

# Shared and Distinct Aspects of the Sepsis Transcriptomic Response to Fecal Peritonitis and Pneumonia

Katie L. Burnham<sup>1</sup>, Emma E. Davenport<sup>1</sup>, Jayachandran Radhakrishnan<sup>1</sup>, Peter Humburg<sup>1</sup>, Anthony C. Gordon<sup>2</sup>, Paula Hutton<sup>3</sup>, Eduardo Svoren-Jabalera<sup>4</sup>, Christopher Garrard<sup>3</sup>, Adrian V. S. Hill<sup>1</sup>, Charles J. Hinds<sup>4</sup>, and Julian C. Knight<sup>1</sup>

<sup>1</sup>Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom; <sup>2</sup>Section of Anaesthetics, Pain Medicine and Intensive Care, Imperial College London, London, United Kingdom; <sup>3</sup>Adult Intensive Care Unit, John Radcliffe Hospital, Oxford, United Kingdom; and <sup>4</sup>William Harvey Research Institute, Barts and The London School of Medicine, Queen Mary University, London, United Kingdom

ORCID IDs: 0000-0001-8680-2933 (K.L.B.); 0000-0002-0377-5536 (J.C.K.).

## Abstract

**Rationale:** Heterogeneity in the septic response has hindered efforts to understand pathophysiology and develop targeted therapies. Source of infection, with different causative organisms and temporal changes, might influence this heterogeneity.

**Objectives:** To investigate individual and temporal variations in the transcriptomic response to sepsis due to fecal peritonitis, and to compare these with the same parameters in community-acquired pneumonia.

**Methods:** We performed genome-wide gene expression profiling in peripheral blood leukocytes of adult patients admitted to intensive care with sepsis due to fecal peritonitis (n = 117) or community-acquired pneumonia (n = 126), and of control subjects without sepsis (n = 10).

**Measurements and Main Results:** A substantial portion of the transcribed genome (18%) was differentially expressed compared with that of control subjects, independent of source of infection, with eukaryotic initiation factor 2 signaling being the most enriched

canonical pathway. We identified two sepsis response signature (SRS) subgroups in fecal peritonitis associated with early mortality ( $P = 0.01$ ; hazard ratio, 4.78). We defined gene sets predictive of SRS group, and serial sampling demonstrated that subgroup membership is dynamic during intensive care unit admission. We found that SRS is the major predictor of transcriptomic variation; a small number of genes (n = 263) were differentially regulated according to the source of infection, enriched for IFN signaling and antigen presentation. We define temporal changes in gene expression from disease onset involving phagosome formation as well as natural killer cell and IL-3 signaling.

**Conclusions:** The majority of the sepsis transcriptomic response is independent of the source of infection and includes signatures reflecting immune response state and prognosis. A modest number of genes show evidence of specificity. Our findings highlight opportunities for patient stratification and precision medicine in sepsis.

**Keywords:** gene expression; septic; immune; patient stratification

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Correspondence and requests for reprints should be addressed to Julian C. Knight, M.A., M.B. Ch.B., D.Phil., F.R.C.P., Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive, Oxford OX3 7BN, UK. E-mail: julian@well.ox.ac.uk

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## At a Glance Commentary

### Scientific Knowledge on the

**Subject:** There is significant heterogeneity in the septic response, which has hindered efforts to understand pathophysiology and develop targeted therapies. Molecular approaches may provide insights into variation in the host response, enabling biomarker development. Recent evidence suggests that transcriptomic sepsis response signatures (SRSs) can define patient subgroups associated with early outcome in sepsis due to community-acquired pneumonia (CAP).

### What This Study Adds to the

**Field:** This study provides the first substantive analysis of the transcriptomic response in patients admitted to intensive care with sepsis due to fecal peritonitis. Comparison with sepsis due to community-acquired pneumonia and with nonseptic control subjects demonstrated a shared sepsis response, independent of source of infection, that involved a significant proportion of the transcribed genome and overlapped with the “genomic storm” following trauma. We found evidence of SRS groups in patients with fecal peritonitis predictive of early mortality, with group membership changing over time in some patients. We show that the major predictor of variation in gene expression between patients with sepsis is SRS group rather than source of infection, with only a small number of genes differentially regulated according to the latter, enriched for IFN signaling and antigen presentation. These findings highlight opportunities for patient stratification in sepsis.

Sepsis is the life-threatening organ dysfunction caused by a dysregulated host response to infection (1). Therapeutic options remain limited despite extensive efforts to develop and refine treatment strategies (2). New insights into pathophysiology and the development of targeted treatments appropriate for individual patients at specific stages of the

illness are urgently required (3). To be successful, this approach requires clearer understanding of heterogeneity in the sepsis response, in which proinflammatory and immunosuppressed states are dynamic and frequently coexist (4). We recently identified distinct transcriptomic sepsis response signatures (SRSs) in peripheral blood leukocytes from patients with community-acquired pneumonia (CAP) that are informative for immune response states and outcome (5). In particular, patients with SRS1 exhibit an immunosuppressed phenotype associated with higher early mortality, with features of endotoxin tolerance, T-cell exhaustion, and downregulation of HLA class II.

It is not yet known whether comparable SRSs are present in sepsis caused by other sources of infection. Additionally, the extent to which the source of infection contributes to heterogeneity in the transcriptomic response is unclear, although there is evidence that gene expression signatures can distinguish between gram-positive, gram-negative, and viral etiologies, and these signatures may be useful in the diagnosis of CAP (6–13). In fecal peritonitis (FP), sepsis is triggered by a polymicrobial infection within the peritoneal cavity, complicated by the release of damage-associated molecular patterns (DAMPs) and the effects of anesthesia (14). Conversely, CAP is caused by specific bacterial or atypical pathogens, and it is commonly preceded or caused by viral infection (15). Antibiotic choice, such as macrolides for CAP, may also have immunomodulatory effects (16, 17). Finally, whereas patients with FP usually have a rapid onset of illness, patients with CAP are often unwell for many days prior to intensive care unit (ICU) admission.

FP is a common cause of sepsis with a high mortality (18) in which longitudinal analysis is tractable, given that a defined time of onset can be estimated. We hypothesized that SRSs similar to those seen in CAP would be present in patients with FP, but that aspects of the transcriptomic host response would be dependent on the source of infection and stage of illness. In the present study, we investigated how patterns of gene expression in leukocytes are influenced by source of infection in FP and CAP, as well as how they vary between individual patients and within patients over time. Some of the results of these studies were previously reported in the form of abstracts (19, 20).

## Methods

### Study Design and Participants

The objective was to characterize the transcriptomic response to sepsis caused by FP, including an analysis of temporal changes and a comparison with sepsis caused by CAP. The subjects were adult patients admitted to the ICU with sepsis as part of the UK Genomic Advances in Sepsis (GAINs) study ([www.ukccg-gains.org](http://www.ukccg-gains.org)) with predefined inclusion and exclusion criteria (described in the online supplement). FP was diagnosed at laparotomy as inflammation of the peritoneal membrane secondary to large bowel perforation and fecal contamination (18). CAP was defined as a febrile illness associated with cough, sputum production, breathlessness, leukocytosis, and radiological features of pneumonia acquired in the community or within 2 days of ICU admission (21, 22).

This was a prospective observational study. The transcriptomic response was investigated in peripheral blood leukocytes and compared between patients and over time, and with control subjects undergoing elective cardiac surgery (described in the online supplement). Discovery and validation cohorts were used to identify shared and specific gene expression patterns among patients with FP and patients with CAP (see Figure E1 in the online supplement).

### Sample Collection

Blood samples (5 ml) were collected (VACUETTE ethylenediaminetetraacetic acid-coated tubes; Greiner Bio-One, Kremsmünster, Austria) on the first, third, and/or fifth day after ICU admission. The total blood leukocyte population was isolated using LeukoLOCK filters (Life Technologies, Carlsbad, CA) and then stabilized using RNAlater (Life Technologies), followed by total RNA extraction (described in the online supplement). Extensive, anonymized clinical information was recorded using an electronic case report form (5).

### Microarray Analysis

Genome-wide gene expression was quantified using Illumina HumanHT-12 v4 Expression BeadChips (47,231 probes; Illumina, San Diego, CA) with sample processing, data preparation, background subtraction, transformation, and normalization using the vsn package (23) (described in the online supplement). Gene

expression data are available through the ArrayExpress database (accession number E-MTAB-5273/E-MTAB-5274).

### Statistical Analysis

Analysis was performed using the R statistical software package (R Foundation for Statistical Computing, Vienna, Austria) unless otherwise specified, with statistical tests, power calculations, differential gene expression, enrichment testing, predictive modeling, and cluster analysis described in the online supplement.

## Results

### Transcriptomic Response to FP: Identification of SRSs

We quantified genome-wide gene expression for 117 adult patients with FP

and 126 adult patients with CAP admitted to the ICU with sepsis, analyzing RNA from blood leukocytes rapidly isolated at the bedside. All patients showed evidence of organ dysfunction based on Sequential Organ Failure Assessment (SOFA) scores during ICU admission. These patients were recruited concurrently and processed in parallel as discovery (64 with FP, 73 with CAP) and validation cohorts (53 with FP, 53 with CAP; the 53 patients with CAP were previously published [5]). We also analyzed samples obtained from 10 subjects prior to elective cardiac surgery as nonseptic control subjects in the discovery cohort.

Demographics and clinical covariates for the discovery set (FP,  $n = 64$  patients; CAP,  $n = 73$  patients; 221 samples; see Figure E1) are shown in Tables 1 and E1A.

We investigated whether sepsis response subgroups based on global patterns

of gene expression were present in FP. The combined FP cohort (147 samples from 117 patients) had a 28-day mortality of 13%; the mean age was 66 years, and 50.4% of patients were male (Table 2, Table E1B). We applied two complementary approaches to this FP cohort: (1) hypothesis-free unsupervised hierarchical clustering based on observed patterns of variation in gene expression and (2) assignment based on expression of a specific seven-gene set previously shown to be predictive of SRS group membership in CAP (5).

We first analyzed variation in global gene expression (2,716 probes representing the top 10% most variably expressed genes) using agglomerative hierarchical clustering followed by consolidation of group membership using  $k$ -means. This approach revealed two distinct patient groups: SRS1\_FP ( $n = 68$  [46%]) and SRS2\_FP

**Table 1.** Comparison of Clinical Characteristics of Patients with Sepsis, Based on Source of Infection in the Discovery and Validation Cohorts for the First Available Sample for Each Patient

	Discovery Cohort			Validation Cohort		
	CAP ( $n = 73$ )	FP ( $n = 64$ )	<i>P</i> Value	CAP ( $n = 53$ )	FP ( $n = 53$ )	<i>P</i> Value
Age, yr	60.6 (15.6)	65.1 (17.5)	NS	68.4 (13.7)	68 (12.7)	NS
Male sex	37 (50.6%)	28 (43.7%)	NS	37 (69.8%)	31 (58.5%)	NS*
APACHE II score on Day 1 in the ICU	18 (6.2)	15 (5.9)	0.005	19.7 (6.1)	15.7 (5.9)	0.0008
SOFA score on day of sampling	6.8 (3.5)	5.4 (4.0)	0.03	6.2 (3.8)	6.3 (3.5)	NS
Mortality						
14 d	8 (11.0%)	6 (9.4%)	NS <sup>†</sup>	7 (13.2%)	6 (11.3%)	NS <sup>†</sup>
28 d	13 (17.8)	8 (12.5%)	NS <sup>†</sup>	7 (13.2%)	7 (13.2%)	NS <sup>†</sup>
6 mo	17 (23.3%)	11 (17.2%)	NS <sup>†</sup>	11 (20.8%)	12 (22.6%)	NS <sup>†</sup>
Infection						
Gram-positive bacteria	14 (19.2%)			7 (13.2%)		
Gram-negative bacteria	8 (11.0%)			5 (9.4%)		
Viral	6 (8.2%)			5 (9.4%)		
Mechanical ventilation	51 (69.9%)	34 (53.1%)	0.05	19 (35.8%)	21 (39.6%)	NS*
Respiratory rate, breaths/min	29.2 (9.2)	20.0 (8.2)	<0.0001	31.1 (8.7)	22.7 (9.0)	<0.0001
Days of respiratory support	9.6 (13.5)	4.7 (8.1)	0.01	8.8 (12.1)	9.3 (14.8)	NS
Oxygenation index	19.3 (9.9)	30.9 (12.3)	<0.0001	21.2 (7.5)	28.2 (12.2)	<0.0001
Vasopressors/inotropes			NS			NS*
No dose	37 (50.7%)	35 (54.7%)		36 (67.9%)	27 (50.9%)	
Low dose	1 (1.4%)	1 (1.6%)		0 (0%)	0 (0%)	
Medium dose	10 (13.7%)	5 (7.8%)		7 (13.2%)	6 (11.3%)	
High dose	24 (32.9%)	23 (35.9%)		10 (18.9%)	20 (37.7%)	
Mean arterial pressure, lowest, mm Hg	65.0 (12.8)	67.7 (12.5)	NS	69.6 (11.1)	67.5 (14.6)	NS
Temperature, low, °C	36.4 (0.8)	36.1 (0.7)	0.018	36.0 (1.0)	36.1 (0.6)	NS
Hematocrit, %	35.4 (6.9)	30 (5.9)	<0.0001	35.0 (7.9)	31.5 (6.6)	0.02
Proportion of leukocytes						
Lymphocytes	9.17%	6.52%	0.008	9.05%	8.77%	NS
Polynucleocytes	84.10%	87.98%	0.029	83.40%	85.30%	NS
Mononucleocytes	6.73%	5.50%	NS	7.51%	5.95%	NS
Bicarbonate, mmol/L	25.0 (6.8)	22.1 (5.1)	0.008	24.6 (6.8)	23.1 (5.0)	NS
Renal replacement therapy	6 (8.2%)	5 (7.8%)	NS	5 (9.4%)	8 (15.1%)	NS*

*Definition of abbreviations:* APACHE II = Acute Physiology and Chronic Health Evaluation II; CAP = community-acquired pneumonia; FP = fecal peritonitis; ICU = intensive care unit; NS = not significant; SOFA = Sequential Organ Failure Assessment.

Data are count (percent) or mean (SD) unless otherwise specified. Statistical analysis was done by  $t$  test unless otherwise specified.

\*Chi-square test.

<sup>†</sup>Log-rank test.

**Table 2.** Characteristics of Patients with Fecal Peritonitis (All Patients) on Day 1

Characteristics	Data
Number of patients	117
Age, yr, mean $\pm$ SD	66.4 ( $\pm$ 15.5)
Male sex, %	50.4%
Median (range) APACHE II score	15 (2–40)
Median (range) SOFA score on day of sampling	5 (0–18)
Mortality	
14 d	12 (10.3%)
28 d	15 (12.8%)
6 mo	23 (19.7%)
Cause of peritonitis	
Diverticular disease	30 (25.6%)
Surgical anastomosis breakdown	28 (23.9%)
Malignancy	14 (12%)
Trauma	3 (2.6%)
Other/unknown	42 (35.9%)
Mechanical ventilation	55 (47%)
Vasopressors	55 (47%)
Renal replacement therapy	13 (11%)
Mean (SD) arterial pressure, lowest, mm Hg	68.2 (14.8)

Definition of abbreviations: APACHE II = Acute Physiology and Chronic Healthy Evaluation II; SOFA = Sequential Organ Failure Assessment. Data are count (percent) unless otherwise specified.

( $n = 79$  [54%]) (Figures 1A and 1B). Following group assignment, 1,075 genes were found to be differentially expressed between these groups (Figure 1C, Table E1C), showing strong correlation with those previously observed in patients with CAP (5) (Pearson's  $r = 0.791$ ) (Figure E2). To determine the functional overlap with SRS groups identified in CAP, we performed pathway, function enrichment, and upstream regulator analysis (Figure 1D, Table E1D). Cell death, apoptosis, necrosis, T-cell activation, and endotoxin tolerance were significantly enriched biological functions in SRS1 for both sources of infection. We proceeded to test for association with outcome in FP and found that SRS1\_FP group membership was associated with higher early mortality (14-d mortality log-rank test  $P = 0.0096$ ; 18.8% vs. 4.3%; hazard ratio, 4.78; 95% confidence interval, 1.29–17.65) (Figure 1E). The effect of SRS group membership remained after inclusion of age, SOFA score, Charlson comorbidity index, and sex in a Cox proportional hazards model ( $P = 0.037$ ; hazard ratio, 4.23; 95% confidence interval, 1.09–16.39).

To further validate our findings, we adopted a second approach, namely assignment of SRS group membership in this FP cohort using the seven-gene set (*DYRK2*, *CCNB1IP1*, *TDRD9*, *ZAP70*, *ARL14EP*, *MDC1*, and *ADGRE3*) we

previously established in patients with CAP (5) (Figure E3). This showed strong concordance with the results obtained by unsupervised analysis (misclassification rate, 21.1%; area under the curve [AUC], 0.923) (Figure 1F). The groups defined using the seven-gene set showed a significant difference in early mortality rates (log-rank test  $P = 0.030$  for patients with FP), a differential gene expression signature strongly correlated with that seen in the original CAP analysis ( $r = 0.845$ ), as well as pathway enrichment comparable to findings in patients with CAP. We also derived a six-gene set (*CD163*, *ZDHHC19*, *MME*, *FAM89A*, *ZBP1*, and *B3GNT2*) from the FP data predictive of SRS\_FP group membership, with a 4.1% misclassification rate based on internal leave-one-out cross-validation (AUC, 0.975) (Figure 1F). When applied to the original CAP cohort (5), this six-gene set again performed well (misclassification rate, 27.9%; AUC, 0.931).

A poor outcome subgroup has previously been reported in children with sepsis (24). We investigated the similarity between pediatric endotype A and SRS1 by comparative differential gene expression analysis. Although there was some overlap in pathway enrichment (e.g., T- and B-cell receptor signaling), the gene expression patterns that distinguish SRS groups were not enriched in the pediatric endotype contrast (Figure E4).

### Temporal Changes in SRSs

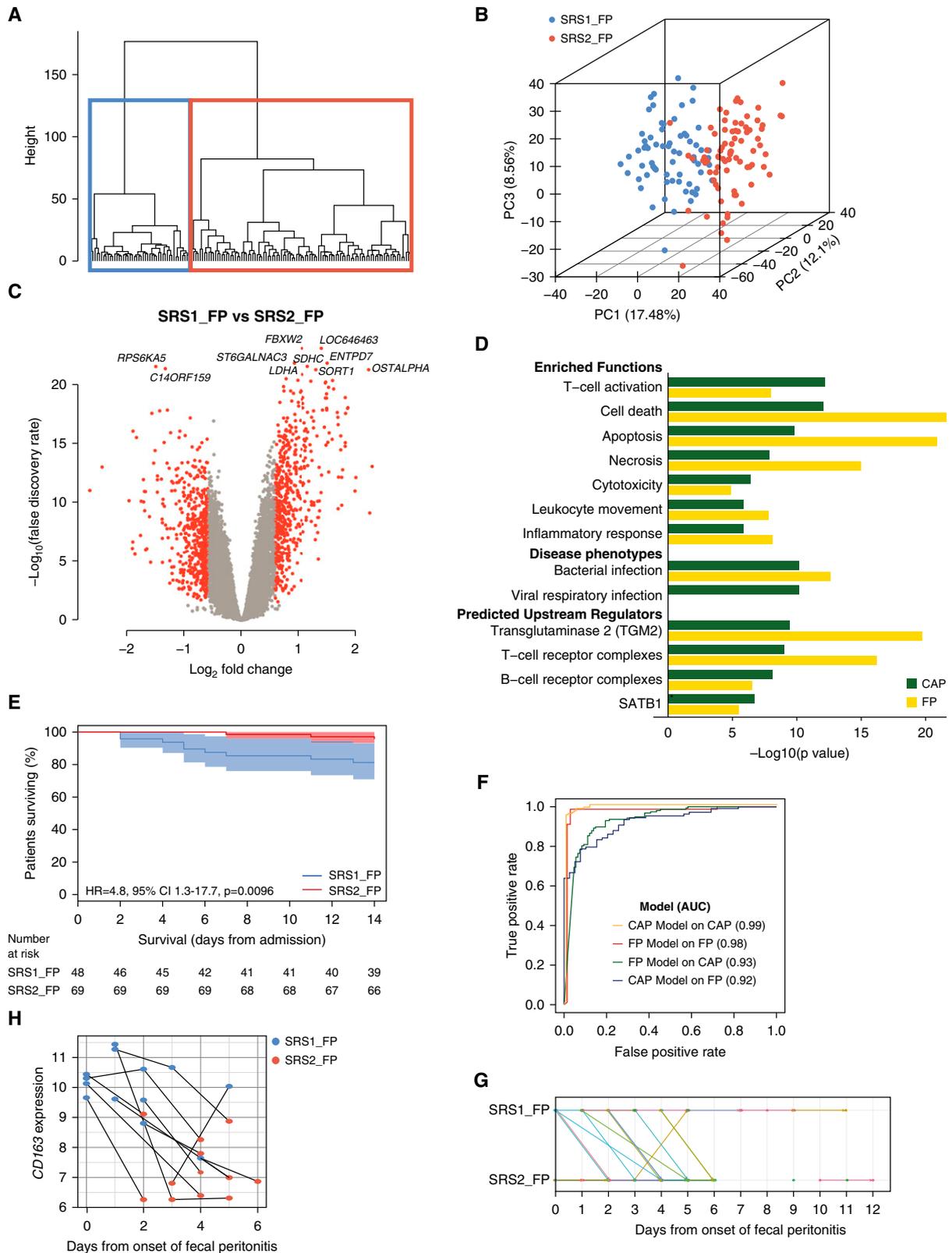
Serial sampling on Day 1, Day 3, and Day 5 following ICU admission provides the opportunity to investigate the relationship between SRS group membership and disease progression. We found that 11 (46%) of 24 patients with FP with serial samples moved between groups over time; 10 moved from SRS1 to SRS2, only 1 of whom died (Figure 1G). Thirteen patients (54%) remained in the same SRS group; three of seven patients remaining in SRS1 died, compared with no deaths among the six remaining in SRS2. This movement between SRS groups involves large changes in gene expression, illustrated by *CD163*, encoding an innate immune sensor for bacteria, and one of the six gene classifiers for SRS\_FP (Figures 1H and E5).

### Influence of Source of Infection on the Transcriptomic Response

We proceeded to further characterize and compare gene expression in patients with FP and patients with CAP to determine the relative importance of shared and specific features of the transcriptomic response. As expected, in the discovery cohort, gastrointestinal comorbidities were more common in patients with FP, whereas patients with CAP had more respiratory comorbidities, higher respiratory rates and oxygenation requirements, and higher lymphocyte counts (Table 1 and Table E1A). Although mortality did not differ significantly, patients with CAP had higher Acute Physiology and Chronic Healthy Evaluation II and SOFA scores than patients with FP (Table 1).

To understand the relationships between source of infection, SRS group, and heterogeneity in the septic response, we performed principal component analysis using the top 10% most variably expressed transcripts. Nonseptic control subjects clustered together and were clearly distinct from septic patients (Figure 2A). Among the combined population of patients with sepsis, there was clear segregation based on SRS group but not source of infection (Figure 2A).

To further elucidate the drivers of variation in the septic response, we calculated correlations between the observed variance in global gene expression for the sepsis samples (the first six principal components of variance, representing approximately 50% of the variance in the



**Figure 1.** Transcriptomic sepsis response signatures (SRSs) in fecal peritonitis (FP). (A) Unsupervised hierarchical clustering analysis of 147 FP samples for the top 10% most variable probes ( $n = 2,716$ ). (B) First three principal components (PCs) plotted with the proportion of variance explained by each component, with samples colored by SRS group membership assigned by hierarchical and  $k$ -means clustering. (C) Volcano plot of differentially expressed probes for SRS1\_FP versus SRS2\_FP (red coloring shows fold change  $> 1.5$ , false discovery rate  $< 0.05$ ). (D) Enriched functions, disease

data), a comprehensive set of clinical covariates, and SRS group (Figure 2B, Table E1E). This showed that variation in gene expression was most strongly correlated with SRS group membership (PC1  $r = 0.77$ ; false discovery rate [FDR],  $< 2.2 \times 10^{-16}$ ). More modest correlation was seen with source of infection (PC1  $r = -0.417$ ; FDR,  $6.12 \times 10^{-9}$ ), bicarbonate levels (PC1  $r = 0.35$ ; FDR,  $2.2 \times 10^{-8}$ ), and neutrophil count (PC1  $r = -0.29$ ; FDR,  $2.0 \times 10^{-3}$ ). When the dataset was divided into CAP and FP samples and the analysis repeated, we found that SRS remained the most significant correlate (Figure E6).

When we compared all patients in the discovery cohort (FP,  $n = 64$ ; CAP,  $n = 73$ ) with nonseptic control subjects ( $n = 10$ ), we found that sepsis was associated with differential regulation of a large proportion of the transcribed genome (18.0% of transcripts assayed; 4,881 probes;  $> 1.5$ -fold change [FC]; FDR,  $< 0.05$ ) (Table E1F). Pathway analysis showed that eukaryotic initiation factor 2 signaling was the top canonical pathway, consistent with the reported role for this translational initiation factor in response to viral and bacterial infection (25), and predicted key upstream regulators IL-13, ATB1, TGF $\beta$ 1, and IL-2 (Table E1G). We used the same methodology to compare gene expression in trauma patients with healthy subjects, using the previously published genomic response to trauma dataset (26) but restricting this to samples from the same time frame as the sepsis cohort (5 d following injury). We found that, of the genes differentially expressed in the sepsis response and measured in the trauma cohort, the majority ( $n = 1,884$  [64%]) were also differentially expressed in trauma (Figure 2C). Commonality was seen with eukaryotic initiation factor 2 signaling and inflammation-related pathways enriched in both sepsis and trauma responses, whereas the inflammatory response to organismal injury was specific to trauma and tumor necrosis factor receptor 1 (TNFR1)

signaling was specific to sepsis (Figure 2C, Table E1H).

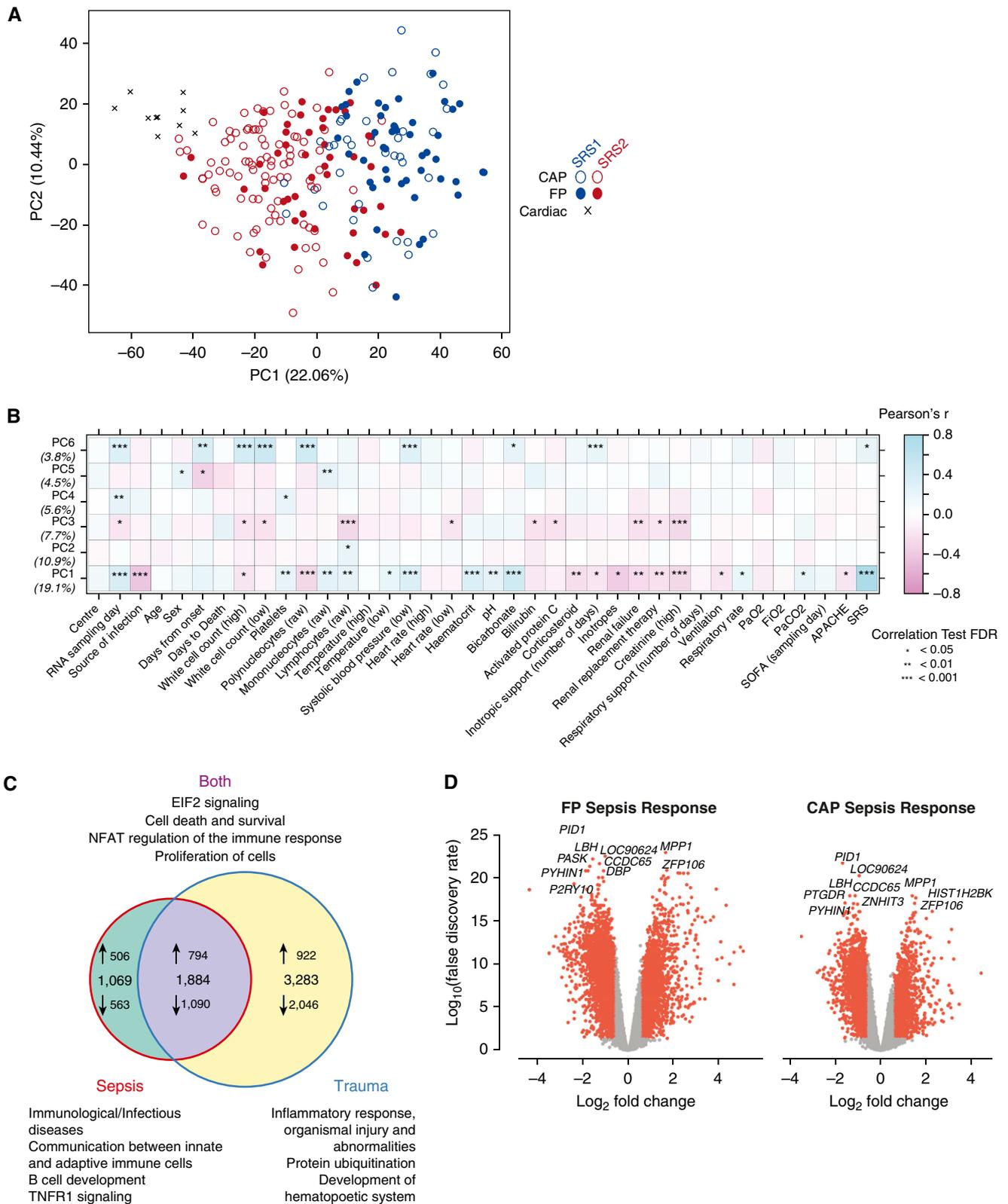
When we compared patients with CAP versus control subjects and patients with FP versus control subjects, we observed that gene expression patterns were highly correlated, demonstrating that most sepsis response pathways are common and independent of source of infection (Figures 2D, 3A, and 3B; Tables E1I and E1J). To further investigate differential gene expression between patients with sepsis due to FP or CAP, we compared the first available samples for each patient following ICU admission (FP,  $n = 64$ ; CAP,  $n = 73$ ). We found 310 probes (263 genes) differentially expressed between the two sources of infection (FC,  $> 1.5$ ; FDR,  $< 0.05$ ) (Figure 3C, Table E1K), significantly more than expected by chance based on permutation analysis (Figure E7). We noted significant differences in total and differential cell counts between CAP and FP. The inclusion of differential cell counts in the regression model had minimal influence, with a similar list of differentially regulated genes and FCs strongly correlated with the original analysis (Figure E8, Table E1K). IFN signaling ( $P = 6.1 \times 10^{-10}$ ) and antigen presentation ( $P = 1.6 \times 10^{-8}$ ) were the most significant enriched canonical pathways, upregulated in patients with CAP compared with FP (Figure 3D, Table E1L). Enriched networks involved IFN- $\alpha/\beta$  and the antimicrobial/inflammatory response ( $P = 1 \times 10^{-39}$ ) (Figure 3E). *IFIT1*, *IFIT2*, and *IFIT3* (IFN-induced antiviral genes), *BTN3A3* (major histocompatibility complex class I gene), *IFIH1* (sensor of viral nucleic acids), and *OAS2* (IFN-induced antiviral enzyme) were all upregulated in patients with CAP. IFNs, endotoxins, and TNF were found to be significant upstream regulators (Figure 3D, Table E1L).

We confirmed our findings in a validation set comprising 53 patients with FP and 53 patients with CAP prospectively recruited to the UK GAINs study (Figure

E1). We found SRS group, source of infection, cell count, and day of sampling were strongly associated with the first six principal components of gene expression; genes differentially expressed between FP/CAP correlated with those found in the discovery cohort (Pearson's  $r = 0.83$ ;  $P < 2.2 \times 10^{-16}$ ) (Table E1M). Pathway analysis demonstrated high concordance of enriched pathways and functions (Figure 3D, Table E1N). We found that a gene set comprising *EPHB1*, *NQO2*, *ARG1*, *HMGB2*, *FGL2*, and *GPR162* was predictive of source of infection (FP or CAP) for the discovery cohort. We applied a sparse regression method to show that, for the discovery cohort, the misclassification rate was 29.9% with internal leave-one-out validation (AUC, 0.760); for the validation cohort, the test error was 28.3% (AUC, 0.798) (Figure E9). There was no difference in the proportion of different sampling days following ICU admission between FP and CAP samples in the analyzed cohorts (chi-square  $P = 0.350$ ) (Figure E10).

Given a prominent viral signature among genes differentially expressed between patients with FP and patients with CAP, we investigated if this came from a subset of patients with CAP with a viral infection. Only 6 of 73 patients with CAP in the discovery cohort had a confirmed viral infection; we therefore analyzed the transcriptomic differences between patients with and without confirmed viral infection in our larger, previously published CAP cohort (25 vs. 240 patients) (5). We identified 39 differentially expressed genes (FDR,  $< 0.05$ ; FC,  $> 1.5$ ) (Figure 3F, Table E1O), including *IFI27*, an IFN- $\alpha$ -inducible protein reported as a marker of influenza infection (27), and *LAMP3*, a dendritic cell glycoprotein induced by influenza A infection (28). Pathway analysis showed the most significant enrichment for pattern recognition receptors, IFN signaling, and IFN regulatory factors (Table E1P). We then compared this gene set to the genes differentially expressed between patients

**Figure 1.** (Continued). phenotypes, and predicted upstream regulators derived from differentially expressed probes in FP SRS groups and compared with the previously published community-acquired pneumonia (CAP) dataset. Enrichment was also seen for an endotoxin tolerance gene expression signature we previously defined (5) using publicly available datasets (42, 43) in SRS1\_FP relative to SRS2\_FP, tested using ROAST (rotation gene set testing), a gene set enrichment test ( $P < 1 \times 10^{-5}$ ). (E) Kaplan-Meier survival plot by SRS group (shaded areas, 95% confidence intervals [CIs]) with a single sample selected at random for those patients with multiple samples to assign SRS membership. (F) The performance of the SRS group assignment models (gene sets) which were derived and tested in the FP and previously published CAP datasets, are shown by receiver operating characteristic curves, and the area under the curve (AUC) is given for each. (G) Time course of patient SRS-FP group membership using serial samples and days from disease onset. (H) Expression of *CD163* over time from disease onset in samples from the 11 patients with FP who moved between SRS groups. Each point represents a sample, colored according to SRS group assignment, with lines linking samples from the same patient. HR = hazard ratio.



**Figure 2.** Transcriptomic response to sepsis. (A) First two principal components (PCs) of gene expression data plotted with the proportion of variance explained by each component shown. *Solid circles* represent fecal peritonitis (FP) discovery samples (n = 94), and *open circles* represent community-acquired pneumonia (CAP) discovery samples (n = 127). Samples are colored according to sepsis response signature (SRS) group assignment, showing that sepsis response states have considerable overlap between sources of infection. Control subjects (n = 10) are indicated by x. (B) Heatmap showing that sepsis response states have considerable overlap between sources of infection. Control subjects (n = 10) are indicated by x. (C) Venn diagram showing that sepsis response states have considerable overlap between sources of infection. Control subjects (n = 10) are indicated by x. (D) Volcano plots showing that sepsis response states have considerable overlap between sources of infection. Control subjects (n = 10) are indicated by x.

with FP and patients with CAP and found significant enrichment of 24 of 39 genes ( $P < 1 \times 10^{-4}$ ).

### Temporal Changes in Gene Expression

Interpretation of gene expression patterns in sepsis may be complicated by dynamic differences in the host response over time (10, 29). We explored temporal changes in gene expression for patients with FP and patients with CAP from whom serial samples following ICU admission were available. We first sought to identify genes that varied over time using a repeated measures regression model. We found that 714 genes were significantly differentially expressed in patients with FP sepsis between Days 1 and 5, compared with only 80 genes in patients with CAP (FDR,  $<0.05$ ; FC,  $>1.5$ ) (Figure 4A, Table E1Q). We further analyzed our data using a multivariate empirical Bayes model that ranks genes based on differential expression for longitudinal series involving multiple biological conditions (30), restricting this analysis to patients from whom samples were available at all three time points. Notably, temporal changes in gene expression were again more pronounced in patients with FP (Table E1R), and the specific genes whose expression changed over time differed between FP and CAP (Figures 4B and C).

In patients with FP, it was possible to estimate with reasonable accuracy the time of onset of the acute event. We calculated days from disease onset for each FP sample and used this to investigate temporal changes. Using a linear model with limma analysis, we found that 140 genes showed significant changes in expression over time (FDR,  $<0.05$ ) (Table E1S), including *AAK1* and *SNN* (Figure 4D). Pathway analysis was significant for genes involved in phagosome formation; natural killer cell and IL-3 signaling with evidence of enrichment for specific biological functions, notably cellular degranulation, chemotaxis, leukocyte activation, macrophage adhesion, phagocytosis, and bacterial infection (Table E1T).

### Discussion

We have characterized the transcriptomic response to sepsis caused by FP and found evidence of SRSs associated with outcome, with patients in SRS1 having higher early mortality. Transcriptomic features of endotoxin tolerance and pathway enrichment for cell death, apoptosis, necrosis, and T-cell exhaustion are consistent with animal models and human studies demonstrating the importance of immune compromise in sepsis pathogenesis and as a determinant of poor outcomes (4, 31, 32). The FP dataset allowed us to explore evolution of SRS membership. We found a significant number of patients (46%) switched SRS groups in the first 5 days of ICU admission, with the majority moving from SRS1 to SRS2. Persistence of SRS1 is associated with a poor outcome, whereas maintenance or recovery of immune competence (SRS2) is associated with survival. These findings further support the concept that SRS group membership reflects clinically important biological differences (5) and suggest that, if used as a biomarker, the transcriptomic response signature should be determined at the time a therapeutic intervention is being considered. Establishing the immune response state of a patient could enable individually tailored immunotherapy and monitoring of the response to treatment.

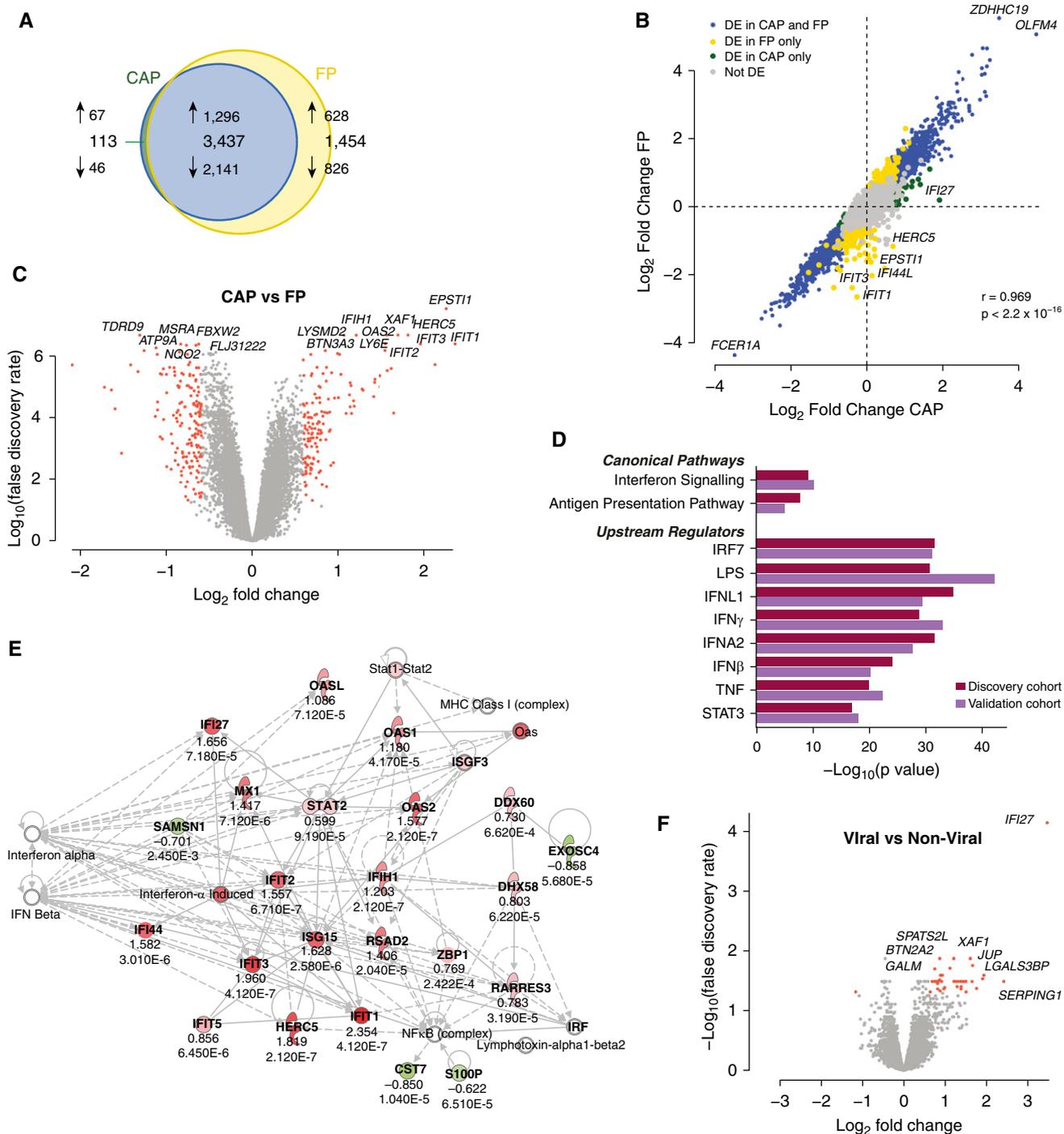
We found that the transcriptomic response is, to a large extent, shared between the two sources of infection we analyzed, with the expression of only a modest number of genes being dependent on source of infection. This shared sepsis response involves a significant proportion of the transcribed genome and overlapped with the “genomic storm” following trauma, although we observed some differences involving, for example, TNFR1 signaling (26). Regarding gene expression between FP and CAP, the most enriched networks involved IFN- $\alpha/\beta$  and the antimicrobial/inflammatory response. These differences seem to be driven predominantly by viral respiratory infection

within the CAP cohort; however, given current difficulties in pathogen detection, the biological and clinical interpretation of such transcriptomic differences remains challenging. Whereas some previous studies have supported a common transcriptional septic response independent of pathogen (33), others have reported that expression signatures can discriminate between infecting organisms (6–8, 13), although these findings remain controversial (9, 10). For patients admitted to the ICU with suspected CAP, a 78-transcript signature has been reported to differentiate cases of CAP from non-CAP, with the *FAIM3:PLAC8* gene expression ratio proposed as a diagnostic biomarker (11).

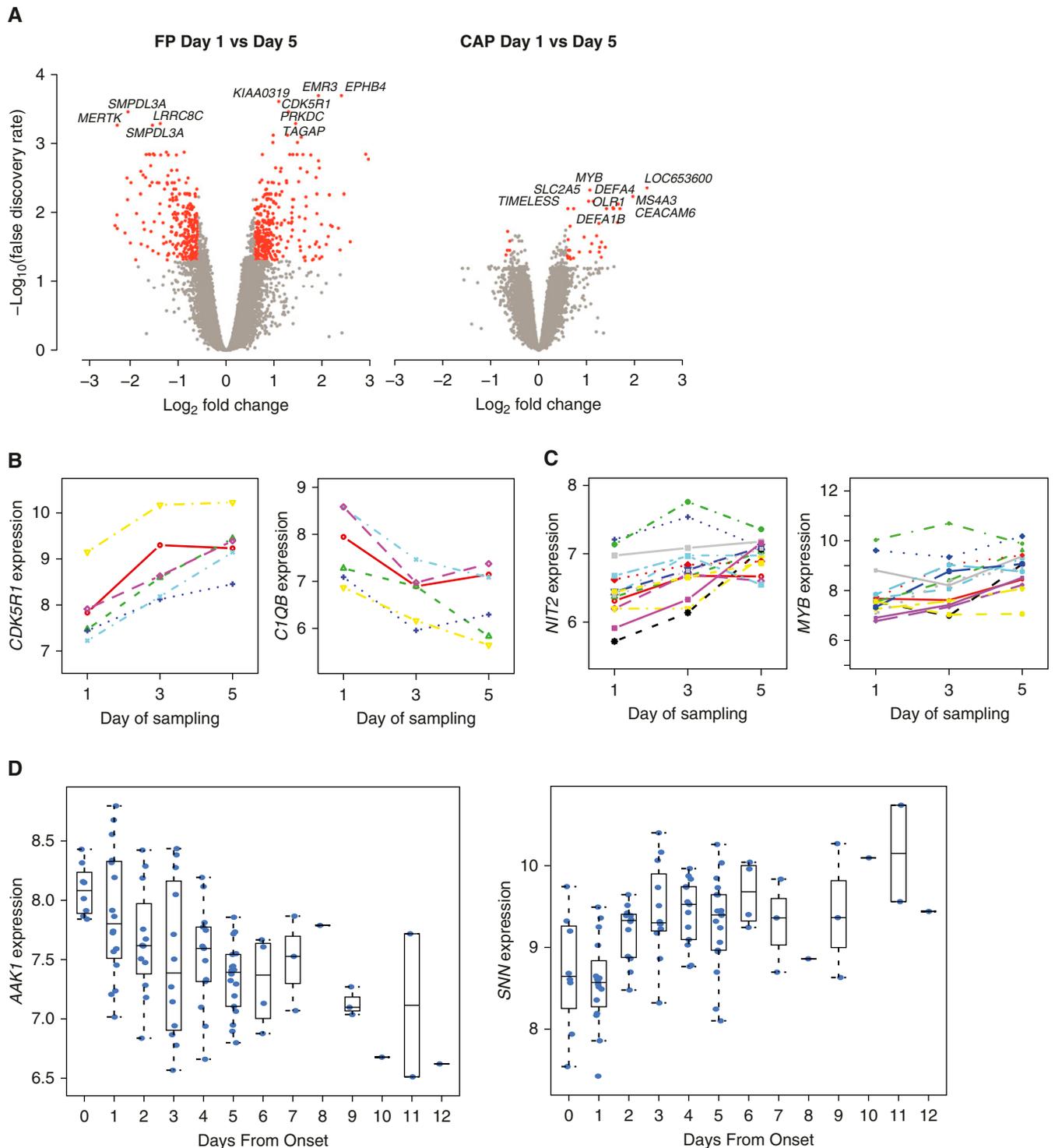
We found that temporal changes were more pronounced in patients with FP, with more than eight times as many genes differentially expressed between admission and Day 5 than in patients with CAP, and these changes involved phagosome formation, natural killer cell signaling, IL-3 signaling, leukocyte activation, mitochondrial damage, and apoptosis. To date, time series analyzing changes in gene expression have been focused on animal models of sepsis (34), the endotoxin response in healthy volunteers (35), the response to trauma and subsequent infection (26), and early events in sepsis (29), and authors of a recent analysis of time-matched cohorts defined a gene set distinguishing sterile inflammation from infectious inflammation (12). In general, clearer resolution of temporal differences is critical to resolving heterogeneity in observed sepsis responses within and between patients.

Future work should include comprehensive pathogen phenotyping, such as by using metagenomic sequencing, given the recognized high proportion of culture-negative patients with sepsis (36, 37). Comparison of patients with FP with specific control subjects, such as a group of patients undergoing laparotomy for noninfectious indications, would control for the effects of damage-associated molecular pattern-mediated signaling due to the surgical procedure as well as any modulating influences on the transcriptome due to general anesthesia, whereas inclusion

**Figure 2.** (Continued). showing correlation between the first six PCs, SRSs, and clinical covariates for sepsis samples (FP,  $n = 94$ ; CAP,  $n = 127$ ). (C) Venn diagram showing the overlap in differential gene expression versus control subjects in the sepsis response and the response to trauma (false discovery rate [FDR],  $<0.05$ ; fold change [FC],  $>1.5$ ; first 5 d). Selected condition-specific enriched pathways and biological functions are noted. (D) Volcano plot of differentially expressed probes for FP ( $n = 94$ ; left) and CAP ( $n = 127$ ; right) versus control subjects ( $n = 10$ ) (red coloring shows FC  $>1.5$ , FDR  $<0.05$ ). APACHE = Acute Physiology and Chronic Health Evaluation; EIF2 = eukaryotic initiation factor 2; NFAT = nuclear factor of activated T cells; SOFA = Sequential Organ Failure Assessment; TNFR1 = tumor necrosis factor receptor 1.



**Figure 3.** Variation in the sepsis transcriptome according to source of infection. (A) Venn diagram showing the overlap in the fecal peritonitis (FP) and community-acquired pneumonia (CAP) sepsis response in terms of the number of differentially expressed genes versus control subjects (FP, n = 64 samples; CAP, n = 73 samples; control subjects, n = 10 samples). (B) Correlation of differential gene expression (DE) between sepsis due to FP (n = 64 samples) and control subjects (n = 10 samples), and sepsis due to CAP (n = 73 samples) and control subjects (n = 10 samples). (C) Volcano plot of differentially expressed probes for CAP versus FP (red coloring shows fold change [FC] >1.5, FDR <0.05; positive fold change indicates relative upregulation in CAP). (D) Enriched pathways and predicted upstream regulators derived from differentially expressed genes for CAP versus FP in the discovery (CAP, n = 73 samples; FP, n = 64 samples) and validation cohorts (CAP, n = 53 samples; FP, n = 53 samples). (E) Most significantly enriched network ( $P = 1 \times 10^{-39}$ ), based on comparison of CAP versus FP with differentially expressed genes shaded (red = upregulation; green = downregulation), and logarithmic FC with P value shown for each gene. (F) Volcano plot of the differentially expressed probes for positive viral diagnosis (n = 25) versus no positive viral diagnosis (n = 240) in 265 patients with CAP. Red coloring indicates FC less than 1.5, FDR less than 0.05. IRF = IFN regulatory factor; MHC = major histocompatibility complex; STAT = signal transducer and activator of transcription; TNF = tumor necrosis factor.



**Figure 4.** Dynamics of gene expression in sepsis due to fecal peritonitis (FP) and community-acquired pneumonia (CAP). (A) Volcano plot of the differentially expressed probes for Day 1 versus Day 5 samples from the same patient in FP ( $n = 9$ ; *left*) and CAP (*right*;  $n = 17$ ) (red coloring indicates fold change  $> 1.5$  and false discovery rate  $< 0.05$ ). (B) Examples of genes showing significant temporal variation in expression over time for patients with sepsis due to FP include the kinase activator *CDK5R1* and complement component *C1QB*; other examples are the adhesion receptor *EMR3* and cysteine protease *CAPN13*. (C) Examples of genes showing variation in expression over time for patients with sepsis due to CAP include cell cycle gene *NIT2* and the hematopoiesis regulatory transcription factor gene *MYB*; other examples are the antimicrobial neutrophil peptidase *CTSG*, inflammatory regulator *CAMP*, defensin *DEFA4*, and neutrophil granule elastase *ELANE*. (D) Further examples of genes differentially expressed in FP over time plotted from time of onset of FP include the adaptor-related protein complex 2-associated kinase 1 (*AAK1*), involved in regulating clathrin-mediated endocytosis (44), which is critical for bacterial entry (45), as well as *SNN* encoding stannin, a mitochondrial damage sensor (46).

of patients undergoing laparotomy for gastric or small bowel perforation would to some extent control for different pathogens. Differences in differential cell count are potential confounders in transcriptomic analysis (10), and, although the results presented were robust to differential leukocyte count, cell-type-specific transcriptomic profiling will be important for future studies (38).

Defining the most robust and informative predictive gene set for SRS membership on the basis of transcriptomics requires prospective large-scale validation using an appropriate point-of-care test. We note that the current seven-gene set established in patients with CAP and validated in patients with FP is consistent with a key role for a dysfunctional immune response in sepsis. This predictive gene set

includes genes involved in cell growth (*DYRK2*), cell cycle (*CCNB1IP1*), stem cell maintenance (*TDRD9*), and DNA damage (*ADGRE3*), consistent with the observed pathway enrichment (cell death, apoptosis, necrosis) and with immune response through lymphocyte activation (*ZAP70*), major histocompatibility complex class II export (*ARL14EP*), and myeloid cell interactions in immunity (*MDC1*). The lack of overlap with pediatric sepsis endotypes that we observed requires further validation and may reflect developmental differences impacting pathophysiology and host immune dysfunction (39, 40).

We have addressed sepsis heterogeneity using a transcriptomic approach in patients with FP and patients with CAP, demonstrating a shared sepsis response with distinct SRS groups that are dynamic, reflect

the underlying biological response, and are informative for prognosis. Our findings also provide some evidence for differential patterns of expression between CAP and FP, two of the most common causes of sepsis. Our analysis, coupled with recent advances in the field (3, 4, 11, 41), highlights the opportunity to develop novel therapeutic interventions that can be targeted and appropriately timed to individual patients on the basis of their transcriptomic signatures, providing opportunities for precision medicine in sepsis. ■

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