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Diagnosis of sepsis from a drop of blood by measurement of spontaneous neutrophil motility in a microfluidic assay

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Table S1. Neutrophil spontaneous migration parameters

Neutrophil migration Parameter	Abbr.	Definition
1. Spontaneously Migrating Neutrophil Count	N	The total number of neutrophils that migrate from the whole blood loading channel into the maze
2. Oscillations	O	The total number of cells that switch direction twice in a channel and migrate for more than 15 μm in each segment
3. Mean and variance Pausing	P	The total number of cells that migrate less than 5 μm for more than three frames (6 minutes)
4. Mean and variance Reverse Migration	R	The average migration distance of neutrophils towards the maze entrance [μm]
5. Average Distance Migrated	AD	The average migration distance of neutrophils in the mazes [μm]
6. Maximum migration Length	MD	The maximum migration distance of neutrophils in the linear portions of the mazes [μm]
7. Mean and Variance of Velocity	V	The average velocity of neutrophils in the mazes [$\mu\text{m}/\text{min}$]
8. Mean and Variance of Acceleration	A	The average acceleration of neutrophils in the mazes [$\mu\text{m}/\text{min}$]
9. Mean Distance of Oscillatory Migration	OD	The average migration distance of oscillating neutrophils [μm]
10. Mean and Variance Forward Migration	F	The average migration distance of neutrophils migrating away from the maze entrance [μm]
11. Mean and Variance Vertical migration Distance	VM	The average migration distance of neutrophils migrating parallel to the channels [μm]
12. Mean and Variance Horizontal Migration Distance	HM	The average migration distance of neutrophils migrating perpendicular to the channels [μm]
13. Mean and Variance of Nucleus Size	S	The average nucleus size of migrating cells

Table S2. Primary, “derivation” cohort - basic characteristics

DERIVATION COHORT			
Characteristic	Sepsis	Non-sepsis	Total
Number of patients	5	18	23
Number of blood samples	31	39	70
Age - mean	54	53.7	53.8
Male sex - no.	30	35	65
Surgical admission - no.	23	37	60
On Vasopressors - no.	11	14	25
Septic Shock - no.	10	0	10
Mechanical Ventilation - no.	26	25	51
Acute renal failure - no.	6	0	6
Death - no.	0	0	0
SIRS - no.	20	17	37
Site of Infection at the time of blood sampling			
Pneumonia	20	N/A	20
Urinary tract infection	0	N/A	0
Intraabdominal infection	0	N/A	0
Skin or soft-tissue infection	0	N/A	0
Catheter-related infection	0	N/A	0
Infection of unknown source	0	N/A	0
Intrathoracic infection	5	N/A	5
Positive blood culture - no.	0	N/A	0
Type of Bacterial Infection			
Gram-negative bacteria	13	N/A	13
Gram-positive bacteria	13	N/A	13
Polymicrobial	0	N/A	0
No pathogen isolated	0	N/A	0

Table S3. Derivation cohort - Detailed characteristics of septic patients

Pneumonia sepsis:	
Patient 1	48-year-old male presenting after being struck by a car. He had multiple injuries including TBI, skull fractures, facial fractures, multiple orthopedic fractures, and clavicle and rib fractures. He was intubated at an outside facility and transferred for further care. He was found to have a <i>Haemophilus influenzae</i> pneumonia on 9/22/15, which he was treated for. He eventually recovered enough from his injuries, and was discharged to rehab.
Patient 2	40-year-old male presenting after a high speed MVC with multiple traumatic brain injuries, pelvic fracture, rib fractures, pulmonary contusion. His fractures were repaired by orthopedic surgery. His post operative and hospital course was complicated by aspiration pneumonia, that eventually speciated to methicillin sensitive <i>Staphylococcus aureus</i> . He was treated for his pneumonia. He also had multiple DVTs and pulmonary emboli, and was anticoagulated for this. Eventually, a tracheostomy and percutaneous endoscopic gastrostomy tube were placed. He was discharged to acute rehab.
Patient 3	65-year-old male with hypertension, obesity, diabetes, hyperlipidemia, admitted with acute gallstone pancreatitis with abdominal compartment syndrome. He was empirically started on antibiotics at another facility, without confirmed infection. He had acute respiratory failure and acute kidney injury secondary to his pancreatitis. In the ICU, he was treated for streptococcal pneumonia. He was extubated on 2/14, but re-intubated the next day. Tracheostomy was performed on 2/18. He developed renal failure and hemodialysis was started on 2/19. His renal function eventually recovered. He had a prolonged hospital course but was eventually discharged to rehab.
Patient 4	49-year-old female who was admitted for repair of a thoraco-abdominal aortic aneurysm. Her operation was uncomplicated, but sputum cultures taken early in her hospital course were positive for <i>Staphylococcus aureus</i> . She was treated with clindamycin and was eventually discharged to rehab.
Intrathoracic sepsis (empyema)	
Patient 1	87-year-old male with a PMH of hypertension, hyperlipidemia, and diabetes who was transferred for an intractable right sided pleural effusion growing <i>Pseudomonas aeruginosa</i> . His course was complicated by renal failure and ongoing pleural effusions. He had additional chest tubes placed for drainage. He developed c. diff colitis during his course and was treated appropriately for this.

Table S4. Secondary, “validation” cohort - basic characteristics

VALIDATION COHORT			
Characteristic	Sepsis	Non-sepsis	Total
Number of patients	10	9	19
Number of blood samples	27	23	50
Age - mean	55.8	69.8	62.3
Male sex - no.	20	12	32
Surgical admission - no.	7	16	23
On Vasopressors - no.	12	7	19
Septic Shock - no.	16	0	16
Mechanical Ventilation - no.	18	16	34
Acute renal failure - no.	11	6	17
Death - no.	2	1	3
SIRS - no.	11	9	20
Site of Infection at the time of blood sampling			
Pneumonia	10	N/A	10
Urinary tract infection	2	N/A	2
Intraabdominal infection	7	N/A	7
Skin or soft-tissue infection	2	N/A	2
Catheter-related infection	1	N/A	1
Infection of unknown source	4	N/A	4
Positive blood culture - no.	12	N/A	12
Type of Bacterial Infection			
Gram-negative bacteria	12	N/A	12
Gram-positive bacteria	8	N/A	8
Polymicrobial	2	N/A	2
No pathogen isolated	4	N/A	4

Table S5. Validation Cohort – Detailed characteristics of septic patients

Pneumonia Sepsis	Patient History
Patient 1	50-year-old male on mechanical ventilation for respiratory failure in the setting of toxic ingestion. He developed septic shock from Methicillin-sensitive <i>Staphylococcus aureus</i> ventilator-associated pneumonia. Respiratory cultures from a bronchoalveolar lavage confirmed infection. He improved with antibiotics and supportive care.
Patient 2	55-year-old male with hypertrophic obstructive cardiomyopathy and chronic obstructive pulmonary disease, status post septal myectomy and mitral valve replacement. The patient's course was complicated by prolonged dependence upon mechanical ventilation. He subsequently developed septic shock from ventilator-associated pneumonia. Respiratory cultures from a bronchoalveolar lavage confirmed infection with <i>Enterobacter cloacae</i> . He improved with antibiotics and supportive care.
Patient 3	57-year-old male with diabetes and chronic renal insufficiency who developed septic shock from <i>Haemophilus influenzae</i> pneumonia and bacteremia, confirmed by sputum and blood cultures. His course was complicated by myocarditis with resultant cardiogenic shock, hypoxic respiratory failure, and cardiac arrest. He required aggressive respiratory and cardiovascular resuscitation with extracorporeal membrane oxygenation in the intensive care unit. He subsequently improved with antibiotics and supportive care.
Urinary Tract Infection Sepsis	
Patient 4	92-year-old male with ischemic cardiomyopathy, chronic renal insufficiency, and pulmonary hypertension, who presented with septic shock. Urine and blood cultures confirmed <i>Escherichia coli</i> infection. He progressed to multi-organ failure, and ultimately expired.
Intraabdominal Infection Sepsis	
Patient 5	48-year-old male with aortic dissection, hypertension, liver disease, chronic obstructive pulmonary disease, and diabetes, who presented with septic shock from severe colitis. Computed tomography scan of the abdomen demonstrated extensive colonic inflammation, and blood cultures confirmed infection with <i>Escherichia coli</i> . He developed end-organ failure; however, he improved with antibiotics and supportive care in the intensive care unit.
Patient 6	74-year-old male with hypertension, diabetes, and ampullary carcinoma admitted with septic shock from ascending cholangitis and hepatic abscess. Magnetic resonance imaging of the liver revealed hepatic abscess, and blood cultures confirmed infection with <i>Escherichia coli</i> . He developed profound end-organ injury; however, he subsequently improved with antibiotics and supportive care in the intensive care unit.
Patient 7	66-year-old female with venous thromboembolism and chronic heart failure who presented with septic shock from fulminant <i>Clostridium difficile</i> colitis with perforation. Computed tomography of the abdomen and pelvis revealed diffuse colonic inflammation, and she underwent emergent colectomy. Intra-operative findings demonstrated a perforated colon. Stool PCR confirmed <i>Clostridium difficile</i> infection. She presented with profound end-organ failure, required a prolonged intensive care unit stay, and ultimately improved post-operatively with antibiotics and supportive care.
Skin or soft tissue infection Sepsis	
Patient 8	56-year-old male with coronary artery disease, who presented with septic shock from right lower extremity cellulites. Blood cultures confirmed infection with Group A <i>Streptococcus</i> and Methicillin-sensitive <i>Staphylococcus aureus</i> . Blood analysis also revealed a high titer of anti-streptolysin O. He ultimately improved with antibiotics and supportive care in the intensive care unit.
Catheter-related infection Sepsis	
Patient 9	46-year-old female with a complicated history of abdominal surgeries, who presented with sepsis from infection of an indwelling central venous line. Her blood cultures confirmed infection with <i>Enterococcus faecalis</i> , <i>Streptococcus bovis</i> , <i>Klebsiella pneumoniae</i> , <i>Aeromonas species</i> , <i>Escherichia coli</i> , <i>Hafnia species</i> , <i>Candida glabrata</i> , <i>Rhodococcus V species</i> , and <i>Arthrobacter species</i> . She subsequently improved with antibiotics and supportive care.
Infection of unknown source Sepsis	
Patient 10	29-year-old male with common variable immune deficiency, status post bilateral lung transplants, and on chronic immunosuppressive therapy, presented with fevers, shortness of breath, and cough. He developed hypoxic respiratory failure. Computed tomography scan of the chest demonstrated pneumonitis, consistent with a viral or bacterial infection. Although cultures were unrevealing, he improved with antibiotics.

Table S6. Activation of neutrophil activity by cytokines.

Treatment	Conc [ng/mL]	Number of samples	Spontaneously migrating cells (N)	Oscillations (O)	Pausing (P)	Retrotaxis (R)	Distance migrated (AD)	Score
IFN-γ	1.8	10	10.8 $\pm$ 12.4	3.8 $\pm$ 5.9	5.6 $\pm$ 6.8	3.3 $\pm$ 4.1	242.3 $\pm$ 147.2	3.2 $\pm$ 3.9
	2.5	3	0 $\pm$ 0.0	-	-	-	-	0
TNF-α	0.1	10	7.2 $\pm$ 13.9	1.4 $\pm$ 3.1	3.8 $\pm$ 8.0	3.3 $\pm$ 5.9	264.6 $\pm$ 323.7	1.9 $\pm$ 3.9
	0.5	3	25.3 $\pm$ 26.1	0.7 $\pm$ 1.2	14 $\pm$ 16.1	13 $\pm$ 12.8	350.8 $\pm$ 47.1	9.4 $\pm$ 9.7
	1.0	3	2.7 $\pm$ 1.2	0.7 $\pm$ 1.2	1.3 $\pm$ 0.6	1.0 $\pm$ 1.7	173.6 $\pm$ 92.4	0.5 $\pm$ 0.3
G-SCF	0.3	10	6.7 $\pm$ 13.6	3.8 $\pm$ 9.2	4.2 $\pm$ 9.2	2.2 $\pm$ 4.5	303.3 $\pm$ 156.1	2.4 $\pm$ 5.0
	0.5	3	2.3 $\pm$ 2.3	0.3 $\pm$ 0.6	2.0 $\pm$ 1.7	0.7 $\pm$ 1.2	231.4 $\pm$ 142.6	0.7 $\pm$ 0.9
IL1-β	0.1	10	3.8 $\pm$ 3.2	1.1 $\pm$ 1.5	1.9 $\pm$ 2.6	1.4 $\pm$ 1.8	217.9 $\pm$ 193.6	1.2 $\pm$ 0.6
	1.0	3	0.3 $\pm$ 0.6	0.3 $\pm$ 0.6	0.3 $\pm$ 0.6	-	84.2 $\pm$ 145.9	0.1 $\pm$ 0.1
LPS	0.2	10	4.8 $\pm$ 5.6	1.6 $\pm$ 2.5	3.5 $\pm$ 5.0	1.3 $\pm$ 1.6	123.7 $\pm$ 101.8	0.9 $\pm$ 1.1
Healthy CONTROL		62	31.4 $\pm$ 35.0	6.3 $\pm$ 8.7	2.4 $\pm$ 3.6	12.1 $\pm$ 14.3	179.3 $\pm$ 148.1	9.15 $\pm$ 12.0
SEPSIS average		58	235.4 $\pm$ 210.1	108.8 $\pm$ 80.4	81.8 $\pm$ 128.0	106.2 $\pm$ 82.6	327.8 $\pm$ 163.3	176.4 $\pm$ 261.5

Values are: Mean $\pm$ Standard Deviation

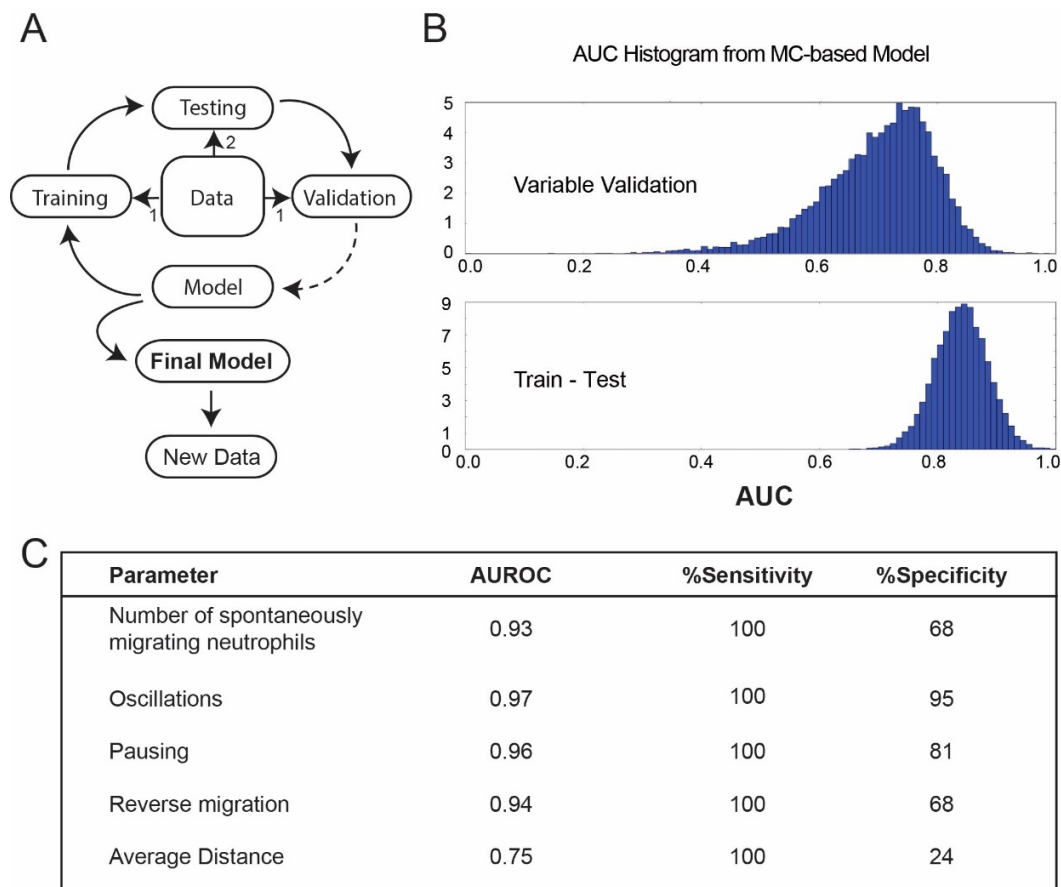


Figure S1: Identification of discriminative motility variables using machine-learning approaches. **A)** Diagram of SVM machine learning approach used. Data was split 1:2:1 for use in training, testing and validation steps to develop an optimized model. **B)** Each variable was validated independently (Variable Validation), and then in combination (Train - Test) to optimize the model. **C)** Predictive values for individual neutrophil migration parameters.

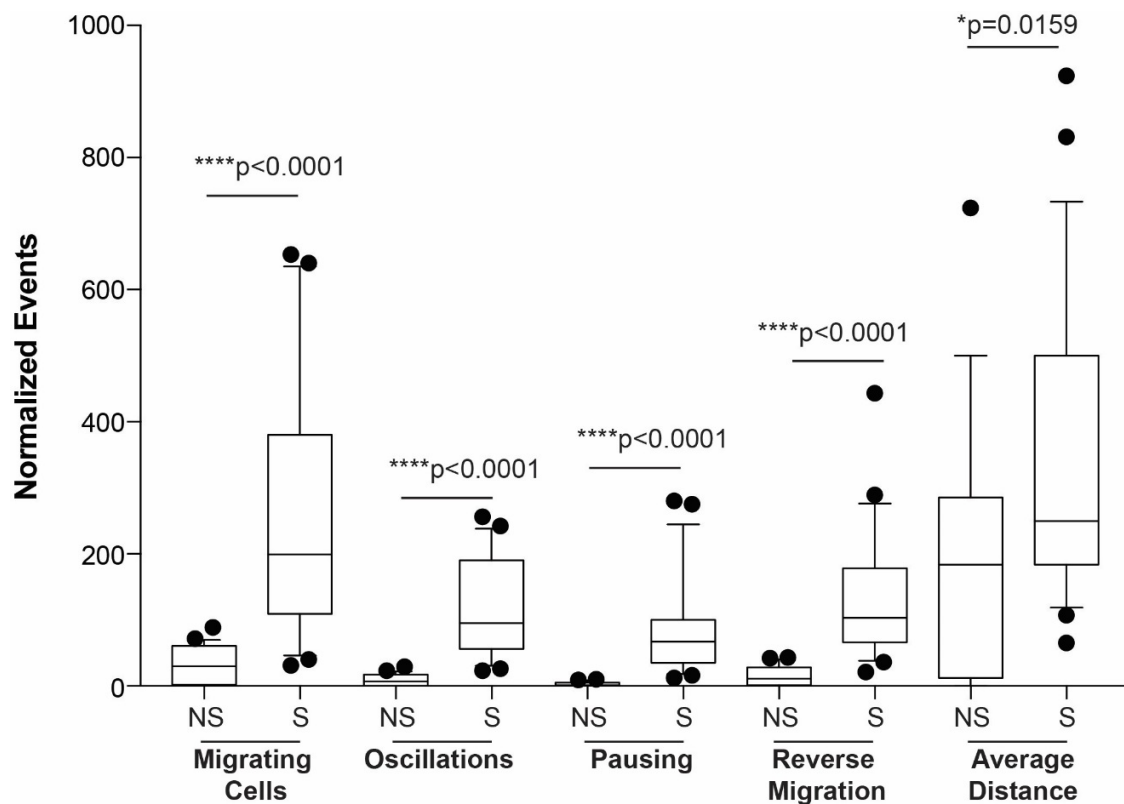


Figure S2: Detailed neutrophil behavior in secondary, “validation” cohort. Graph shows comparison of neutrophil behaviors used to calculate the Sepsis Score for Non-Septic (NS) and Septic (S) patients from the second patient cohort. Significant differences were observed for the number of migrating cells as well as oscillation and reverse migration events. Box and whisker plot: Tukey’s boxplot (see Methods). Statistics: Unpaired t-test. N = 50 samples, N = 19 patients.

Video S1. Spontaneous motility of neutrophils within a microfluidic device. Comparison of spontaneous neutrophil motility between non-sepsis (left panel) and sepsis (right panel) samples during our microfluidic assay for whole blood.