Enriched endotypes for sepsis

Julian Knight
University of Oxford, UK

wellcome centre human genetics

Interdisciplinary symposium on sepsis, Paris 8th September 2022

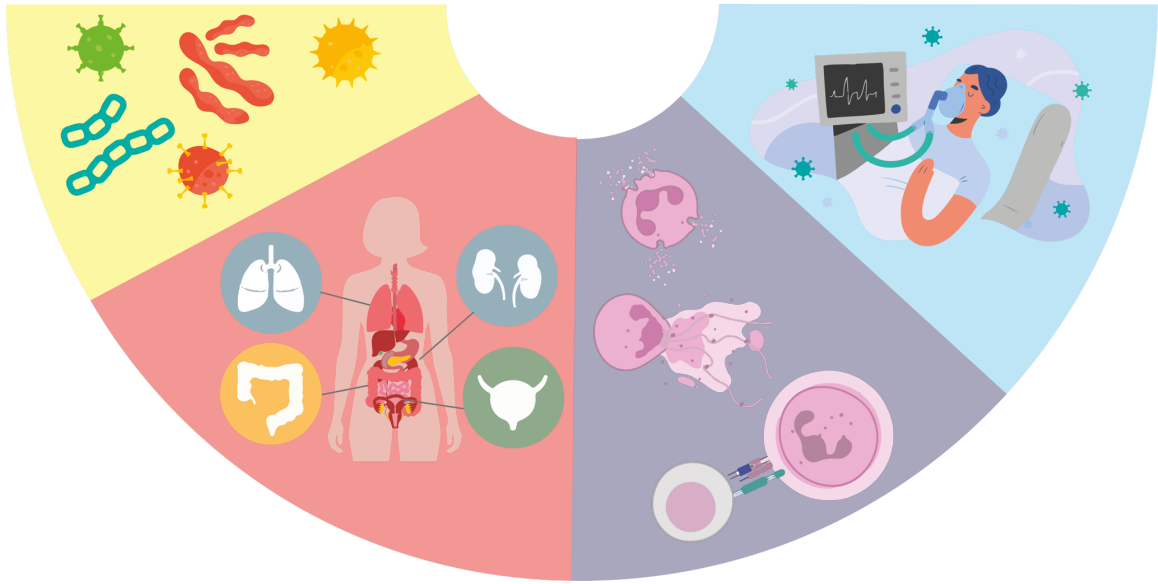
Sepsis: life-threatening organ dysfunction caused by a dysregulated host response to infection

Our individual response to infection is highly **heterogeneous** and not well captured by sepsis as a clinical syndrome

Complexity of sepsis pathophysiology, incomplete knowledge

Organ dysfunction and risk of death results from a **maladaptive host immune response to infection**

Currently, **clinical trials** and **development of targeted immunomodulatory therapies** is limited by incomplete understanding of the drivers of sepsis and how to more effectively stratify patients



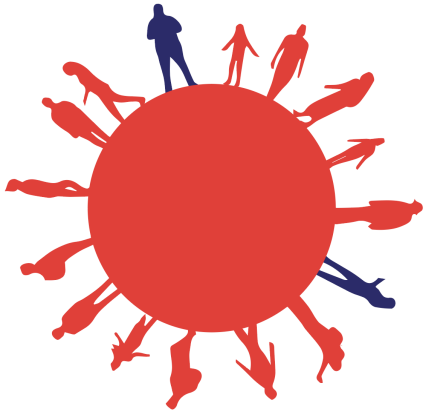
Singer M *et al*. 2016 The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA* **315**, 801-10; Marshall JC. 2014 Why have clinical trials in sepsis failed? *Trends Mol Med* 20:195-203; van der Poll *et al.* 2021 The immunology of sepsis. *Immunity*, **54,** 2450-2464.

Sepsis: moving towards a more precision medicine approach

RECOVERY
Randomised Evaluation of COVID-19 Therapy

The more homogenous severe COVID-19 disease shows potential for immunotherapy in extreme response to infection

Delivering the right treatment to the right patient at the right time in critical illness



A precision medicine approach based on patient characteristics informative for one or more **pathophysiological mechanisms/ processes/ states** predominant in a given patient (occurring or predicted to occur) that are therapeutically relevant for that patient at the time of assessment and clinical decision making to guide targeted intervention

Horby et al., *Dexamethasone in Hospitalized Patients with Covid-19.* N Engl J Med, 2021. **384,** 693-704

Achieving greater precision within sepsis syndrome: terminology

- **subphenotypes** (subgroups of patients) based on patient characteristics, clinical and or molecular (-omic, multi-omic) which individually may be informative for a specific state but that state may only be identifiable by looking, for example in the plasma proteome
- **endotype** = where subphenotype (subgroup) characteristics/biomarkers define or associate with a specific pathophysiological mechanism
- **treatable trait** = where the subphenotype (subgroup) characteristics/biomarkers identify a group of patients with a specific pathophysiological derangement and predictable response to a specific therapy

Maslove DM et al (2022). Redefining critical illness. *Nature Medicine* **28**, 1141-1148

Precision medicine in sepsis

Long term goal: a disease classification based on pathophysiology, with patient groupings (classifiers) that will likely span current overlapping clinical syndromic definitions (sepsis, ARDS, AKI…)

Progress….

- currently mainly **uni-modal, single lens view** (subphenotyping based on clinical/laboratory features, circulating cytokines, single –omic viewpoints)

JAMA | Original Investigation | CARING FOR THE CRITICALLY ILL PATIENT

Derivation, Validation, and Potential Treatment Implications of Novel Clinical Phenotypes for Sepsis

Christopher W. Seymour, MD, MSc; Jason N. Kennedy, MS; Shu Wang, MS; Chung-Chou H. Chang, PhD; Corrine F. Elliott, MS; Zhongying Xu, MS; Scott Berry, PhD; Gilles Clermont, MD, MSc; Gregory Cooper, MD, PhD; Hernando Gomez, MD, MPH; David T. Huang, MD, MPH; John A. Kellum, MD, FACP, MCCM; Qi Mi, PhD; Steven M. Opal, MD; Victor Talisa, MS; Tom van der Poll, MD, PhD; Shyam Visweswaran, MD, PhD; Yoram Vodovotz, PhD; Jeremy C. Weiss, MD, PhD; Donald M. Yealy, MD, FACEP; Sachin Yende, MD, MS; Derek C. Angus, MD, MPH

JAMA. 2019;321(20):2003-2017. doi:10.1001/jama.2019.5791

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- **unsupervised approaches (clustering)** powerful but inconsistency within molecular phenotyping across studies – different subphenotypes, platforms, variable power, covariates; progress towards defining endotypes and treatable traits remains limited

Genomic expression profiling across the pediatric systemic inflammatory response syndrome, sepsis, and septic shock spectrum*

Hector R. Wong, MD; Natalie Cvijanovich, MD; Geoffrey L. Allen, MD; Richard Lin, MD; Nick Anas, MD; Keith Meyer, MD; Robert J. Freishtat, MD; Marie Monaco, BSN; Kelli Odoms, BS; Bhuvaneswari Sakthivel, MS; Thomas P. Shanley, MD; for the Genomics of Pediatric SIRS/Septic Shock Investigators

Crit Care Med 2009 Vol. 37, No. 5

Genomic landscape of the individual host response and outcomes in sepsis: a prospective cohort study

Emma E Davenport, Katie L Burnham*, Jayachandran Radhakrishnan*, Peter Humburg, Paula Hutton, Tara C Mills, Anna Rautanen, Anthony C Gordon, Christopher Garrard, Adrian V S Hill, Charles J Hinds, Julian C Knight

Lancet Respir Med 2016 4: 259–71

Predicting sepsis severity at first clinical presentation: The role of endotypes and mechanistic signatures

Arjun Baghela,[a,b] Olga M. Pena,[a] Amy H. Lee,[c] Beverlie Baquir,[a] Reza Falsafi,[a] Andy An,[a] Susan W. Farmer,[a] Andrew Hurlburt,[d] Alvaro Mondragon-Cardona,[e,f] Juan Diego Rivera,[e,f] Andrew Baker,[g] Uriel Trahtemberg,[g] Maryam Shojaei,[h] Carlos Eduardo Jimenez-Canizales,[e,f] Claudia C. dos Santos,[g] Benjamin Tang,[h] Hialmar R. Bouma,[i,j] Gabriela V. Cohen Freue,[k] and Robert E.W. Hancock[a,*]

eBioMedicine 2022;75:103776

Prospective clinical testing and experimental validation of the Pediatric Sepsis Biomarker Risk Model

Hector R. Wong[1,2*], J. Timothy Caldwell[1], Natalie Z. Cvijanovich[3], Scott L. Weiss[4], Julie C. Fitzgerald[4], Michael T. Bigham[5], Parag N. Jain[6], Adam Schwarz[7], Riad Lutfi[8], Jeffrey Nowak[9], Geoffrey L. Allen[10], Neal J. Thomas[11], Jocelyn R. Grunwell[12], Torrey Baines[13], Michael Quasney[14], Bereketeab Haileselassie[15], Christopher J. Lindsell[16]

Sci. Transl. Med. **11**, eaax9000 (2019)

Unsupervised Analysis of Transcriptomics in Bacterial Sepsis Across Multiple Datasets Reveals Three Robust Clusters

Timothy E. Sweeney, MD, PhD[1,2]; Tej D. Azad[1,2]; Michele Donato, PhD[1,2]; Winston A. Haynes[1,2]; Thanneer M. Perumal, PhD[3]; Ricardo Henao, PhD[4,5]; Jesús F. Bermejo-Martin, MD, PhD[6]; Raquel Almansa, PhD[6]; Eduardo Tamayo, MD, PhD[6]; Judith A. Howrylak, MD[7]; Augustine Choi, MD[8]; Grant P. Parnell, PhD[9]; Benjamin Tang, MD[9–12]; Marshall Nichols, MS[4]; Christopher W. Woods, MD[4,13,14]; Geoffrey S. Ginsburg, MD, PhD[4]; Stephen F. Kingsmore, MD, DSc[15]; Larsson Omberg, PhD[3]; Lara M. Mangravite, PhD[3]; Hector R. Wong, MD[16,17]; Ephraim L. Tsalik, MD[4,13,14]; Raymond J. Langley, PhD[18]; Purvesh Khatri, PhD[1,2]

Critical Care Medicine 2018 • Volume 46 • Number 6

Classification of patients with sepsis according to blood genomic endotype: a prospective cohort study

Brendon P Scicluna, Lonneke A van Vught, Aeilko H Zwinderman, Maryse A Wiewel, Emma E Davenport, Katie L Burnham, Peter Nürnberg, Marcus J Schultz, Janneke Horn, Olaf L Cremer, Marc J Bonten, Charles J Hinds, Hector R Wong, Julian C Knight, Tom van der Poll, on behalf of the MARS consortium*

Lancet Respir Med 20 http://dx.doi.org/10.1016/

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Progress….

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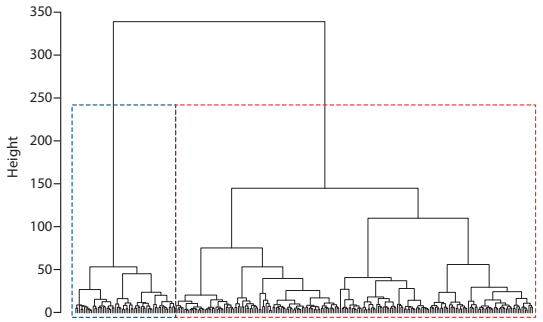
- **unsupervised approaches (clustering)** powerful but inconsistency within molecular phenotyping – different subphenotypes reported, different platforms, variable power, covariates; progress towards defining endotypes and treatable traits remains limited
- **need for** standardization of terminology, goals, collaborative systematic approaches; multi-modal data integration; high quality mechanistic work; addressing in clinical trial setting; feasibility of point of care testing….
- here I describe our work with sepsis response signatures and **progress towards a sepsis endotype**

Structure of talk:

- Whole blood leukocyte transcriptomics identifies sepsis response signatures
- An SRS quantitative score applicable to a variety of infections
- Towards a mechanistic basis for SRS

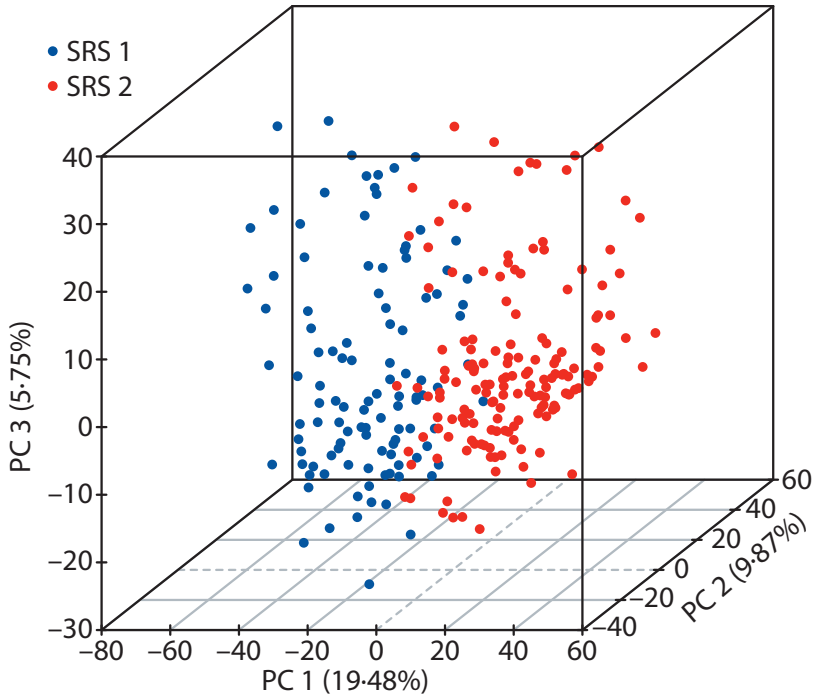
Transcriptomics-led approach defines sepsis subphenotypes associated with response state and outcome

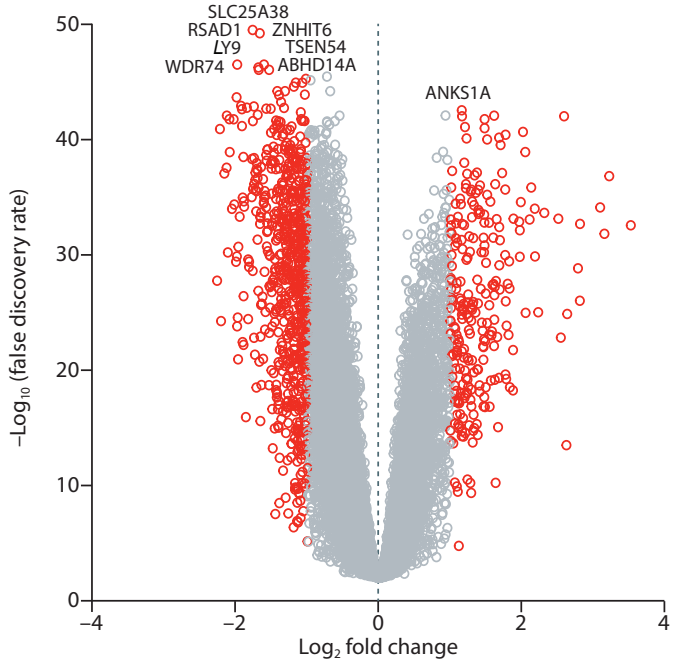


UK Genomic Advances in Sepsis (GAinS) study

Unsupervised hierarchical cluster analysis 10% most variable genes in whole blood leukocytes from sepsis due to community acquired pneumonia (discovery cohort n=265)

Sepsis response signatures. SRS1: expression signatures of endotoxin tolerance, T-cell exhaustion, down-regulation of HLA class II






Emma Davenport


Katie Burnham


Charles Hinds

Davenport *et al* 2016 Genomic landscape of the individual host response and outcomes in sepsis: a prospective cohort study. *Lancet Respiratory Medicine* **4**, 259-271

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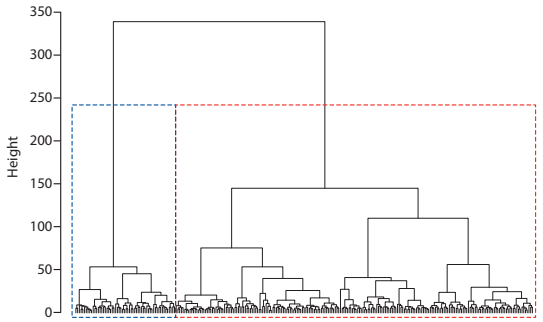

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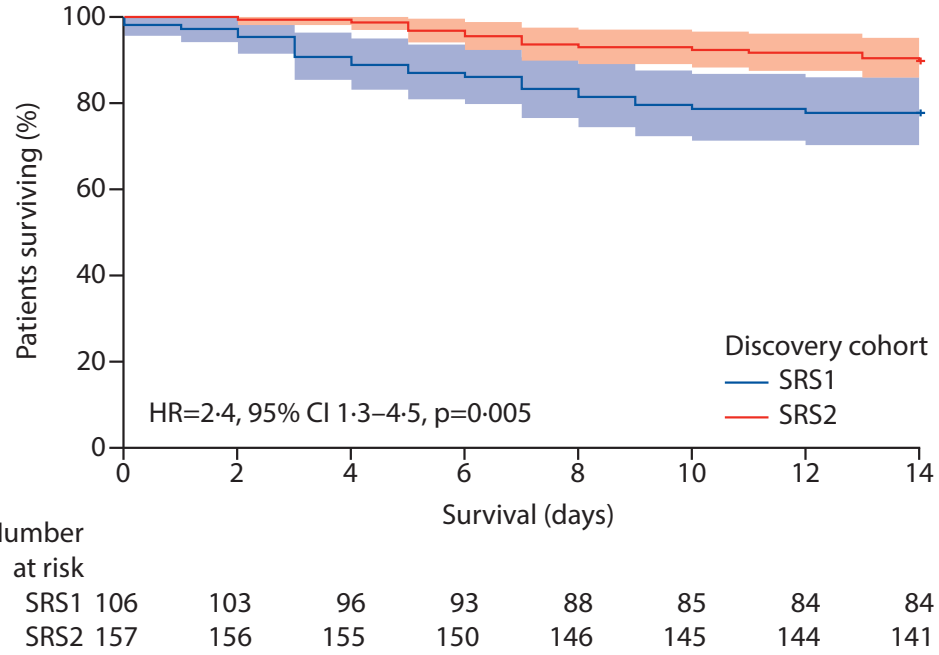
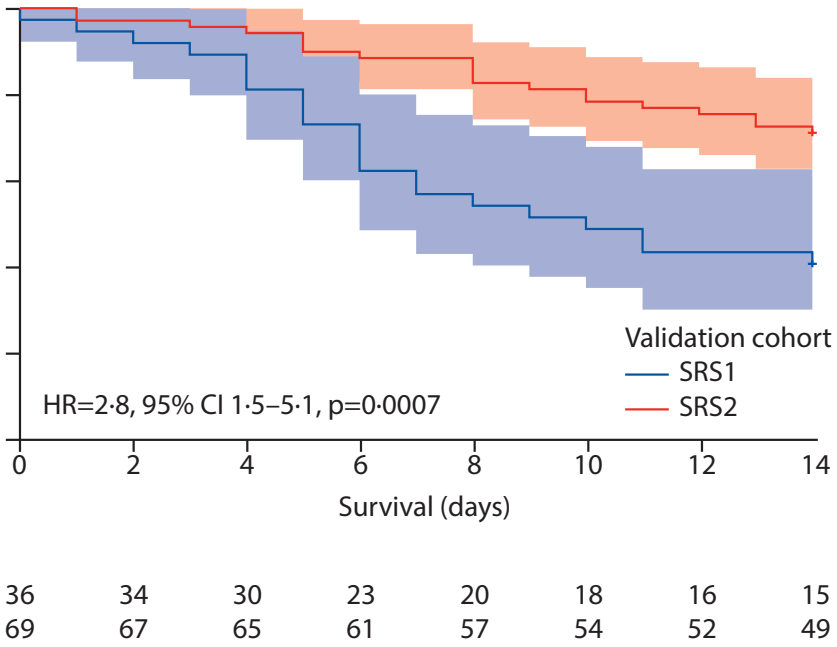


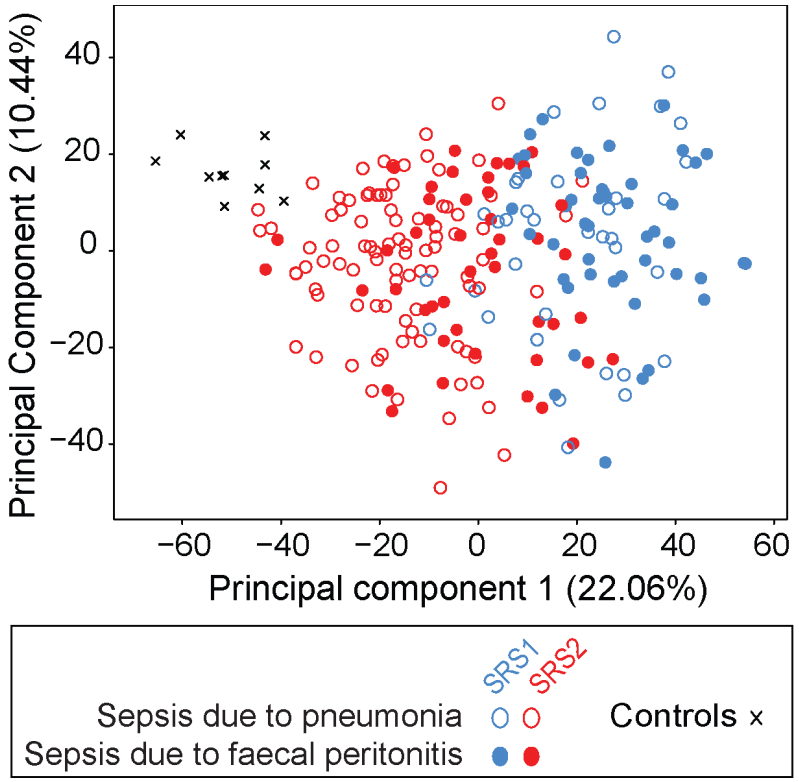


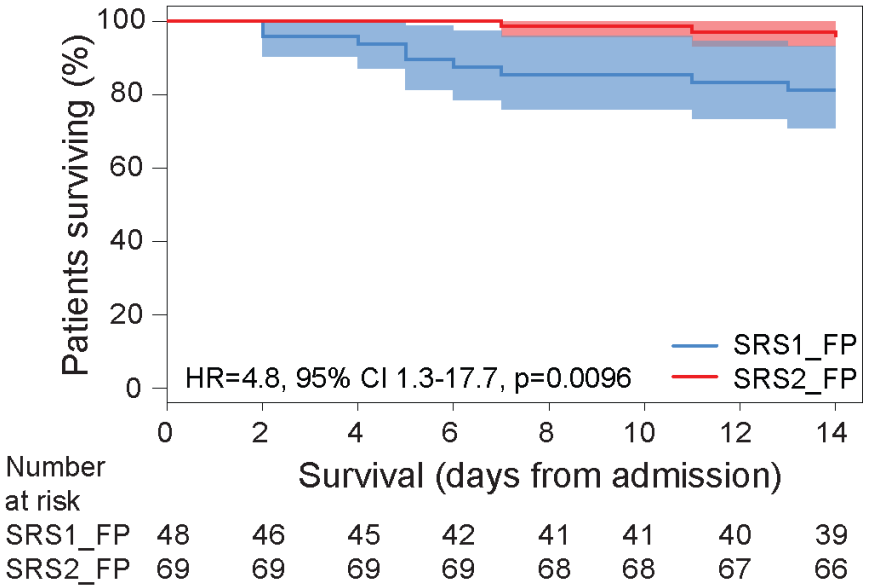
SRS1 associated with **higher early mortality** and more severe illness but not age, sex or microbiology

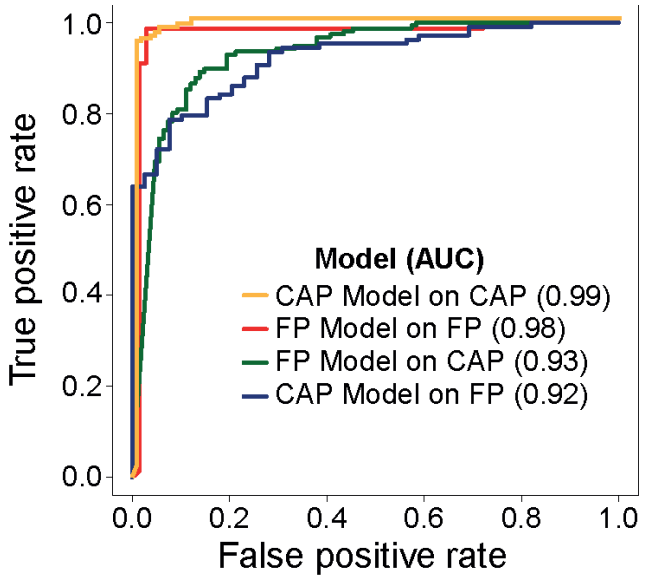
Expression of seven genes **predictive of SRS group membership**

Clinical covariates limited efficacy to predict SRS

SRS in sepsis due to faecal peritonitis







DYRK2, CCNB1IP1, TDRD9, ZAP70, ARL14EP, MDC1, ADGRE3

SRS main driver of variance in gene expression rather than aetiology. Sepsis due to faecal peritonitis (FP) (n=117) or community acquired pneumonia (CAP) (n=126), and non-septic controls (n=10)

SRS and sepsis due to **fecal peritonitis** shows same association with mortality

ROC curves for SRS assignment using predictive gene sets derived in CAP or FP

Burnham K *et al* 2017 Shared and Distinct Aspects of the Sepsis Transcriptomic Response to Fecal Peritonitis and Pneumonia. *Am J Respir Crit Care Med* 196, 328-339

Is knowledge of SRS potentially useful in guiding therapy?

Steroids and patient survival in sepsis

- variation between trials with differences in the mortality effects

Approach

- post-hoc analysis of a double-blind randomized clinical trial in septic shock (VANISH)
- 18 UK intensive care units
- adult patients <6 hours of onset of shock, randomised to norepinephrine or vasopressin followed by hydrocortisone or placebo
- primary outcome survival at 28 days
- SRS determined using pre-defined endotype definitions (expression of seven discriminant genes)


David Antcliffe

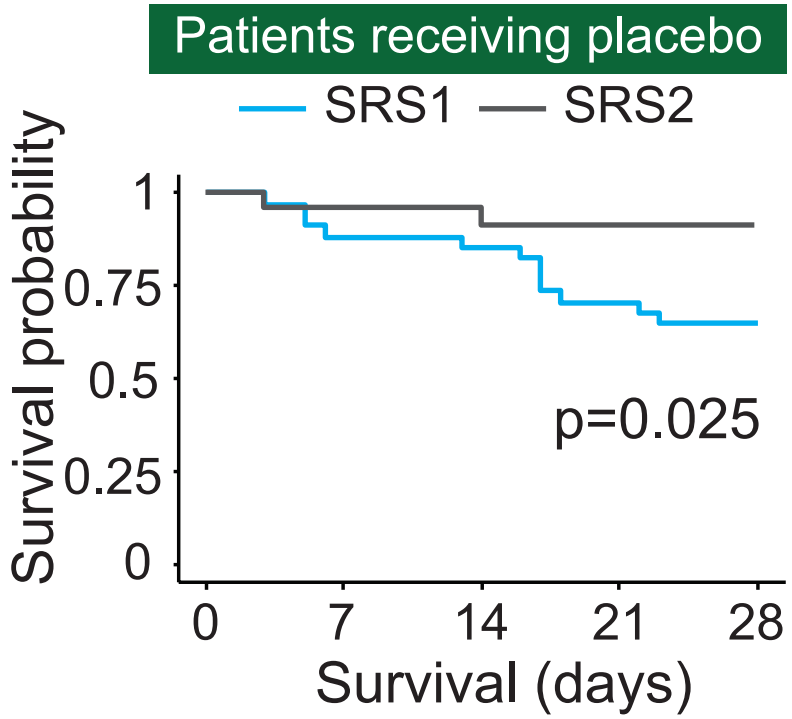

Katie Burnham


Tony Gordon

Antcliffe DB *et al*. 2019 Transcriptomic Signatures in Sepsis and a Differential Response to Steroids. From the VANISH Randomized Trial. *Am J Respir Crit Care Med* **199**, 980-986.

SRS associated with outcome

176 patients, 83 assigned to SRS1 and 93 to SRS2 endotype

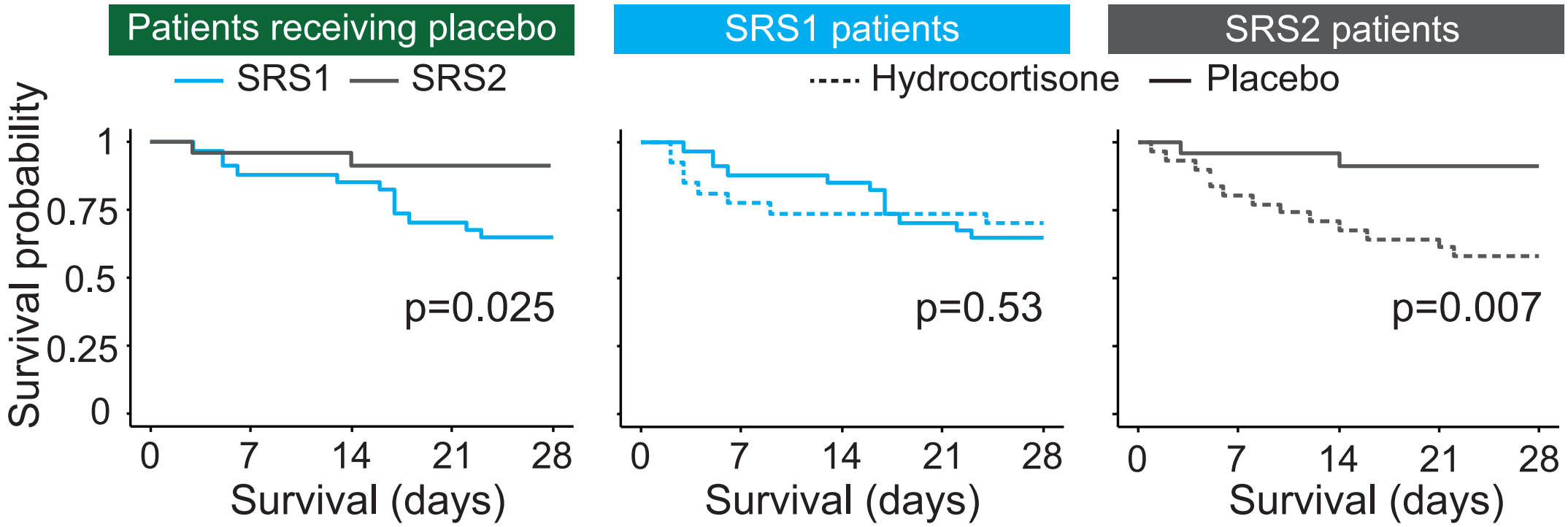


In patients who received **placebo**, mortality was lower in those with the SRS2 compared to SRS1

- 28-day mortality SRS2 (8%) compared with SRS1 (37%)
- odds ratio 0·15 95%CI 0·03-0·76, p=0.02
- consistent with mortality differences associated with SRS endotypes in GAinS

Hydrocortisone use associated with increased mortality in SRS2 patients

Interaction between assignment to **hydrocortisone or placebo, and SRS endotype (p=0·02)**

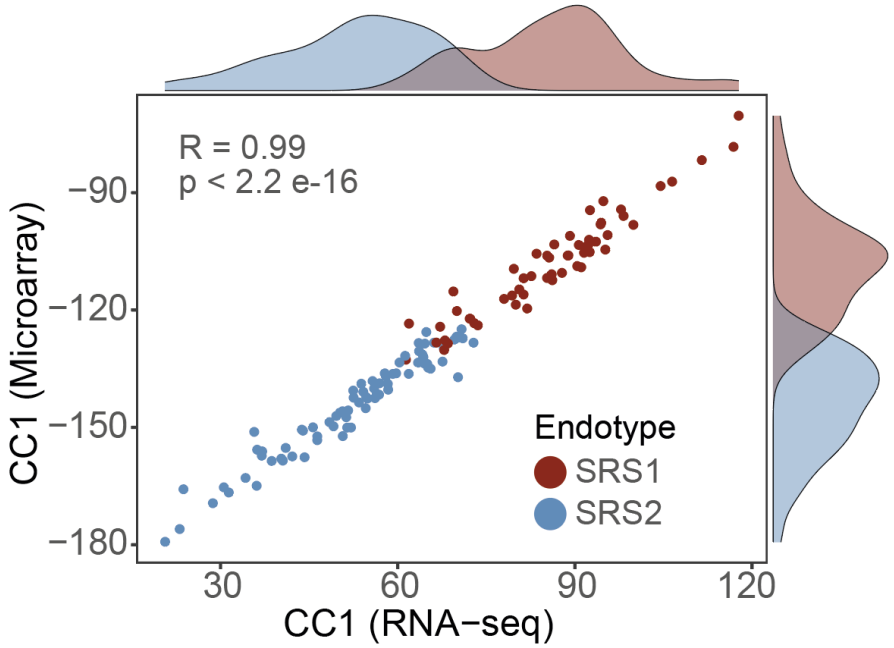


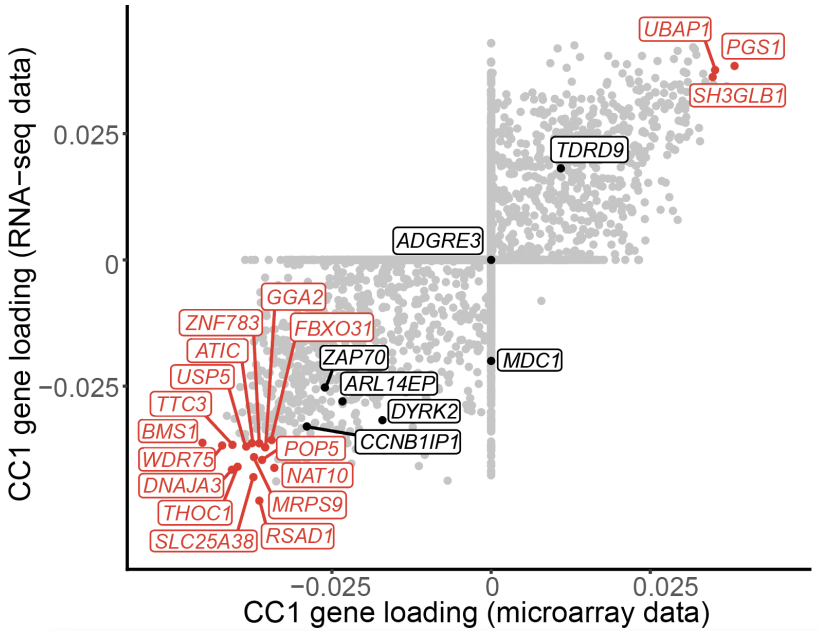
SRS1 OR 0·85 (95% CI 0·30-2·43)

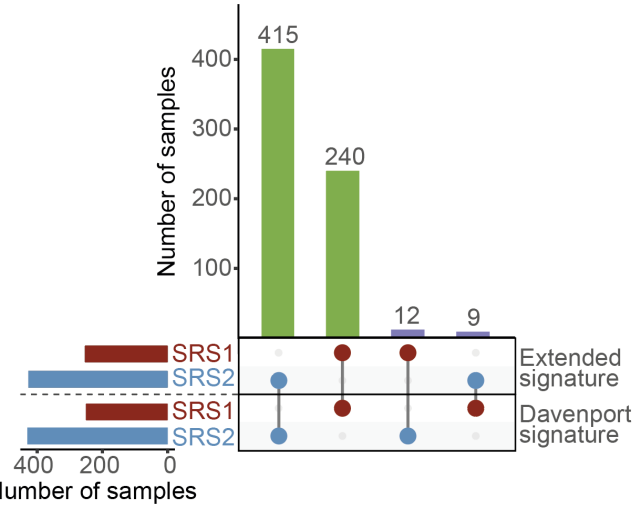
SRS2 OR 7·9 (95%CI 1·6-39·9)

Findings support use of SRS in future **biomarker guided trials of corticosteroids in septic shock**

SRS assignment using different assays of gene expression







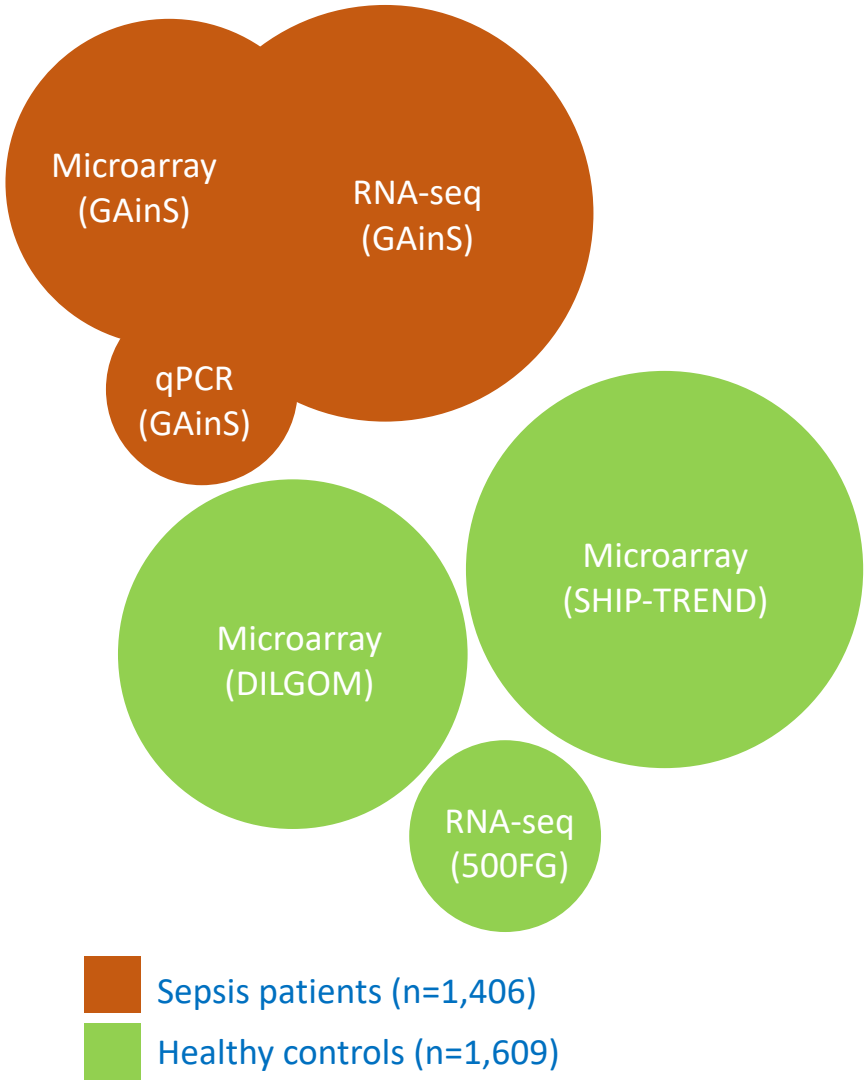
SRS assignment using gene expression assayed using microarray or RNA-seq is highly correlated (canonical correlation analysis) (n=134 GAinS patients)

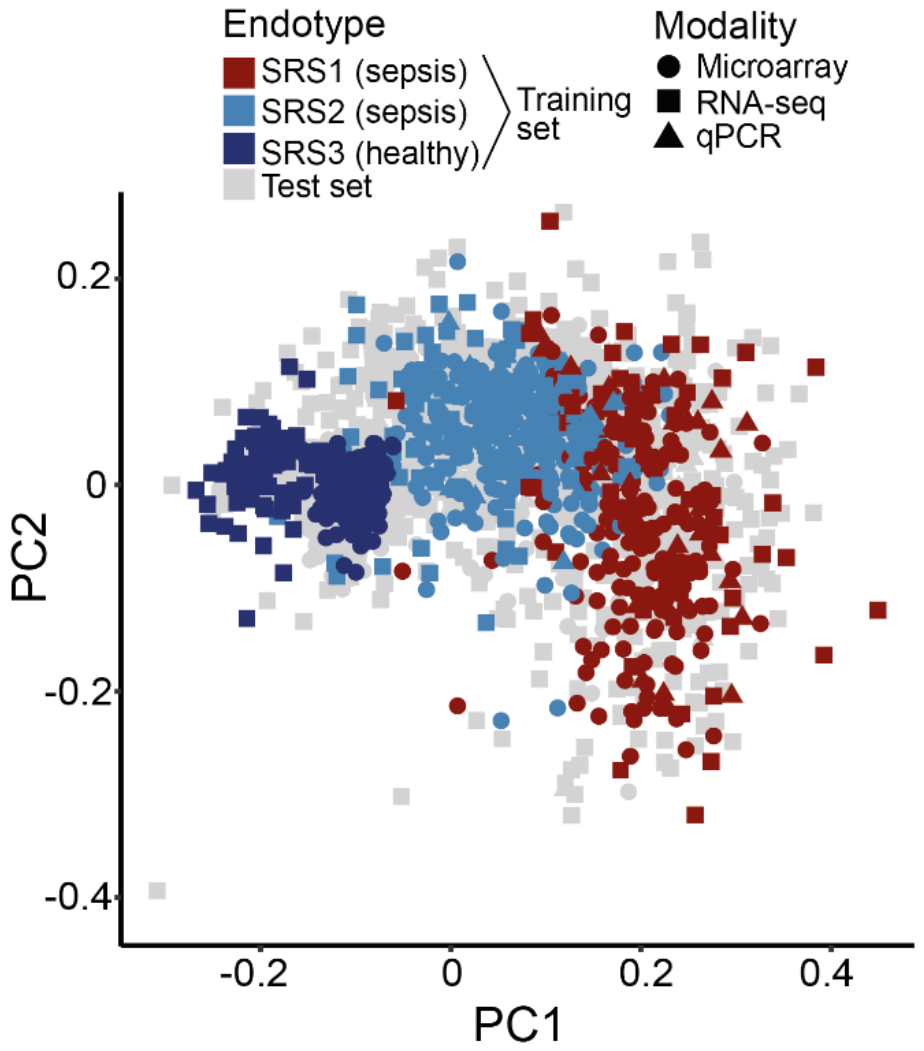
Refined original assignment gene set (n=7 genes) with additional 12 genes ranked amongst the top 1% with highest CC1 contribution (robust to technological variation)

Eddie Cano-Gamez

Cano-Gamez E et al (2022). An immune dysfunction score for stratification of patients with acute infection based on whole blood gene expression. *medRxiv*, 2022.2003.2017.22272427.

Constructing a cross-platform reference map of gene expression

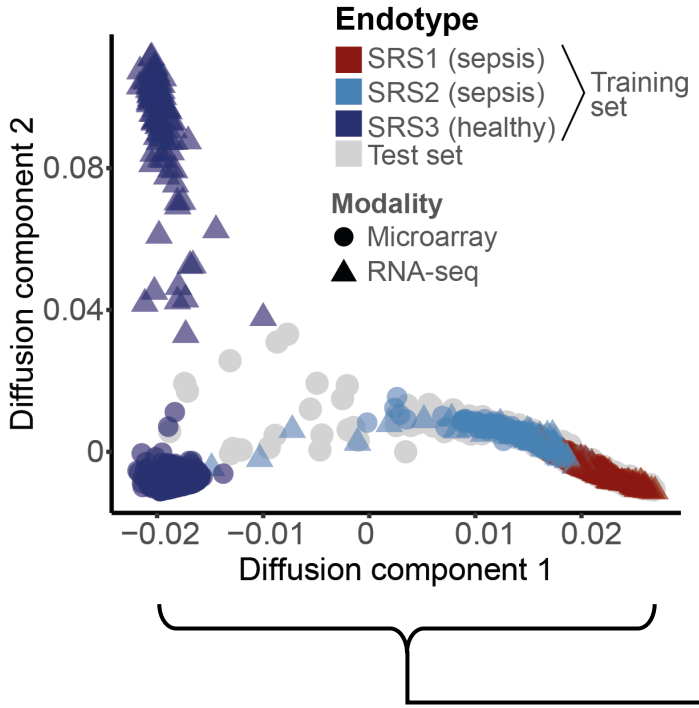




Cross-platform reference map of gene expression in sepsis anchored with reference to three cohorts of healthy individuals

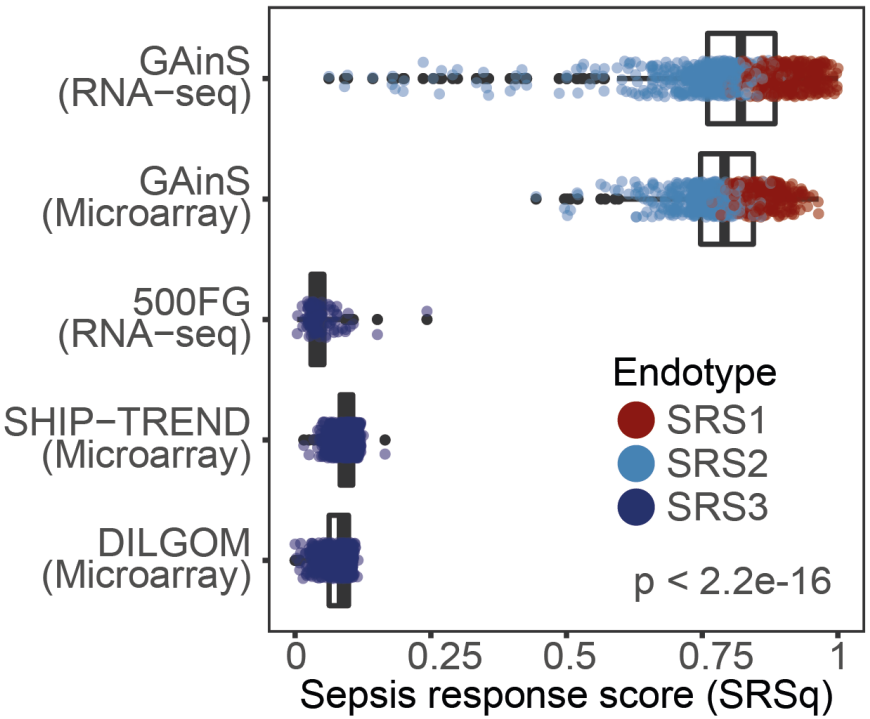
- Training set (n=909, known SRS plus healthy individuals) and test set (n=2,355) used to train random forest classifiers
- SRS3 (healthy individuals and patients in the low severity/recovery spectrum)

SRSq: a quantitative sepsis response signature score



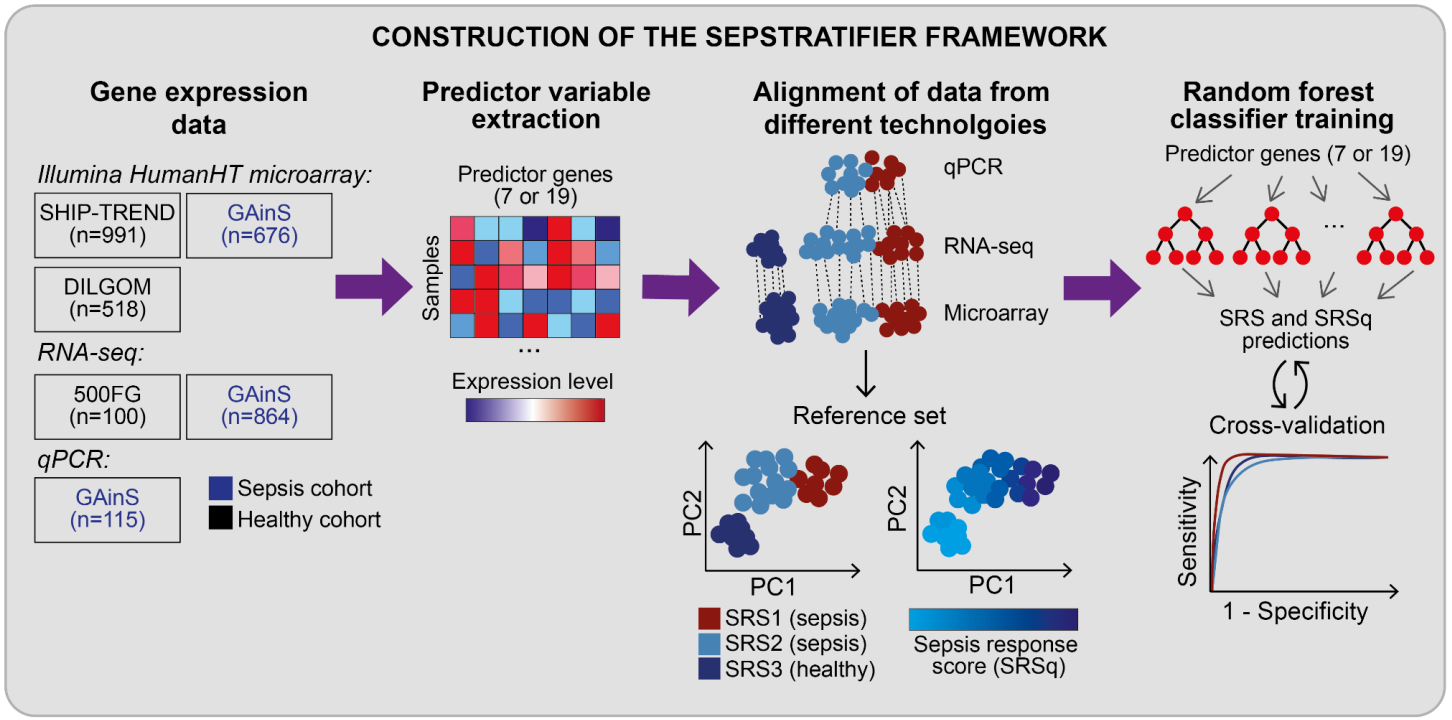
Modelling patients as a continuum using diffusion maps reflecting original connectivity - first diffusion component (DC1) separates samples from SRS3 to SRS2 and SRS1

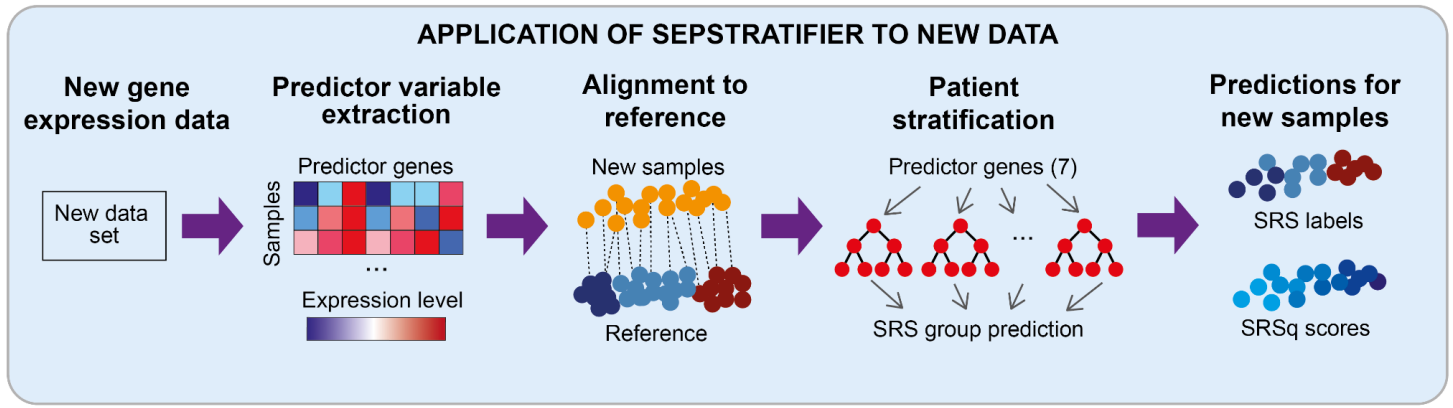
DC1 used to derive a quantitative metric reflective of the position of individuals along this continuum, the **quantitative sepsis response signature score** (SRSq) (0-1)



Distribution of SRSq across cohorts - lower values indicating patient is transcriptionally closer to health and higher values indicating similarity to the **highest extreme of SRS1**

SepstratifieR: a machine learning framework for patient stratification





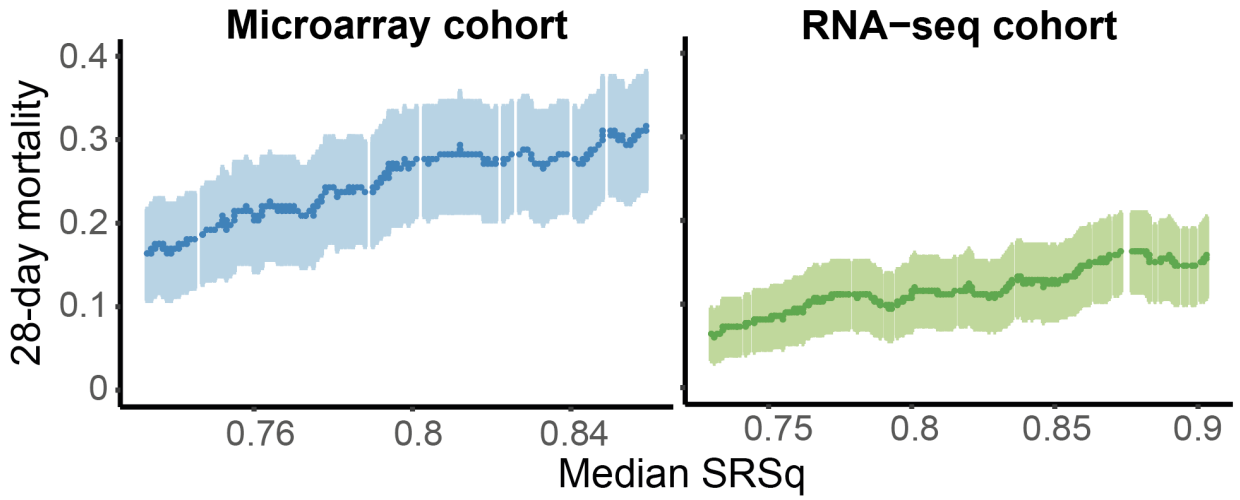
SRS/SRSq prediction in new data sets

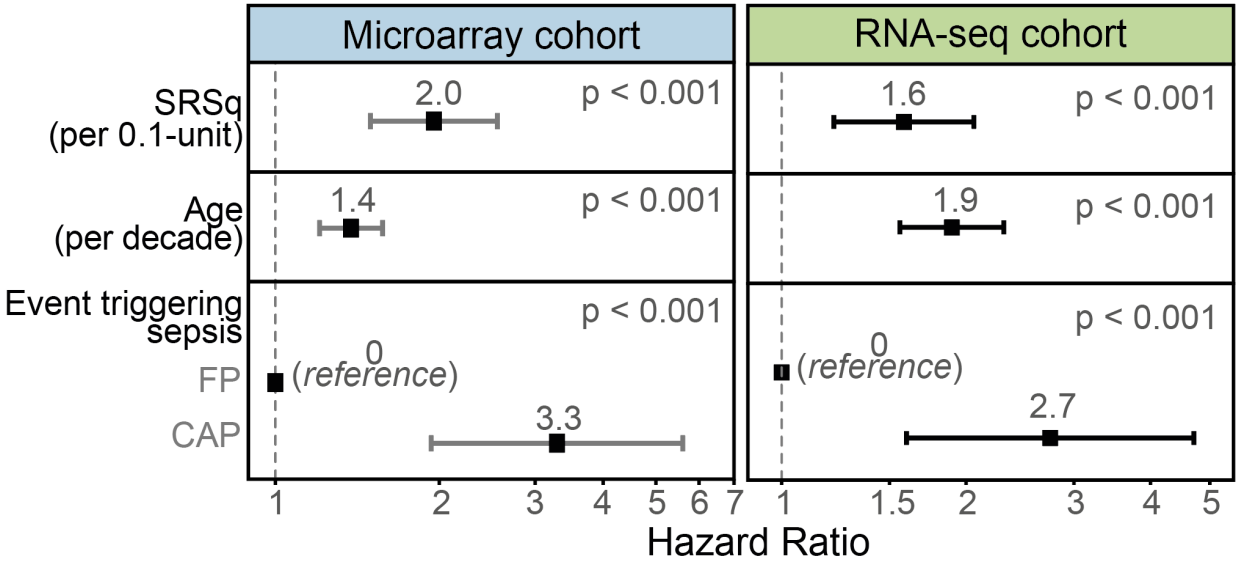
SepstratifieR algorithmic framework

- extracts expression measurements of signature genes
- aligns samples to the corresponding reference map using mutual nearest neighbour
- predicts SRS and SRSq using random forest classifiers

https://github.com/jknightlab/SepstratifieR

Mortality increases proportionally to SRSq



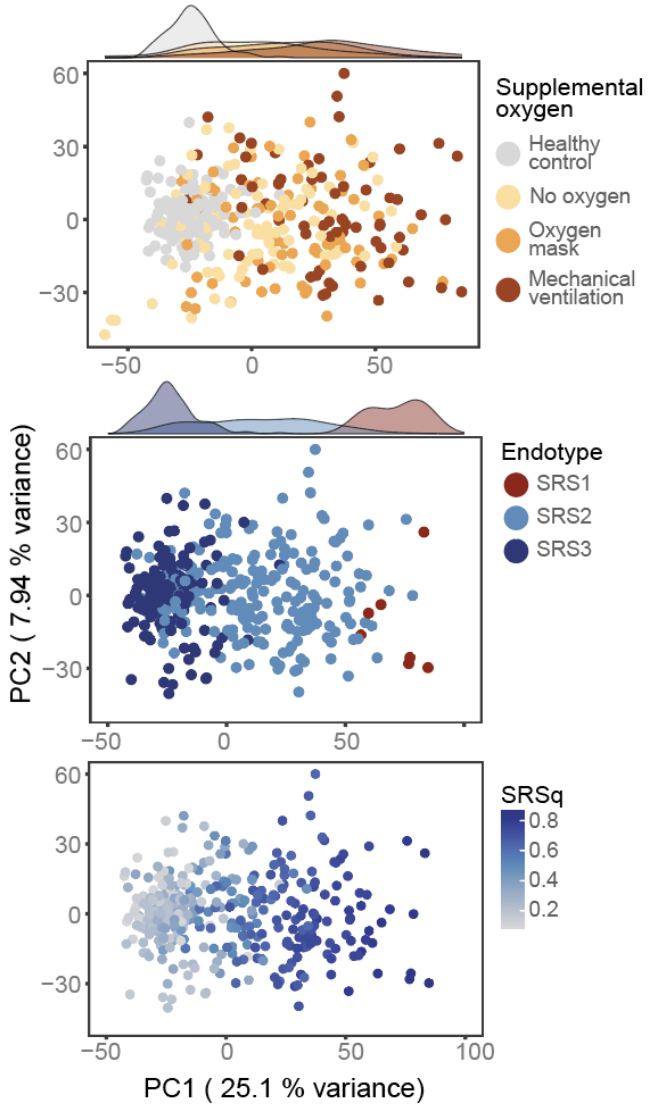


Significant associations between **SRSq and 28-day mortality** (Cox Proportional-Hazards model) including when accounting for age and source of sepsis (UK GAinS cohort)

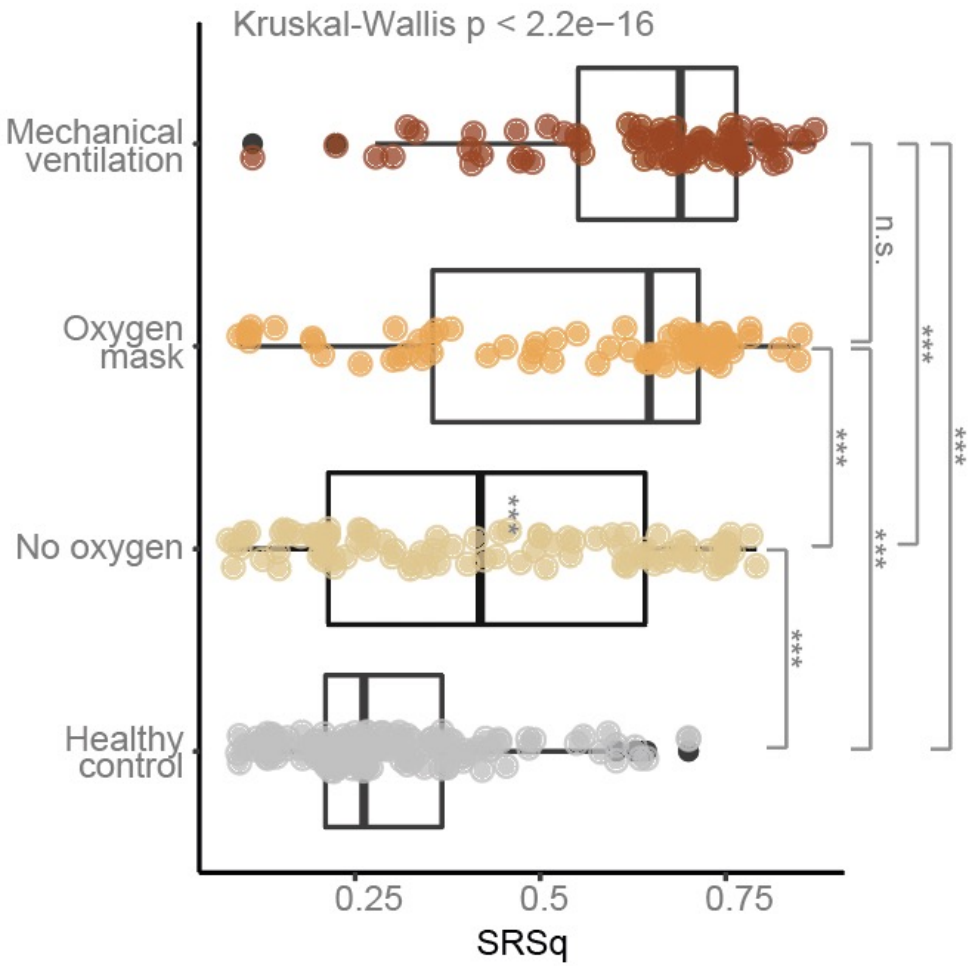
- a 0.1 increase in SRSq decreased patient survival as much as if the patient were a decade older

SRSq and H1N1 influenza

Mechanisms of Severe Acute Influenza Consortium (MOSAIC)



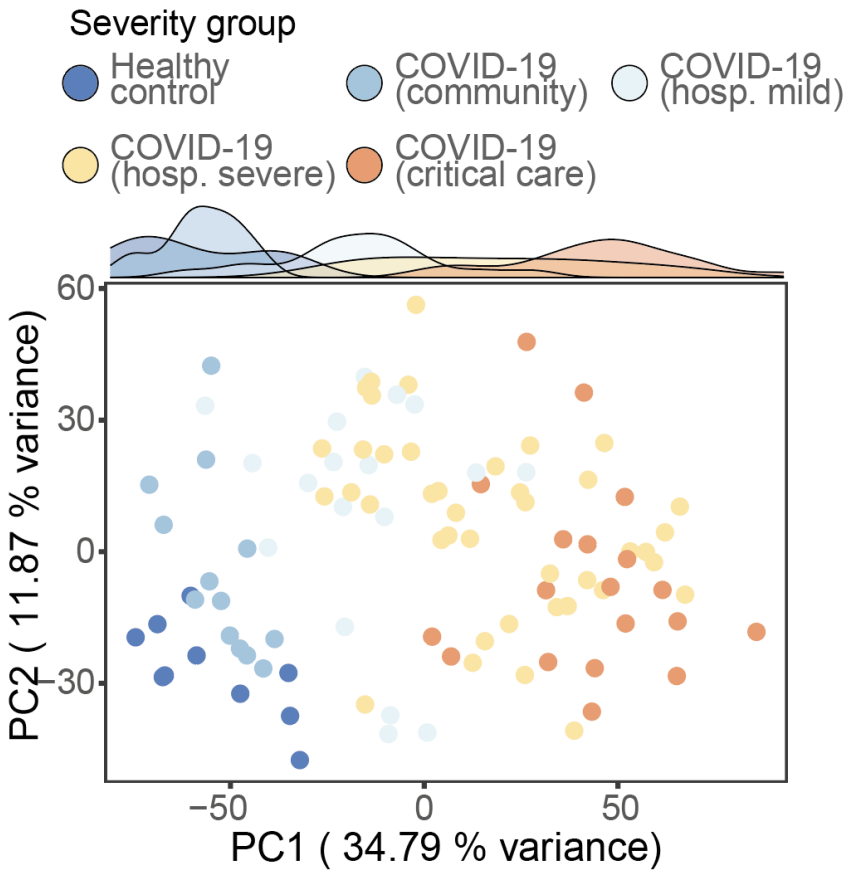
PCA plots based on whole blood transcriptomes – graded illness severity correlated with SRSq



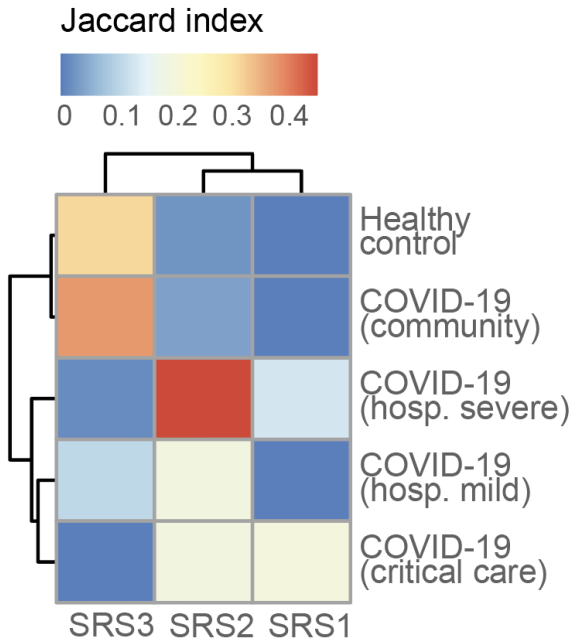
Box plots showing the association between SRSq and supplemental oxygen requirement

Dunning J et al MOSAIC Investigators. Progression of whole-blood transcriptional signatures from interferon-induced to neutrophil-associated patterns in severe influenza. Nat. Immunol. 19, 625–635 (2018)

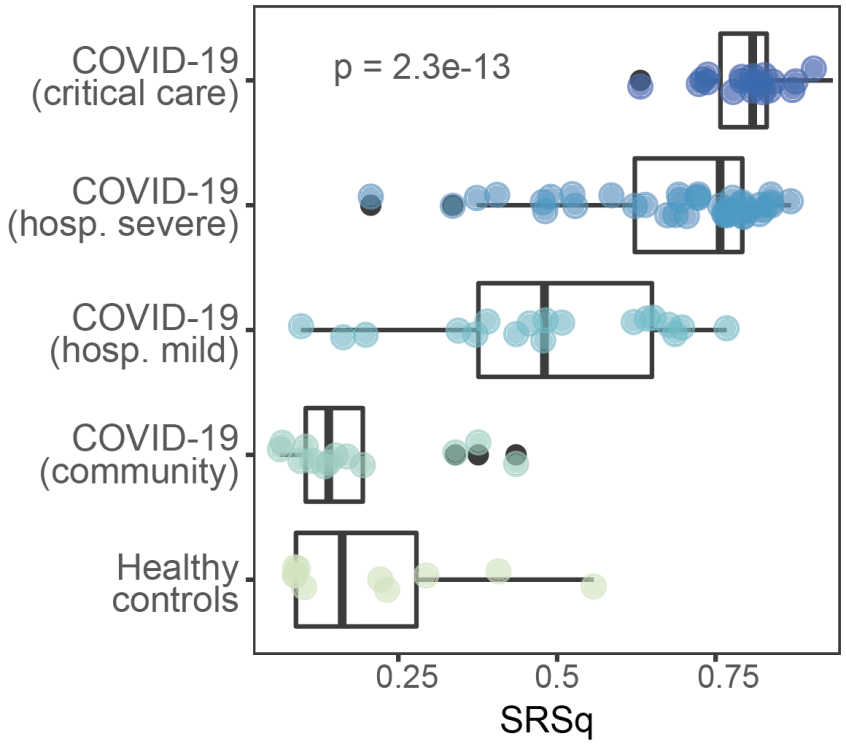
SRSq and COVID-19



PCA plot whole blood transcriptomes



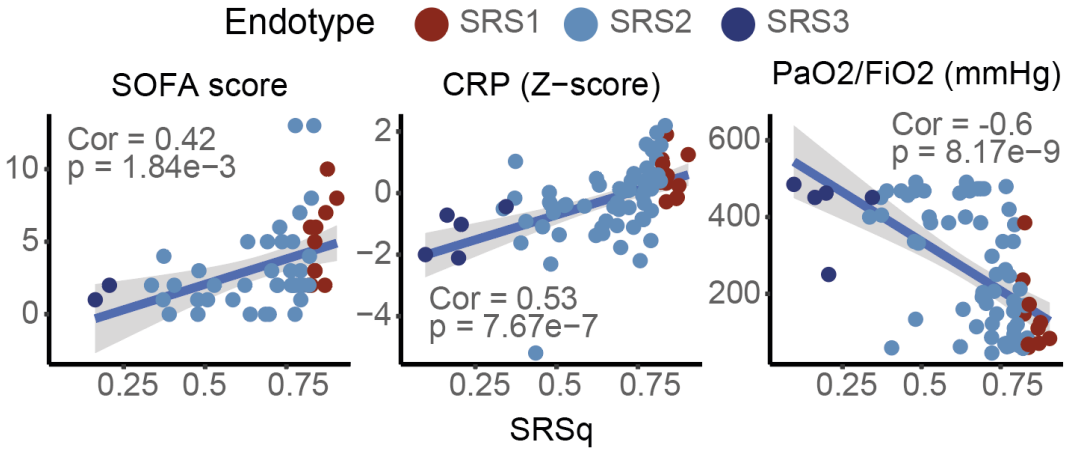
Heatmap showing overlap (Jaccard index) between sepsis endotypes and clinical severity groups



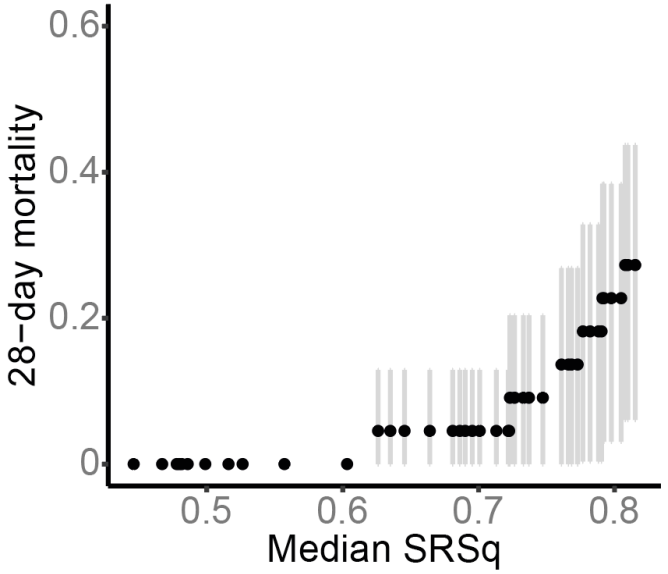
SRSq stratified by clinical severity

COMBAT Consortium. 2022. A blood atlas of COVID-19 defines hallmarks of disease severity and specificity. *Cell* **185**, 916-938

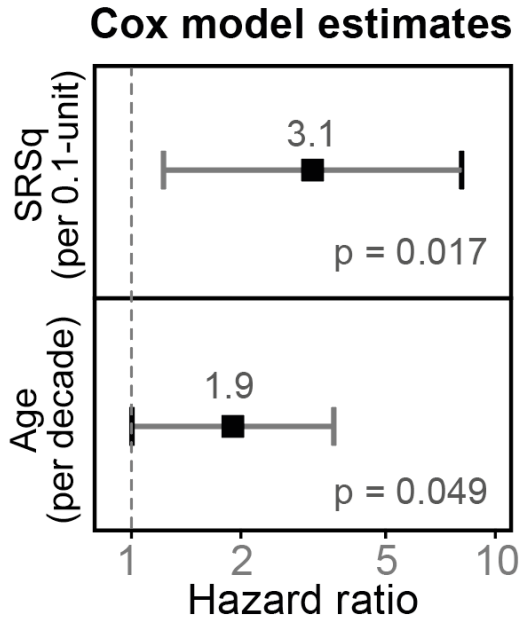
SRSq and COVID-19



Association between SRSq and clinical variables

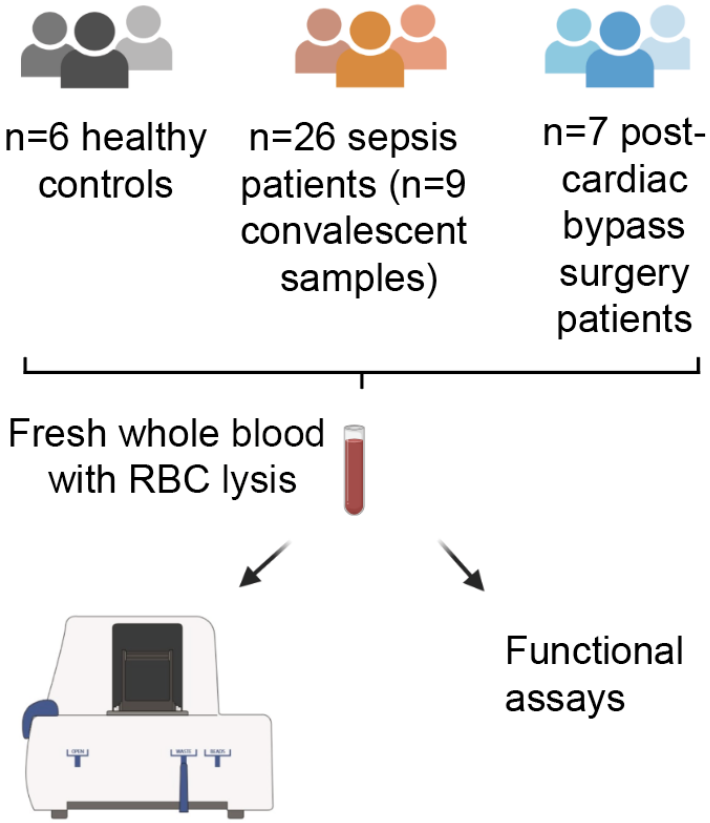


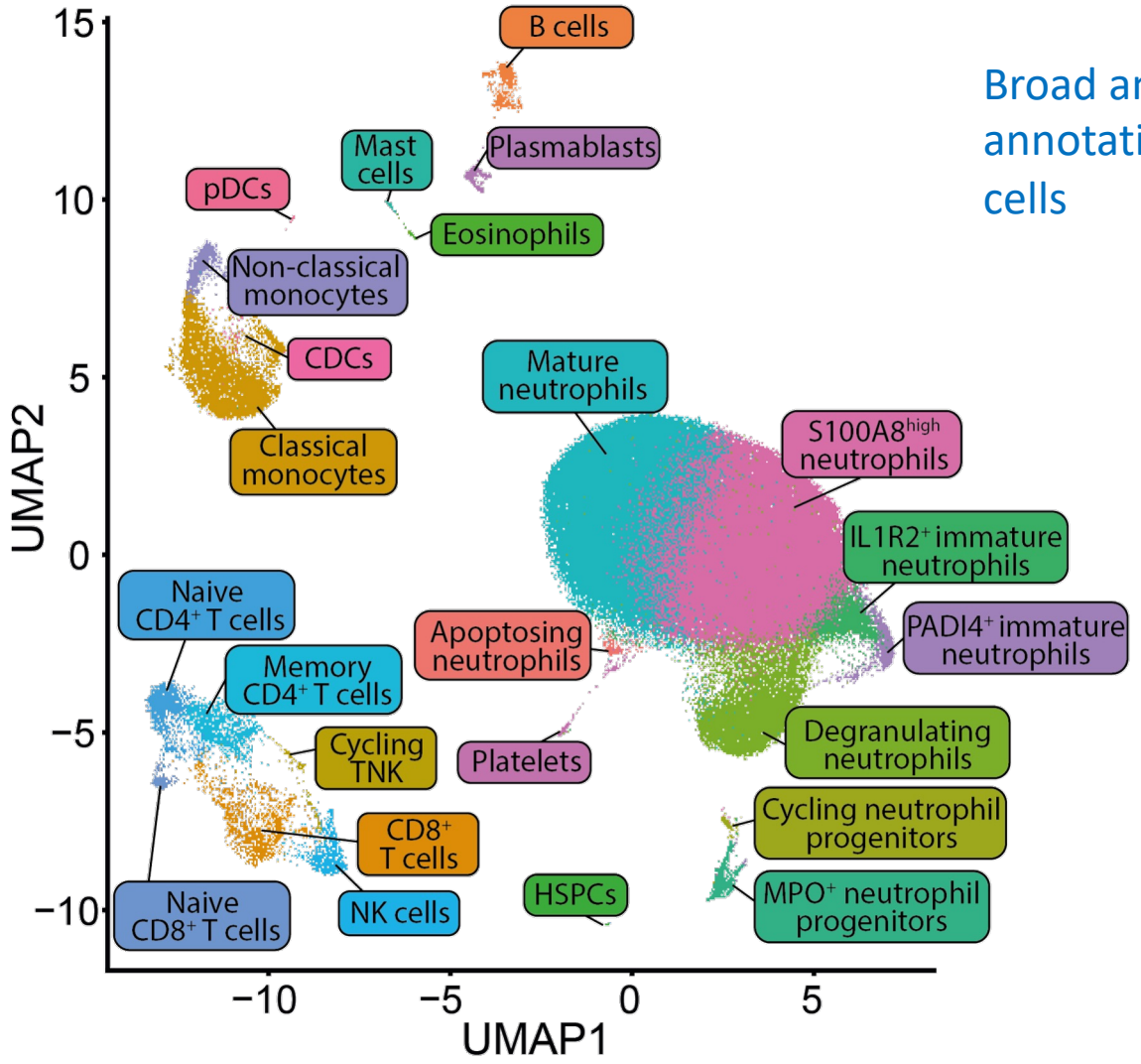
Association between SRSq and mortality



Hazard ratio estimates

An unbiased single-cell atlas of peripheral blood leukocytes in sepsis



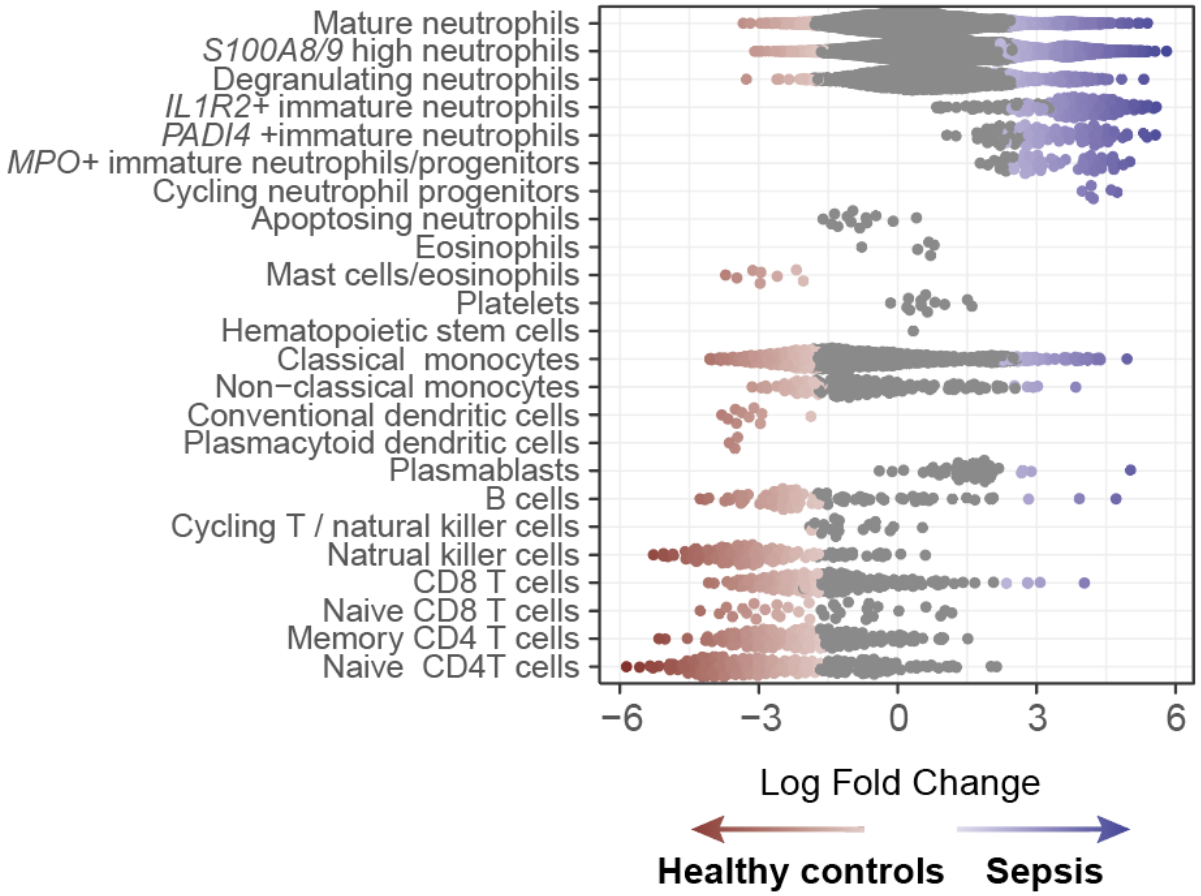


Broad and fine level annotations for 272,993 cells

Andrew Kwok

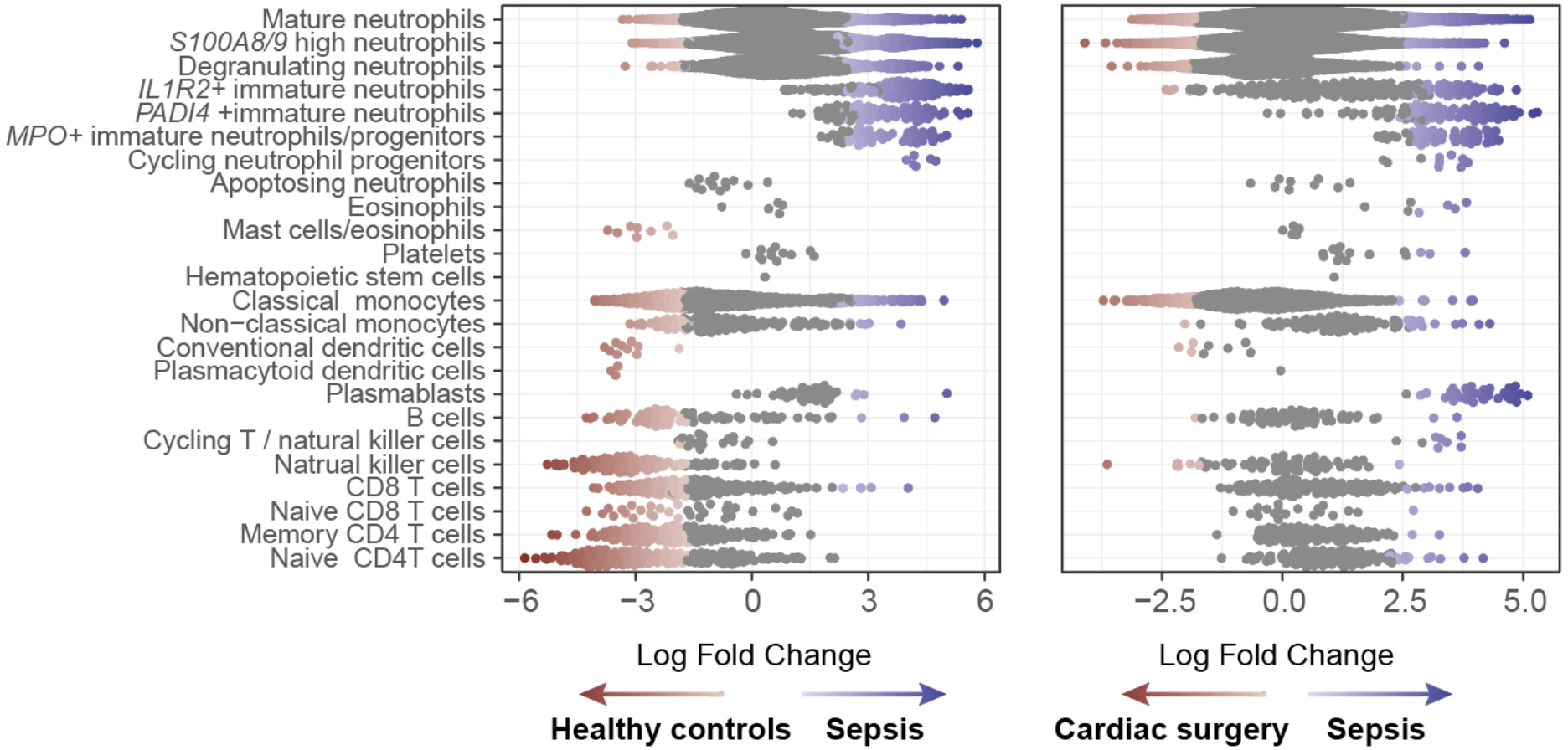
Kwok A et al (2022). Identification of deleterious neutrophil states and altered granulopoiesis in sepsis. *medRxiv*, 2022.03.22.22272723v1.

An unbiased single-cell atlas of peripheral blood leukocytes in sepsis



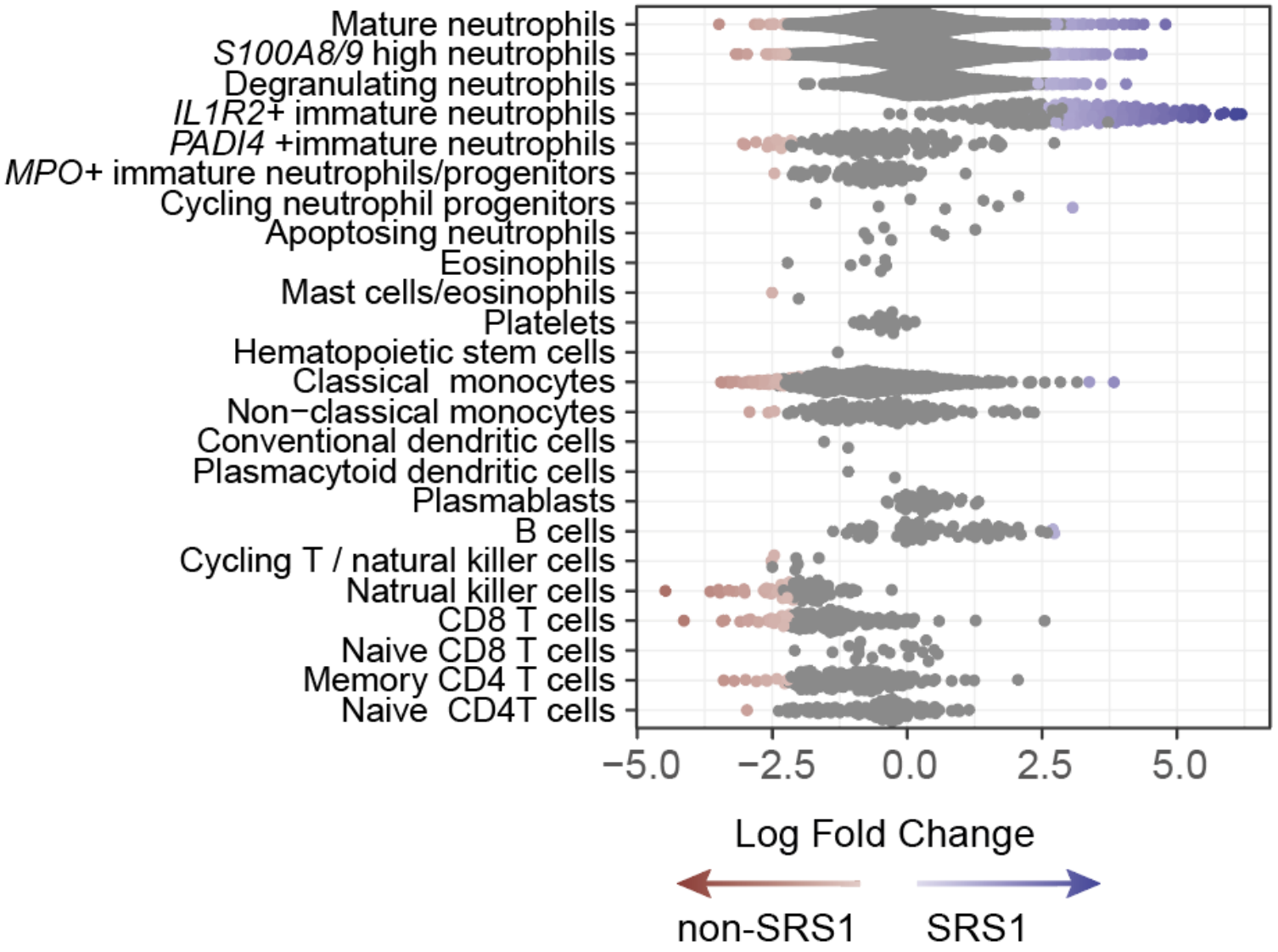
Sampling neighborhoods of cells showed proportionally **more degranulating and S100A8/9 high neutrophils in sepsis** compared to healthy controls, while **all mononuclear cell subsets except plasmablasts were reduced**

An unbiased single-cell atlas of peripheral blood leukocytes in sepsis



Comparable differences were seen for CS versus HC, suggesting these were non-specific features of inflammation. By contrast, higher abundance of the **immature neutrophil populations** was specific to sepsis

Expansion of IL1R2+ immature neutrophils and neutrophil progenitors in SRS1

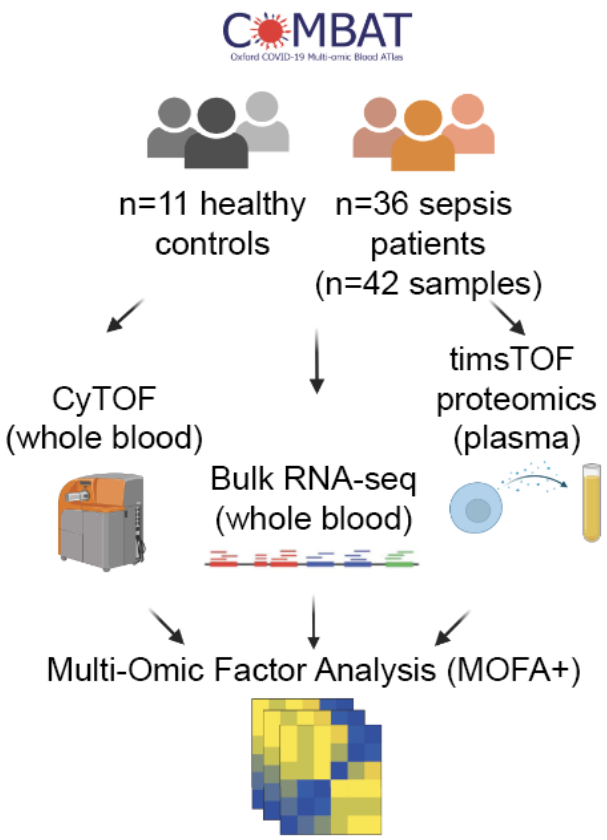


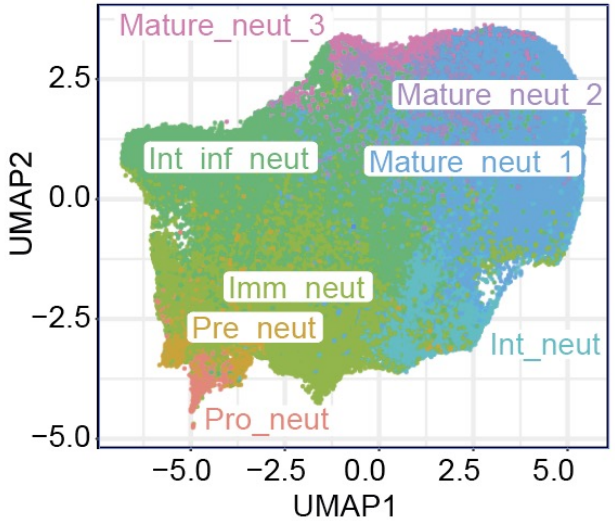
SRS1

- expansion of IL1R2+ immature neutrophils and increased cycling neutrophil progenitors
- depletion of MNCs including cMonos, NK, and memory T cells

Patient separation by SRS group on principal components analysis for differential gene expression in mature, S100A8/9 high, degranulating, and IL1R2+ immature neutrophils with minimal differences between SRS in the MNC subsets

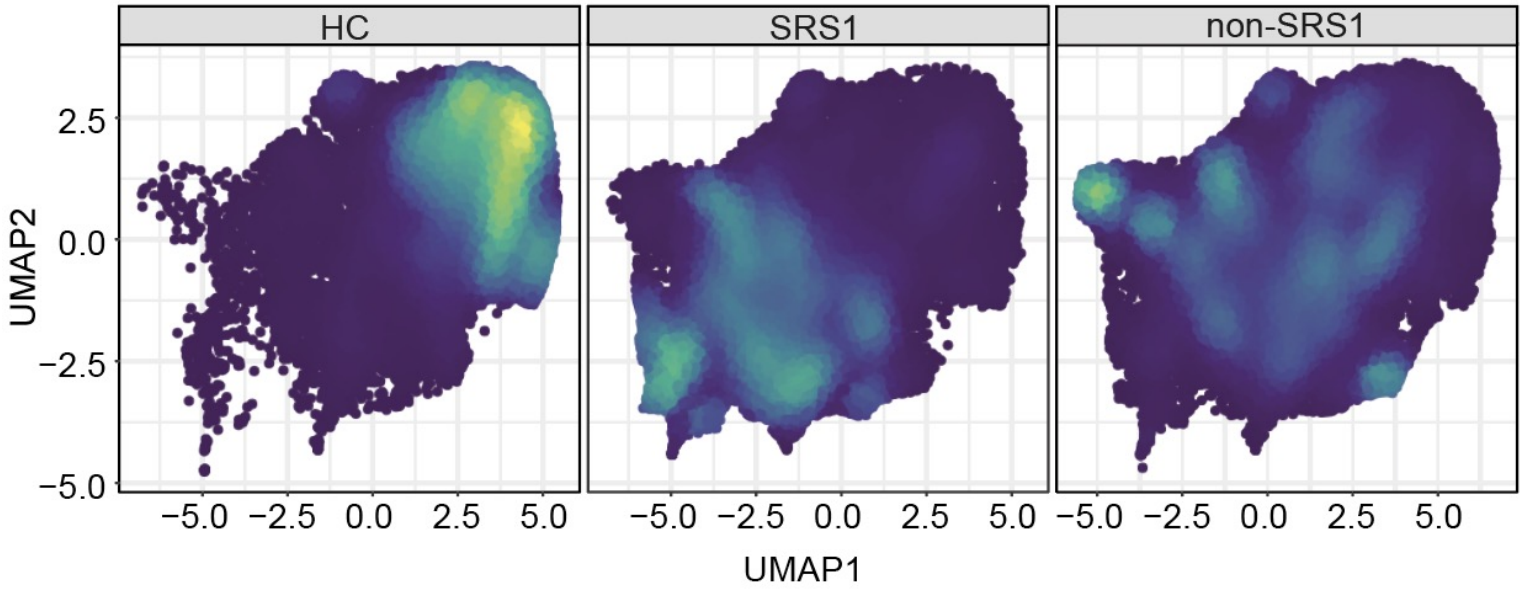
Neutrophil differences in SRS



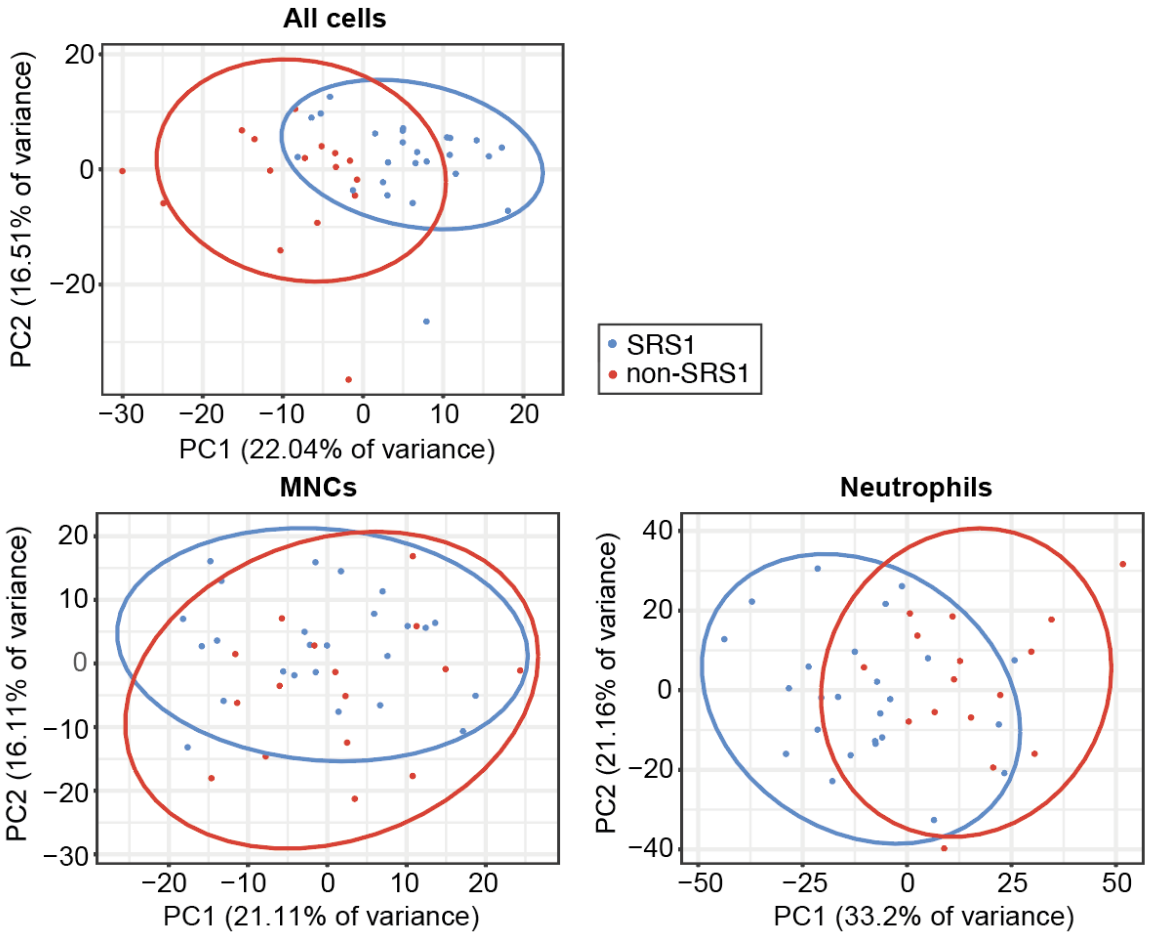


Eight neutrophil clusters in the CyTOF dataset

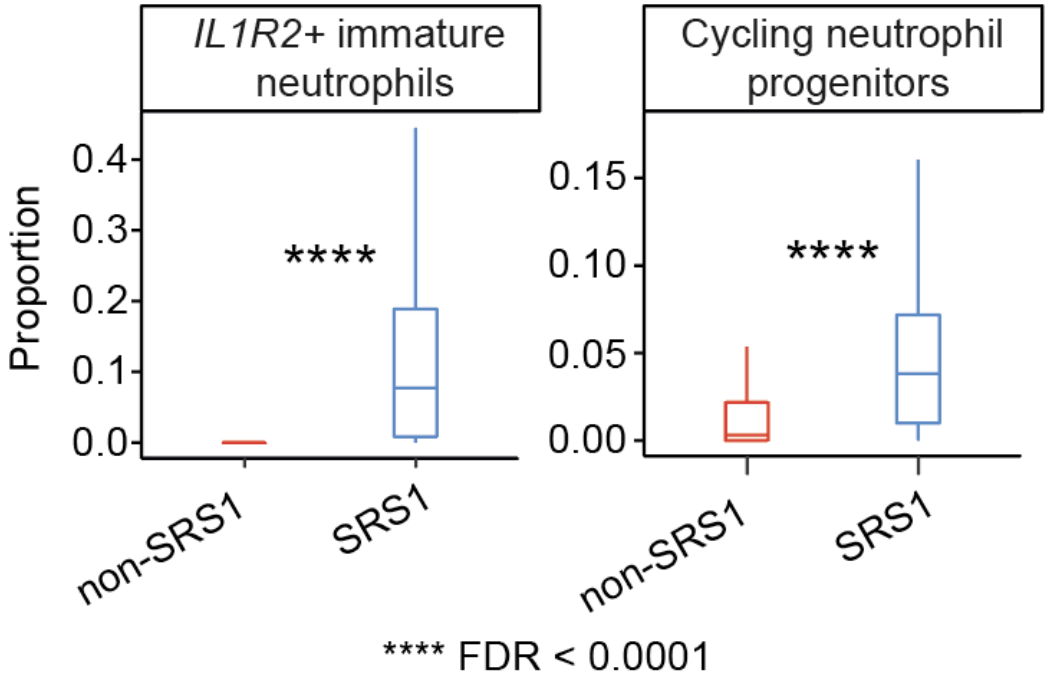
More immature and pro/pre-neutrophils in SRS1



Neutrophil differences in SRS



CyTOF: PCA on cell proportions showed separation by **neutrophil** but not MNC compartments for SRS groups



Sepsis whole blood transcriptomic data (n=667 patients):

- applied cell type/state proportion deconvolution
- significant **IL1R2+ immature neutrophil and cycling neutrophil progenitor expansion in SRS1**

Conclusions and future directions

Whole blood leukocyte transcriptomics enables subphenotyping with evidence for a disease endotype driven by altered neutrophil biology and granulopoiesis informative for underlying immune response state, outcome and therapy

Further **mechanistic work** is required to establish function and opportunities for targeted intervention

SRSq shows applicability across infectious aetiologies to date and is amenable to point of care testing (potentially 1hr minute assay turnaround with automated nested multiplex PCR system)

Ongoing work with **other –omic** approaches including plasma proteomics and deep clinical phenotyping will likely reveal further complexity in subphenotyping

Need to understand therapeutic potential and whether this is informative for a treatable trait

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