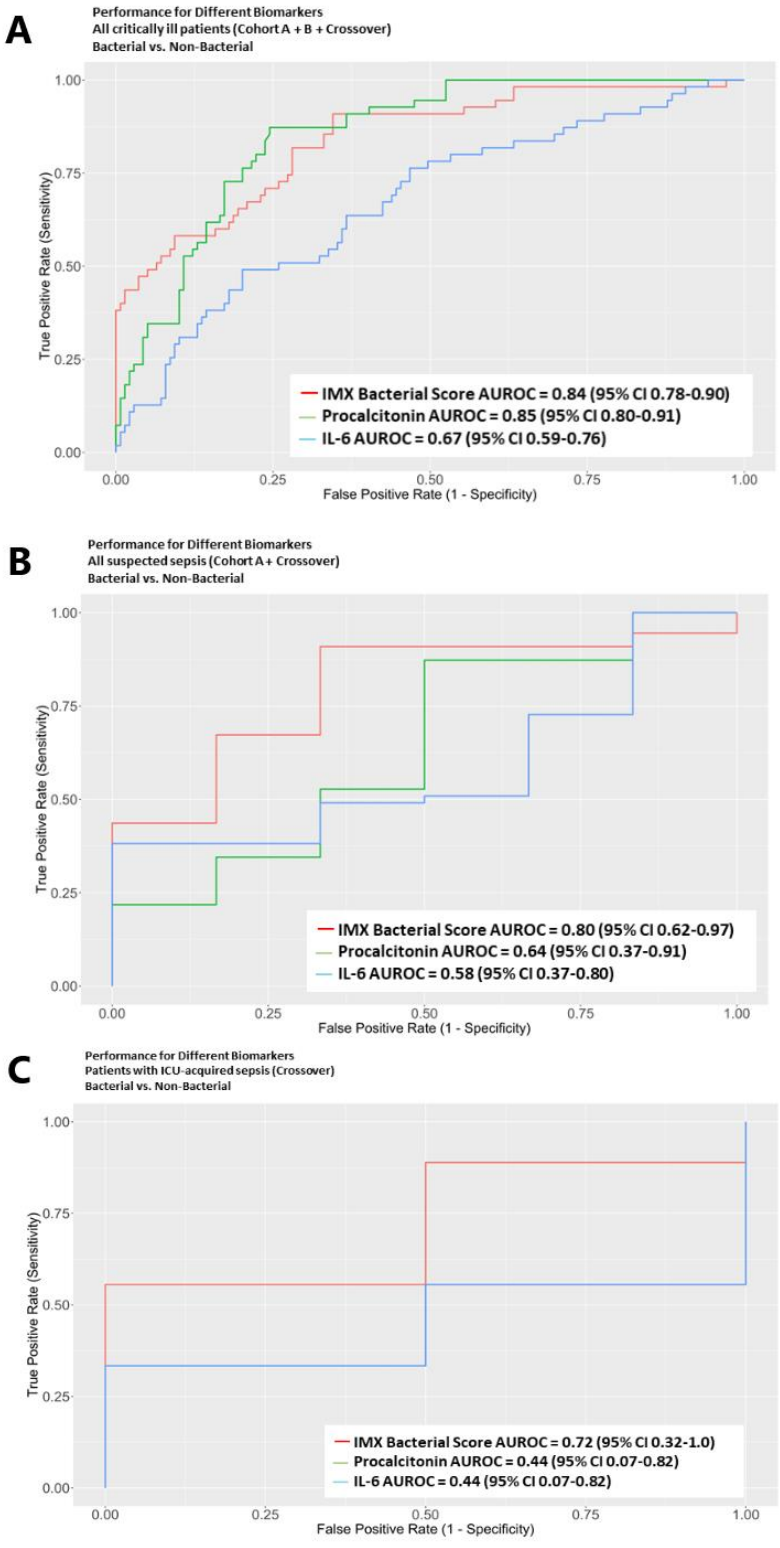
eTable 3. Spearman Correlation Coefficients for the Total Cohort at Day 0 and Total Samples

This supplementary material has been provided by the authors to give readers additional information about their work.

eTable 1. List of Genes Used in the IMX Transcriptomic Metric

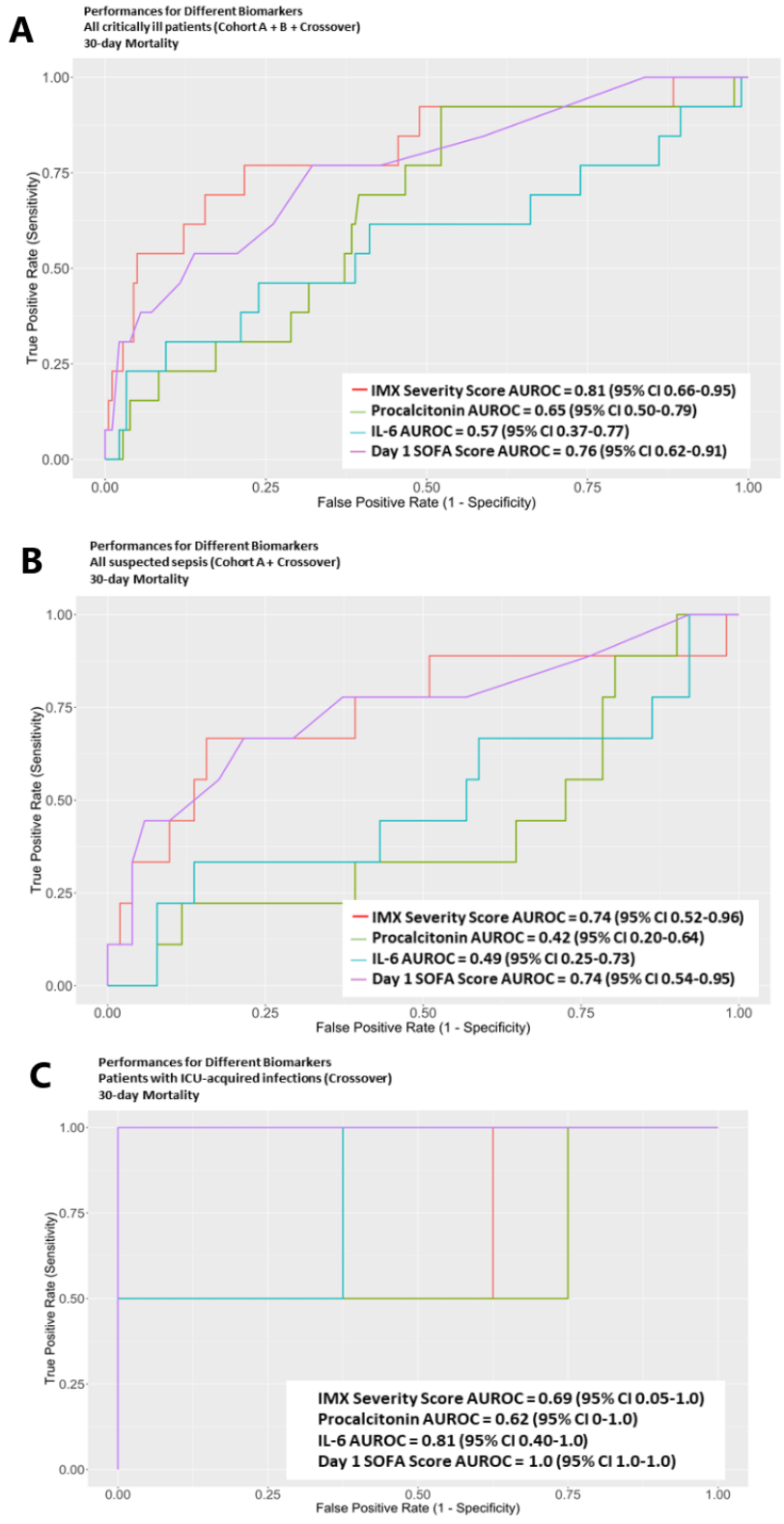
Gene ID	Name
ARG1	Arginase 1
BATF	Basic leucine zipper transcription factor, ATF-like
C3AR1	Complement component 3a receptor 1
CD163	High affinity scavenger receptor for the hemoglobin-haptoglobin complex
CEACAM1	CEA Cell Adhesion Molecule 1
CTSB	Cathepsin B
CTSL	Cathepsin L1
DEFA4	Defensin alpha 4
FAM214A	Family with sequence similarity 214 member A
FURIN	Furin, paired basic amino acid cleaving enzyme
GAD45A	Growth arrest and DNA damage, 45A
GNA15	G protein subunit alpha 15
HK3	Hexokinase isoform 3
HLADMB	Major Histocompatibility Complex, Class II, DM Beta
IFI27	Interferon alpha inducible protein 27
ISG15	Ubiquitin-like modifier/interferon stimulated gene 15
JUP	Junction plakoglobin
KCNJ2	Potassium inwardly rectifying channel subfamily J member 2
LY86	Lymphocyte antigen 86
NMRK1	Nicotinamide riboside kinase 1
OASL	2,5 Oligoadenylate synthetase 1
OLFM4	Olfactomedin 4
PDE4B	Phosphodiesterase 4B
PER1	Period circadian regulator 1
PSMB9	Proteasome 20S subunit beta 9
RAPGEF1	Rap guanine nucleotide exchange factor 1
S100A12	S100 calcium binding protein A12
TGFBI	Transforming growth factor B induced
ZDHHC19	Zinc finger DHHC-type palmitoyltransferase 19

eFigure 1. AUROC Figures of Discrimination of Bacterial Infection for IMX-BVN-3 and Procalcitonin and IL-6 Levels



Panel A: all critically ill study patients (Cohort A + B + Crossover). Panel B: all patients with suspected sepsis (Cohort A + Crossover). Panel C: Cohort B “crossover” patients with hospital-acquired sepsis.

eFigure 2. AUROC Figures of Discrimination of 30-Day Mortality for IMX-BVN-3 and Procalcitonin and IL-6 Levels



Panel A: all critically ill study patients (Cohort A + B + Crossover). Panel B: all patients with suspected sepsis (Cohort A + Crossover). Panel C: Cohort B “crossover” patients with hospital-acquired sepsis.

eTable 2. Sensitivity, Specificity, and Odds Ratios for the Various Tests and Models
Panel A. Performance for bacterial infection (A1, IMX-BVN-3; A2, procalcitonin, A3, IL-6; A4, CRP; A5, CBC; A6, PMN:ALC). Panel B. Performance for severity (IMX-SEV-3).

A(1)

IMX-BVN-3

Total Subjects

	Bacterial infection		% in band	Sensitivity	Specificity	LR
	Yes	No				
Very likely bacterial	35	27	0.315	0.636	0.810	3.347
Possibly bacterial	17	59	0.386	0.309	0.585	0.744
Unlikely bacterial	3	53	0.284	0.945	0.373	0.146
Very unlikely bacterial	0	3	0.015	1.000	0.021	0.000

Cohort A

	Bacterial infection		% in band	Sensitivity	Specificity	LR
	Yes	No				
Very likely bacterial	30	2	0.627	0.652	0.600	1.630
Possibly bacterial	14	3	0.333	0.304	0.400	0.507
Unlikely bacterial	2	0	0.039	0.957	0.000	Inf
Very unlikely bacterial	0	0	0	1.000	0.000	NaN

Cohort A + Crossover Cohort

	Bacterial infection		% in band	Sensitivity	Specificity	LR
	Yes	No				
Very likely bacterial	35	2	0.597	0.636	0.714	2.227
Possibly bacterial	17	4	0.339	0.309	0.429	0.541
Unlikely bacterial	3	1	0.065	0.945	0.143	0.382
Very unlikely bacterial	0	0	0	1.000	0.000	NaN

Crossover Cohort

	Bacterial infection		% in band	Sensitivity	Specificity	LR
	Yes	No				
Very likely bacterial	5	0	0.455	0.556	1	Inf
Possibly bacterial	3	1	0.364	0.333	0.5	0.667
Unlikely bacterial	1	1	0.182	0.889	0.5	0.222
Very unlikely bacterial	0	0	0	1	0	-

A(2)

Procalcitonin

Total Subjects

	Bacterial infection		% in band	Sensitivity	Specificity	LR
	Yes	No				
PCT > 0.5 ng/mL	40	27	0.345	0.727	0.806	3.744
0.25 ng/mL < PCT ≤ 0.5 ng/mL	8	20	0.144	0.145	0.856	1.011
0.1 ng/mL < PCT ≤ 0.25 ng/mL	7	41	0.247	0.873	0.295	0.431
PCT ≤ 0.1 ng/mL	0	51	0.263	1.000	0.367	0.000

Cohort A

	Bacterial infection		% in band	Sensitivity	Specificity	LR
	Yes	No				
PCT > 0.5 ng/mL	33	1	0.68	0.717	0.750	2.870
0.25 ng/mL < PCT ≤ 0.5 ng/mL	6	1	0.14	0.130	0.750	0.522
0.1 ng/mL < PCT ≤ 0.25 ng/mL	7	2	0.18	0.848	0.500	0.304
PCT ≤ 0.1 ng/mL	0	0	0	1.000	0.000	NaN

Cohort A + Crossover Cohort

	Bacterial infection		% in band	Sensitivity	Specificity	LR
	Yes	No				
PCT > 0.5 ng/mL	40	3	0.705	0.727	0.500	1.455
0.25 ng/mL < PCT ≤ 0.5 ng/mL	8	1	0.148	0.145	0.833	0.873
0.1 ng/mL < PCT ≤ 0.25 ng/mL	7	2	0.148	0.873	0.333	0.382
PCT ≤ 0.1 ng/mL	0	0	0	1.000	0.000	NaN

Crossover Cohort

	Bacterial infection		% in band	Sensitivity	Specificity	LR
	Yes	No				
PCT > 0.5 ng/mL	7	2	0.818	0.778	0	0.778
0.25 ng/mL < PCT ≤ 0.5 ng/mL	2	0	0.182	0.222	1	Inf
0.1 ng/mL < PCT ≤ 0.25 ng/mL	0	0	0	1	0	-
PCT ≤ 0.1 ng/mL	0	0	0	1	0	-

A(3)

IL-6

Total Subjects

	Bacterial infection		% in band	Sensitivity	Specificity	LR
	Yes	No				
IL-6 > 35 pg/mL	46	91	0.706	0.836	0.345	1.278
10.5 pg/mL < IL-6 ≤ 35 pg/mL	9	42	0.263	0.164	0.698	0.542
1.8 pg/mL < IL-6 ≤ 10.5 pg/mL	0	6	0.031	1.000	0.043	0.000
IL-6 ≤ 1.8 pg/mL	0	0	0	1.000	0.000	NaN

Cohort A

	Bacterial infection		% in band	Sensitivity	Specificity	LR
	Yes	No				
IL-6 > 35 pg/mL	41	3	0.88	0.891	0.250	1.188
10.5 pg/mL < IL-6 ≤ 35 pg/mL	5	1	0.12	0.109	0.750	0.435
1.8 pg/mL < IL-6 ≤ 10.5 pg/mL	0	0	0	1.000	0.000	NaN
IL-6 ≤ 1.8 pg/mL	0	0	0	1.000	0.000	NaN

Cohort A + Crossover Cohort

	Bacterial infection		% in band	Sensitivity	Specificity	LR
	Yes	No				
IL-6 > 35 pg/mL	46	5	0.836	0.836	0.167	1.004
10.5 pg/mL < IL-6 ≤ 35 pg/mL	9	1	0.164	0.164	0.833	0.982
1.8 pg/mL < IL-6 ≤ 10.5 pg/mL	0	0	0	1.000	0.000	NaN
IL-6 ≤ 1.8 pg/mL	0	0	0	1.000	0.000	NaN

Crossover Cohort

	Bacterial infection		% in band	Sensitivity	Specificity	LR
	Yes	No				
IL-6 > 35 pg/mL	5	2	0.636	0.556	0	0.556
10.5 pg/mL < IL-6 ≤ 35 pg/mL	4	0	0.364	0.444	1	Inf
1.8 pg/mL < IL-6 ≤ 10.5 pg/mL	0	0	0	1	0	-
IL-6 ≤ 1.8 pg/mL	0	0	0	1	0	-

A(4)

CRP

Total Subjects

	Bacterial infection		% in band	Sensitivity	Specificity	LR
	Yes	No				
CRP > 40 mg/L	43	8	0.911	0.977	0.333	1.466
20 mg/L < CRP ≤ 40 mg/L	1	3	0.071	0.023	0.750	0.091
10 mg/L < CRP ≤ 20 mg/L	0	0	0	1.000	0.000	NaN
CRP ≤ 10 mg/L	0	1	0.018	1.000	0.083	0.000

Cohort A

	Bacterial infection		% in band	Sensitivity	Specificity	LR
	Yes	No				
CRP > 40 mg/L	37	4	0.953	0.974	0.200	1.217
20 mg/L < CRP ≤ 40 mg/L	1	1	0.047	0.026	0.800	0.132
10 mg/L < CRP ≤ 20 mg/L	0	0	0	1.000	0.000	NaN
CRP ≤ 10 mg/L	0	0	0	1.000	0.000	NaN

Cohort A + Crossover Cohort

	Bacterial infection		% in band	Sensitivity	Specificity	LR
	Yes	No				
CRP > 40 mg/L	43	5	0.96	0.977	0.167	1.173
20 mg/L < CRP ≤ 40 mg/L	1	1	0.04	0.023	0.833	0.136
10 mg/L < CRP ≤ 20 mg/L	0	0	0	1.000	0.000	NaN
CRP ≤ 10 mg/L	0	0	0	1.000	0.000	NaN

Crossover Cohort

	Bacterial infection		% in band	Sensitivity	Specificity	LR
	Yes	No				
CRP > 40 mg/L	6	1	1	1.000	0.000	1.000
20 mg/L < CRP ≤ 40 mg/L	0	0	0	0.000	1.000	NaN
10 mg/L < CRP ≤ 20 mg/L	0	0	0	1.000	0.000	NaN
CRP ≤ 10 mg/L	0	0	0	1.000	0.000	NaN

A(5)

WBC (drawn standard of care)

Total Subjects

	Bacterial infection		% in band	Sensitivity	Specificity	LR
	Yes	No				
WBC > 20K cells/mm^3	14	15	0.154	0.264	0.889	2.377
15K cells/mm^3 < WBC ≤ 20K cells/mm^3	17	22	0.207	0.321	0.837	1.968
11K cells/mm^3 < WBC ≤ 15K cells/mm^3	10	37	0.25	0.811	0.274	0.688
WBC ≤ 11K cells/mm^3	12	61	0.388	0.774	0.452	0.501

Cohort A

	Bacterial infection		% in band	Sensitivity	Specificity	LR
	Yes	No				
WBC > 20K cells/mm^3	13	0	0.255	0.283	1.000	Inf
15K cells/mm^3 < WBC ≤ 20K cells/mm^3	13	1	0.275	0.283	0.800	1.413
11K cells/mm^3 < WBC ≤ 15K cells/mm^3	9	0	0.176	0.804	0.000	Inf
WBC ≤ 11K cells/mm^3	11	4	0.294	0.761	0.800	0.299

Cohort A + Crossover Cohort

	Bacterial infection		% in band	Sensitivity	Specificity	LR
	Yes	No				
WBC > 20K cells/mm^3	14	0	0.237	0.264	1.000	Inf
15K cells/mm^3 < WBC ≤ 20K cells/mm^3	17	1	0.305	0.321	0.833	1.925
11K cells/mm^3 < WBC ≤ 15K cells/mm^3	10	0	0.169	0.811	0.000	Inf
WBC ≤ 11K cells/mm^3	12	5	0.288	0.774	0.833	0.272

Crossover Cohort

	Bacterial infection		% in band	Sensitivity	Specificity	LR
	Yes	No				
WBC > 20K cells/mm^3	1	0	0.125	0.143	1.000	Inf
15K cells/mm^3 < WBC ≤ 20K cells/mm^3	4	0	0.5	0.571	1.000	Inf
11K cells/mm^3 < WBC ≤ 15K cells/mm^3	1	0	0.125	0.857	0.000	Inf
WBC ≤ 11K cells/mm^3	1	1	0.25	0.857	1.000	0.143

A(6)

PMN:ALC (from CBC, drawn standard of care)

Total Subjects

	Bacterial infection		% in band	Sensitivity	Specificity	LR
	Yes	No				
Ratio > 18	13	4	0.179	0.260	0.911	2.925
9 < Ratio ≤ 18	26	15	0.432	0.520	0.667	1.560
6 < Ratio ≤ 9	7	12	0.2	0.860	0.267	0.525
Ratio ≤ 6	4	14	0.189	0.920	0.311	0.257

Cohort A

	Bacterial infection		% in band	Sensitivity	Specificity	LR
	Yes	No				
Ratio > 18	13	0	0.277	0.302	1.000	Inf
9 < Ratio ≤ 18	20	1	0.447	0.465	0.750	1.860
6 < Ratio ≤ 9	6	0	0.128	0.860	0.000	Inf
Ratio ≤ 6	4	3	0.149	0.907	0.750	0.124

Cohort A + Crossover Cohort

	Bacterial infection		% in band	Sensitivity	Specificity	LR
	Yes	No				
Ratio > 18	13	0	0.236	0.260	1.000	Inf
9 < Ratio ≤ 18	26	1	0.491	0.520	0.800	2.600
6 < Ratio ≤ 9	7	0	0.127	0.860	0.000	Inf
Ratio ≤ 6	4	4	0.145	0.920	0.800	0.100

Crossover Cohort

	Bacterial infection		% in band	Sensitivity	Specificity	LR
	Yes	No				
Ratio > 18	0	0	0	0.000	1.000	NaN
9 < Ratio ≤ 18	6	0	0.75	0.857	1.000	Inf
6 < Ratio ≤ 9	1	0	0.125	0.857	0.000	Inf
Ratio ≤ 6	0	1	0.125	1.000	1.000	0.000

CBC, complete blood cell count with differential; PMN, polymorphonuclear leukocytes; ALC, absolute lymphocyte count.

B

IMX-SEV-3

Total Subjects

	30-day mortality		% in band	Sensitivity	Specificity	LR
	Yes	No				
High severity	8	15	0.117	0.571	0.918	6.971
Moderate severity	6	151	0.797	0.429	0.175	0.519
Low severity	0	17	0.086	1.000	0.093	0.000

Cohort A

	30-day mortality		% in band	Sensitivity	Specificity	LR
	Yes	No				
High severity	6	10	0.314	0.750	0.767	3.225
Moderate severity	2	32	0.667	0.250	0.256	0.336
Low severity	0	1	0.020	1.000	0.023	0.000

Cohort B

	30-day mortality		% in band	Sensitivity	Specificity	LR
	Yes	No				
High severity	1	4	0.037	0.250	0.969	8.187
Moderate severity	3	111	0.844	0.750	0.153	0.885
Low severity	0	16	0.119	1.000	0.122	0.000

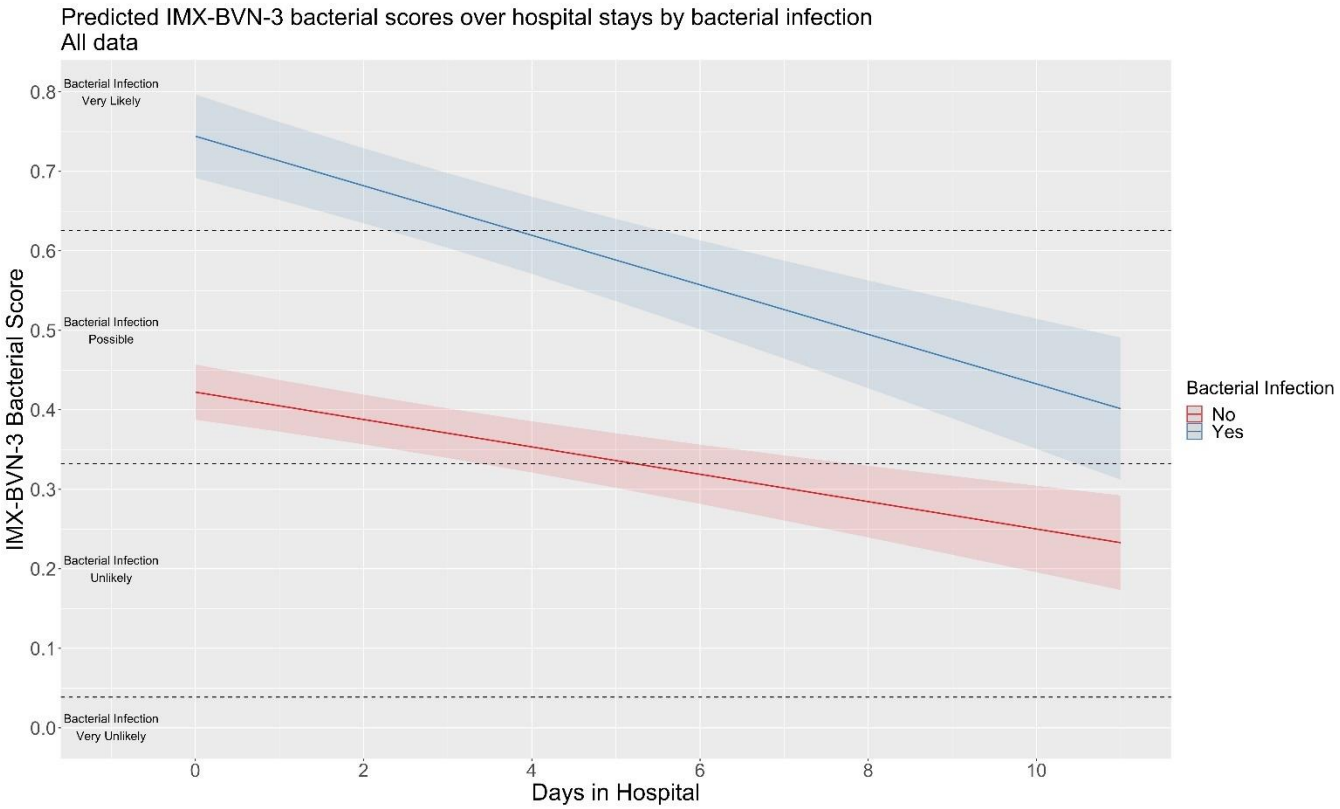
Cohort A + Crossover Cohort

	30-day mortality		% in band	Sensitivity	Specificity	LR
	Yes	No				
High severity	7	11	0.290	0.700	0.788	3.309
Moderate severity	3	40	0.694	0.300	0.231	0.390
Low severity	0	1	0.016	1.000	0.019	0.000

Crossover Cohort

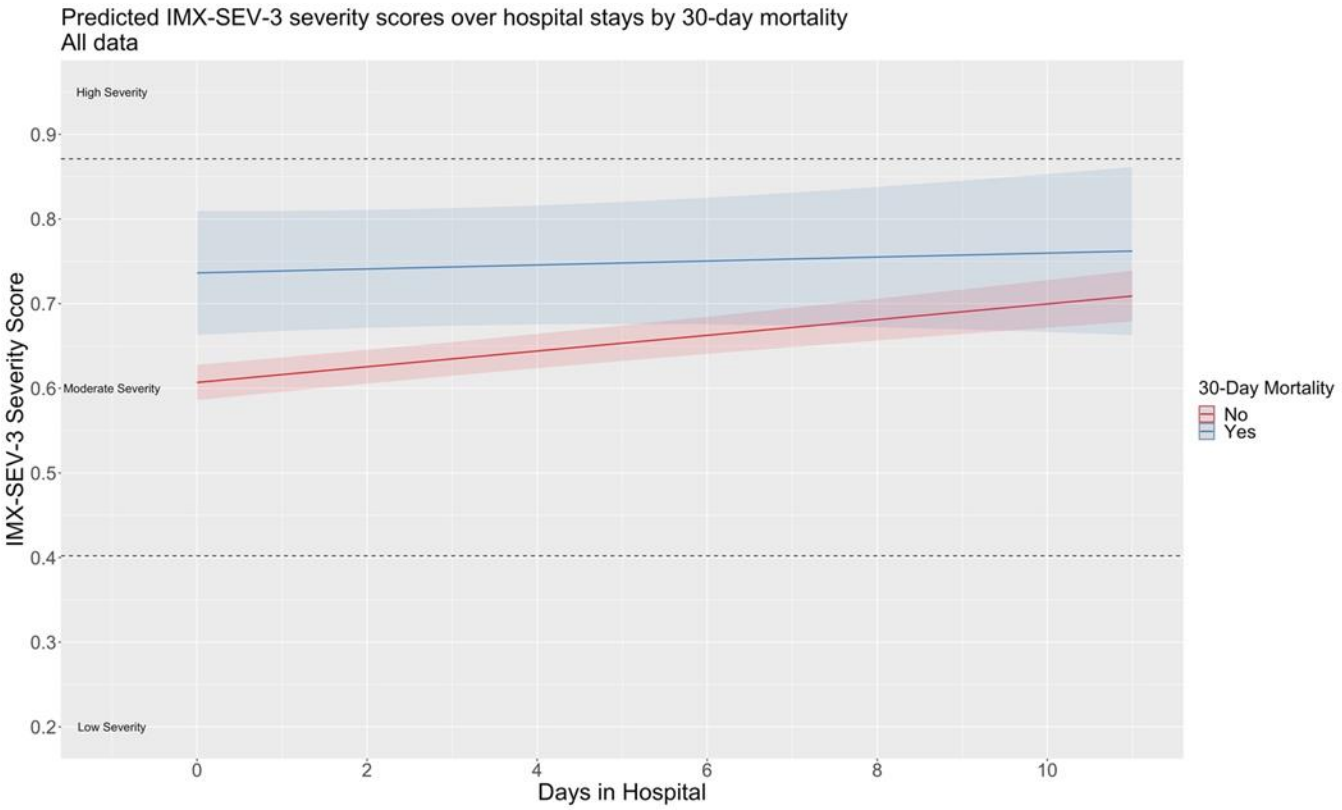
	30-day mortality		% in band	Sensitivity	Specificity	LR
	Yes	No				
High severity	1	1	0.182	0.500	0.889	4.500
Moderate severity	1	8	0.818	0.500	0.111	0.562
Low severity	0	0	0.000	1.000	0.000	NaN

eFigure 3. Linear Mixed-Effects Modeling of IMX-BVN-3 Scores Over Time in the Patients With Sepsis (Cohort A [Blue]) and Patients at Risk of Sepsis (Cohort B [Red])



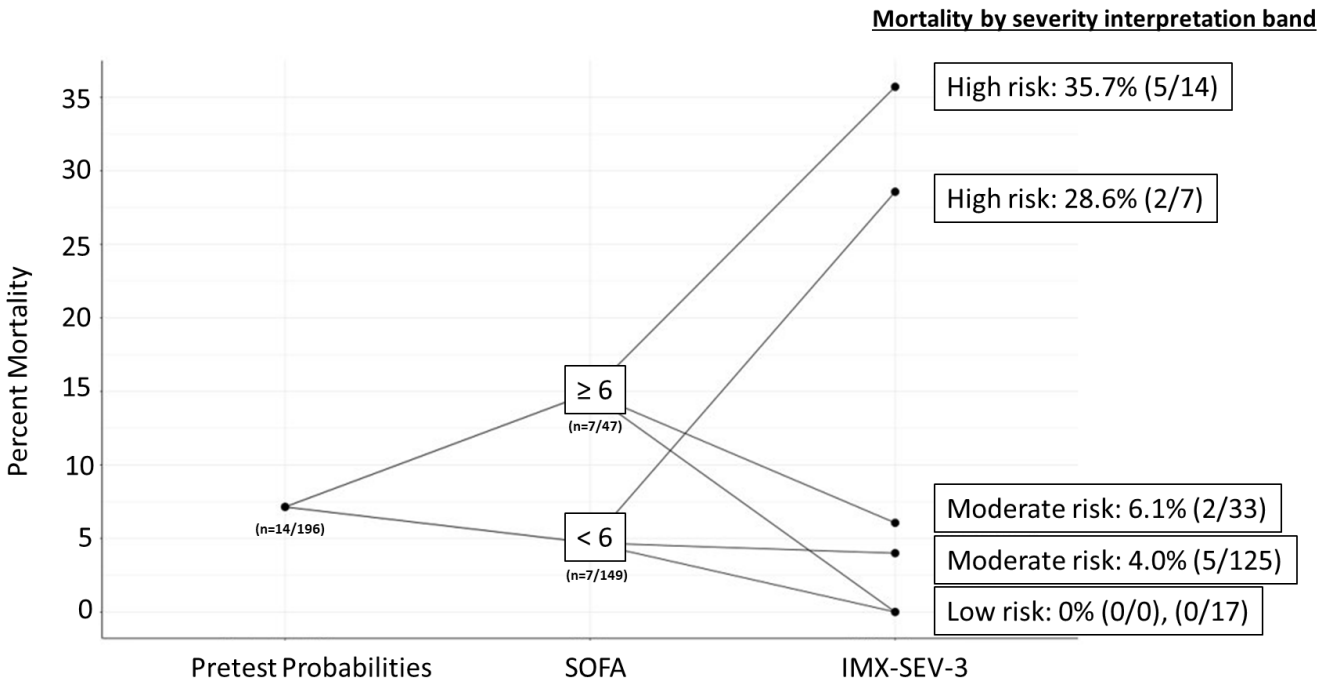
eFigure 4. Linear Mixed-Effects Modeling of IMX-SEV-3 Scores in Patients With Those Dead (Blue Lines) and Alive (Red Lines) at 30 Days

Initial severity scores were greater among patients who suffered 30-day mortality ($p<0.00001$). Severity scores increased over time in both groups, consistent with early discharge for surviving patients, whose data were subsequently censored. Data is represented as a linear mixed effects model.



eFigure 5. A Framework for Using IMX-SEV-3 Severity Scores to Further Stratify Risk for 30-day Mortality

Patients were stratified initially by sequential organ failure assessment (SOFA) score cutoffs and then by IMX-SEV-3 severity scores indicating that 30-day mortality was “high”, “moderate”, and “low”.



eTable 3. Spearman Correlation Coefficients for the Total Cohort at Day 0 and Total Samples

Day 0 n=200

	BVN-3 bacterial	BVN-3 viral	SEV-3 severity	IL6	PCT	SOFA
BVN-3 bacterial	1					
BVN-3 viral	-0.36	1				
SEV-3 severity	0.54	-0.61	1			
IL6	0.38	-0.49	0.42	1		
PCT	0.55	-0.4	0.58	0.52	1	
SOFA	0.26	-0.3	0.39	0.34	0.32	1

All values are $p<0.0001$

A univariable logistic regression model using procalcitonin alone to predict infection had AUROC of 0.85 (0.80 - 0.91), whereas a multivariable model using procalcitonin plus the IMX-BVN-3 bacterial score had AUROC 0.87 (0.81 - 0.92), which was not significantly greater than procalcitonin alone ($p=0.596$). A univariable logistic regression model using SOFA alone to predict 30-day mortality had AUROC 0.77 (0.63 - 0.90). A multivariable model using SOFA plus the IMX-SEV-3 severity score had AUROC 0.83 (0.70 - 0.97), which was not significantly greater than SOFA alone ($p=0.092$). This is not surprising, since correlation coefficients among the different biomarkers, including IMX-SEV-3, and SOFA scores were all highly significant ($p<0.001$).