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Blood leukocyte transcriptomes in gram-positive and gram-negative community-acquired pneumonia

Liza Pereverzeva[1], Fabrice Uhel[1], Hessel Peters Sengers[1], Joe Butler[1], Lonneke A. van Vught[1], Katie L. Burnham[2], Emma E. Davenport[2], Julian C. Knight[3], Olaf L. Cremer[4], Marcus J. Schultz[5,6,7], Marc M. J. Bonten[8,9], Brendon P. Scicluna[1,10], and Tom van der Poll[1,11]; on behalf of the MARS consortium.

Affiliations: [1]Center for Experimental and Molecular Medicine, Amsterdam University Medical Centers, location Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands; [2]Wellcome Sanger Institute, Cambridge, UK; [3]Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK; [4]Department of Intensive Care Medicine, University Medical Center Utrecht, Utrecht, the Netherlands; [5]Department of Intensive Care, Amsterdam University Medical Centers, location Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands; [6]Mahidol-Oxford Tropical Medicine Research Unit (MORU), Mahidol University, Bangkok, Thailand; [7]Nuffield Department of Medicine, University of Oxford, Oxford, UK; [8]Department of Medical Microbiology, University Medical Center Utrecht, Utrecht, the Netherlands; [9]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, the Netherlands; [10]Department of Clinical Epidemiology, Biostatistics, and Bioinformatics, Amsterdam University Medical Centers, location Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands; [11]Division of Infectious Diseases, Amsterdam University Medical Centers, location Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands.

Correspondence: Liza Pereverzeva, MD. Amsterdam UMC, location AMC, Meibergdreef 9, G2-129, 1105 AZ Amsterdam, the Netherlands. Phone: +31205666378. Mail: e.pereverzeva@amsterdamumc.nl

Summary: Outcome and host response in critically ill patients with community-acquired pneumonia are similar when caused by Gram-positive compared to Gram-negative bacteria.

Abstract

Background: Gram-positive and Gram-negative bacteria are the most common causative pathogens in community-acquired pneumonia (CAP) on the intensive care unit (ICU). The aim of this study was to determine whether the host immune response differs between Gram-positive and Gram-negative CAP upon ICU admission.

Methods: Sixteen host response biomarkers providing insight in pathophysiological mechanisms implicated in sepsis and blood leukocyte transcriptomes were analysed in patients with CAP upon ICU admission in two tertiary hospitals in the Netherlands.

Results: 309 patients with CAP with a definite or probable likelihood (determined by predefined criteria) were included. A causative pathogen was determined in 74.4% of admissions. Patients admitted with Gram-positive CAP (n = 90) were not different from those admitted with Gram-negative CAP (n = 75) regarding demographics, chronic comorbidities, severity of disease and mortality. Host response biomarkers reflective of systemic inflammation, coagulation activation and endothelial cell function, as well as blood leukocytes transcriptomes, were largely similar between Gram-positive and Gram-negative CAP. Blood leukocyte transcriptomes were also similar in Gram-positive and Gram-negative CAP in two independent validation cohorts. On a pathogen-specific level, *Streptococcus pneumoniae* and *Escherichia coli* induced the most distinct host immune response.

Conclusion: Outcome and host response are similar in critically ill patients with CAP due to Gram-positive bacteria compared to Gram-negative bacteria.

Introduction

Community-acquired pneumonia (CAP) is the leading infectious cause of death worldwide, accounting for 3 million deaths annually [1]. In high-income countries, pneumonia is the most common cause of sepsis, responsible for roughly half of all sepsis cases [2]. Despite treatment with antimicrobial therapy, 10-20% of all adult patients hospitalised with CAP require supportive care at an intensive care unit (ICU) [3, 4].

The Gram-positive bacterium *Streptococcus pneumoniae* has been reported as the most common causative pathogen of CAP [5]. Recent studies show that the global aetiology of CAP is changing. In Asia, the incidence of Gram-negative causative pathogens is increasing [6]. On the ICU, Gram-negative enteric bacteria and *Staphylococcus aureus* are more prevalent [7], and these pathogens, together with *Pseudomonas aeruginosa,* have been associated with higher mortality rates [8]. In spite of thorough microbiological testing on the ICU, the causative pathogen remains unknown in 45% of CAP cases in critically ill patients [7, 9].

Many investigations have reported on host response biomarkers in patients with CAP [10-13]. In general, these studies sought to determine the diagnostic and/or prognostic value of biomarkers with little attention to pathophysiological implications [10-13]. Numerous investigations aimed to determine the capacity of biomarkers such as C-reactive protein (CRP) and procalcitonin in discriminating between bacterial and viral respiratory tract infection. Only few studies compared the host response in CAP caused by different causative bacterial pathogens [14, 15]. These investigations only included CAP patients admitted to a general hospital ward (i.e., not ICU), reported a limited number of biomarkers and primarily focused on plasma cytokine responses. Importantly, the host response during CAP requiring intensive care is likely to be different from that in CAP patients admitted to a general hospital ward, not only because of the different spectrum of causative microorganisms [7], but also considering the

profoundly disturbed immunological and inflammatory homeostasis associated with critical illness per se [16, 17].

The aim of our study was to investigate whether the host immune response during CAP in critically ill patients varies between causative pathogens. We focused on differences between Gram-positive and Gram-negative causative bacteria, considering the more prominent role of enteric bacteria in CAP on the ICU [7, 18] and considering that lipopolysaccharide (one of the most potent biological products capable of activating immune cells) is uniquely expressed by Gram-negative bacteria [19, 20]. To this end we measured 16 biomarkers providing insight into deregulation of key host response pathways implicated in the pathogenesis of severe CAP (i.e., systemic inflammation, coagulation activation and endothelial dysfunction) and – in an unbiased way – analysed genome-wide transcriptomes in blood leukocytes.

Methods

Study design, setting and patient identification

This study was part of the "Molecular Diagnosis and Risk Stratification of Sepsis" (MARS) project, a prospective observational cohort study conducted in the ICUs of two hospitals in the Netherlands (ClinicalTrials.gov identifier NCT01905033) [21, 22]. Between January 2011 and January 2014, all patients above 18 years of age admitted with an expected length of stay longer than 24 hours were included via an opt-out method approved by the ethical committees of both participating hospitals.

For the current analysis, all consecutive patients suspected of CAP and for which the attending clinician initiated antibiotic treatment were included. Exclusion criteria were transfer to the ICU more than 48 hours after admission to the ward, a history of respiratory symptoms longer than 10 days before ICU admission, readmissions within the same hospital stay or within 30 days after discharge, and transfers from another ICU, except when on the same day of presentation to the first ICU. For every patient, the likelihood of CAP was assessed by dedicated researchers making use of all clinical, radiological and

microbiological data and categorised in a four-point scale (ascending from *none*, *possible*, *probable* to *definite*) using criteria as described in detail (supplementary table S1) [21, 23]. For the final study population, admissions with the highest likelihood of CAP, i.e. probable or definite, were selected. For the comparison of Gram-positive to Gram-negative CAP, CAP caused by mixed Gram-positive – Gram-negative infection were excluded from analysis. For the comparison of the host response in CAP due to the five most common bacteria, only mono-infections were selected.

Clinical variables

Shock was defined by the use of vasopressors (norepinephrine, epinephrine or dopamine) in a norepinephrine-equivalent dose of more than 0.1 µg/kg/min. Comorbidities were defined as specified in the online supplement. Acute kidney injury and acute respiratory distress syndrome (ARDS) were defined using strict pre-set criteria [24, 25]. ICU-acquired complications were defined when they occurred more than 48 hours after ICU admission.

Plasma biomarker measurements

Measurements were done in EDTA anticoagulated plasma collected on ICU admission as described in the online supplement.

Blood gene expression microarrays

Blood leukocyte gene profiles were determined in patients enrolled during the first 1.5 years of the study. Whole blood was drawn in PAXgene^{TM} tubes (Becton-Dickinson, Breda, the Netherlands) within 24 hours after the ICU admission and from 42 healthy controls (median age 35 years [interquartile range 30-

63]; 57% male) after having obtained written informed consent. Total RNA was isolated, processed, and hybridized to the Affymetrix Human Genome U219 96-array plate and analysed as described in the online supplement.

Validation cohorts

Blood leukocyte transcriptome data were validated in two independent cohorts [26, 27]. For details see the online supplement.

Statistical analysis

Continuous variables are presented as means ± standard deviation (SD) and were compared using Student's *t*-test or ANOVA when normally distributed, and presented as medians [interquartile ranges, IQR] and analysed using parametric Mann-Whitney *U* or Kruskal-Wallis tests when not. Categorical variables are presented as numbers (percentages) and were analysed using a Chi square or Fisher's exact test. For multiple group comparisons, post-hoc testing was done using a Dunn's Test of multiple comparisons using rank sums (continuous variables) or a pairwise test for a multi-level 2-dimensional matrix (categorical variables). Kaplan-Meier curves were used to plot 30-day survival. For the plasma biomarker outcomes of specific causative pathogens, linear regression was used with contrast dummy coding for causative pathogen categories. To meet normality assumption, log10 or Box-Cox transformation was used. Overall group difference was tested by Wald χ^2 statistics, adjusted for multiple testing with the Benjamini-Hochberg (BH) false discovery rate approach. If the overall group difference was deemed significant, each category of the causative pathogen was compared to the reference category to identify particular differences between pairs. Calculation of principal component analysis plots was done by a singular value decomposition of the centred and scaled data matrix including gene expression.

All analyses were performed in R studio version 4.0.2 (R Core Team 2020, Vienna, Austria) with packages survival (3.1-8), ggplot2 (3.3.0), prcomp, pheatmap (1.0), and limma (3.46). Nominal and multiple-comparison-adjusted *P* values < .05 were considered to be of statistical significance. For differential gene expression and pathway enrichment, BH-adjusted *P* values < .05 defined significance.

Results

Patients, microbiology and outcome

The 3-year study period entailed 954 ICU-admissions with suspected CAP (figure 1). Of these, 239 admissions (25.1%) were excluded because of a preceding stay on the ward for more than 48 hours (n=186), respiratory symptoms for more than 10 days before ICU admission (n=63), readmission within the same hospital stay or within 30 days after discharge (n=50) or transfer from another ICU (n=45). Of the 715 remaining admissions, the likelihood of CAP was classified as definite in 96 (13.4%) cases, probable in 213 (30.0%), possible in 271 (37.9%), and the diagnosis was refuted (likelihood none) in 135 (18.9%). The final cohort consisted of 213 admissions for probable CAP and 96 admissions for definite CAP.

A causative pathogen could be identified in 74.4% of these admissions (supplementary figure S1, supplementary table S2). In total, 358 pathogens were isolated; more than one causative pathogen was found in 13.6% of cases and were mostly coinfections of Gram-positive and Gram-negative bacteria (n=16), or bacteria and viruses (n=13). Gram-positive bacteria were cultured in 36.6% of cases, Gram-negative bacteria in 31.1% (supplementary table S2). *Streptococcus (S.) pneumoniae* was the most common causative pathogen (18.4%), followed by *Staphylococcus (S.) aureus* (9.8%) and *Haemophilus (H.) influenzae* (7.5%).

Patients admitted with Gram-positive bacterial CAP and those admitted with Gram-negative bacterial CAP were not different in terms of demographics and chronic comorbidities (table 1). Patients with Gram-negative CAP were more often admitted from an assisted living facility (*P*=.010). The severity of disease on ICU admission was comparable between groups, as indicated by similar APACHE IV and SOFA scores, as well as similar percentages of mechanical ventilation requirement. Patients with Gram-positive CAP tended to have shock more often (64.4% vs. 48.0%, *P*=.049). Outcomes including 30-day mortality were not different between groups (table 1, supplementary figure S2).

Plasma host response biomarkers

Plasma biomarkers reflecting aberrations in key pathways implicated in sepsis pathogenesis were measured on ICU admission in 65 patients with Gram-positive and 53 with Gram-negative bacterial CAP (figure 2, supplementary table S3). Compared to healthy controls, patients with either Gram-positive or Gram-negative bacterial CAP showed enhanced systemic inflammatory and cytokine responses (illustrated by elevated levels of CRP, interleukin (IL)-6, IL-8, IL-10 and matrix metalloproteinase (MMP)-8), activation of the coagulation system (elevated D-Dimer, prolonged prothrombin time (PT), prolonged activated partial thromboplastin time (APTT) and reduced protein C and antithrombin levels) and endothelial cell activation and dysfunction (increased soluble E-selectin, soluble intercellular adhesion molecule (ICAM)-1, fractalkine and angiopoietin-2/angiopoietin-1 ratio). These responses were similar between patients with Gram-positive or Gram-negative bacterial CAP with the exception of MMP-8 levels, which were significantly higher in Gram-positive bacterial CAP (*P*<.001). The plasma levels of tumor necrosis factor-α, IL-1β, IL-13 and interferon-γ were low or not detectable, and not different between groups (data not shown).

To obtain insight in the pathogen-specific host response in CAP, we further analysed CAP caused by one of the five most common bacterial pathogens: *S. pneumoniae* (n=49), *S. aureus* (n=22), *H. influenzae* (n=17), *Pseudomonas (P.) aeruginosa* (n=15) and *Escherichia (E.) coli* (n=13). While demographics, chronic comorbidities and severity of disease on ICU admission were comparable between patients with CAP caused by these five pathogens, patients with CAP due to *E. coli* showed an increased early mortality (ICU mortality 46.2% versus up to 18.2% for other pathogens (overall *P*=.039, supplementary table S4), and highest 30-day mortality (supplementary figure S3)). Comparison of host response biomarkers between CAP cases caused by these specific pathogens showed differences between groups with regard to IL-8, IL-10, MMP-8, soluble E-selectin, angiopoietin-2 and angiopoietin 2/1 ratio (figure 3). These differences were driven by *S. pneumoniae* and *E. coli.*

Blood leukocyte transcriptome analysis

Blood leukocyte genome-wide RNA profiles were determined on ICU admission in the subgroup of patients enrolled during the first 1.5 years of the study period (n = 74, of whom 37 with Gram-positive CAP and 37 with Gram-negative CAP, supplementary table S5). Principal component analysis revealed a large overlap in overall gene expression between Gram-positive and Gram-negative CAP (supplementary figure S4). Relative to healthy controls (n = 42), patients with either Gram-positive or Gram-negative CAP displayed strong blood transcriptome alterations, encompassing 66-69% of all genes present on the array (figure 4A). Of the altered transcriptomes, 79% were common to patients with CAP due to Gram-negative or Gram-positive bacteria (figure 4A) and this common transcriptional response showed strongly correlated gene expression fold changes (supplementary figure S5). In agreement, the top 10 most differentially regulated genes largely overlapped between Gram-positive and Gram-negative CAP (supplementary table S6). Consistent with earlier studies in CAP patients [28], pathway analysis of the common response revealed a typical overexpression of genes involved in both proinflammatory (IL-1, IL-

8, inflammasome, TREM-1 signalling) and anti-inflammatory (IL-10 signalling) innate immune responses and metabolic pathways (mitochondrial dysfunction, HIF-1α signalling), and a concomitant underexpression of genes of lymphocyte (B-cell development, Th1 and Th2 activation, T-cell receptor signalling pathways), antigen presentation and mTOR pathways (supplementary figure S6). Genes involved in those pathways are depicted in supplementary figure S7. Differential gene expression analysis of patients with CAP due to Gram-positive relative to Gram-negative bacteria revealed limited differences between groups, encompassing 74 significantly altered genes (multiple-comparison adjusted $P < .05$) (Figure 4B). Pathway analysis showed that underexpressed genes in patients with CAP due to Gram-positive as compared with Gram-negative bacteria were significantly associated with a more severe impairment of pathways linked to the adaptive immunity, especially pathways involving lymphocytes (supplementary figure S8). Overexpressed genes were not significantly associated to specific pathways.

Comparison of blood leukocyte transcriptomes of patients with CAP caused by the five most common causative pathogens (Gram-positive: *S. pneumoniae* and *S. aureus*; Gram-negative: *H. influenzae*, *P. aeruginosa* and *E. coli*) revealed marked differences with leukocyte transcriptomes from healthy controls (supplementary figure S9, supplementary figure S10, supplementary table S7). Direct comparison between causative pathogens within the Gram-negative group also disclosed some differences in blood leukocyte transcriptomes (supplementary figure S10D, supplementary table S8), but not within the Gram-positive group (supplementary figure S10C).

Validation of blood leukocyte transcriptome data

Validation of gene expression profiles in blood leukocytes from critically ill CAP patients caused by Gram-positive or Gram-negative pathogens was done in two independent cohorts (supplementary tables S9 and S10) [26, 27]. These data confirmed a large overlap in gene expression between Gram-positive and Gram-negative CAP as compared to healthy controls (figure 4C). Moreover, pathway analysis of the common response revealed underexpression of genes of lymphocyte and metabolic pathways (supplementary figure S11). However, direct comparison between the two groups did not reveal significantly altered genes (Figure 4D). The absence of significant differential gene expression of patients with CAP due to Gram-positive (n=60) relative to Gram-negative bacteria (n=35) was also found in the second validation cohort (the GAinS cohort, supplementary figure S12). Comparing leukocyte transcriptomes between individual pathogens was not possible in the validation cohorts due to the low sample size.

Discussion

The aim of the present study was to determine whether differences exist between the host response elicited by Gram-positive versus Gram-negative causative organisms in patients with CAP requiring intensive care. By measuring 16 biomarkers in the circulation and by analysing genome-wide mRNA expression profiles in blood leukocytes we show that the host response in Gram-positive and Gram-negative CAP is largely similar on admission to the ICU. Likewise, clinical presentation and mortality were comparable in patients with Gram-positive or Gram-negative CAP. On a pathogen-specific level, *S. pneumoniae* and *E. coli* induced the most distinct host immune response.

While most previous studies conducted in the ICU reported an unknown aetiology in approximately 45% of CAP patients [7], a causative pathogen was determined in 74% of our patients. This could be explained

by our strict diagnostic criteria for CAP, applied by a dedicated team of researchers that scored the likelihood of CAP making use of all relevant data collected after admission [21, 23]. This led to refutation of the diagnosis in 18.9% of suspected CAP, and the exclusion of another 271 cases in which the likelihood of CAP was only scored as possible. Similar to other studies [7, 8, 29, 30], *S. pneumoniae* was the most common causative pathogen in our ICU cohort and the proportion of other causative pathogens also was akin to earlier surveys on the ICU, making the results generalizable to other populations. Only 24 patients (7.8%) were diagnosed with viral infection and 13 patients (4.2%) with mixed viral-bacterial CAP. Of note, this study was purely observational, reflecting common clinical practice, in which only 40% of patients were tested for respiratory viruses, suggesting that viruses might have been underreported [31]. Indeed, studies in which both bacterial and viral testing was performed, systematically reported that respiratory viruses are isolated at least as often as bacteria from pneumonia patients on the ICU [3, 32, 33].

Patients with CAP caused by Gram-positive bacteria presented with similar comorbidities, disease severity and mortality rates compared to patients with Gram-negative CAP. Moreover, the evolution was similar between Gram-positive and Gram-negative bacterial CAP, with comparable lengths of hospital stay and incidence of ICU-acquired complications. We found no significant difference in the incidence of ARDS between patients with CAP due to different pathogens, neither on admission nor acquired during ICU stay. In accordance, ARDS was reported to occur in 29% of mechanically ventilated CAP patients, independent of its aetiology [29]. Previous investigations on biomarkers in CAP have mostly been performed in the emergency room or general hospital ward and have focused on their value in discriminating bacterial from viral disease and prognosis [10-13]. Our study is different in that we did not seek to evaluate biomarkers as potential diagnostic or prognostic tools, but rather to obtain insight into differences between pathophysiological mechanisms at play during Gram-negative and Gram-positive CAP upon admission to the ICU, i.e., in the context of critical illness. In a targeted approach we focused

on biomarkers reflecting aberrations in host response pathways considered to be involved in the pathogenesis of sepsis [34, 35]. As reported previously [10, 23, 36-38], CAP patients, relative to healthy subjects, presented with signs of systemic inflammation, activation of coagulation and endothelial cell dysfunction irrespective of the type of causative microorganism. Most of these responses were not different between Gram-positive and Gram-negative CAP with the exception of increased MMP-8 levels in the former group, along with a trend in higher soluble E-selectin, which was significant prior to adjustment for multiple testing. Of interest, in a previous investigation from our group, critically ill patients with CAP were reported to have higher MMP-8 and soluble E-selectin levels as compared to patients with hospital-acquired pneumonia (HAP) [23]. Considering that the proportion of Gram-positive infection was much higher in CAP than in HAP patients [23], this previous study also hints at stronger induction of MMP-8 and endothelial cell activation by Gram-positive bacteria than by Gram-negative microorganisms during pneumonia. A study with hospitalized CAP patients reported similar CRP, TNF and IL-6 levels in patients with documented Gram-positive or Gram-negative infection; only IL-8 differed between groups, with higher levels in patients with Gram-negative CAP, which was driven by infections caused by *Enterobacteriaceae*. In our cohort, we observed that *S. pneumoniae* and *E. coli* elicited the strongest systemic responses.

To analyse the host response between Gram-positive and Gram-negative CAP in the ICU in an unbiased way, we assessed the genome-wide transcriptomes in blood leukocytes. Previous studies have documented that blood leukocyte gene expression profiles are strongly altered in critically ill patients admitted to the ICU, but largely similar between different conditions such as Gram-positive and Gram-negative infections in general, community- and hospital-acquired infections and even sepsis and trauma [23, 39, 40]. In agreement, in our cohort gene expression in Gram-positive and Gram-negative bacterial CAP was largely common, characterised by an upregulated innate immune response, as well as downregulated genes related to the adaptive immunity. However, we found Gram-positive bacteria to

be associated with a more severe impairment of lymphocyte and IL-3 signalling. Interestingly, a study analysing plasma biomarkers to distinguish blood stream infections caused by different species, found IL-3 levels to be increased in patients with Gram-positive infection as compared to Gram-negative infection [41]. Of note, however, we did not confirm these differences in leukocyte transcriptomes of patients with Gram-positive versus Gram-negative CAP in two independent validation cohorts. These validations did confirm the similar gene expression pattern in blood leukocytes from patients with Gram-positive and Gram-negative bacterial CAP.

Our study has strengths and limitations. This is the first study to investigate the influence of the causative pathogen on the host immune response in ICU patients with CAP, by using a large set of host response plasma biomarkers and whole-genome blood leukocyte transcriptome analysis. Moreover, this is the first study to compare clinical characteristics and outcome based on the causative pathogen of CAP in patients admitted to the ICU. Our investigation was purely observational and therefore does not establish a causal relationship between causative pathogen and host response. Furthermore, data on the blood leukocyte transcriptiomes in CAP caused by individual microorganisms should be considered with caution due to low sample sizes.

In conclusion, critically ill patients with CAP caused by Gram-positive bacteria had similar outcomes and a largely overlapping host response as compared to CAP caused by Gram-negative bacteria.

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Tables

Table 1. Baseline characteristics and outcome of patients admitted to the intensive care unit with Gram-positive or Gram-negative bacterial community-acquired pneumonia

	Gram-positive bacterial CAP	Gram-negative bacterial CAP	*P* value
Admissions	**90**	**75**	
Demographics			
Age, years, median [IQR]	62 [47, 72]	64 [52, 74]	.37
Gender male, n (%)	58 (64.4)	47 (62.7)	.94
Body mass index, median [IQR]	24 [21, 26]	24 [22, 27]	.42
Race, white, n (%)	77 (85.6)	65 (86.7)	>.99
Medical admission, n (%)	83 (92.2)	70 (93.3)	>.99
Time between hospital presentation and ICU admission, hours, median [IQR]	15 [10, 21]	16 [12, 22]	.50
Readmission[a], n (%)	2 (2.2)	2 (2.7)	>.99
Assisted living facility, n (%)	0 (0.0)	7 (9.3)	.010
Chronic comorbidity, n (%)			
None	25 (27.8)	26 (34.7)	.43
Cardiovascular disease	31 (34.4)	15 (20.0)	.06
COPD	25 (27.8)	16 (21.3)	.44
Diabetes	12 (13.3)	10 (13.3)	>.99
Immunocompromised state	21 (23.3)	16 (21.3)	.91
Liver cirrhosis	2 (2.2)	0 (0.0)	.56
Malignancy	17 (18.9)	11 (14.7)	.61
Renal insufficiency	9 (10.0)	11 (14.7)	.50
Respiratory insufficiency	29 (32.2)	20 (26.7)	.54
Charlson comorbidity index	4 [2, 6]	4 [2, 5]	.64
Severity of disease on ICU admission			
APACHE IV Score, median [IQR]	82 [64, 108]	78 [62, 98]	.51
SOFA Total, median [IQR]	7 [6, 9]	7 [4, 9]	.09
Mechanical ventilation, n (%)	76 (84.4)	65 (86.7)	.86
Shock, n (%)	58 (64.4)	36 (48.0)	.049
Organ failure, n (%)	87 (96.7)	72 (96.0)	>.99
Acute kidney injury, n (%)	38 (42.2)	23 (30.7)	.17
Acute respiratory distress syndrome, n (%)	23 (25.6)	27 (36.0)	.20
Acute myocardial infarction, n (%)	1 (1.1)	1 (1.3)	>.99
Outcome			
Length of ICU stay, days, median [IQR]	5 [3, 9]	5 [3, 9]	.97
Length of hospital stay, days, median [IQR]	14 [7, 24]	14 [6, 28]	.86
ICU-acquired complications, n (%)			

None	77 (85.6)	61 (81.3)	.60
Acute kidney injury	8 (8.9)	7 (9.3)	>.99
Acute respiratory distress syndrome	6 (6.7)	3 (4.0)	.68
Mortality[b], n (%)			
ICU	13 (14.8)	15 (20.5)	.45
Hospital	20 (22.7)	22 (30.1)	.38
30 days	21 (23.9)	22 (30.1)	.47
60 days	25 (28.4)	28 (38.4)	.24
90 days	29 (33.0)	31 (42.5)	.28
1 year	35 (39.8)	36 (49.3)	.29

Abbreviations: APACHE, Acute Physiology and Chronic Health Evaluation; COPD, chronic obstructive pulmonary disease; ICU, intensive care unit; IQR, interquartile range; SOFA, Sequential Organ Failure Assessment.

[a] Readmissions > 30 days after hospital discharge.

[b] Mortality was calculated using the first ICU-admission for each patient; readmissions were not included in this analysis.

Figure legends

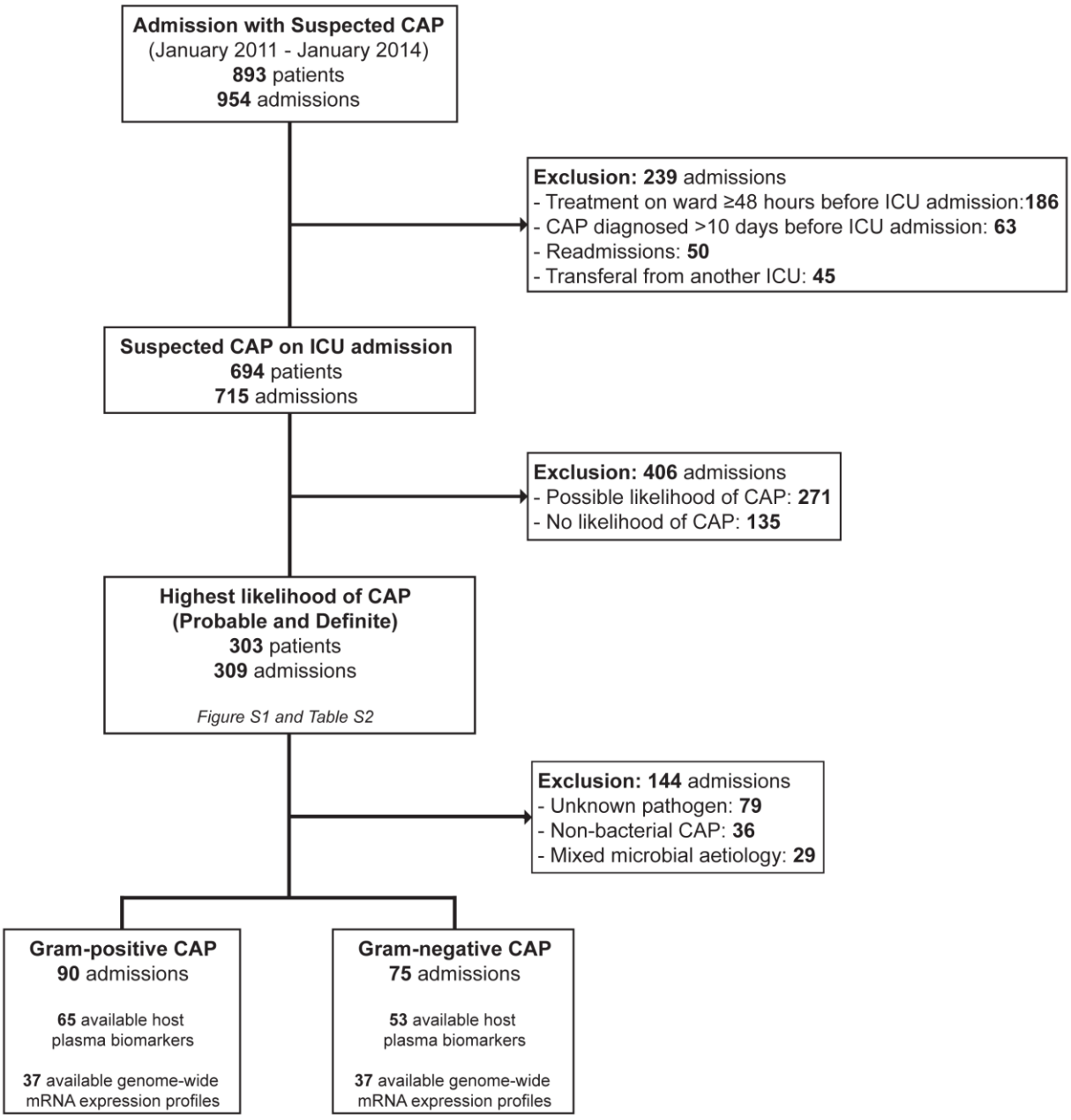

Figure 1. Flow chart of included patients and their likelihood of community-acquired pneumonia.

In 79 admissions multiple exclusion criteria were met. Abbreviations: CAP, community-acquired pneumonia; ICU, intensive care unit.

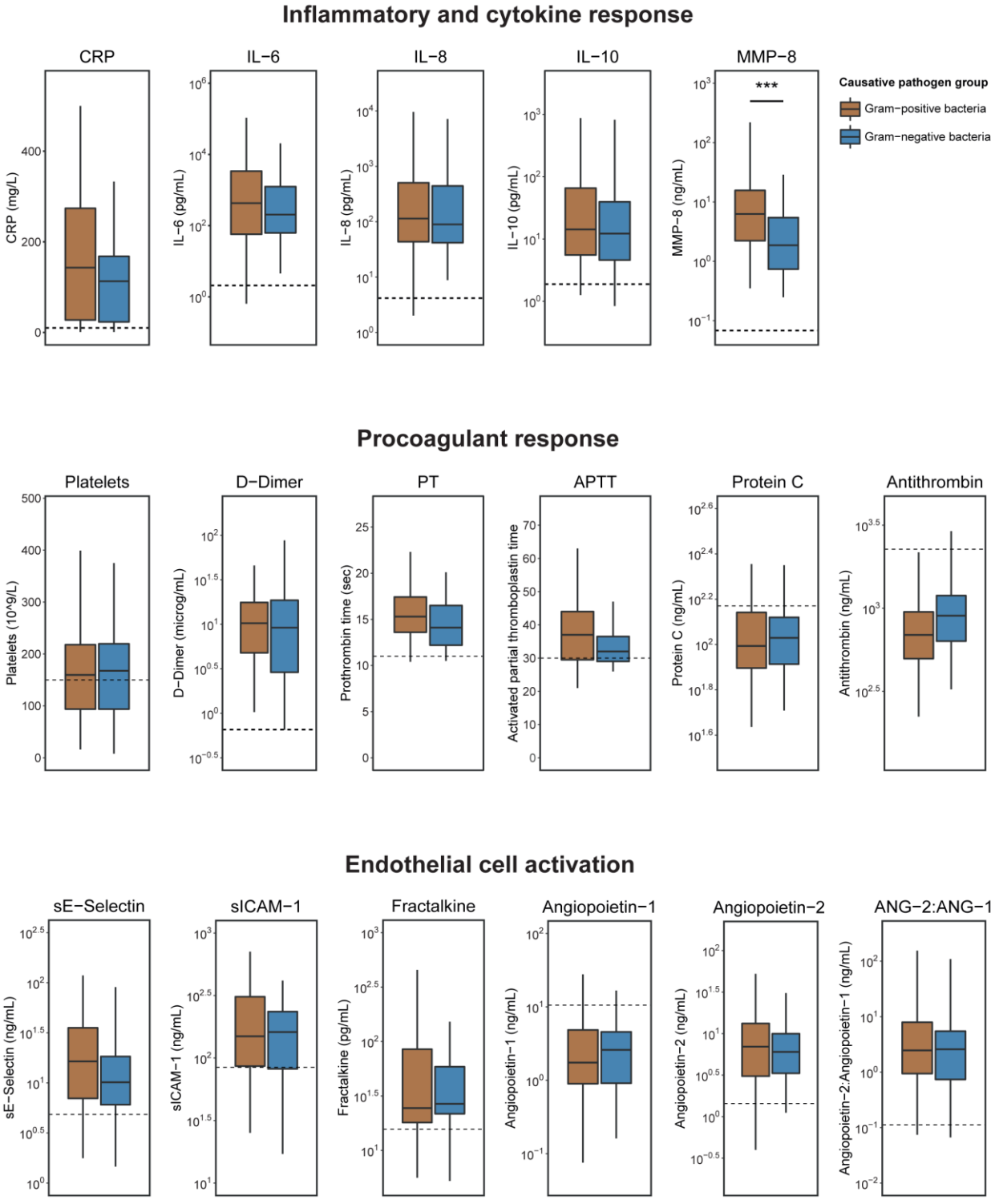
Inflammatory and cytokine response
CRP
IL−6
IL−8
IL−10
MMP−8
CRP (mg/L)
IL−6 (pg/mL)
IL−8 (pg/mL)
IL−10 (pg/mL)
MMP−8 (ng/mL)
***
Causative pathogen group
Gram−positive bacteria
Gram−negative bacteria
Procoagulant response
Platelets
D−Dimer
PT
APTT
Protein C
Antithrombin
Platelets (10^9/L)
D−Dimer (microg/mL)
Prothrombin time (sec)
Activated partial thromboplastin time
Protein C (ng/mL)
Antithrombin (ng/mL)
Endothelial cell activation
sE−Selectin
sICAM−1
Fractalkine
Angiopoietin−1
Angiopoietin−2
ANG−2:ANG−1
sE−Selectin (ng/mL)
sICAM−1 (ng/mL)
Fractalkine (pg/mL)
Angiopoietin−1 (ng/mL)
Angiopoietin−2 (ng/mL)
Angiopoietin−2:Angiopoietin−1 (ng/mL)

Figure 2. Host response plasma biomarkers in critically ill patients with Gram-positive or Gram-negative bacterial community-acquired pneumonia.

Plasma biomarkers were measured on intensive care unit admission. Parameters are classified as inflammatory responses, endothelial cell activation, and coagulation activation biomarkers. Data are expressed as box-and-whisker plots depicting the median with the lower and upper quartiles, and whiskers extending to the farthest points that are not outliers, which are defined as measurements that are within 1.5 times the interquartile range of the lowest and highest quartile. Dotted lines indicate median values obtained in 27 healthy subjects. Values in patients were all significantly different from those in healthy controls, except for platelet counts. Asterisks indicate differences between patients with community-acquired pneumonia caused by Gram-positive bacteria compared to Gram-negative bacteria analysed using Mann-Whitney *U* test, adjusted for multiple testing with the Benjamini-Hochberg false discovery rate approach. *** $P<.001$. Abbreviations: ANG, angiopoietin; APTT, activated partial thromboplastin time; CRP, C-reactive protein; sICAM, soluble intercellular adhesion molecule; IL, interleukin; MMP, matrix metalloproteinase; PT, prothrombin time.

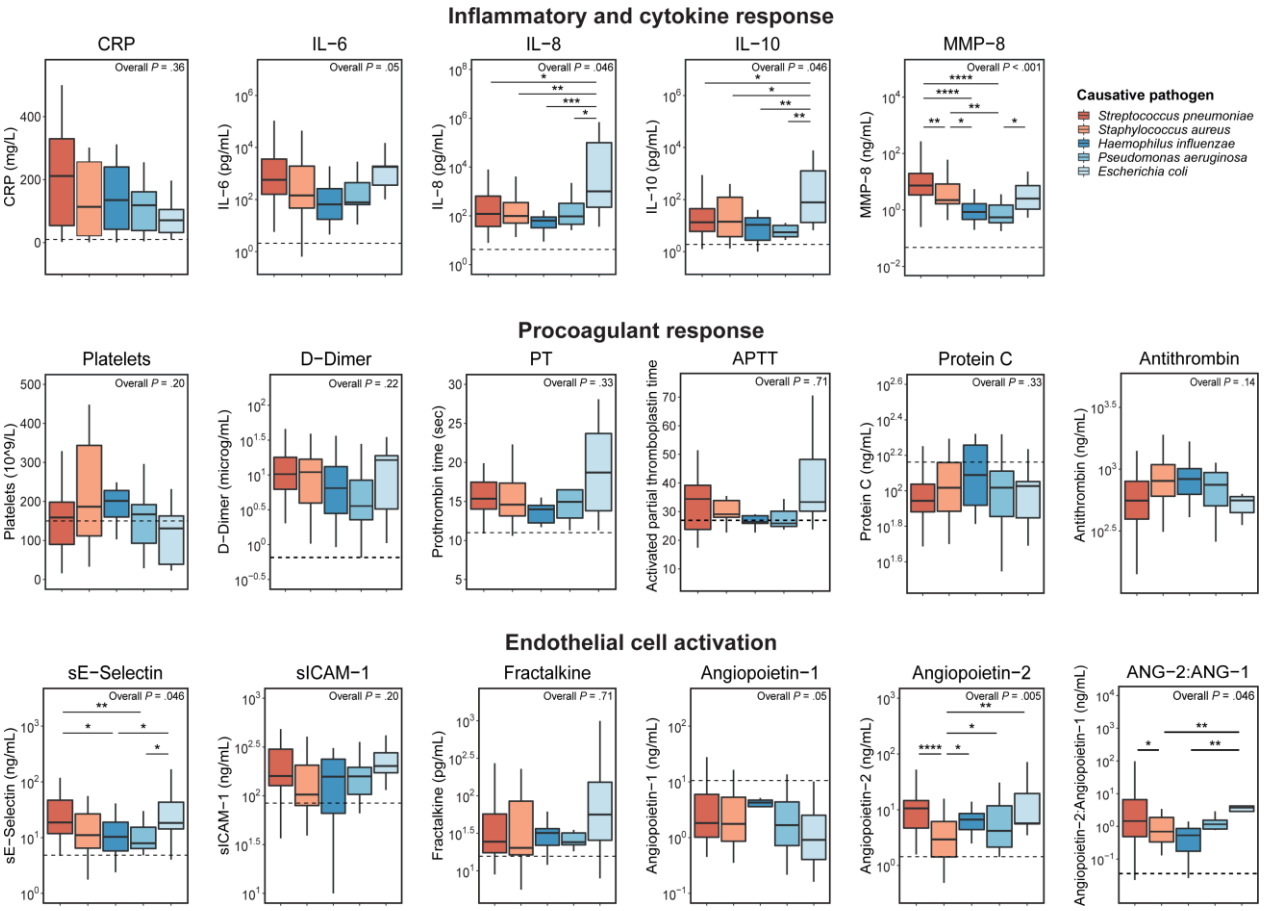

Figure 3. Host response plasma biomarkers in critically ill patients with community-acquired pneumonia caused by one of five most common bacterial causative pathogens.

Plasma biomarkers were measured on ICU admission in patients with CAP due to *S. pneumoniae* (n=33), *S. aureus* (n=18), *H. influenzae* (n=12), *P. aeruginosa* (n=10) and *E. coli* (n=9). Parameters are classified as inflammatory responses, endothelial cell activation, and coagulation activation biomarkers. Data are expressed as box-and-whisker plots depicting the median with the lower quartile, upper quartile and their 1.5 IQR as whiskers. Dotted lines indicate median values obtained in 27 healthy subjects. Overall group difference was tested by Wald χ^2 statistics, adjusted for multiple testing with the Benjamini-Hochberg false discovery rate approach. * *P*<.05, ** *P*<.01, *** *P*<.001, **** *P*<.0001 using linear

regression with contrast dummy coding for causative pathogen categories. Abbreviations: ANG, angiopoietin; APTT, activated partial thromboplastin time; CRP, C-reactive protein; sICAM, soluble intercellular adhesion molecule; IL, interleukin; MMP, matrix metalloproteinase; PT, prothrombin time.

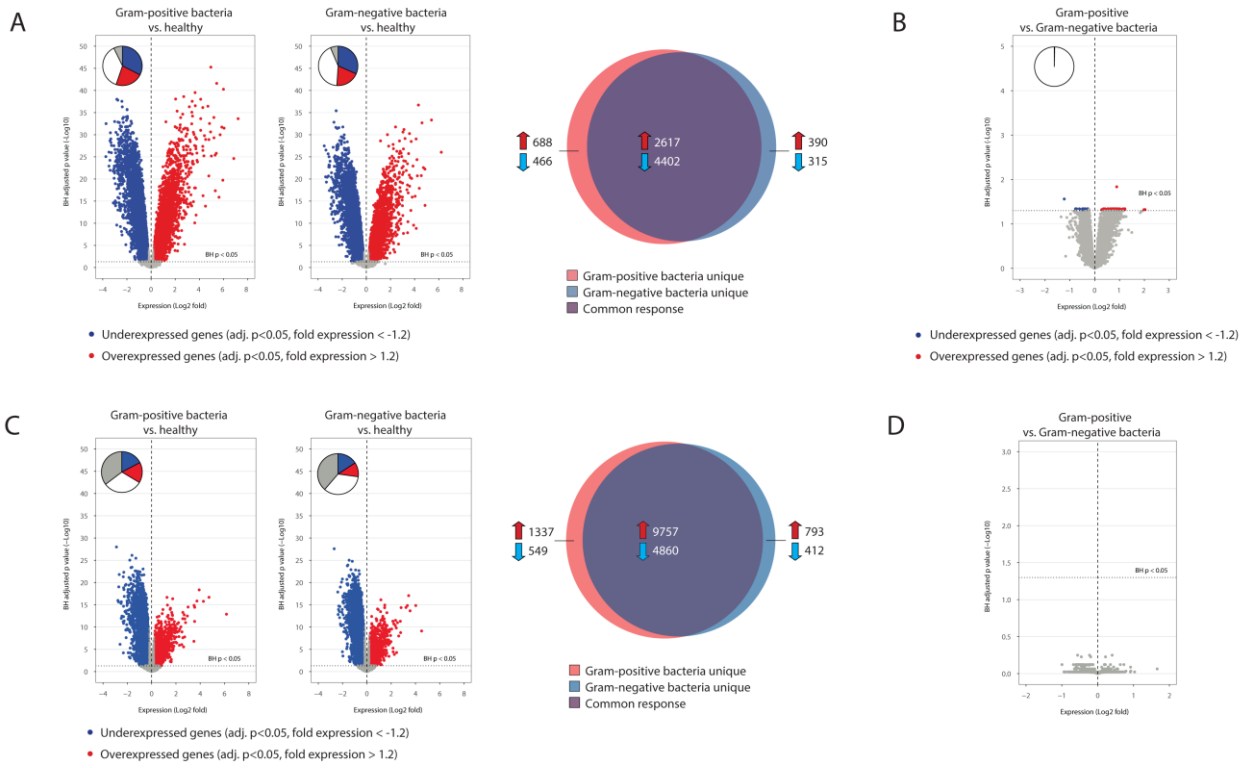

Figure 4. Leukocyte genomic responses upon admission in patients with Gram-positive or Gram-negative bacterial community-acquired pneumonia.

(A) and (C) Volcano plots illustrating the differences in leukocyte genomic responses (integrating log2 fold changes and multiple-test adjusted probabilities) between patients with community-acquired pneumonia (CAP) due to Gram-positive bacteria and healthy subjects (left), and between patients with CAP due to Gram-negative bacteria and healthy subjects (right) in the original (A) and validation (C) cohort. Blue dots represent significantly underexpressed genes (adjusted *P*<.05, fold expression <-1.2) whereas red dots represent significantly overexpressed genes (adjusted *P*<.05, fold expression >1.2) in patients relative to healthy controls. Horizontal dotted line indicates multiple-test adjusted Benjamini-Hochberg (BH) *P*<.05 threshold. Within plots, pie charts show the extent of gene expression changes: blue slices show significantly underexpressed genes (adjusted *P*<.05 and expression more than 1.2-times

decreased compared with healthy controls), red slices show significantly overexpressed genes (adjusted *P*<.05 and expression more than 1.2-time increased compared with healthy controls), and grey slices show significantly different gene expression (adjusted *P*<.05 and expression less than 1.2-time increased or decreased compared with healthy controls). Venn-Euler representation of differentially expressed genes on admission in patients with CAP due to Gram-positive or Gram-negative bacteria vs healthy subjects (adjusted *P*<.05). Red arrows denote overexpressed genes, blue arrows denote underexpressed genes. (B) and (D) Volcano plot illustrating the differences in leukocyte genomic responses on admission between patients with CAP due to Gram-positive bacteria and patients with CAP due to Gram-negative bacteria in the original (B) and validation (D) cohort. (A) Considering adjusted *P*<.05, 8173, and 7724 genes were identified as differentially expressed in patients with CAP due to Gram-positive or Gram-negative bacteria vs healthy subjects, respectively. (B) Considering adjusted *P*<.05, 74 genes were differentially expressed. Within plots, pie charts show the extent of gene expression changes in Gram-positive compared to Gram-negative pneumonia. (C) Considering adjusted *P*<.05, 16503, and 15822 genes were identified as differentially expressed in patients with CAP due to Gram-positive or Gram-negative bacteria vs healthy subjects, respectively. (D) Considering adjusted *P*<.05, no genes were differentially expressed. Within plots, pie charts show the extent of gene expression changes in Gram-positive compared to Gram-negative pneumonia. –log (Benjamini-Hochberg (BH)) *P* value, negative log10-transformed *P* value corrected for multiple comparisons.

Online Data Supplement

Blood leukocyte transcriptomes in gram-positive and gram-negative community-acquired pneumonia

Liza Pereverzeva, Fabrice Uhel, Hessel Peters Sengers, Joe Butler, Lonneke A. van Vught, Katie L. Burnham, Emma E. Davenport, Julian C. Knight, Olaf L. Cremer, Marcus J. Schultz, Marc M. J. Bonten, Brendon P. Scicluna, and Tom van der Poll; on behalf of the MARS consortium.

Supplemental Methods

Definition of demographics and comorbidities

Race was defined on admission by the nurse on call and categorised into white (Caucasian), black (African) and Asian.

Cardiovascular disease was defined as having a medical history of congestive heart failure, chronic cardiovascular disease, peripheral vascular disease or cerebrovascular disease. Immunocompromised state was defined as a medical history of immune deficiency, human immune deficiency virus (HIV) or acquired immune deficiency syndrome (AIDS), or by the use of corticosteroids (prednisone or an equivalent of prednisone of 75 mg/day during 1 week or >0.1 mg/kg/day during 3 months prior to admission) or antineoplastic medication. Malignancy was defined as a medical history of either non-metastatic solid tumor, metastatic malignancy or hematologic malignancy. Renal insufficiency was defined as a history of chronic renal insufficiency, or treatment with chronic intermittent hemodialysis or continuous ambulatory peritoneal dialysis. Respiratory insufficiency was defined as chronic obstructive pulmonary disease or respiratory insufficiency in the medical history.

Chronic comorbid conditions were scored using the Charlson comorbidity index [1].

Plasma biomarker measurements

Plasma biomarkers were measured in patients enrolled during the first 2.5 years of the study. EDTA anticoagulated blood was collected on admission and stored within 4 hours at -80°C until use. Tumor necrosis factor-α, interleukin (IL)-6, IL-8, IL-1β, IL-10, IL-13, interferon-γ, soluble E-selectin, soluble intercellular adhesion molecule-1 (ICAM-1) and fractalkine were measured by FlexSet cytometric bead array (BD Biosciences, San Jose, CA) using FACS Calibur flow cytometer (Becton Dickenson, Franklin Lakes, NJ). Matrix metalloproteinase-8 (MMP-8), angiopoietin-1, angiopoietin-2, protein C, antithrombin, (all R&D systems, Abingdon, UK) and D-dimer (Procartaplex, eBioscience, San Diego, CA) were measured by

Luminex multiplex assay using BioPlex 200 (BioRad, Hercules, CA). C-reactive protein was determined by immunoturbidimetric assay (Roche diagnostics). Prothrombin time (PT) and activated partial thromboplastin time (APPT) were determined by using a photometric method with Dade Innovin Reagent or by Dade Actin FS Activated PTT Reagent, respectively (both Siemens Healthcare Diagnostics). Normal plasma protein values were acquired from EDTA plasma from 27 age- and gender-matched healthy volunteers (from whom written informed consent was obtained), with the exception of C-reactive protein, PT and APTT for which routine laboratory reference values were used.

Microarray analysis and bioinformatics

Whole blood was drawn in PAXgeneTM tubes (Becton-Dickinson, Breda, the Netherlands) within 24 hours after ICU admission. PAXgeneTM blood samples were also collected from 42 healthy controls (median age 35 years [interquartile range 30-63]; 57% male) after obtaining written informed consent. Total RNA was extracted using the PAXgene blood mRNA kit (Qiagen, Venlo, the Netherlands), according to manufacturer's instructions. Total RNA (RNA integrity number > 6.0) was processed and hybridized to the Affymetrix Human Genome U219 96-array and scanned by using the GeneTitan instrument at the Cologne Center for Genomics (CCG, Cologne, Germany), as described by the manufacturer (Affymetrix).

Raw data scans (.CEL files) were read into the R language and environment for statistical computing (version 2.15.1; R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org/). Pre-processing and quality control were performed by using the Affy package (version 1.36.1) [2]. Array data were background corrected by robust multi-array average, quantile-normalized and summarized by median polish using the expresso function. The resultant 49,386 log-transformed probe intensities were filtered by means of a 0.5 variance cutoff using the genefilter method [3] to recover 24,646 expressed probes in at least one sample. The occurrence of non-experimental chip-effects was evaluated by means of the Surrogate Variable Analysis (R package version 3.4.0) and corrected by the empirical Bayes Method

ComBat [4, 5]. The non-normalized and normalized MARS gene expression data sets are available at the Gene Expression Omnibus public repository of NCBI under the accession number GSE65682.

The 24,646 probes were assessed for differential abundance across healthy subjects and patient samples using the limma method (version 3.36.5) [6]. Supervised analysis (comparison between pre-defined groups) was performed by moderated t-statistics. Throughout Benjamini-Hochberg (BH) multiple comparison adjusted probabilities, correcting for the 24,646 probes (false discovery rate < 5%), defined significance. Ingenuity pathway analysis (Ingenuity Systems IPA, http://www.ingenuity.com) was used to identify the association with canonical signaling pathways, stratifying genes by over- and under-expressed patterns using fold changes. The ingenuity knowledgebase was selected as reference and human species specified. All other parameters were default. Genes with a log2 fold change of >1 or <-1 involved in the top over- and under-expressed pathways respectively were depicted in heatmaps. Multiple comparison adjusted (Benjamini-Hochberg) Fisher test probabilities <.05 defined significance.

Microarray analysis validation cohorts

Firstly, transcriptomic results from a second severe CAP cohort measured during the last 1.5 years of the MARS study were used for validation. Gene transcripts from the Affymetrix Human Transcriptome Array 2.0 platform (Thermo-Fisher) are publicly available at the Gene Expression Omnibus public repository of NCBI under the accession number GSE134364, and were processed as described earlier [7]. For the current analysis, only protein coding genes were selected. In total, 22 patients with Gram-positive CAP, 23 Gram-negative CAP patients and 25 healthy controls (median age 54 years [interquartile range 40-60]; 20% male) were included in this validation cohort.

Secondly, transcriptomic data from an external CAP cohort (the GAinS cohort) [8] were used for the second validation cohort. Illumina Human-HT-12 version 4 Expression BeadChips with 47 231 probes (Illumina, San Diego, CA, USA) was used to do genome-wide transcription profiling. Gene expression data were

available through ArrayExpress (accession: E-MTAB-4421 and E-MTAB-4451), including 60 Gram-positive CAP and 35 Gram-negative CAP patients. No healthy controls were included in this cohort. Comparisons between study groups were done using the limma method (version 3.36) as described above.

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Table S1. Diagnostic criteria for community-acquired pneumonia

Likelihood	Patients presenting with respiratory symptoms within 48 hours of hospital admission
Possible	● Abnormal chest radiograph of uncertain cause **and** low clinical suspicion of pneumonia with at least one of the following symptoms/signs: a) cough b) new onset of purulent sputum or change in character of sputum c) fever or hypothermia d) leukocytosis e) elevated CRP (>30 mg/l) f) hypoxemia (pO2<60 mmHg)
Probable	● Evident new or progressive radiographic infiltrate, consolidation, cavitation, or pleural effusion **and** high clinical suspicion of pneumonia with at least two of the criteria at possible **and** one or more of the following: a) rales or dullness to percussion on physical examination of the chest b) positive rapid diagnostic tests such as Legionella or pneumococcal
Definite	● Evident new or progressive radiographic infiltrate, consolidation, cavitation, or pleural effusion **and** high clinical suspicion of pneumonia and at least two of the criteria at probable **and** isolation of a likely pulmonary pathogen, with at least one of the following symptoms/signs: a) pathogen cultured from blood b) pathogen in high concentration from a quantitative lower respiratory tract sample c) isolation of virus from or detection of viral antigen in respiratory secretions d) diagnostic single antibody titer (IgM) or fourfold increase in paired sera (IgG) for pathogen e) histopathologic evidence of pneumonia

Table S2. Causative pathogens in critically ill patients admitted with community-acquired pneumonia with probable or definite likelihood

Assigned Pathogens (n = 358)					
Gram-positive bacteria	**120**	**33.5%**	**Gram-negative bacteria**	**106**	**29.7%**
Admissions	**113**	**36.6%**	**Admissions**	**96**	**31.1%**
Streptococcus pneumoniae	66	18.4%	*Haemophilus influenzae*	27	7.5%
Staphylococcus aureus	35	9.8%	*Pseudomonas aeruginosa*	21	5.9%
Streptococcus pyogenes	6	1.7%	*Escherichia coli*	21	5.9%
Other Streptococcus species	5	1.4%	*Moraxella catarrhalis*	9	2.5%
Streptococcus agalactiae	3	0.8%	*Klebsiella pneumoniae*	8	2.2%
Coagulase-negative Staphylococcus	2	0.6%	*Enterobacter aerogenes*	3	0.8%
Streptococcus viridans	2	0.6%	*Legionella pneumophila*	3	0.8%
Enterococcus faecium	1	0.3%	*Serratia marcescens*	3	0.8%
Virus	**38**	**10.6%**	*Enterobacter cloacae*	2	0.6%
Admissions	**37**	**12.0%**	*Klebsiella oxytoca*	2	0.6%
Influenza virus (incl. H1N1)	24	6.7%	*Proteus mirabilis*	2	0.6%
Other respiratory viruses*	9	2.5%	*Acinetobacter baumannii*	1	0.3%
Human metapneumovirus	3	0.8%	*Citrobacter species*	1	0.3%
Adenovirus	1	0.3%	*Coxiella burnetti*	1	0.3%
Herpes simplex virus	1	0.3%	*Neisseria meningitides*	1	0.3%
Yeast/Fungi	**13**	**3.6%**	*Stenotrophomonas maltophilia*	1	0.3%
Admissions	**13**	**4.2%**	**Other pathogens****	**2**	**0.6%**
Aspergillus fumigatus	5	1.4%	**Admissions**	**2**	**0.6%**
Pneumocystis jirovecii	4	1.1%	**Unknown**		
Yeast not further specified	3	0.8%	**Admissions**	**79**	**25.6%**
Other Aspergilles species	1	0.3%			

* Respiratory syncytial virus 4 (1.1%), Coronavirus 2 (0.6%), Para-infuenza virus III 2 (0.6%), Rhinovirus 1 (0.3%)
** *Achromobacter xylosoxidans* 1 (0.3%), *Mycobacterium kansasii* 1 (0.3%)

Numbers and percentages depict the total number of isolated pathogens and the proportion of all isolated pathogens. Number and percentage of admissions describe the proportion of CAP caused by at least one pathogen from the correlating group within all CAP admission (n=309). In 42 (13.6%) of all CAP patients more than one pathogen was assigned as causative.

Table S3. Baseline characteristics and outcome of patients admitted with community-acquired pneumonia due to Gram-positive or Gram-negative bacteria included in the analyses of host response plasma biomarkers

Patients	Gram-positive bacterial CAP 65	Gram-negative bacterial CAP 53	P value
Demographics			
Age, years, median [IQR]	62 [46, 71]	64 [56, 72]	.38
Gender male, n (%)	42 (64.6)	33 (62.3)	.94
Body mass index, median [IQR]	24 [21, 26]	25 [22, 28]	.50
Race, white, n (%)	54 (83.1)	47 (88.7)	.55
Medical admission, n (%)	60 (92.3)	48 (90.6)	>.99
Time between hospital presentation and ICU admission, hours, median [IQR]	14 [8, 20]	19 [14, 24]	.039
Readmission[a], n (%)	2 (3.1)	1 (1.9)	>.99
Assisted living facility, n (%)	0 (0.0)	4 (7.5)	.08
Chronic comorbidity, n (%)			
None	18 (27.7)	14 (26.4)	>.99
Cardiovascular disease	25 (38.5)	10 (18.9)	.034
COPD	19 (29.2)	12 (22.6)	.55
Diabetes	8 (12.3)	9 (17.0)	.65
Immunocompromised state	15 (23.1)	13 (24.5)	>.99
Liver cirrhosis	2 (3.1)	0 (0.0)	.57
Malignancy	11 (16.9)	8 (15.1)	.99
Renal insufficiency	8 (12.3)	9 (17.0)	.65
Respiratory insufficiency	22 (33.8)	13 (24.5)	.37
Charlson comorbidity index	4 [2, 6]	4 [2, 5]	.95
Severity of disease on ICU admission			
APACHE IV Score, median [IQR]	85 [64, 109]	77 [63, 97]	.38
SOFA Total, median [IQR]	8 [6, 9]	7 [5, 9]	.27
Mechanical ventilation, n (%)	57 (87.7)	47 (88.7)	>.99
Shock, n (%)	43 (66.2)	29 (54.7)	.28
Organ failure, n (%)	63 (96.9)	52 (98.1)	>.99
Acute kidney injury, n (%)	28 (43.1)	14 (26.4)	.09
Acute respiratory distress syndrome, n (%)	17 (26.2)	23 (43.4)	.08
Acute myocardial infarction, n (%)	1 (1.5)	1 (1.9)	>.99
Outcome			
Length of ICU stay, days, median [IQR]	5 [4, 9]	6 [3, 11]	.47
Length of hospital stay, days, median [IQR]	14 [8, 24]	16 [9, 32]	.48
ICU-acquired complications, n (%)			
None	54 (83.1)	42 (79.2)	.77
Acute kidney injury	8 (12.3)	5 (9.4)	.84
Acute respiratory distress syndrome	5 (7.7)	2 (3.8)	.61
Mortality[b], n (%)			
ICU	11 (17.5)	8 (15.4)	.96
Hospital	17 (27.0)	14 (26.9)	>.99
30 days	18 (28.6)	13 (25.0)	.83
60 days	21 (33.3)	18 (34.6)	>.99
90 days	24 (38.1)	21 (40.4)	.95

1 year	28 (44.4)	26 (50.0)	.68

Abbreviations: APACHE, Acute Physiology and Chronic Health Evaluation; COPD, chronic obstructive pulmonary disease; ICU, intensive care unit; IQR, interquartile range; SOFA, Sequential Organ Failure Assessment.

[a] Readmissions > 30 days after hospital discharge.

[b] Mortality was calculated using the first ICU-admission for each patient; readmissions were not included in this analysis.

172 ***Table S4. Baseline characteristics, severity of disease scores and mortality of patients admitted to the ICU with community-acquired pneumonia***
173 ***due to the five most common bacterial causative pathogens***
174

	Streptococcus pneumoniae	*Staphylococcus aureus*	*Haemophilus influenzae*	*Pseudomonas aeruginosa*	*Escherichia coli*	P value
Patients	**49**	**22**	**17**	**15**	**13**	
Demographics						
Age, years, median [IQR]	65 [54, 72]	60 [50, 74]	68 [59, 77]	64 [48, 72]	64 [59, 76]	.84
Gender male, n (%)	32 (65.3)	13 (59.1)	9 (52.9)	10 (66.7)	9 (69.2)	.86
Body mass index, median [IQR]	23 [21, 26]	25 [23, 27]	25 [22, 27]	26 [23, 28]	24 [23, 25]	.15
Race, white, n (%)	44 (89.8)	18 (81.8)	15 (88.2)	13 (86.7)	13 (100.0)	.59
Medical admission, n (%)	47 (95.9)	19 (86.4)	17 (100.0)	13 (86.7)	13 (100.0)	.21
Time between hospital presentation and ICU admission, hours, median [IQR]	15 [12, 20]	12 [4, 21]	19 [14, 27]	19 [15, 27]	15 [8, 19]	.22
Readmission[a], n (%)	2 (4.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	.59
Assisted living facility, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	3 (20.0)	1 (7.7)	.003
Chronic comorbidity, n (%)						
None	13 (26.5)	6 (27.3)	5 (29.4)	3 (20.0)	6 (46.2)	.62
Cardiovascular disease	20 (40.8)	8 (36.4)	3 (17.6)	3 (20.0)	3 (23.1)	.28
COPD	16 (32.7)	7 (31.8)	7 (41.2)	3 (20.0)	1 (7.7)	.28
Diabetes	7 (14.3)	3 (13.6)	1 (5.9)	5 (33.3)	0 (0.0)	.10
Immunocompromised state	9 (18.4)	6 (27.3)	1 (5.9)	6 (40.0)	3 (23.1)	.18
Liver cirrhosis	2 (4.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	.59
Malignancy	9 (18.4)	5 (22.7)	1 (5.9)	4 (26.7)	3 (23.1)	.59
Renal insufficiency	5 (10.2)	4 (18.2)	2 (11.8)	2 (13.3)	3 (23.1)	.76
Respiratory insufficiency	18 (36.7)	9 (40.9)	7 (41.2)	4 (26.7)	2 (15.4)	.50
Charlson comorbidity index	5 [3, 6]	5 [2, 8]	4 [3, 5]	4 [2, 6]	4 [2, 5]	.76
Severity of disease on ICU admission						
APACHE IV Score, median [IQR]	85 [66, 105]	74 [64, 94]	77 [61, 82]	81 [66, 100]	104 [62, 124]	.39
SOFA Total, median [IQR]	7 [6, 9]	7 [6, 9]	6 [5, 8]	7 [4, 8]	8 [4, 11]	.43
Mechanical ventilation, n (%)	41 (83.7)	17 (77.3)	16 (94.1)	12 (80.0)	11 (84.6)	.70
Shock, n (%)	29 (59.2)	16 (72.7)	8 (47.1)	5 (33.3)	8 (61.5)	.16
Organ failure, n (%)	47 (95.9)	21 (95.5)	17 (100.0)	15 (100.0)	12 (92.3)	.74
Acute kidney injury, n (%)	19 (38.8)	9 (40.9)	4 (23.5)	4 (26.7)	6 (46.2)	.61
Acute respiratory distress syndrome, n (%)	10 (20.4)	6 (27.3)	5 (29.4)	3 (20.0)	6 (46.2)	.41

Acute myocardial infarction, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (6.7)	0 (0.0)	.15
Outcome						
Length of ICU stay, days, median [IQR]	5 [2, 11]†	4 [3, 6]	6 [3, 9]	7 [4, 20]†	2 [2, 3]	.004
Length of hospital stay, days, median [IQR]	13 [7, 22]†	11 [6, 15]	21 [9, 34]†	16 [12, 34]†	3 [2, 9]	<.001
ICU-acquired complications, n (%)						
None	40 (81.6)	22 (100.0)	17 (100.0)	10 (66.7)	13 (100.0)	.004
Acute kidney injury	5 (10.2)	0 (0.0)	0 (0.0)	3 (20.0)	0 (0.0)	.07
Acute respiratory distress syndrome	5 (10.2)	0 (0.0)	0 (0.0)	2 (13.3)	0 (0.0)	.18
Mortality[b], n (%)						
ICU	6 (12.8)	4 (18.2)	2 (11.8)	1 (6.7)	6 (46.2)	.039
Hospital	9 (19.1)‡	7 (31.8)	3 (17.6)	3 (20.0)	8 (61.5)	.028
30 days	9 (19.1)‡	8 (36.4)	2 (11.8)	5 (33.3)	8 (61.5)	.016
60 days	12 (25.5)	8 (36.4)	4 (23.5)	6 (40.0)	8 (61.5)	.13
90 days	14 (29.8)	9 (40.9)	4 (23.5)	7 (46.7)	8 (61.5)	.17
1 year	18 (38.3)	11 (50.0)	5 (29.4)	8 (53.3)	8 (61.5)	.33

Abbreviations: APACHE, Acute Physiology and Chronic Health Evaluation; COPD, chronic obstructive pulmonary disease; ICU, intensive care unit; IQR, interquartile range; SOFA, Sequential Organ Failure Assessment.

† Significant vs *Escherichia coli* using a Dunn's Test of multiple comparisons using rank sums.

‡ Significant vs *Escherichia coli* using a pairwise test for a multi-level 2-dimensional matrix.

[a] Readmissions > 30 days after hospital discharge.

[b] Mortality was calculated using the first ICU-admission for each patient; readmissions were not included in this analysis.

177 ***Table S5. Baseline characteristics and outcome of patients admitted to the ICU with community-acquired***
178 ***pneumonia due to Gram-positive bacteria and Gram-negative bacteria included in the blood whole***
179 ***genome analyses***
180

	Gram-positive bacterial CAP	Gram-negative bacterial CAP	P value
Patients	37	37	
Demographics			
Age, years, median [IQR]	61 [45, 67]	68 [60, 76]	.012
Gender male, n (%)	23 (62.2)	24 (64.9)	>.99
Body mass index, median [IQR]	24 [21, 26]	24 [21, 27]	.88
Race, white, n (%)	32 (86.5)	31 (83.8)	>.99
Medical admission, n (%)	34 (91.9)	34 (91.9)	>.99
Time between hospital presentation and ICU admission, hours, median [IQR]	16 [12, 22]	15 [12, 22]	.59
Readmission[a], n (%)	0 (0.0)	1 (2.7)	>.99
Assisted living facility, n (%)	0 (0.0)	2 (5.4)	.47
Chronic comorbidity, n (%)			
None	11 (29.7)	12 (32.4)	>.99
Cardiovascular disease	13 (35.1)	8 (21.6)	.30
COPD	7 (18.9)	12 (32.4)	.29
Diabetes	4 (10.8)	5 (13.5)	>.99
Immunocompromised state	10 (27.0)	8 (21.6)	.79
Liver cirrhosis	0 (0.0)	0 (0.0)	NA
Malignancy	7 (18.9)	6 (16.2)	>.99
Renal insufficiency	3 (8.1)	6 (16.2)	.48
Respiratory insufficiency	7 (18.9)	12 (32.4)	.29
Charlson comorbidity index	3 [2, 5]	4 [3, 5]	.06
Severity of disease on ICU admission			
APACHE IV Score, median [IQR]	93 [68, 110]	80 [65, 95]	.11
SOFA Total, median [IQR]	9 [7, 11]	7 [5, 9]	.11
Mechanical ventilation, n (%)	33 (89.2)	34 (91.9)	>.99
Shock, n (%)	25 (67.6)	21 (56.8)	.47
Organ failure, n (%)	35 (94.6)	37 (100.0)	.47
Acute kidney injury, n (%)	16 (43.2)	9 (24.3)	.14
Acute respiratory distress syndrome, n (%)	15 (40.5)	16 (43.2)	>.99
Acute myocardial infarction, n (%)	1 (2.7)	0 (0.0)	>.99
Outcome			
Length of ICU stay, days, median [IQR]	8 [5, 11]	6 [4, 9]	.42
Length of hospital stay, days, median [IQR]	15 [9, 24]	14 [9, 24]	.86
ICU-acquired complications, n (%)			
None	32 (86.5)	29 (78.4)	.54
Acute kidney injury	2 (5.4)	4 (10.8)	.67
Acute respiratory distress syndrome	3 (8.1)	0 (0.0)	.24
Mortality[b], n (%)			
ICU	6 (16.2)	7 (19.4)	.96
Hospital	9 (24.3)	10 (27.8)	.94
30 days	9 (24.3)	10 (27.8)	.94
60 days	9 (24.3)	13 (36.1)	.40
90 days	10 (27.0)	15 (41.7)	.28

1 year	12 (32.4)	19 (52.8)	.13

Abbreviations: APACHE, Acute Physiology and Chronic Health Evaluation; COPD, chronic obstructive pulmonary disease; ICU, intensive care unit; IQR, interquartile range; NA, not applicable; SOFA, Sequential Organ Failure Assessment.

[a] Readmissions > 30 days after hospital discharge.

[b] Mortality was calculated using the first ICU-admission for each patient; readmissions were not included in this analysis.

Table S6. Top 10 most up- and downregulated genes in blood leukocytes of patients with Gram-positive and Gram-negative CAP compared to healthy subjects

	Upregulated genes	Log2 fold change	Adjusted *P* value	Downregulated genes	Log2 fold change	Adjusted *P* value
Gram-positive CAP						
	C19orf59	4.950591	5.33E-46	***P2RY10***	-2.8336	9.79E-39
	ARG1	5.991461	5.34E-41	***FAM102A***	-2.75245	1.55E-38
	S100A12	3.64287	3.04E-40	***BCL2***	-2.41767	2.82E-38
	C5orf32	2.703758	2.47E-39	***RASGRP1***	-2.38377	4.99E-37
	S100A8	2.031046	8.61E-39	***GATA3***	-1.98835	1.96E-36
	GADD45A	4.107651	9.74E-39	***NMT2***	-1.98371	2.31E-36
	IRAK3	3.657076	2.83E-37	***FLT3LG***	-1.95729	7.04E-35
	ANXA3	4.705634	3.61E-37	***FBXL16***	-2.05564	9.69E-35
	UPP1	3.165969	4.85E-37	***EPHX2***	-2.28807	1.15E-34
	SERPINB1	2.156021	4.55E-36	***SBK1***	-1.95311	1.95E-34
Gram-negative CAP						
	C19orf59	4.31606	1.94E-37	***BCL2***	-2.50047	3.87E-36
	ARG1	5.391317	4.74E-34	***P2RY10***	-2.67998	1.49E-33
	C5orf32	2.420808	1.77E-32	***GATA3***	-1.94382	1.77E-32
	S100A12	3.116728	6.63E-32	***FAM102A***	-2.51973	2.18E-32
	GADD45A	3.571859	5.47E-31	***RASGRP1***	-2.27164	2.58E-32
	S100A8	1.747296	9.71E-31	***NMT2***	-1.83278	1.06E-30
	IRAK3	3.20263	7.20E-30	***CD96***	-2.07433	2.89E-30
	ANXA3	4.120777	9.18E-30	***FBXL16***	-1.95304	3.96E-30
	CDKN2D	1.176051	2.47E-29	***FLT3LG***	-1.8391	6.66E-30
	UPP1	2.743875	2.49E-29	***DDHD2***	-2.04022	2.23E-29

Table S7. Top 10 most up- and downregulated genes in blood leukocytes of patients with CAP due to one of the five most common bacteria compared to healthy subjects

	Upregulated genes	Log2 fold change	Adjusted *P* value	Downregulated genes	Log2 fold change	Adjusted *P* value
S.pneumoniae						
	C19orf59	5.61	3.97E-31	***FAM102A***	-2.96	1.33E-30
	GADD45A	4.27	6.98E-28	***TNFRSF25***	-2.96	9.87E-27
	ARG1	6.15	3.73E-27	***RASGRP1***	-2.51	3.01E-26
	CD177	7.80	5.61E-27	***BCL11B***	-3.67	9.36E-26
	GYG1	3.53	2.10E-26	***SIRPG***	-2.17	2.89E-25
	CLEC4D	5.41	2.36E-26	***DPP4***	-3.27	3.60E-25
	GPR84	5.70	4.13E-26	***FLT3LG***	-2.04	3.64E-25
	C5orf32	3.53	7.37E-26	***KIAA0748***	-2.95	3.75E-25
	SERPINB1	2.20	8.14E-26	***P2RY10***	-2.82	1.46E-24
	ANKRD22	5.31	8.39E-26	***BCL2***	-2.48	1.46E-24
S.aureus						
	C19orf59	4.69	7.19E-21	***P2RY10***	-2.87	3.36E-17
	IRAK3	3.77	1.06E-17	***RASGRP1***	-2.24	2.56E-16
	ARG1	5.56	3.36E-17	***BCL2***	-2.40	2.56E-16
	UPP1	3.21	3.36E-17	***FLT3LG***	-1.91	2.85E-16
	CD177	6.95	8.70E-17	***FAM102A***	-2.20	4.02E-16
	GADD45A	3.61	1.36E-16	***CD96***	-2.04	5.90E-16
	C5orf32	2.57	2.56E-16	***CDC25B***	-2.49	2.17E-15
	GYG1	3.12	2.56E-16	***SIRPG***	-1.86	6.40E-15
	ANXA3	4.48	2.85E-16	***NPIP***	-1.46	9.23E-15
	S100A12	3.45	2.85E-16	***SBK1***	-1.79	1.41E-14
H.influenzae						
	C19orf59	3.95	4.74E-20	***RASGRP1***	-2.38	4.74E-20
	ARG1	5.23	5.19E-19	***CD96***	-2.14	2.12E-19
	GADD45A	3.35	6.14E-18	***BCL2***	-2.42	3.17E-19
	MAPK14	2.72	4.91E-17	***P2RY10***	-2.73	4.19E-19
	PGS1	3.61	8.46E-17	***FAM102A***	-2.15	2.67E-18
	CDKN2D	1.22	8.88E-17	***PRKCQ***	-2.52	5.82E-18
	C5orf32	2.80	1.77E-16	***NLRC3***	-2.48	8.83E-18
	CD59	2.56	1.77E-16	***KIAA0748***	-2.55	2.78E-17
	ANXA3	3.91	1.98E-16	***TNFRSF25***	-2.33	5.24E-17
	CD177	5.81	2.41E-16	***SBK1***	-1.79	5.48E-17
P.aeruginosa						
	C5orf32	2.71	7.72E-08	***FAM102A***	-2.61	1.17E-11

	C19orf59	3.02	1.86E-07	***TNFRSF25***	-2.75	3.24E-10
	ADAMTS15	0.88	1.86E-07	***P2RY10***	-2.88	3.24E-10
	CD59	2.35	2.37E-07	***BCL2***	-2.49	5.06E-10
	TMED8	1.15	3.07E-07	***NELL2***	-3.18	8.75E-10
	GADD45A	2.78	3.44E-07	***GPR183***	-3.24	2.12E-09
	ZDHHC3	1.71	3.91E-07	***RASGRP1***	-2.21	2.12E-09
	UBTD1	1.14	6.86E-07	***CD96***	-2.05	2.69E-09
	UPP1	2.30	8.00E-07	***STMN3***	-2.64	3.02E-09
	MAP1LC3B	1.36	9.39E-07	***FBXL16***	-2.12	3.02E-09
E.coli						
	C19orf59	6.04	4.10E-17	***FAM102A***	-2.76	9.48E-14
	CD59	3.61	9.48E-14	***P2RY10***	-3.10	1.05E-12
	GADD45A	4.33	1.04E-13	***RASGRP1***	-2.46	3.17E-12
	ANXA3	5.42	1.72E-13	***NMT2***	-2.29	4.65E-12
	CLEC4D	5.76	1.72E-13	***LFNG***	-2.34	4.65E-12
	C5orf32	3.76	4.39E-13	***SKAP1***	-3.21	4.68E-12
	GYG1	3.66	4.39E-13	***HLA-DPA1***	-3.49	1.09E-11
	CEACAM1	4.66	4.39E-13	***KIAA0748***	-2.93	1.09E-11
	UGCG	3.85	1.05E-12	***SIRPG***	-2.13	1.16E-11
	ARG1	5.94	1.10E-12	***CDC25B***	-2.77	1.20E-11

Table S8. Top 10 most up- and downregulated genes in blood leukocytes of patients with CAP due to the five most common bacteria compared within the Gram-positive and Gram-negative bacterial group

	Upregulated genes	Log2 fold change	Adjusted *P* value	Downregulated genes	Log2 fold change	Adjusted *P* value
H.influenzae* vs. *P.aeruginosa						
	NARF	1.248352	0.003831	***APITD1***	-1.16124	0.000713
	SNX18	1.604487	0.020707	***ADAMTS15***	-0.7192	0.004983
	CCNY	1.305219	0.020707	***PTPLA***	-0.97336	0.005281
	GALNT1	1.292478	0.020707	***TRIM14***	-0.93863	0.005281
	GMFG	1.083433	0.020707	***PSMB5***	-0.93212	0.012188
	NARF	0.954502	0.025966	***CDKL1***	-1.09868	0.013673
	ACTR2	1.357769	0.026385	***CMKLR1***	-0.8289	0.013673
	KPNA1	1.138626	0.026385	***FXYD1***	-0.69749	0.013673
	RHOQ	1.630425	0.026641	***C4BPA***	-2.03756	0.020707
	H3F3B	1.284211	0.026641	***HIRIP3***	-0.92527	0.020707
H.influenzae* vs. *E.coli						
	YY1	1.416466	0.002062	***CEACAM1***	-2.44916	0.000241
	MPZL1	1.85388	0.002332	***WSB2***	-1.41197	0.000518
	SULF2	2.82953	0.00328	***TCTEX1D1***	-1.67483	0.000799
	OSBPL1A	2.161853	0.003763	***HNRPLL***	-1.66878	0.000799
	YY1	1.097573	0.003763	***BCAP29***	-1.15272	0.000799
	EIF2C2	1.256294	0.006181	***RMI1***	-0.89676	0.000799
	CNTNAP3	1.358175	0.006996	***COCH***	-1.60216	0.001193
	IDS	1.177313	0.007028	***CD300LF***	-1.38788	0.001193
	FOXJ2	0.909103	0.007028	***AMPD3***	-1.11426	0.001193
	TREM1	2.827085	0.007403	***C9orf46***	-1.42772	0.001282
P.aeruginosa* vs. *E.coli						
	ADAMTS15	1.024941	0.000325	***IDI1***	-4.07853	0.000451
	HIC2	1.196938	0.000697	***CEACAM1***	-3.4823	0.000451
	C2CD4C	1.034213	0.000832	***CD58***	-2.88484	0.000451
	YJEFN3	1.119453	0.001038	***CCPG1***	-2.82082	0.000451
	BBC3	1.171342	0.001161	***ATP6V1C1***	-2.73536	0.000451
	GNAI2	1.078061	0.001161	***RAB27A***	-2.61184	0.000451
	BTN2A3	1.043968	0.001161	***GPR160***	-3.06828	0.000501
	EGR3	0.992362	0.001161	***AZIN1***	-2.11388	0.000501
	MT1M	1.274148	0.001316	***C4orf3***	-1.96762	0.000501
	APITD1	1.117652	0.00137	***ACN9***	-2.33047	0.000521

Table S9. Baseline characteristics and outcome of patients admitted to the ICU with community-acquired pneumonia due to Gram-positive bacteria and Gram-negative bacteria included in the internal validation cohort of blood whole genome transcriptomic analyses

	Gram-positive bacterial CAP	Gram-negative bacterial CAP	P value
Patients	22	23	
Demographics			
Age, years, median [IQR]	63 [52, 72]	60 [46, 72]	.63
Gender male, n (%)	16 (72.7)	15 (65.2)	.82
Body mass index, median [IQR]	24 [21, 27]	25 [24, 28]	.15
Race, white, n (%)	17 (77.3)	20 (87.0)	.65
Medical admission, n (%)	21 (95.5)	21 (91.3)	>.99
Time between hospital presentation and ICU admission, hours, median [IQR]	1 (4.5)	1 (4.3)	>.99
Readmission[a], n (%)	15 [13, 21]	18 [13, 25]	.64
Assisted living facility, n (%)	0 (0.0)	4 (17.4)	.13
Chronic comorbidity, n (%)			
None	2 (9.1)	10 (43.5)	.023
Cardiovascular disease	8 (36.4)	6 (26.1)	.67
COPD	9 (40.9)	2 (8.7)	.03
Diabetes	4 (18.2)	3 (13.0)	.95
Immunocompromised state	6 (27.3)	4 (17.4)	.66
Liver cirrhosis	2 (9.1)	0 (0.0)	.45
Malignancy	6 (27.3)	2 (8.7)	.22
Renal insufficiency	3 (13.6)	2 (8.7)	.96
Respiratory insufficiency	11 (50.0)	4 (17.4)	.045
Charlson comorbidity index	6 [4, 8]	4 [1, 5]	.016
Severity of disease on ICU admission			
APACHE IV Score, median [IQR]	76 [66, 90]	74 [60, 98]	.71
SOFA Total, median [IQR]	7 [5, 8]	5 [3, 7]	.19
Mechanical ventilation, n (%)	17 (77.3)	18 (78.3)	>.99
Shock, n (%)	13 (59.1)	8 (34.8)	.18
Organ failure, n (%)	21 (95.5)	21 (91.3)	>.99
Acute kidney injury, n (%)	9 (40.9)	5 (21.7)	.29
Acute respiratory distress syndrome, n (%)	2 (9.1)	6 (26.1)	.27
Acute myocardial infarction, n (%)	0 (0.0)	1 (4.3)	>.99
Outcome			
Length of ICU stay, days, median [IQR]	4 [2, 8]	5 [3, 8]	.52
Length of hospital stay, days, median [IQR]	10 [7, 25]	15 [8, 34]	.41
ICU-acquired complications, n (%)			
None	18 (81.8)	19 (82.6)	>.99
Acute kidney injury	3 (13.6)	1 (4.3)	.57
Acute respiratory distress syndrome	2 (9.1)	3 (13.0)	>.99
Mortality[b], n (%)			
ICU	2 (9.5)	2 (9.1)	>.99
Hospital	5 (23.8)	4 (18.2)	.94
30 days	4 (19.0)	4 (18.2)	>.99
60 days	6 (28.6)	7 (31.8)	>.99
90 days	7 (33.3)	7 (31.8)	>.99

1 year	10 (47.6)	7 (31.8)	.46

Abbreviations: APACHE, Acute Physiology and Chronic Health Evaluation; COPD, chronic obstructive pulmonary disease; ICU, intensive care unit; IQR, interquartile range; NA, not applicable; SOFA, Sequential Organ Failure Assessment.

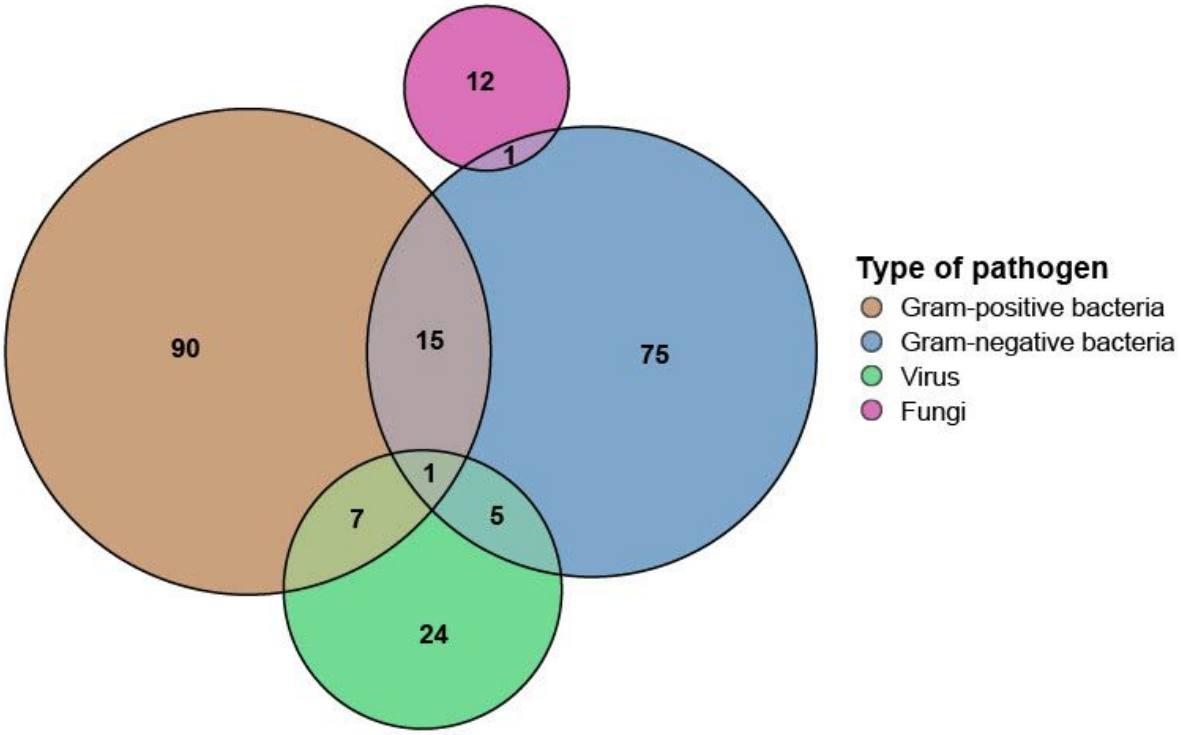
[a] Readmissions > 30 days after hospital discharge.

[b] Mortality was calculated using the first ICU-admission for each patient; readmissions were not included in this analysis.

Table S10. Baseline characteristics and outcome of patients admitted to the ICU with community-acquired pneumonia due to Gram-positive bacteria and Gram-negative bacteria included in the external validation cohort (the GAinS cohort) of blood whole genome transcriptomic analyses

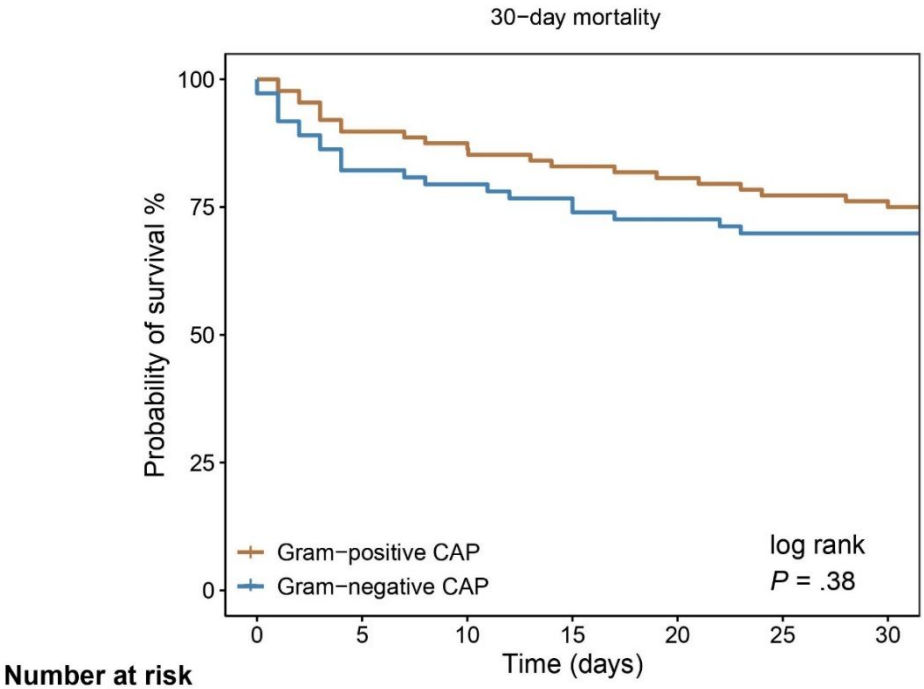
	Gram-positive bacterial CAP	**Gram-negative bacterial CAP**	**P value**
Patients	60	35	
Demographics			
Age, years, median [IQR]	61 [51, 74]	64 [56, 74]	.37
Gender male, n (%)	32 (53)	23 (66)	.34
Severity of disease on ICU admission			
APACHE II Score, median [IQR]	20 [14, 24]	19 [15, 26]	.99
SOFA Total, median [IQR]	7 [4, 11]	8 [4, 11]	.90
Mechanical ventilation, n (%)	50 (83)	28 (80)	.90
Outcome			
Length of ICU stay, days, median [IQR]	8 [3, 14]	10 [5, 19]	.31
Mortality, n (%)			
28 days	20 (33)	8 (23)	.39
6 months	21 (35)	9 (26)	.48

Figure S1. Causative pathogens in critically ill patients admitted with culture proven community-acquired pneumonia grouped by microorganism type



Venn-Euler diagram representing the number of patients in whom a specific pathogen was detected. A total of 279 pathogens were isolated in 230 of 309 patients admitted with a community-acquired pneumonia with a definite or probable likelihood. The number of patients in whom multiple pathogens were detected are depicted in the overlapping areas.

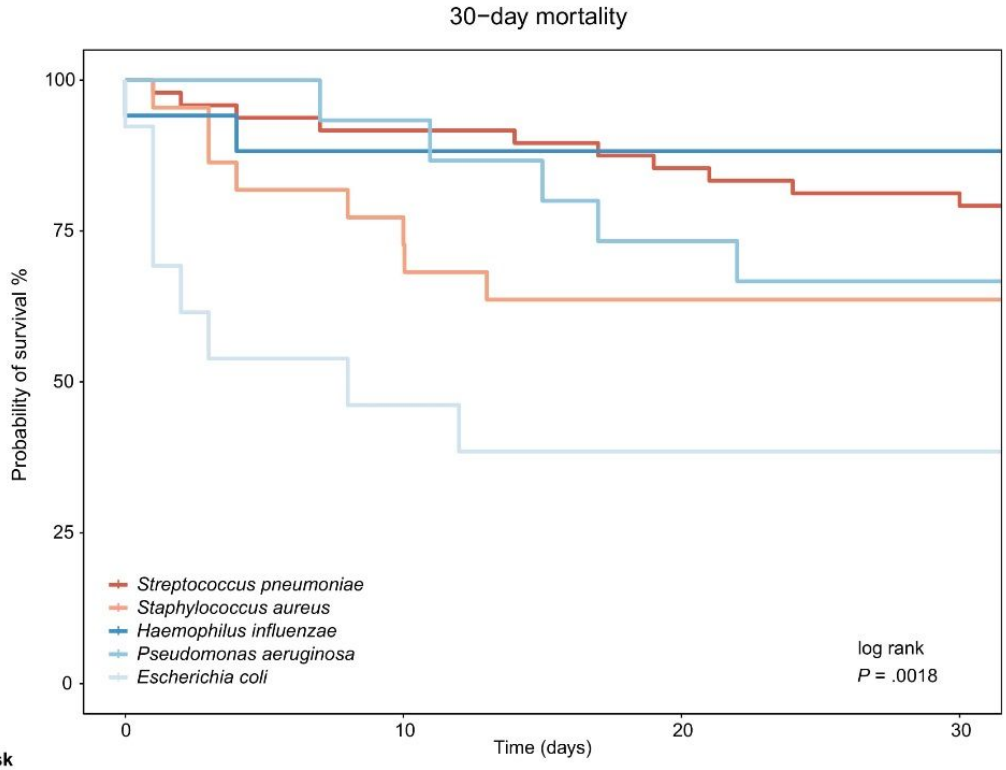
Figure S2. Kaplan-Meier 30-day survival plot of patients admitted with community-acquired pneumonia due to Gram-positive or Gram-negative bacteria



Number at risk

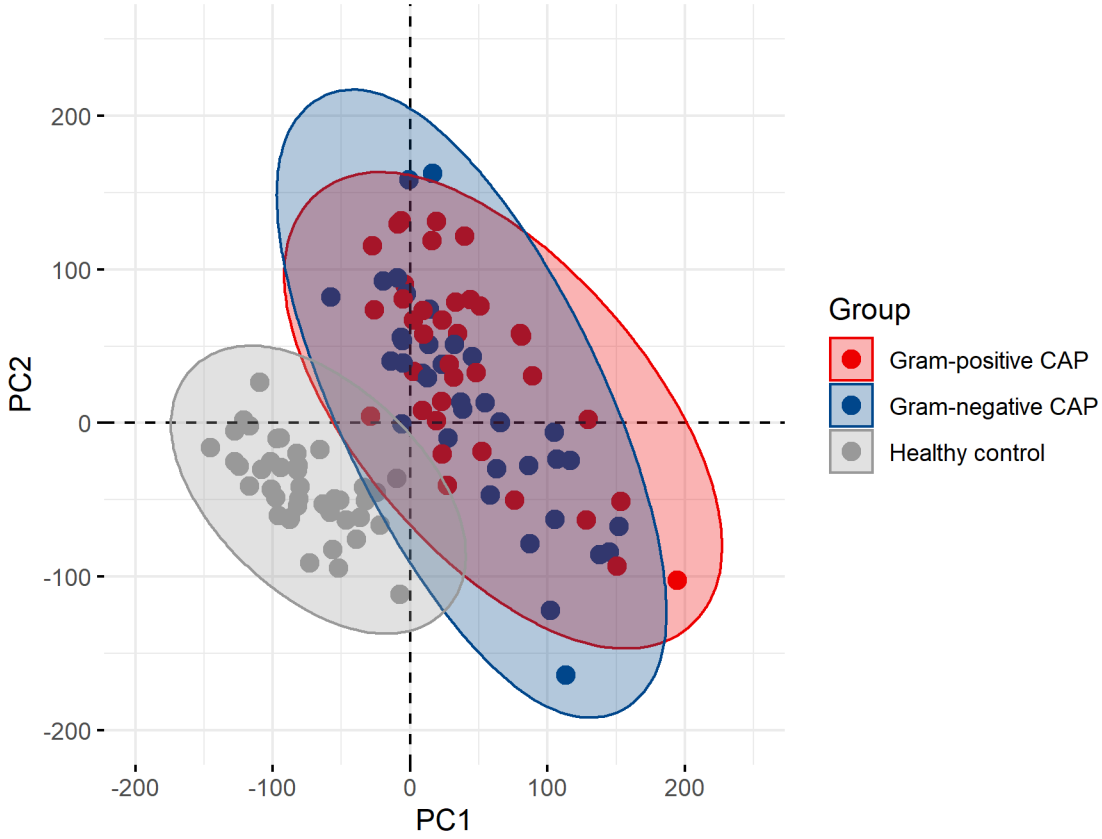
Gram−positive CAP	88	79	77	73	71	68	67
Gram−negative CAP	73	60	58	56	53	51	51

Figure S3. Kaplan-Meier 30-day survival plot of patients admitted with community-acquired pneumonia due to the five most common bacterial causative pathogens



Number at risk				
Streptococcus pneumoniae	48	44	41	39
Staphylococcus aureus	22	17	14	14
Haemophilus influenzae	17	15	15	15
Pseudomonas aeruginosa	15	14	11	10
Escherichia coli	13	6	5	5

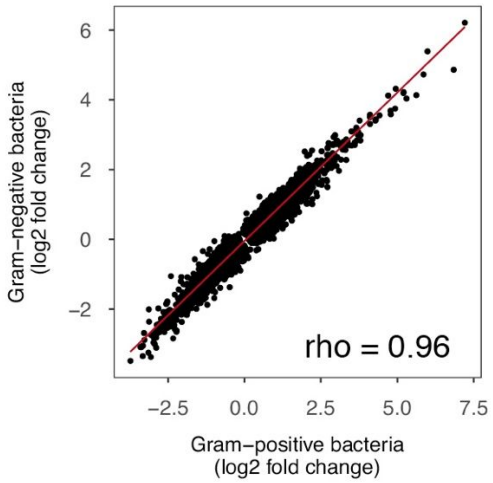
Figure S4. Principal component analysis plot of transcriptional response in blood leukocytes of patients with community-acquired pneumonia due to Gram-positive or Gram-negative bacteria



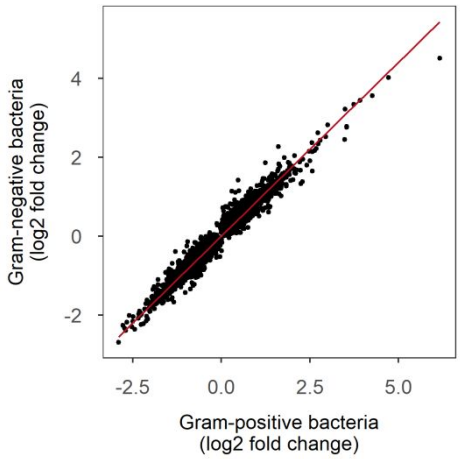
Ellipse circles in PCA plots are drawn around patient data points, wherein the centroid is the barycentre of the patient data points belonging to the same group.

Figure S5. Common transcriptional response of patients with community-acquired pneumonia due to Gram-positive or Gram-negative bacteria

A.

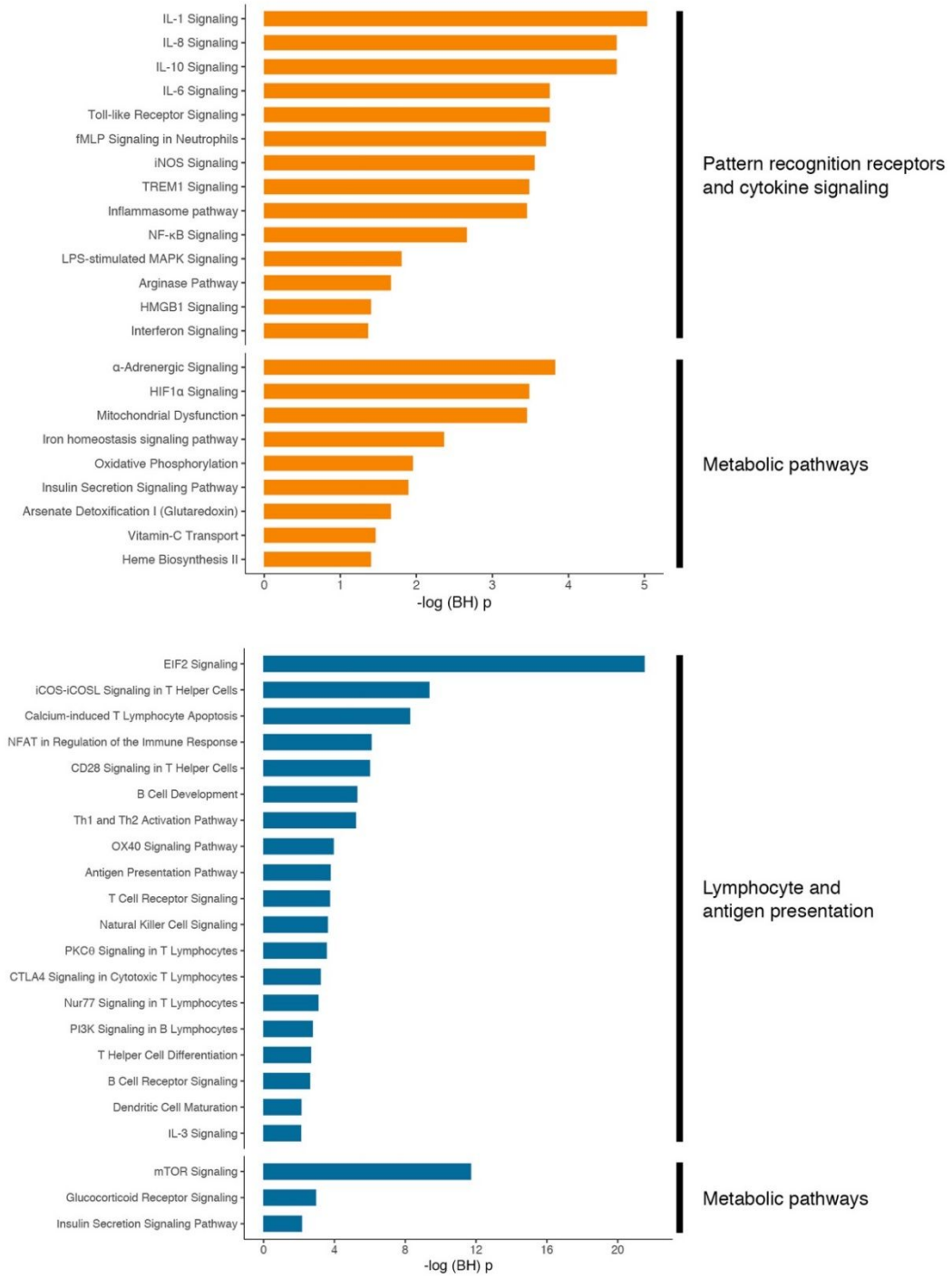


B.



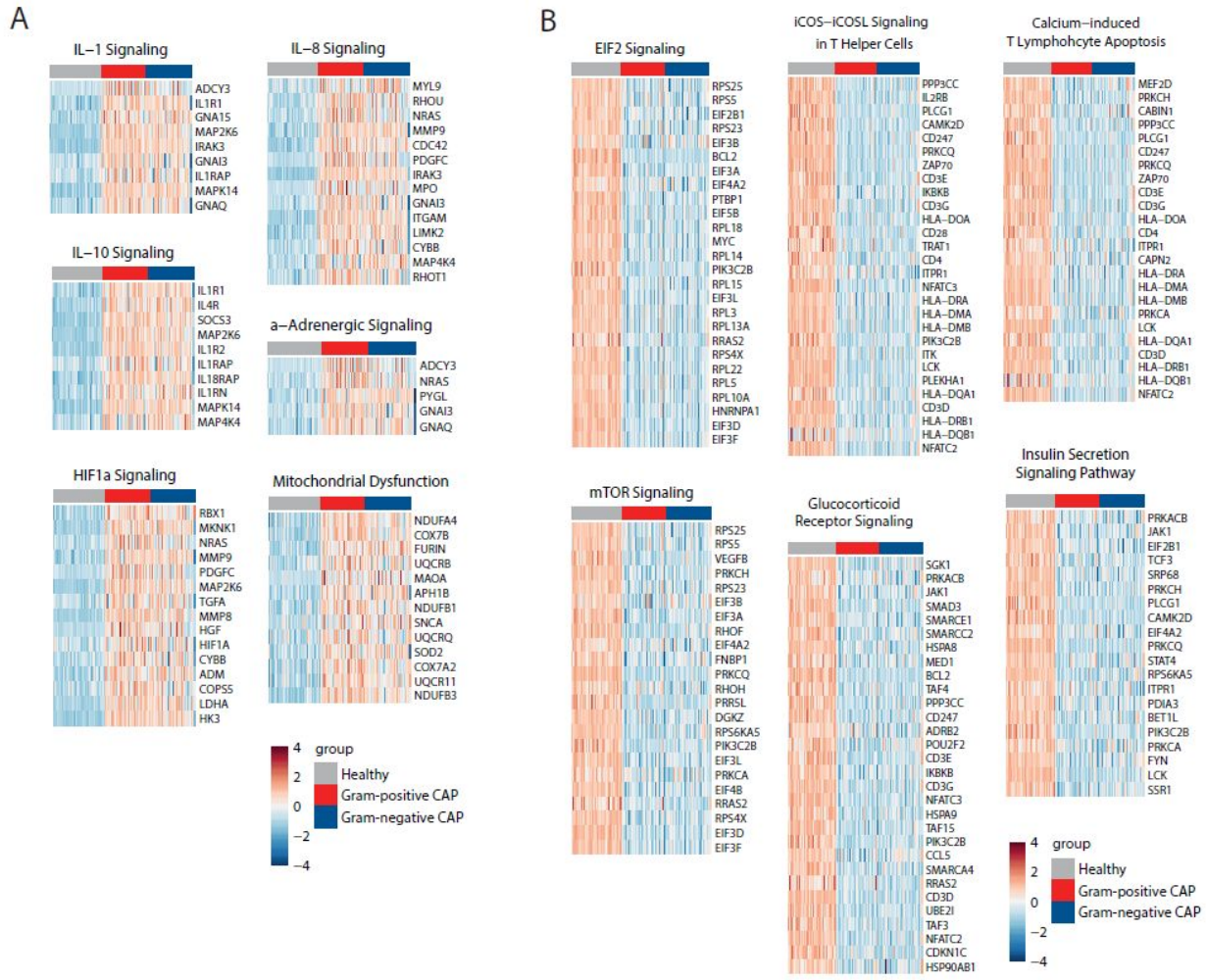
Dot plot depicting the common transcriptional response (log2 fold changes) of patients with CAP due to Gram-positive or Gram-negative bacteria as compared with healthy subjects in (A) the derivation cohort and (B) the internal validation cohort. Rho, Spearman's correlation coefficient.

Figure S6. Pathway analysis of the common transcriptional response in blood leukocytes obtained on admission in patients with community-acquired pneumonia due to Gram-positive or Gram-negative bacteria relative to health



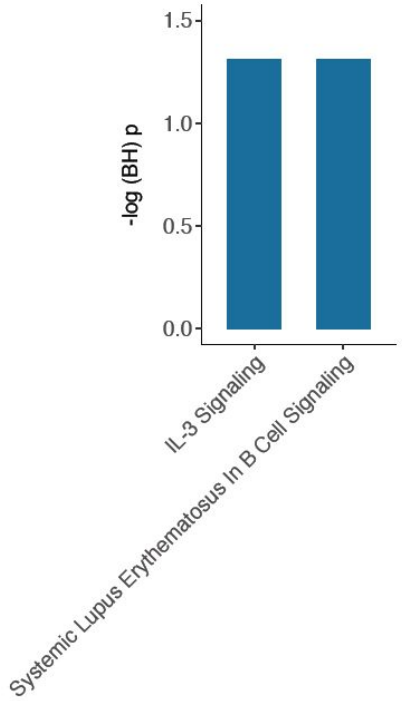
Considering Benjamini-Hochberg's adjusted *P*<.05, over-expressed (orange, top), and under-expressed (blue, bottom) genes were analyzed for association with canonical signaling pathways by Ingenuity pathway analysis (IPA, www.ingenuity.com). Significance was gauged by BH-adjusted Fisher exact probability. –log (BH) *P*, negative log transformed BH-adjusted *P* value

Figure S7. Genes involved in the common transcriptional response pathways in blood leukocytes obtained on admission in patients with community-acquired pneumonia due to Gram-positive or Gram-negative bacteria relative to health



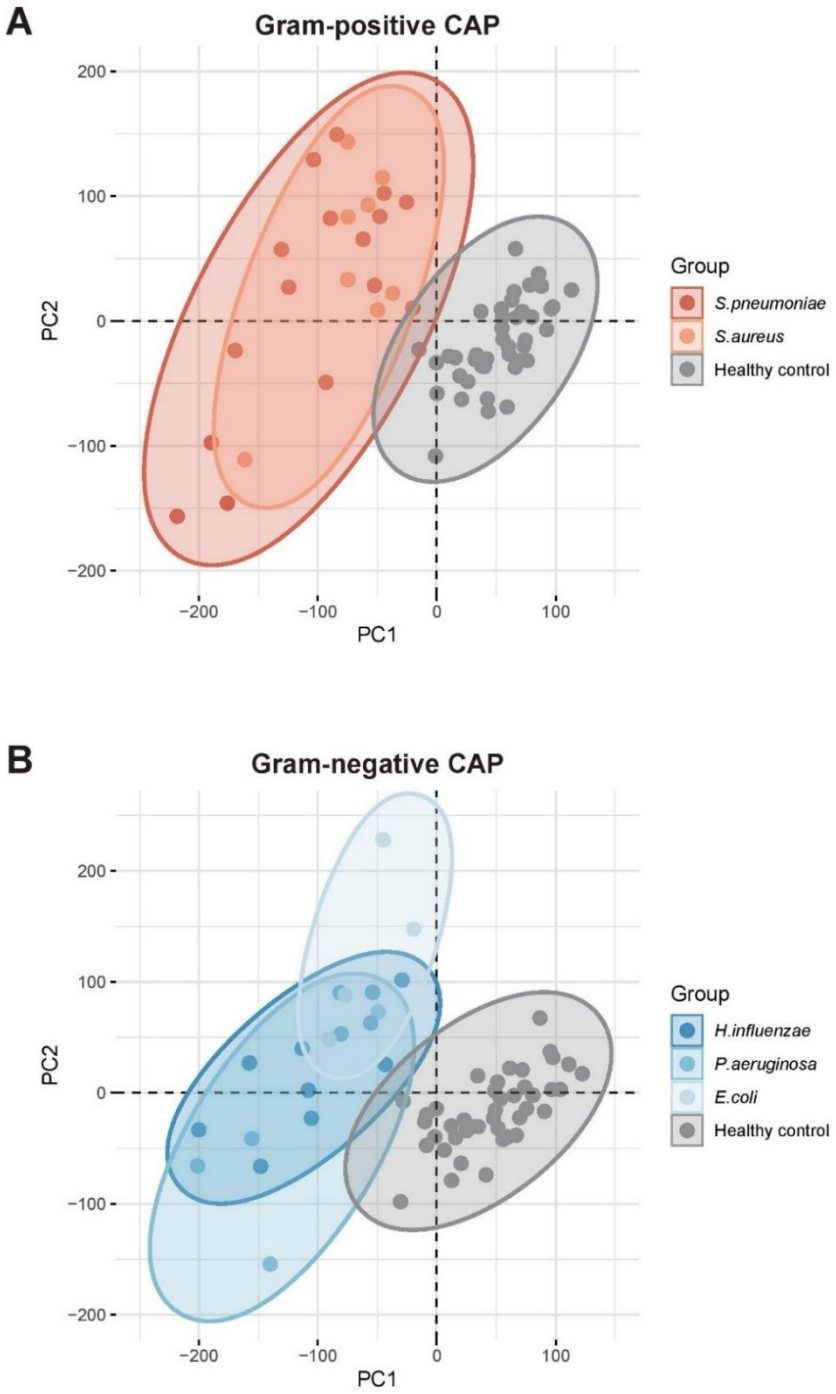
(A) Heatmaps of genes with log2 fold change of >1 involved in the top-3 overexpressed pathways within the main subgroups ('Pattern recognition receptors and cytokine signaling' and 'Metabolic pathways') as presented in Figure S6 upper panel. (B) Heatmaps of genes with log2 fold change of <-1 involved in the top-3 underexpressed pathways within the main subgroups ('Lymphocyte and antigen presentation' and 'Metabolic pathways') as presented in Figure S6 lower panel.

Figure S8. Pathway analysis of commonly under-expressed genes in patients with CAP due to Gram-positive vs. Gram-negative bacteria.



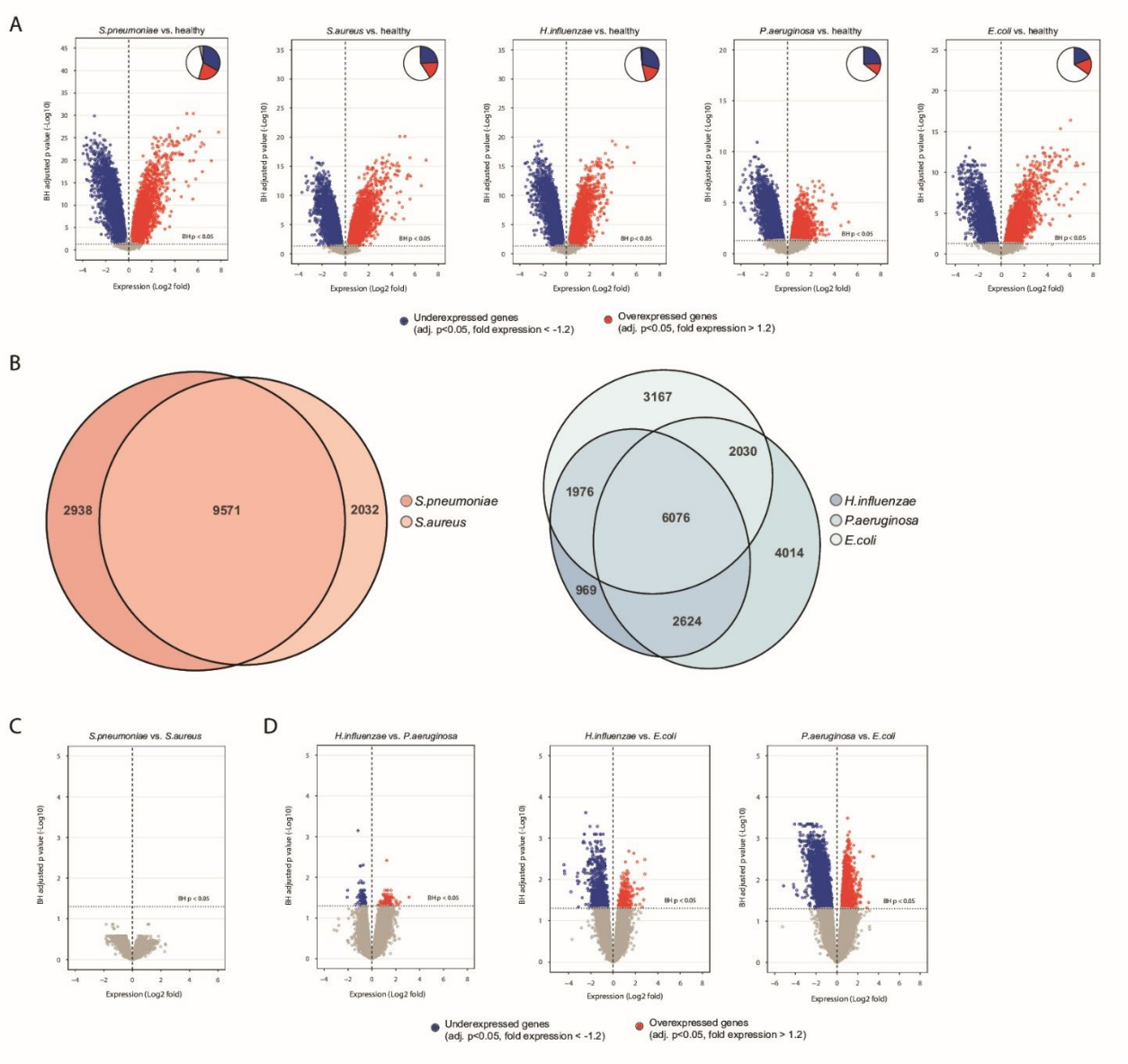
Ingenuity pathway analysis of commonly under-expressed genes in patients with CAP due to Gram-positive vs. Gram-negative bacteria as depicted in figure 4B. –log (Benjamini-Hochberg (BH)) *P* value, negative log10-transformed *P* value corrected for multiple comparisons.

Figure S9. Principal component analysis plot of transcriptional response in blood leukocytes of patients with community-acquired pneumonia due to the five most common bacteria



Ellipse circles of causative pathogens in PCA plots are drawn around patient data points, wherein the centroid is the barycentre of the patient data points belonging to the same group within Gram-positive (A) and Gram-negative (B) community-acquired pneumoniae (CAP).

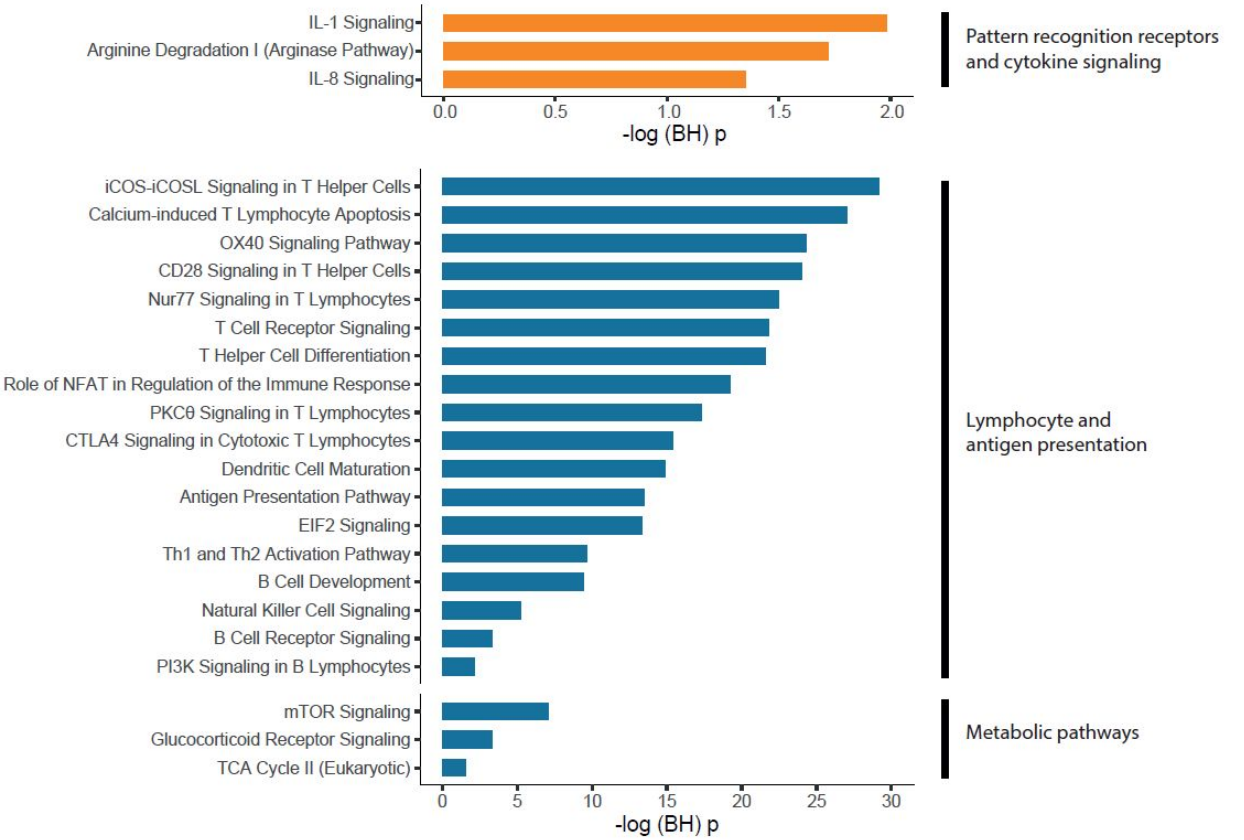
Figure S10. Leukocyte genomic responses upon admission in patients with community-acquired pneumonia due to the five most common bacterial causative pathogens



(A) Volcano plots illustrating the differences in leukocyte genomic responses (integrating log2 fold changes and multiple-test adjusted probabilities) between patients with community-acquired pneumonia (CAP) due to one of the five most common bacterial pathogens (*S.pneumoniae* (n=16)*, S.aureus* (n=8)*, H.influenzae* (n=12)*, P.aeruginosa* (n=4) *and E.coli* (n=4)) and healthy subjects (n=42). Blue dots represent significantly underexpressed genes (adjusted *P*<.05, fold expression <-1.2) whereas red dots represent significantly overexpressed genes (adjusted *P*<.05, fold expression >1.2) in patients relative to healthy controls. Horizontal dotted line indicates multiple-test adjusted Benjamini-Hochberg (BH) *P*<.05 threshold. Within plots, pie charts show the extent of gene expression changes: blue slices show significantly underexpressed genes (adjusted *P*<.05 and expression more than 1.2-times decreased compared with healthy controls), red slices show significantly overexpressed genes (adjusted *P*<.05 and expression more than 1.2-time increased compared with healthy controls), and grey slices show significantly different gene expression (adjusted *P*<.05 and expression less than 1.2-time increased or decreased compared with healthy controls). (B) Venn-Euler diagram illustrating the shared and distinct leukocyte transcriptional responses between the two Gram-positive bacteria (left) and the three Gram-negative bacteria (right) to health (with differential expressed genes according to high effect size >0.8 with Hedges g). (D) Volcano plots illustrating the differences in leukocyte genomic responses on admission between patients with

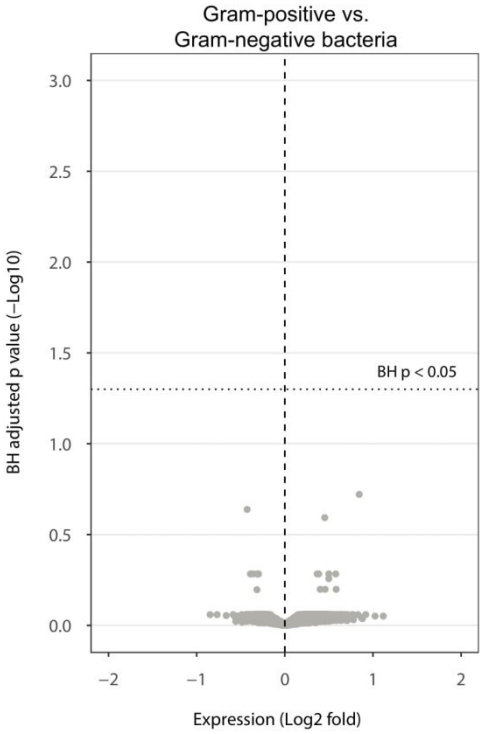
CAP due to pathogens indicated. –log (Benjamini-Hochberg (BH)) *P* value, negative log10-transformed *P* value corrected for multiple comparisons.

Figure S11. Pathway analysis of the common transcriptional response in blood leukocytes obtained on admission in patients with community-acquired pneumonia due to Gram-positive or Gram-negative bacteria relative to health, in the (internal) validation cohort



Considering Benjamini-Hochberg's adjusted *P*<.05, over-expressed (orange, top), and under-expressed (blue, bottom) genes were analyzed for association with canonical signaling pathways by Ingenuity pathway analysis (IPA, www.ingenuity.com). Significance was gauged by BH-adjusted Fisher exact probability. –log (BH) *P*, negative log transformed BH-adjusted *P* value

Figure S12. Validation of the transcriptional response in blood leukocytes of patients with community-acquired pneumonia due to Gram-positive or Gram-negative bacteria in the (external) GAinS cohort



Volcano plot illustrating the differences in leukocyte genomic responses on admission between patients with CAP due to Gram-positive bacteria (n=60) and patients with CAP due to Gram-negative bacteria (n=35). Considering adjusted P<.05, no genes were differentially expressed. –log (Benjamini-Hochberg (BH)) P value, negative log10-transformed P value corrected for multiple comparisons.