Appendix with supplementary material. This appendix was part of the submitted manuscript and has been peer reviewed. It is posted as supplied by the authors.

Supplementary Tables

Table S1. Definitions of infection in the ICU, modified from the

ISF criteria (International sepsis forum consensus conference of infection in the ICU)

	Pneumonia	Catheter-related infection	Secondary peritonitis	Skin- and soft tissue infection	Intra abdominal abscess
Microbiologically confirmed:	1. Clinically present and isolation of a pathogen; (1) a probable etiologic agent from an uncontaminated specimen e.g. blood, pleural fluid, (2) respiratory secretions of a pathogen that does not colonize the upper airways, (3) a possible pathogen in high concentrations using quantitative cultures of a lower respiratory tract sample e.g. BAL, PSB or (4) positive serology.	4. At least one peripheral positive blood culture and positive tip culture for the same pathogen.	7. Isolation of one or more microbial pathogens found in the peritoneum or the blood > 24 hrs. after a gastrointestinal perforation of the stomach, oesophagus or duodenum, or any perforation of the small bowel distal the ligament of treitz. A penetrating abdominal wound or documented perforation that is surgically repaired within 12 hrs. o fits occurrence is not sufficient evidence.	10. Isolation by culture of a microorganism from a wound or skin lesion that has drained pus, or from a skin aspirate or biopsy of the subcutaneous tissues of an erythematous skin lesion or wound.	13. Clinical, radiographic and surgical confirmation of an inflammatory collection within the peritoneal space or surrounding structures with isolation of microbial pathogens from the fluid collection. The fluid collection needs to be under sterile technique or direct surgical observation with acquisition of culture material directly from the abscess cavity or the blood.
Probable:	2. Clinically present plus (1) detection of a likely pulmonary pathogen in respiratory secretions e.g. sputum, endotracheal aspirate or (2) quantitatively cultured BAL fluid or PSB but in concentrations below diagnostic threshold or (3) presence of a negative respiratory tract culture if collected within 72 hrs. after starting a new antibiotic regimen.	5. A hub or exit site culture growing the same microorganism as peripheral blood	8. Compatible clinical illness associated with documented evidence of perforation (free air in the abdomen on radiographic studies or surgical confirmation of peritoneal inflammation in the absence of microbiologically confirmed peritonitis).	11. Clinical and laboratory evidence (such as spreading erythema or drainage of purulent material on opening surgical wounds, or leucocytosis) of the presence of a skin and soft tissue infection based on radiographic, clinical and surgical findings without microbiological confirmation.	14. The presence of an abnormal collection of fluid in the intra-abdominal contents or surrounding structures with evidence of inflammatory cells but with negative cultures from fluid accumulation or blood.
Possible:	3. Low or moderate clinical suspicion but with microbiological or serological evidence of pneumonia.	6. No positive blood culture but positive culture of tip, exit site or hub	9. Upper gastrointestinal perforation or penetrating abdominal trauma that is surgically repaired, or a	12.Clinical, laboratory or radiographic findings suggestive of a skin and soft tissue infection but with insufficient evidence to confirm	15. Clinical or radiographic evidence of an abnormal fluid accumulation within the abdominal contents or

			finding of peritoneal fluid in the absence of microbiological confirmation.	diagnosis.	surrounding structures but without microbiologic or surgical confirmation..
Endocarditis	Primary bloodstream infection	Meningitis	Urosepsis	Secondary bloodstream infection	Epiglottitis
16. Typical microorganism consistent with infective endocarditis demonstrated by two separate blood cultures or histologic examination of a vegetation or a intracardiac abscess *or* single positive blood culture for Coxiella burnetti *and* echocardiogram positive for endocarditis	17. Microorganism in blood culture, not usually regarded as skin contaminant, i.e. diphteroids, Bacillus species, Proprionebacterium species, coagulase-negative staphylococci or micrococci *or* above mentioned skin contaminants cultured from two or more blood cultures drawn separately *and* the organism cultured is not related to infection at another site.	18. Cerebrospinal fluid (CSF) with elevated white blood cells, elevated proteins and/or decreased glucose *and* isolation of a microbial pathogen from a sample of the CSF using culture, PCR, direct microscopy or antigen detection.	19. Microorganism isolated from urine and from fluid other than urine *or* radiographic, surgical or histopathological evidence of infection in the urinary tract.	20. Microorganism in blood culture ,not usually regarded as skin contaminant, i.e. diphteroids, Bacillus species, Proprionebacterium species, coagulase-negative staphylococci or micrococci. The organism cultured is related to an infection with the same organism at another site.	21 Visualisation of the supraglottal region shows inflammatory oedema of the arytenoids, aryepiglottic folds and epiglottis *and* isolation of a pathogen *or* *r*adiographic evidence of epiglottitis.

Table S2. SIRS and Sepsis classification

Modified SIRS criteria	
Patients had to meet at least three of the following four criteria:	
1	Core temperature of > 38°C (100.4°F) or < 36°C (96.8°F)
2	Heart rate of > 90 beats/min, except in patients with a medical condition known to increase the heart rate or those receiving treatment that would prevent tachycardia
3	Respiratory rate of > 20 breaths/min or a $PaCO_2$ of < 4.3 kPa (32 mm Hg) or the use of mechanical ventilation for an acute respiratory process
4	White Blood cell Count of > 12,000/mm^3 or < 4,000/mm^3
Criteria for organ dysfunction	
One or more of the following five criteria:	
1	Cardiovascular dysfunction: Arterial systolic blood pressure < 90 mm Hg or mean arterial pressure < 70 mm Hg for at least 1 hour despite adequate fluid resuscitation, adequate intravascular volume status or the use of vasopressors in an attempt to maintain a systolic blood pressure of > 90 mm Hg or a mean arterial pressure of > 70 mm Hg
2	Kidney dysfunction: Urine output < 0.5 ml/kg of body weight/hour for 2 hour, despite adequate fluid resuscitation
3	Respiratory dysfunction: Ratio of PaO_2 to FiO_2 < 27 kPa
4	Hematologic dysfunction: Platelet count < 80,000/mm^3 or to have decreased by 50 % in the 3 days preceding enrolment
5	In the case of unexplained metabolic acidosis, the pH had to be < 7.30 or the base deficit had to be > 5.0 mmol/L in association with a plasma lactate level that was > 3.0 mmol/L
Sepsis Syndrome, Severe Sepsis and Septic Shock	
	Sepsis Syndrome: At least three SIRS criteria and a possible, probable or confirmed infection, as evidenced by the ISF criteria
	Severe Sepsis: Sepsis Syndrome and at least one criteria for organ dysfunction
	Septic Shock: Sepsis Syndrome and fulfilment of the criteria for cardiovascular dysfunction

$PaCO_2$ partial pressure of arterial carbon dioxide, PaO_2 partial pressure of arterial oxygen, FiO_2 fraction of inspired oxygen and ISF international sepsis forum consensus conference of infection in the ICU.

Table S3. Number of significant positive blood cultures and other cultures.

Pathogen	Blood culture (n=56)	Other culture (n=58)
Staph aureus	5	11
Klebsiella pneumoniae	1	6
Streptococcus pneumoniae	1	5
Escherichia coli	1	4
Haemophilus influenzae	0	7
Enterobacter cloacae	0	3
Streptococcus milleri	0	2
Pseudomonas aeruginosa	0	2
Streptococcus pyogenes	0	2
Bacteroides fragilis	0	1
Serratia marcescens	0	1
Proteus mirabilis	0	1
Moraxella catarrhalis	0	1
Mycoplasma pneumoniae	0	1

Table S4 Likelihood, primary site and severity of infection.

	n = 58
Time from ICU admission to antibiotic treatment, days	2.6 (1, 4)
Likelihood of infection according to ISF	
Confirmed infection (ISF 1, 4, 7, 10, 13, 16, 17, 18, 19, 20)	38 (65.5 %)
Probable infection (ISF 2, 5, 8, 11, 14)	13 (22.4 %)
Possible infection (ISF 0, 3, 6, 9, 12, 15)	7 (12.1 %)
Primary site of infection	
Pneumonia (ISF 1, 2, 3)	44 (75.9 %)
Catheter-related infection (ISF 4, 5, 6)	1 (1.7 %)
Peritonitis (ISF 7, 8, 9)	0 (0 %)
Skin and soft-tissue infection (ISF 10, 11, 12)	4 (6.9 %)
Intraabdominal abscess (ISF 13, 14, 15)	0 (0 %)
Endocarditis (ISF 16)	1 (1.7 %)
Primary bloodstream infection (ISF 17)	0 (0 %)
Meningitis (ISF 18)	1 (1.7 %)
Urosepsis (ISF 19)	1 (1.7 %)
Unknown (ISF not applicable)	6 (10.3 %)
Microbiological findings	
Positive blood culture	8 (14 %)
Positive PBS or BAL	32 (55 %)
Positive urine culture	6 (10 %)
Positive CSF, tissue, abscess, endotracheal	28 (48 %)
Any positive culture	47 (81 %)
Gram stain negative growth in culture	26 (45 %)
Worst SIRS/sepsis severity in infected patients in ICU	
No SIRS	2 (3.4 %)
SIRS*	3 (5.2 %)
Sepsis	4 (6.9 %)
Severe sepsis	24 (41.4 %)
Septic shock	25 (43.1 %)
Worst SOFA/sepsis severity in infected patients in ICU	
<2	0 (0 %)
2-4	1 (2 %)
5-10	18 (31 %)
11-16	33 (57 %)
17-24	6 (10 %)

Vasopressor dependent & hyperlactatemia (>2 mmol/L)	31 (53 %)
Median (IQR) or n (%). *SIRS before but not during possible, probable or confirmed infection.	

Table S5. Univariable and multivariable logistic regression analysis, showing the association with having a possible, probable or confirmed bacterial infection on the day of antibiotic prescription.

		Univariable analysis		Multivariable analysis	
Explanatory variable	Obs	Odds ratio (95 % CI)	P	Odds ratio (95 % CI)	P[a]
Female gender	110	0.35 (0.14 - 0.86)	0.02	0.7 (0.01 - 0.35)	0.001
APACHE II (per point increment)	110	1.09 (1.03 - 1.15)	0.003	1.13 (1.02 - 1.25)	0.02
GI/Liver disease	110	4.00 (0.81 - 19.77)	0.09	6.6 (0.53 - 82.36)	0.14
SIRS at admission	109	3.04 (1.12 - 8.20)	0.03	5.9 (0.98 - 35.16)	0.05
Calprotectin mg/L on day of antibiotic prescription	78	1.51 (1.16 - 1.98)	0.002	2.0 (1.32 - 3.14)	0.001

[a]Variables with a $P < 0.10$ in the univariable analysis were included in the multivariable analysis. APACHE II: Acute Physiology and Chronic health Evaluation II. GI/Liver: Gastrointestinal or liver disease. SIRS: Systemic Inflammatory response Syndrome.

Sensitivity analysis; in calculations below patients with possible and probable infection were excluded (ISF 0, 3, 6, 9, 12, 15 + ISF 2, 5, 8, 11, 14).

Table S6. ROC area with 95% CI, optimal cut-off, sensitivity, specificity at the time of antibiotic prescription and one day before antibiotic prescription. In non-infected patients sampling was 48-72 hours and 24-48 hours post admission, respectively. P-value for the test of equality between the ROC area of each biomarker vs. calprotectin.

Biomarker	ROC area	Cut-off	Sensitivity	Specificity	P-value
Diagnostic value of biomarker (same day as antibiotic prescription)					
White blood cell count	0.52 (0.39 - 0.65)	10.8	42 %	74 %	0.04
Procalcitonin	0.65 (0.48 - 0.81)	0.66	73 %	58 %	0.59
C-reactive protein	0.71 (0.58 - 0.84)	133	82 %	54 %	0.96
Calprotectin	0.70 (0.57 - 0.83)	3.5	55 %	90 %	-
Prognostic value of biomarker (One day before antibiotic prescription)					
White blood cell count	0.44 (0.31 - 0.57)	8.4	67 %	41 %	0.0006
Procalcitonin	0.53 (0.35 - 0.71)	0.77	58 %	58 %	0.06
C-reactive protein	0.69 (0.50 - 0.88)	130	58 %	94 %	0.34
Calprotectin	0.82 (0.70 - 0.94)	1.6	66 %	93 %	-

Table S7. ROC area with 95 % CI, optimal cut-off, sensitivity, specificity at the time of antibiotic prescription and one day before antibiotic prescription. For a non-infected patient, the biomarker level obtained on the corresponding ICU day was selected using greedy matching. The same matched pairs were used for calculating diagnostic value and prognostic value. P-value for the test of equality between the ROC area of each biomarker vs. calprotectin.

Biomarker	ROC area	Cut-off	Sensitivity	Specificity	P-value
Diagnostic value of biomarker (same day as antibiotic prescription)					
White blood cell count	0.50 (0.38 - 0.59)	10.8	41 %	67 %	0.003
Procalcitonin	0.70 (0.58 - 0.82)	0.68	65 %	65 %	0.97
C-reactive protein	0.67 (0.59 - 0.83)	121	77 %	52 %	0.46
Calprotectin	0.70 (0.60 - 0.80)	2.8	54 %	80 %	-
Prognostic value of biomarker (One day before antibiotic prescription)					
White blood cell count	0.48 (0.31 - 0.57)	8.9	57 %	47 %	0.007
Procalcitonin	0.61 (0.48 - 0.74)	0.55	62 %	67 %	0.68
C-reactive protein	0.61 (0.50 - 0.72)	73	73 %	48 %	0.31
Calprotectin	0.68 (0.57 - 0.78)	1.9	62 %	77 %	-

Supplementary Figures

Figure S1. Peak calprotectin levels according to worst SIRS/sepsis severity during ICU stay. Tukey boxplot with dots representing outliers above 1.5 x IQR from the median.