SUPPLEMENTAL DIGITIAL CONTENT

Supplemental Methods

Samples were collected and processed from 335 newly adjudicated surgical sepsis patients, and blood samples were collected at <12 (n=2) and 24 hours (n=333). Upon presumption of sepsis (to be adjudicated at a later point of time), patients were transferred to the surgical ICU (if already not there) where they received implementation of evidence-based sepsis management protocols, including fluid resuscitation, broad spectrum antibiotics, and inotropes (as required). Blood lactate levels were measured every four hours. The estimated time from MEWS screening/warning to presumption of sepsis and initiation of sepsis management protocols was generally less than 4 hours.

Overall cohort inclusion criteria included: 1) age ≥18 years; 2) clinical diagnosis of sepsis as defined by 2001 consensus guidelines; and 3) entrance into the electronic medical record-(EMR) based sepsis clinical management protocol.[1] Exclusion criteria included of any of the following: 1) refractory shock (death <24 hours from sepsis protocol initiation) or inability to achieve source control (e.g., total bowel ischemic necrosis); 2) pre-admission expected lifespan <3 months; 3) patient/proxy not committed to aggressive management; 4) severe congestive heart failure (CHF) (NYHA Class IV); 5) Child-Pugh Class C liver disease or pre-liver transplant; 6) known HIV with $CD4^+$ count <200 cells/mm^3; 7) patients receiving chronic corticosteroids or immunosuppressive agents, including organ transplant recipients; 8) pregnancy; 9) institutionalized patients; 10) inability to obtain informed consent within 96 hours of enrollment; 11) chemotherapy or radiotherapy within 30 days; 12) severe traumatic brain injury (TBI); and 13) spinal cord injury (SCI) resulting in permanent sensory and/or motor deficits.

The IMX-BVN-2 and IMX-SEV-2 algorithms were evaluated on 335 subjects in whom whole blood RNA collections were available. Of those, 333 samples were obtained the following

morning after diagnosis of sepsis (T1 = 24 hours) and institution of treatment bundles. Two samples were not available at 24 hours and were replaced with samples obtained at less than 12 hours post institution of treatment bundles. 289 samples had been stored in the original PAXgene collection tubes, while 46 samples were stored at -80ºC as isolated total cellular RNA.

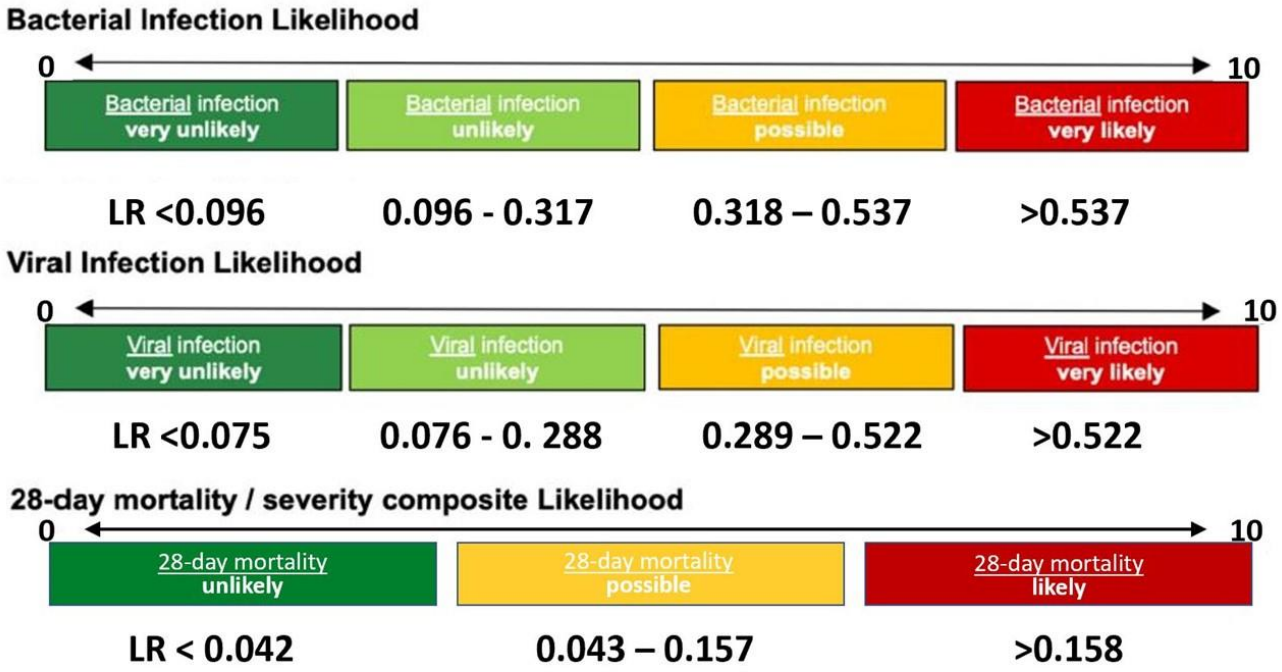
The IMX-SEV-2™ metric was analyzed on a NanoString nCounter FLEX® using the analytical protocol, completed July 2020, using a standard 18-hour incubation period. The analyses were conducted at the University of Florida Department of Pathology Molecular Pathology Laboratory at the Rocky Point Laboratory of UF Health Shands Hospital. The Rocky Point Laboratory is CLIA-certified, CAP-accredited, and the samples were all run by a single ASCP-certified medical technologist. Quality control and quality assurances were provided according to the analytical protocol and verified by a College of Medicine faculty certified by the American Board of Pathology.

Supplemental Table 1. List of 29 genes used in IMX-BVN-2 and IMX-SEV-2. The scores use individual levels of mRNAs originally described in several sepsis diagnostic/prognostic modules. The mRNA modules are: (1) infection-up: *CEACAM1, ZDHHC19, C9orf95, GNA15, BATF, C3AR1;* (2) infection-down: *KIAA1370, TGFBI, MTCH1, RPGRIP1, HLA-DPB1*; (3) bacterial-viral-up: *HK3, TNIP1, GPAA1, CTSB*; (4) bacterial-viral-down: *IFI27, JUP, LAX1*; (5) mortality-up: *DEFA4, CD163, RGS1, PER1, HIF1A, SEPP1, C11orf74, CIT*; and (6) mortality-down*: LY86, TST, KCNJ2.*

Blood samples collected in PAXgene™ Blood RNA tubes had total RNA isolated as described in the primary manuscript, and RNA expression levels from 29 genes determined (Department of Pathology Molecular Pathology Laboratory at Rocky Point, Shands UF Health) (**Supplemental Table 1**). Completely blinded raw data from the NanoString nCounter FLEX® were transferred electronically to Inflammatix for generation of the IMX-BVN-2 and IMX-SEV-2 scores, as previously described.[2-4] IMX-SEV-2 was derived from transcriptomic datasets obtained from 33 independent cohorts with a total of 3288 patients, and was validated in 348 patients, where it had an AUROC of 0.840 for predicting 30-day mortality.[5]

Diagnostic thresholds were set based on prespecified criteria so that the bacterial, viral and severity scores would be separated into discrete interpretation bands each (**Supplemental Figure 1**). The thresholds were originally set in training data targeting likelihood ratios (LRs) of 0.05 in the low bands, and 10 in the upper bands. The selected cutoffs were 0.096, 0.317, and 0.537 for the bacterial score, and 0.075, 0.288, and 0.502 for the viral score.

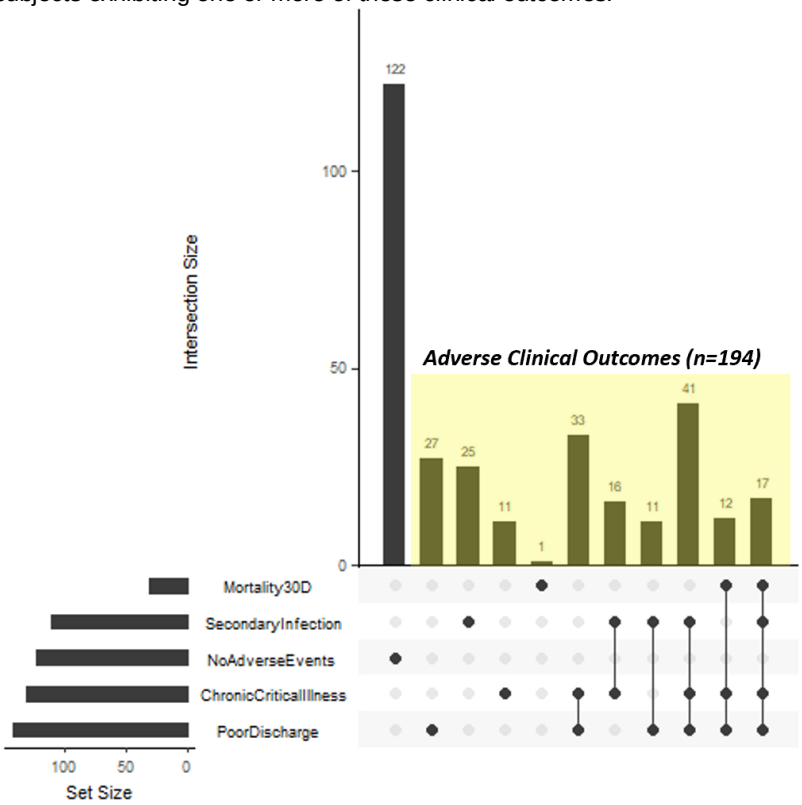
Supplemental Figure 1. Diagnostic thresholds for likelihood of a bacterial or viral infection, or 28 (30) day mortality.



The primary clinical outcome variable was 30-day (all-cause) mortality. Secondary clinical outcome variables included: 1) development or absence of chronic critical illness (CCI), 2) hospital discharge disposition, 3) presence or absence of secondary infections and 4) an overall adverse clinical outcome. Inpatient clinical trajectory was defined as 'early death', 'rapid recovery' (RAP), or 'chronic critical illness' (CCI). Early death was defined as death within 14 days of sepsis onset. CCI was defined as an ICU length of stay (LOS) greater than or equal to

14 days with evidence of persistent organ dysfunction based upon components of the SOFA score.[6] Hospitalized patients who died after an ICU length of stay >14 days from the index hospitalization were classified as CCI.[7] Rapid recovery (RAP) patients were those discharged

Supplemental Figure 2. Outcome overlaps among Sepsis-3 patients. Subjects were assigned to different categories based on whether they exhibited one or more adverse clinical outcomes. The definition of an adverse clinical outcome was based on subjects exhibiting one or more of these clinical outcomes.



from the ICU within 14 days with resolution of organ dysfunction.

Supplemental Figure 2 presents the distribution of Sepsis-3 patients with different clinical outcomes. Patients were defined as having an 'adverse clinical outcome' if they experienced a secondary infection, CCI, poor discharge disposition and/or mortality within the first 30 days. Outcomes overlaps were plotted with UpSetR™ for R software (https://doi.org/10.1093/bioinformatics/btx364). One hundred, twenty four Sepsis-3 patients had no adverse clinical outcomes, while 192 patients experienced one or more adverse clinical

events. One hundred and three patients developed chronic critical illness (CCI) and had either an adverse discharge disposition or death.

Supplemental Results

Supplemental Table 2. Cohort demographics

Characteristics	Overall cohort (n=335)	Sepsis-3 cohort (n=316)
Male, n (%)	178 (53.1)	169 (53.5)
Age in years, median (25th, 75th)	62 (49, 71)	62 (51, 71)
Race, n (%)		
Caucasian	299 (89.3)	284 (89.9)
African American	32 (9.6)	28 (8.9)
Other	4 (1.2)	4 (1.3)
Charlson comorbidity index, median (25th, 75th)	3 (1, 5)	3 (1, 5)
Active cancer diagnosis	47 (14)	44 (13.9)
Reason for hospital admission, n (%)		
Active infection	211 (63)	199 (63)
Non-infectious complication	31 (9.3)	30 (9.5)
Planned surgery	59 (17.6)	56 (17.7)
Trauma	34 (10.1)	31 (9.8)
Inter-facility hospital transfer, n (%)	144 (43)	136 (43)
Primary sepsis diagnosis, n (%)		
Intra-abdominal	98 (29.3)	96 (30.4)
SSI	76 (22.7)	67 (21.2)
Pneumonia	56 (16.7)	55 (17.4)
Urosepsis	37 (11)	34 (10.8)
NSTI	40 (11.9)	40 (12.7)
CLABSI	4 (1.2)	4 (1.3)
Other	24 (7.2)	20 (6.3)
Sepsis severity by Sepsis-2, n (%)		
Sepsis	100 (29.9)	81 (25.6)
Severe sepsis	145 (43.3)	145 (45.9)
Septic shock	90 (26.9)	90 (28.5)
Sepsis severity by Sepsis-3, n (%)		
Infection	19 (5.7)	0 (0)
Sepsis	235 (70.1)	235 (74.4)
Septic shock	81 (24.2)	81 (25.6)
APACHE II, median (25th, 75th)	17 (11, 23)	17 (12, 24)
Maximum SOFA score, median (25th, 75th)	7 (5, 10)	8 (5, 10)
Acute Kidney Injury, n (%)		
KDIGO Stage 1	79 (23.6)	78 (24.7)

KDIGO Stage 2	63 (18.8)	62 (19.6)
KDIGO Stage 3	45 (13.4)	44 (13.9)
MOF, n (%)	145 (43.3)	145 (45.9)
ICU LOS, median (25th, 75th)	7 (3, 17)	8 (4, 17)
Hospital LOS, median (25th, 75th)	16 (8, 26)	16.5 (9, 27)

SSI, surgical site infection; NSTI, necrotizing soft tissue infection; CLABSI, central line associated blood stream infection; APACHE II, acute physiology and chronic health evaluation 2 score assessed at 24 hours after sepsis onset; MOF, multiple organ failure; LOS, length of stay

Supplemental Table 3. Secondary Infections, Length of Hospitalization, and Discharge Disposition in the Overall and Sepsis-3 Cohorts. The two cohorts are further divided into subjects who experienced an early death, rapid recovery (RAP), or chronic critical illness (CCI).

Outcomes	Overall cohort (n=335)	Early Death (n=11)	CCI (n=120)	RAP (n=204)	Sepsis -3 cohort (n=316)	Early Death (n=11)	CCI (n=119)	RAP (n=186)
2º infections/patient, mean (SD)	0.5 (0.8)	0.4 (0.5)	1 (1)	0.2 (0.5)	0.5 (0.8)	0.4 (0.5)	1 (1)	0.2 (0.5)
2º infections/100 hospital days, mean (SD)	2 (3.6)	4.3 (7)	3 (3.5)	1.3 (3.2)	2.1 (3.6)	4.3 (7)	3 (3.5)	1.4 (3.3)
ICU LOS, median (25th, 75th)	7 (3, 17)	6 (5, 9)	20 (15, 28.5)	4 (2, 8)	8 (4, 17)	6 (5, 9)	20 (15, 29)	5 (3, 8)
Hospital LOS, median (25th, 75th)	16 (8, 26)	6 (5, 13)	28 (20, 40)	10.5 (7, 17.5)	16.5 (9, 27)	6 (5, 13)	28 (20, 40)	11 (8, 18)
Discharge disposition, n (%)								
“Good” disposition	193 (57.6)	0 (0)	27 (22.5)	166 (81.4)	175 (55.4)	0 (0)	27 (22.7)	148 (79.6)
Home	66 (19.7)	N/A	1 (0.8)	65 (31.9)	58 (18.4)	0 (0)	1 (0.8)	57 (30.6)
Home healthcare services	102 (30.4)	N/A	15 (12.5)	87 (42.6)	93 (29.4)	0 (0)	15 (12.6)	78 (41.9)
Inpatient rehab facility	25 (7.5)	N/A	11 (9.2)	14 (6.9)	24 (7.6)	0 (0)	11 (9.2)	13 (7)
“Poor” disposition	142 (42.4)	11 (100)	93 (77.5)	38 (18.6)	141 (44.6)	11 (100)	92 (77.3)	38 (20.4)
LTAC	52 (15.5)	N/A	48 (40)	4 (2)	51 (16.1)	0 (0)	47 (39.5)	4 (2.2)
Skilled Nursing facility	44 (13.1)	N/A	11 (9.2)	33 (16.2)	44 (13.9)	0 (0)	11 (9.2)	33 (17.7)
Another Hospital	13 (3.9)	N/A	12 (10)	1 (0.5)	13 (4.1)	0 (0)	12 (10.1)	1 (0.5)
Hospice	8 (2.4)	N/A	7 (5.8)	0 (0)	8 (2.5)	0 (0)	7 (5.9)	0 (0)
Death	26 (7.8)	11 (100)	15 (12.5)	0 (0)	26 (8.2)	11 (100)	15 (12.6)	0 (0)

CCI, chronic critical illness; RAP, rapid recovery; LOS, length of stay; LTAC, long-term acute care facility. Early death, CCI and RAP percentages are those of their respective cohort (Overall, Sepsis-3).

Supplemental Table 4. Correlation Coefficients of Biochemical and Clinical Markers obtained at 24 hours Post Sepsis. For patients meeting Sepsis-3 criteria, measurements were analyzed by Spearman's correlation tests, as appropriate. Values present *rho* value, probability and sample size.

		Transcriptomic Severity metric	APACHE II	Charlson comorbidity index	Age	Plasma IL-6	Plasma CRP	ALC	WBC	Plasma GLP-1
Transcriptomic Severity metric	(ρ)	1								
	(p)									
	(n)	316								
APACHE II	(ρ)	**0.338**	1							
	(p)	**<0.0001**								
	(n)	**316**	316							
Charlson comorbidity index	(ρ)	0.047	**0.347**	1						
	(p)	0.406	**<.0001**							
	(n)	314	**314**	314						
Age	(ρ)	0.089	*NI*	*NI*	1					
	(p)	0.1126								
	(n)	316			316					
Plasma IL-6	(ρ)	**0.432**	**0.243**	0.007	0.075	1				
	(p)	**<.0001**	**<.0001**	0.8992	0.1909					
	(n)	**304**	**304**	302	304	304				
Plasma CRP	(ρ)	**0.173**	-0.020	-0.123	-0.087	**0.288**	1			
	(p)	**0.0092**	0.7638	0.0658	0.1938	**<.0001**				
	(n)	**226**	226	224	226	**225**	226			
ALC	(ρ)	**-0.300**	**-0.212**	-0.121	**-0.266**	**-0.343**	-0.087	1		
	(p)	**<.0001**	**0.0006**	0.054	**<.0001**	**<.0001**	0.2371			
	(n)	**255**	**255**	253	**255**	**246**	188	255		
+WBC	(ρ)	**0.310**	**0.204**	0.055	0.025	0.051	-0.018	**0.231**	1	
	(p)	**<.0001**	**0.0003**	0.3307	0.6583	0.3791	0.7871	**0.0002**		
	(n)	**314**	**314**	312	314	303	225	**255**	314	
Plasma GLP-1	(ρ)	**0.436**	**0.218**	**0.174**	**0.129**	**0.379**	0.104	**-0.151**	**0.160**	1
	(p)	**<.0001**	**0.0001**	**0.0025**	**0.0247**	**<.0001**	0.1204	**0.018**	**0.0053**	
	(n)	**304**	**304**	**302**	**304**	**303**	225	**246**	**303**	304

ρ, Spearman correlation coefficient; p, p-value; n, number of available patients for analysis.

ALC, absolute lymphocyte count; CRP, c-reactive protein; WBC, total white blood cell count; GLP-1, glucagon-like peptide-1.*NI,* not independent variables since the Charlson comorbidity index and APACHE II contain age as a component.

Likelihood of a Viral Infection. None of the patients were clinically adjudicated to have a viral infection. Routine viral DNA or antigen titers were not performed unless clinically justified, and none of the patients were considered to have a concurrent viral infection. Expectedly, the IMX-BVN-2 metric identified 333 of 335 subjects as being either 'Unlikely' or 'Very Unlikely'. Only four subjects were deemed to have a 'Possible' or 'Very Likely' chance of having a viral infection (**Supplemental Table 5**). Since viral DNA or antigen titers were not conducted on any patient, it is impossible to determine whether a subclinical viral infection (or viral reactivation in the setting of critical illness) was present in these four patients with possible or very likely scores.

Supplemental Table 5. Distribution of IMX-BVN-2 Viral Infection Scores.

IMX-BVN-2 Viral Band →	Very likely	Possible	Unlikely	Very unlikely	Total
All patients (assumed non-viral)	**2 (1%)**	**2 (1%)**	**32 (10%)**	**299 (89%)**	**335**

Likelihood of a Bacterial Infection. Importantly, this study was not designed to identify whether the metric could predict whether the patients had or did not have a bacterial infection (**Supplemental Table 6**). All of the patients were clinically adjudicated to have a microbial infection, either bacterial or fungal at the time of initiation of the sepsis management bundles. In addition, all of the patients had received broad spectrum antibiotics upon initiation of sepsis management bundles. In most cases, this was initiated 12-18 hours prior to blood sampling. At the time the study sample was collected, blood or exudate samples were not collected, so it would not be possible to determine how many of these subjects

Supplemental Table 6. Distribution of IMX-BVN-2 Bacterial Infection Scores.

IMX-BVN-2 Bacterial Band →	Very likely	Possible	Unlikely	Very unlikely	Total
Fungal infection	**7 (78%)**	**2 (22%)**	**0 (0%)**	**0 (0%)**	**9**
Bacterial infection	**258 (79%)**	**63 (19%)**	**5 (2%)**	**0 (0%)**	**326**

would still be infected, or the temporal dynamics of the IMX-BVN-2 score with regard to pre/post antibiotics timing.

The IMX-BVN-2 metric identified both adjudicated fungal and bacterial infections as being ‘Very Likely’ or ‘Possible’ bacterial infections. Only 5 of the 335 included subjects were scored to be “Unlikely” whereas both the subjects with bacterial and fungal infections had close to 80% being very likely and nearly 20% as possible.

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