

Supplemental Digital Content

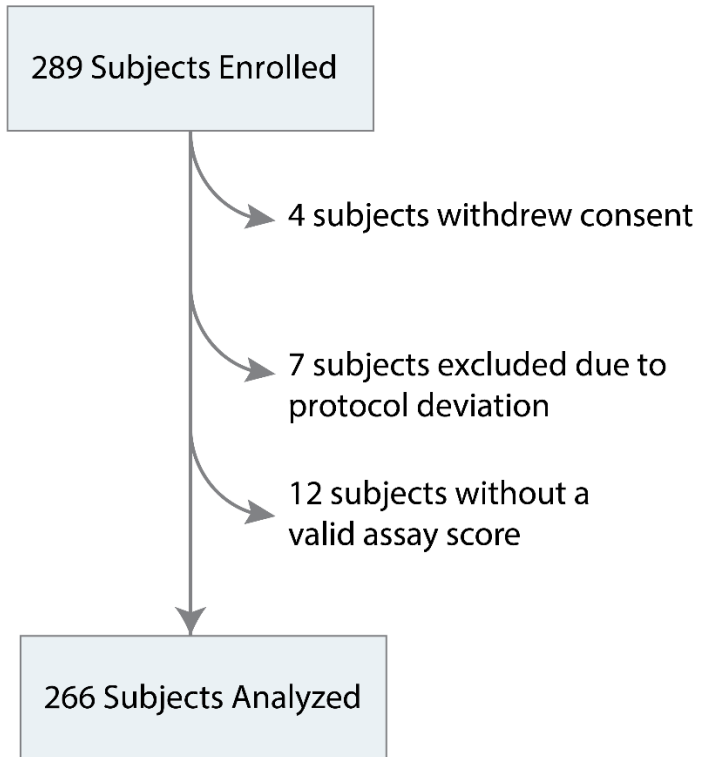


Figure S1: Flow chart for selection of evaluable subjects.

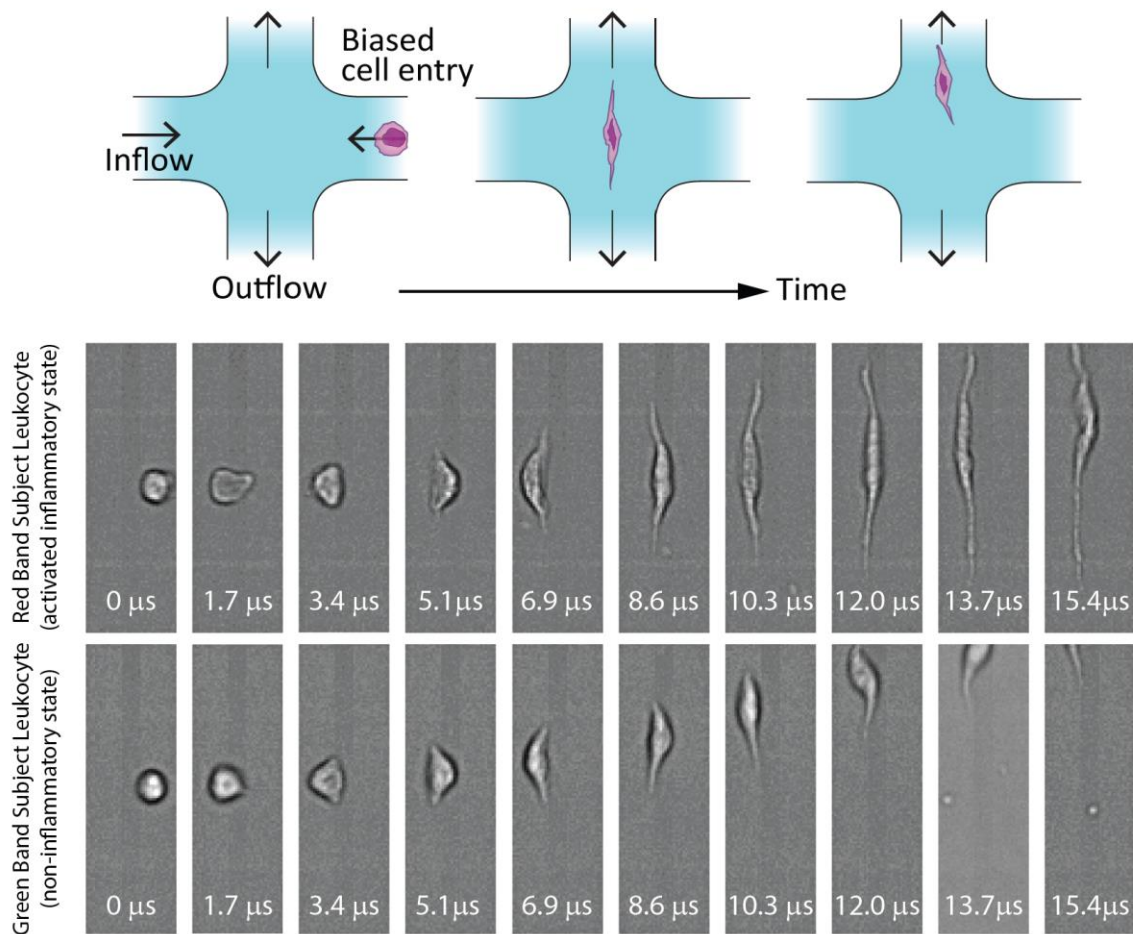
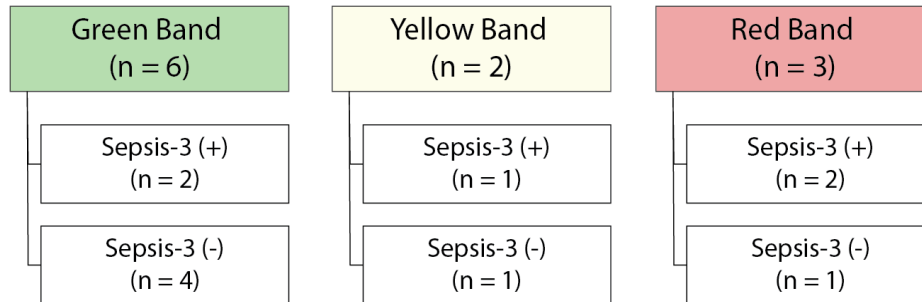


Figure S2: Blood cell movement through microfluidic deformation junction. Time series of cell deformation for representative leukocyte of a red band subject (top) and green band subject (bottom). The IntelliSep test uses microfluidic technology combined with high-speed imaging and machine learning techniques to assess the biophysical properties of human leukocytes – specifically, neutrophils and monocytes, providing a sample to result workflow in less than 10 minutes. The operator introduces 100 microliters of fresh whole blood into the Sample Preparation Module (SPM), where an automated lyse and wash module removes erythrocytes, and washes the purified leukocytes in a diluent, resulting in a total volume of 1mL. The operator then transfers this solution into the IntelliSep Cartridge and inserts it into the Cell Imaging Module (CIM) to interrogate single-cell biophysical properties via deformability cytometry. High-speed Video data, which captures tens of thousands of cells in seconds (a representative video sample is included in the digital supplemental material), is analyzed using custom algorithms in the Imaging and Analysis Module (IAM) allowing for detection of intracellular and nuclear changes that occur during leukocyte activation. The measured leukocytes’ biophysical parameters are combined into a single score between 0.1-10.0, inclusive. To allow for easier clinical interpretation the range is divided into three Interpretation Bands (Green, Yellow, Red) that correspond to increasing disease severity. The score and Interpretation Band are reported to the operator.



Green Band (N = 6)	<ul style="list-style-type: none"> • Age range: 60 – 70 • History of idiopathic pulmonary fibrosis and congestive heart failure • Enrolled in study on SIRS 2+ and blood culture ordered • Received antibiotics and steroids in the ED • Admitted to hospital for acute on chronic respiratory failure • Blood cultures negative • Organ system associated with suspicion of infection: Respiratory • Maximum SOFA score over 3-days following presentation (baseline subtracted) = 2 • Length of stay 7 days, following which patient was transferred to receive a lung transplant • Adjudicators disagreed on the presence of infection • Forced label: not septic
	<ul style="list-style-type: none"> • Age range: 60 – 70 • History of arterial fibrillation, deep venous thrombosis, and chronic obstructive pulmonary disease • Enrolled in study on blood culture ordered • Received antibiotics the ED • Not admitted to hospital • Blood cultures negative • Organ system associated with suspicion of infection: Gastrointestinal / Abdominal • Maximum SOFA score over 3-days following presentation (baseline subtracted) = 1 • Adjudicators disagreed on the presence of organ dysfunction • Forced label: not septic
	<ul style="list-style-type: none"> • Age range: 60 – 70 • History of meningitis and suspected chronic obstructive pulmonary disease • Enrolled in study on SIRS 2+ • Did not receive antibiotics in the ED • Admitted to hospital for encephalitis due to human herpes simplex virus • Blood cultures negative • Organ system associated with suspicion of infection: Central nervous system • Maximum SOFA score over 3-days following presentation (baseline subtracted) = 2 • Length of stay 4 days, following which patient was discharged home • Adjudicators disagreed on whether infection caused organ dysfunction • Forced label: not septic
	<ul style="list-style-type: none"> • Age range: 30 – 40 • History of pulmonary embolism and insulin resistance • Enrolled in study on SIRS 2+ • Did not receive antibiotics in the ED • Not admitted to hospital • No cultures drawn • Organ system associated with suspicion of infection: Respiratory • Maximum SOFA score over 3-days following presentation (baseline subtracted) = 2 • Adjudicators disagreed on the presence of infection • Forced label: not septic
	<ul style="list-style-type: none"> • Age range: 30 – 40 • History of chronic systolic heart failure secondary to premature coronary artery disease, lupus nephritis, and nephrolithiasis • Enrolled in study on SIRS 2+ • Received antibiotics the ED

	<ul style="list-style-type: none"> Admitted to hospital for kidney stone Urine culture positive for Escherichia coli Organ system associated with suspicion of infection: Genitourinary Maximum SOFA score over 3-days following presentation (baseline subtracted) = 1 Length of stay 3 days, following which patient was discharged home Adjudicators disagreed on the presence of organ dysfunction Forced label: septic
	<ul style="list-style-type: none"> Age range: > 80 History of cerebral vascular accident/stroke and recurrent urinary tract infections Enrolled in study on blood and urine cultures ordered Received antibiotics the ED Not admitted to hospital Blood cultures negative, urine culture positive for Escherichia coli Organ system associated with suspicion of infection: Genitourinary Maximum SOFA score over 3-days following presentation (baseline subtracted) = 2 Adjudicators disagreed on the presence of organ dysfunction Forced label: septic
Yellow Band (N = 2)	<ul style="list-style-type: none"> Age range: 50 – 60 History of pulmonary sarcoidosis Enrolled in study on SIRS 2+ and blood culture ordered Received antibiotics the ED Admitted to hospital for sepsis Blood cultures negative, urine culture positive for Staph Aureus Organ system associated with suspicion of infection: Skin and skin structure Maximum SOFA score over 3-days following presentation (baseline subtracted) = 3 Length of stay 20 days, following which patient was discharged home Adjudicators disagreed on the presence of organ dysfunction Forced label: not septic
	<ul style="list-style-type: none"> Age range: 30 – 40 History of insulin dependent diabetes mellitus Enrolled in study on SIRS 2+ Did not receive antibiotics in the ED Admitted to hospital for acute gastroenteritis No cultures drawn Organ system associated with suspicion of infection: Gastrointestinal / Abdominal Maximum SOFA score over 3-days following presentation (baseline subtracted) = 1 Length of stay 1 day, following which patient was discharged home Adjudicators disagreed on the presence of infection Forced label: septic
Red Band (N = 3)	<ul style="list-style-type: none"> Age range: > 80 History of advanced dementia, schizoaffective disorder, and depression Enrolled in study on SIRS 2+ and blood and urine cultures ordered Received antibiotics the ED Admitted to hospital for severe dehydration Blood cultures negative, urine culture positive for Escherichia coli Organ system associated with suspicion of infection: Central nervous system Maximum SOFA score over 3-days following presentation (baseline subtracted) = 7 Length of stay 3 days, following which patient was back to nursing home Adjudicators disagreed on the presence of infection Forced label: not septic
	<ul style="list-style-type: none"> Age range: 60 – 70 History of cerebral vascular accident/stroke and trach/PEG-dependent Enrolled in study on SIRS 2+ and blood culture ordered Received antibiotics the ED Admitted to hospital for sepsis Blood and urine cultures negative Organ system associated with suspicion of infection: Genitourinary Maximum SOFA score over 3-days following presentation (baseline subtracted) = 5

	<ul style="list-style-type: none"> • Length of stay 5 days, following which patient was discharged home • Adjudicators disagreed on whether infection caused organ dysfunction • Forced label: septic
	<ul style="list-style-type: none"> • Age range: 50 – 60 • History of diverticulitis • Enrolled in study on SIRS 2+ and blood culture ordered • Received antibiotics the ED • Patient left hospital against medical advice • Blood and urine cultures negative • Organ system associated with suspicion of infection Gastrointestinal / Abdominal • Maximum SOFA score over 3-days following presentation (baseline subtracted) = 1 • Adjudicators disagreed on the presence of organ dysfunction • Forced label: septic

Figure S3: Details of the patients requiring a forced adjudication.

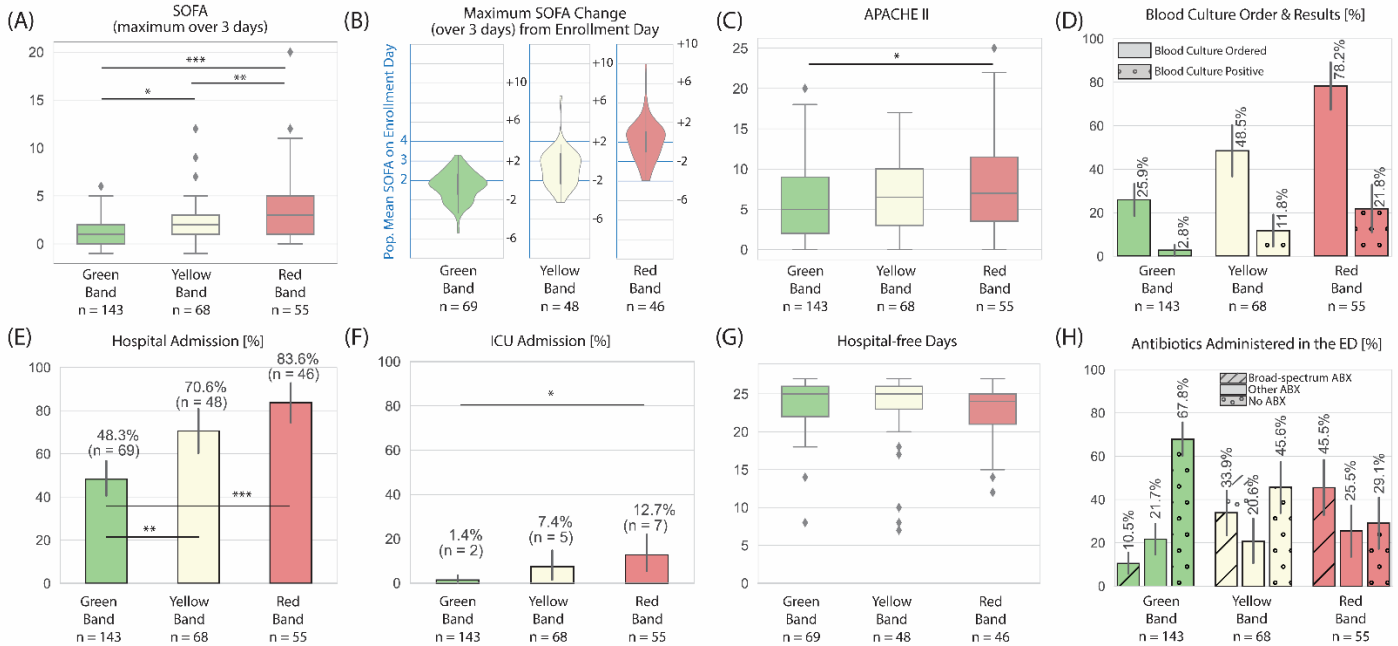


Figure S4: Trends in severity of illness scores, antibiotic and hospital utilization across ISI interpretation bands for all subjects. Hospital free days is shown for subpopulation of subjects admitted to the hospital.

Box plots: lines in the boxes, medians; the box ends, interquartile ranges (IQR); whiskers, 1.5x IQR; diamonds, outliers. Violin plots: thick line, interquartile range; thin line, 1.5x IQR; width, density plot (frequency). Bar graphs: Bars, percentages; error bars, 95% confidence intervals. p-values were obtained from an unpaired two-sample Welch's t-test (except for hospital free days, where the Mann-Whitney U was due to the non-normal distribution), with the null hypothesis that the mean of the two samples are equal. p-values reported as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. Population means for SOFA on enrollment day are shown on the left axis (blue) of Panel B. To reduce figure complexity, the significance is not shown for Panels D & H; please refer to Table 1. Percentage values shown in panels are of the total within the Band.

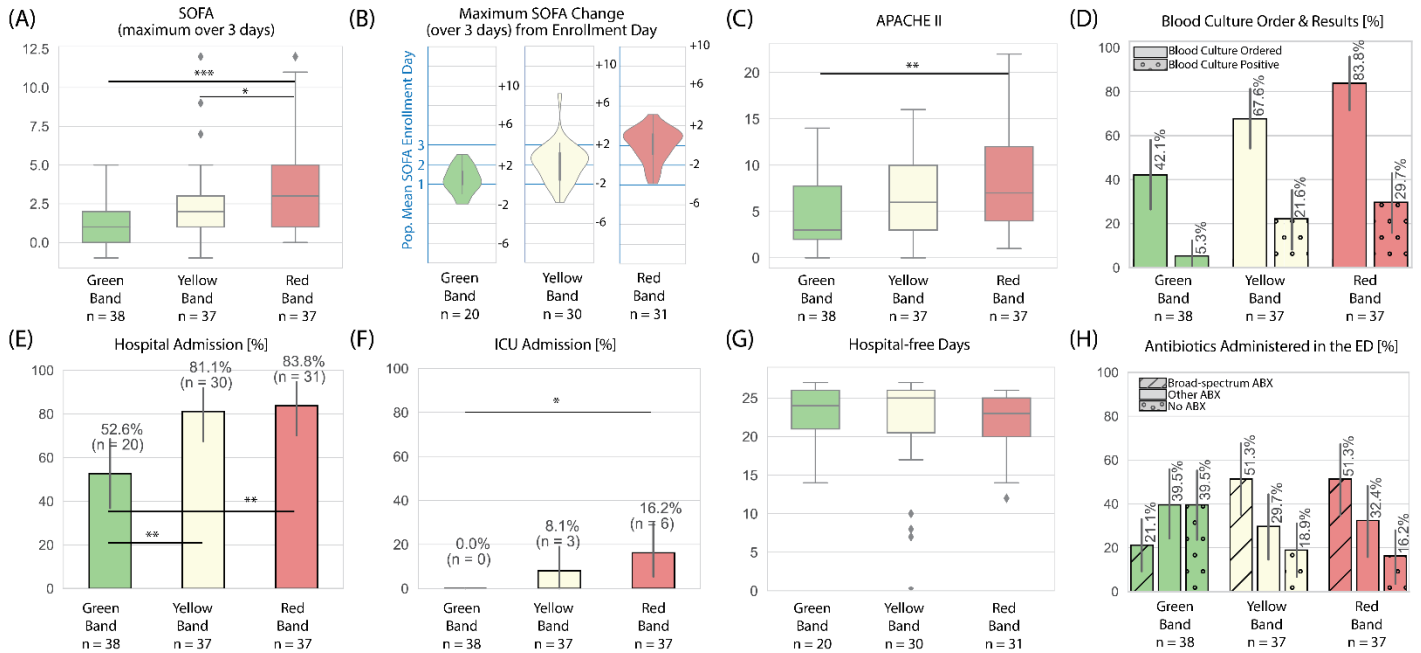


Figure S5: Trends in severity of illness scores, antibiotic and hospital utilization across ISI interpretation bands for subjects adjudicated as infected. Hospital free days is shown for subpopulation of subjects admitted to the hospital.

Box plots: lines in the boxes, medians; the box ends, interquartile ranges (IQR); whiskers, 1.5x IQR; diamonds, outliers. Violin plots: thick line, interquartile range; thin line, 1.5x IQR; width, density plot (frequency). Bar graphs: Bars, percentages; error bars, 95% confidence intervals. p-values were obtained from an unpaired two-sample Welch's t-test (except for hospital free days, where the Mann-Whitney U was due to the non-normal distribution), with the null hypothesis that the mean of the two samples are equal. p-values reported as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. Population means for SOFA on enrollment day are shown on the left axis (blue) of Panel B. To reduce figure complexity, the significance is not shown for Panels D & H. Percentage values shown in panels are of the total within the Band.

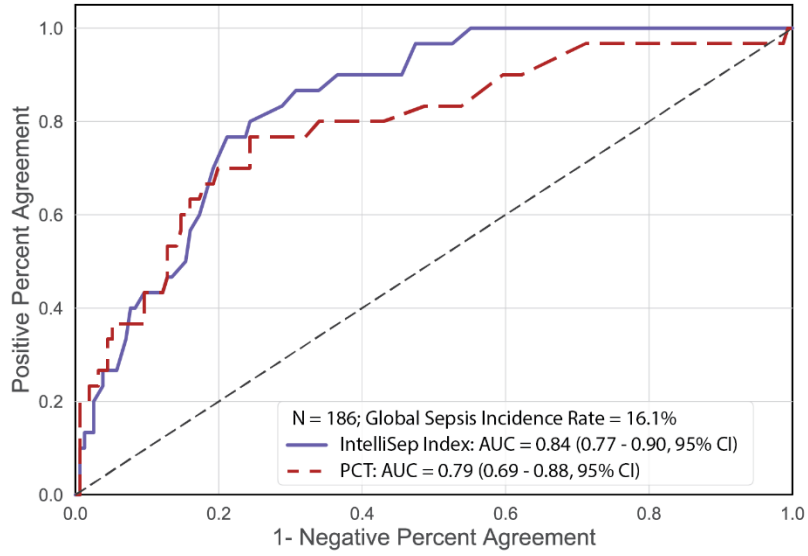


Figure S6: Comparison of the ISI with procalcitonin for the diagnosis of Sepsis-3 in the Emergency Department.

Receiving-Operating-Characteristic Curve for the ISI (solid blue line) and procalcitonin (dashed red line) for the diagnosis of sepsis. AUC, Area Under the Curve.

Table S1: Comparison of the ISI with a selection of other commonly-assessed indicators of sepsis.

Abbreviations: N, number. AUC, area under the receiver operating characteristic curve. CI, confidence interval. NPV, negative predictive value. PPV, positive predictive value. DOR, diagnostic odds ratio. SIRS, systemic inflammatory response syndrome. qSOFA, quick SOFA. WBC, white blood cell count. APACHE II, acute physiology and chronic health evaluation II. SOFA, sequential organ failure assessment.

	SIRS 2+	qSOFA	WBC (< 4 or > 12 $\times 10^3$ cells/ μ L)	Lactate	APACHE II	SOFA (day of ED presentation)	IntelliSep Index
N; Sepsis Incidence Rate in Population	255; 16.8%	255; 16.8%	255; 16.8%	129; 32.5%	255; 16.8%	255; 16.8%	255; 16.8%
AUC (95% CI)	0.60 (0.53 – 0.66)	0.74 (0.66 – 0.81)	0.58 (0.50 – 0.65)	0.61 (0.50 – 0.71)	0.71 (0.63 – 0.79)	0.82 (0.76 – 0.87)	0.84 (0.79 – 0.90)
Cutoff(s)	n/a	2	n/a	2 & 4 (mmol/L)	10	2	5.5 & 6.8
Sensitivity (95% CI)	83.7 (69.3 – 93.2)	37.2 (23.0 – 53.3)	72.1 (56.3 – 84.7)	46.2 (30.1 – 62.8)	41.9 (27.0 – 57.9)	93.0 (80.9 – 98.5)	90.7 (77.9 – 97.4)
Specificity (95% CI)	35.8 (21.0 – 50.9)	90.1 (74.9 – 96.1)	43.9 (27.0 – 57.9)	95.1 (82.7 – 99.4)	78.8 (61.4 – 88.2)	60.8 (44.4 – 75.0)	86.3 (72.1 – 94.7)
NPV (95% CI)	91.6 (77.9 – 97.4)	87.6 (72.1 – 94.7)	88.6 (74.9 – 96.1)	71.6 (49.8 – 86.2)	87.0 (72.1 – 94.7)	97.7 (87.7 – 99.9)	97.1 (81.0 – 99.9)
PPV (95% CI)	20.9 (10.0 – 36.0)	43.2 (27.0 – 57.9)	20.7 (8.4 – 33.4)	57.1 (35.3 – 74.5)	28.6 (15.3 – 43.7)	32.5 (17.2 – 46.1)	42.2 (22.4 – 61.2)
DOR	2.9	5.4	2.0	3.4	2.7	20.7	26.4