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Identification of host endotypes using peripheral blood transcriptomics in a prospective cohort of patients with endocarditis

Israel David Duarte-Herrera[1,2], Cecilia López-Martínez[1,2,3], Raquel Rodríguez-García[1,2,3,4], Diego Parra[1,2,4], Paula Martín-Vicente[1,2,3], Sara M. Exojo-Ramirez[1,2], Karla Miravete-Lagunes[2], Lisardo Iglesias[4], Marcelino González-Iglesias[3], Margarita Fernández-Rodríguez[3], Marta Carretero-Ledesma[5], Inés López-Alonso[1,2,3,6], Juan Gómez[1,2,7], Eliecer Coto[2,7,8], Rebeca González Fernández[9], Belén Prieto García[2,9], Javier Fernández[1,2,10], Laura Amado-Rodríguez[1,2,3,4,8,*], Guillermo M. Albaiceta[1,2,3,4,11]

[1] *Centro de Investigación Biomédica en Red (CIBER)-Enfermedades Respiratorias, Madrid, Spain*
[2] *Instituto de Investigación Sanitaria del Principado de Asturias, Oviedo, Spain*
[3] *Instituto de Oncología del Principado de Asturias, Universidad de Oviedo, Oviedo, Spain*
[4] *Unidad de Cuidados Intensivos Cardiológicos, Hospital Universitario Central de Asturias, Oviedo, Spain*
[5] *Unidad de Enfermedades Infecciosas, Microbiología y Parasitología. Hospital Universitario Virgen del Rocío. Instituto de Biomedicina de Sevilla, Sevilla, Spain*
[6] *Departamento de Morfología y Biología Celular, Universidad de Oviedo, Oviedo, Spain*
[7] *Servicio de Genética, Hospital Universitario Central de Asturias, Oviedo, Spain*
[8] *Departamento de Medicina, Universidad de Oviedo, Oviedo, Spain*
[9] *Servicio de Bioquímica Clínica, Hospital Universitario Central de Asturias, Oviedo, Spain*
[10] *Servicio de Microbiología, Hospital Universitario Central de Asturias, Oviedo, Spain*
[11] *Departamento de Biología Funcional, Universidad de Oviedo, Oviedo, Spain*

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ABSTRACT

Objectives: Host responses to infection are a major determinant of outcome. However, the existence of different response profiles in patients with endocarditis has not been addressed. Our objective was to apply transcriptomics to identify endotypes in patients with infective endocarditis.

Methods: A total of 32 patients with infective endocarditis were studied. Clinical data and blood samples were collected at diagnosis and RNA sequenced. Gene expression was used to identify two clusters (endocarditis endotype 1 [EE1] and endocarditis endotype 2 [EE2]). RNA sequencing was repeated after surgery. Transcriptionally active cell populations were identified by deconvolution. Differences between endotypes in clinical data, survival, gene expression, and molecular pathways involved were assessed. The identified endotypes were recapitulated in a cohort of COVID-19 patients.

Results: A total of 18 and 14 patients were assigned to EE1 and EE2, respectively, with no differences in clinical data. Patients assigned to EE2 showed an enrichment in genes related to T-cell maturation and a decrease in the activation of the signal transducer and activator of transcription protein family pathway, with higher counts of active T cells and lower counts of neutrophils. A total of 14 patients (nine in EE1 and five in EE2) were submitted to surgery. Surgery in EE2 patients shifted gene expression toward a EE1-like profile. In-hospital mortality was higher in EE1 (56% vs 14%, $P = 0.027$), with an adjusted hazard ratio of 12.987 (95% confidence interval 3.356-50). Translation of these endotypes to COVID-19 and non–COVID-19 septic patients yielded similar results in cell populations and outcome.

Conclusions: Gene expression reveals two endotypes in patients with acute endocarditis, with different underlying pathogenetic mechanisms, responses to surgery, and outcomes.

* Corresponding author: Unidad de Cuidados Intensivos Cardiológicos, Hospital Universitario Central de Asturias, Avenida del Hospital Universitario s/n, 33011 Oviedo, Spain. Tel.: +34 985 652 433.
E-mail address: laura.amado@sespa.es (L. Amado-Rodríguez).

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Introduction

Infective endocarditis is a severe disease caused by the infection of heart valves and endocardium by a pathogenic germ. The incidence of endocarditis in a non-selected population is around 15-80 cases per million inhabitants and year and increases up to 5-10 cases per 1000 inhabitants and year in high-risk groups, such as those with a prosthetic heart valve [1]. Despite these relatively low incidences, endocarditis remains a major health issue due to its elevated mortality (in-hospital mortality of 20-30% [2,3]) and the associated resource consumption. Antimicrobial therapy and surgery remain the basis of treatment, and up to 50% of the patients require surgical replacement of the affected valves to control the infectious source and restore hemodynamics [4].

The outcome in infective endocarditis is conditioned by the interaction between the causing pathogen and the host [5]. Virulent or resistant microorganisms show higher morbidity and mortality rates. Host risk factors include previous comorbidities, history of cardiac diseases, and existing intracardiac devices. Moreover, endocarditis triggers a systemic host response that may contribute to pathogenesis and outcome. The most evident cases of this exacerbated systemic response fall within the diagnosis of sepsis and are linked to the high mortality rates [6].

Recent evidence shows that critically ill patients with a common set of symptoms and signs may have very different underlying pathogenetic mechanisms. These specific, mechanistic-driven groups are termed endotypes [7]. The different pathogenesis may result in specific responses to therapeutic measures that cannot be detected in trials including an unselected population. Several systemic endotypes with different immune features and mortality have been described in sepsis [8,9] and in severe infections caused by the coronavirus [10].

The objective of this work is to identify the existence of endotypes in a prospective cohort of patients with infective endocarditis. Bulk RNA sequencing (RNA-seq) from peripheral blood allows patient clustering according to their transcriptomic profiles at diagnosis and during their follow-up. Clinical data, outcomes, and responses to surgery were assessed in a cluster-specific manner to identify the differences in the pathogenesis that could help to find personalized treatments and, ultimately, improve the outcome in this fragile population.

Methods

Study design

The study protocol was reviewed and approved by Comité de Ética de la Investigación Clínica del Principado de Asturias (reference 2021.122) and registered in *clinicaltrials.gov* (NCT04838938). Informed consent was obtained from all patients or their next of kin. The inclusion criteria were age 18 years or older and a diagnosis of definite infective endocarditis according to the Duke criteria [11]. The exclusion criteria were refusal to participate, immunosuppression, terminal status, or do-not-resuscitate orders.

Due to the absence of preliminary data, no formal calculation of the sample size was performed. Instead, we planned to include all patients for 1 year. Thus, all consecutive patients from April 2021 to March 2022 were prospectively included.

All patients followed up by the hospital endocarditis team were screened. Once a diagnosis of definite endocarditis was done, informed consent was obtained, clinical data were collected, and a blood sample for RNA-seq was drawn. An additional 5-ml sample was collected in a Vacutainer serum tube (BD Biosciences), isolated by centrifugation, and stored at –80°C until analysis. Serum interleukin-6 concentration was determined by electrochemiluminescence immunoassay using a Cobas PRO analyzer (Roche Diagnostics). The included patients were followed up to hospital discharge. In those patients in whom surgery was performed, a second blood sample for RNA-seq was taken the day after the intervention. The primary end point was hospital death.

RNA sequencing

Peripheral blood RNA was purified and sequenced in an Ion Torrent platform, as previously described [12]. Raw fastQ files were pseudoaligned against an index (built using the Genome Reference Consortium Human Build 38 38 Organism genome as reference) using Salmon 1.9 [13]. The resulting transcript counts were imported into R using the packages *Annotationhub* and *tximport* [14] to obtain gene counts.

Clustering

Patients were classified into clusters at diagnosis using log2-transformed expression of the 5% genes with the largest variance. Euclidean distances were calculated, and Ward clustering algorithm was applied. The two first emerging clusters were termed endocarditis endotype 1 (EE1) and endocarditis endotype 2 (EE2). Clusters were represented in a two-dimensional space using the uniform manifold approximation and projection (UMAP) algorithm.

Analysis of differentially expressed genes

Differences in gene expression between groups of interest (either endotypes or before and after surgery) were assessed using the software package *DEseq2* for R [15]. The $\log_2$ (fold change) for each gene between endotypes, with the adjusted *P*-value (corrected using a false discovery rate of 0.05) were calculated. Enriched pathways corresponding to genes with differential expression were identified by gene set enrichment analysis (GSEA) using the R package *clusterprofiler* [16].

Deconvolution of cell populations

Peripheral blood gene expression was also used to identify the proportion of circulating, transcriptionally active cell populations by deconvolution of bulk RNA-seq using a previously validated reference matrix (*Immunostates* [17]), after removing cell types not present in peripheral blood.

External validation

The identified endotypes were validated in two external cohorts. Because there are no other publicly available data sets reporting host gene expression from patients with endocarditis, we tested our findings in a cohort of patients with COVID-19 and in a cohort of patients with sepsis. First, a transcriptomic score was calculated as the geometric mean of the normalized expression of the 20 genes with top statistical differences (based on the Wald statistic) between EE1 and EE2. The resulting raw scores were normalized to z-scores to aid comparisons. A cut-off value of −0.5 defined both endotypes with no overlap. Then, the same score was calculated in a cohort of 56 patients with severe COVID-19 (see reference [10] for details), using transcriptomic data obtained in the first 72 hours after intensive care unit (ICU) admission. Similarly, the score was calculated in a cohort of 19 patients with severe sepsis, included in an ongoing multicentric study (Precision Medicine for PostIntensive Care Syndrome [PreMed4PICS]). This study has been registered in *ClinicalTrials.gov* (NCT05518786). Transcriptomes were obtained at ICU admission. Patients from each external cohorts were divided in two clusters using the previously defined cut-off point. Peripheral blood cell counts were obtained using deconvolution and ICU survival was modeled.

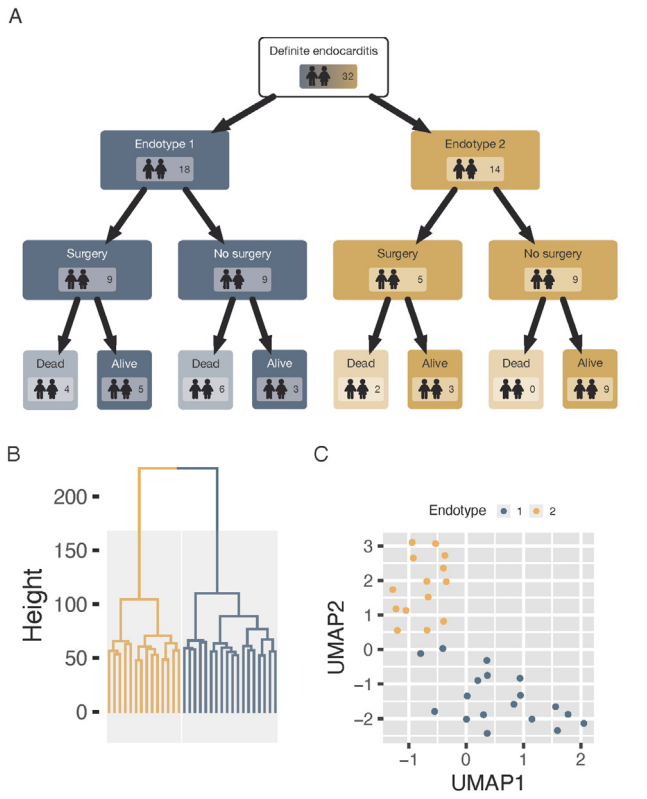

Figure 1. Study flow and clustering. (a) Study flow diagram. (b) Hierarchical clustering tree, showing the two main endocarditis endotypes (EE1/EE2). (c) UMAP with a bidimensional representation of each transcriptome at diagnosis.
UMAP, uniform manifold approximation and projection.

Statistical analysis

Data were collected by the research team in a dedicated database. No imputation of missing data was performed. Results are shown as median (interquartile range) or absolute count (percentage). Differences between endotypes in clinical variables or laboratory data were analyzed using a two-tailed Wilcoxon or a chi-square test (for quantitative and qualitative data respectively). For survival assessment, a competing risks model was constructed using death and hospital discharge alive as competing events. A competing risk model was applied given that censoring is informative in this setting [18–20]. The hazard ratio (with its 95% confidence interval) for each outcome, after adjusting by age and sex, was calculated. All the analyses were performed using the R statistical language (version 4.2.0) with packages *ggplot2* [21], *pheatmap* [22], and *survival* [23], in addition to those previously cited. All the code and raw data can be found at https://github.com/Crit-Lab/endocarditis_endotypes.

Role of the funding source

The funding sources had no role on study design, data acquisition, analysis or interpretation, or manuscript submission.

Results

A total of 32 consecutive cases of endocarditis (age 69 [62-77] years and 26 males and six females) were included in the study. Overall, the in-hospital mortality was 37.5% (12 cases). Figure 1a shows the patient flow across the study. Table 1 shows the main demographical and clinical data at diagnosis. All patients received antibiotic treatment covering the identified germs (Supplementary Table 1).

Clustering

Whole blood RNA was obtained from samples drawn at diagnosis and sequenced. Patients were clustered according to their RNA profiles. Using the 5% genes with the highest variance, two different clusters (EE1 and EE2), with 18 and 14 patients, respectively, emerged (Figure 1b), with a clear separation in the UMAP projection of their transcriptomes (Figure 1c). Supplementary Figure 1 shows the heatmap of these genes by cluster.

Clinical data at admission were then compared. There were no significant differences between clusters in the collected variables (Table 1).

Differential expression analysis

There were 6577 genes with differential expression between clusters (Figure 2a). The complete list of differentially expressed genes is provided in Supplementary file 1. Supplementary Figure 2 shows the heatmap with the genes with the most significant differential expression (*P*-value lower than 10^{-5}), illustrating the opposite responses in both groups.

A GSEA was performed in these genes, revealing 199 gene ontology terms (Supplementary file 2), with an adjusted *P*-value lower than 0.01. Figure 2b shows the main gene ontologies enriched in each endotype, and Figure 2c shows the tree plot with the differences between endotypes. Notably, several gene sets related to T-cell selection and maturation were upregulated in EE2, whereas the signal transducer and activator of transcription protein family signaling pathway was downregulated. The specific genes of inflammatory cytokines and chemokines and their comparison between endotypes are provided in Supplementary file 3.

There were no significant differences between groups in peripheral cell counts (Table 1) or the neutrophil/lymphocyte ratio. Bulk transcriptomes were also deconvoluted to estimate the underlying transcriptionally active cell populations. Patients assigned to EE2 showed lower proportions of functional neutrophils and higher counts of T and B lymphocytes, suggesting an adaptative response to the disease (Figure 2d and Supplementary Figure 3).

Differences according to microbiological results

We assessed the differences in gene expression between patients with (n = 18) and without (n = 14) an isolation of a virulent germ (i.e. *Staphylococci* or *Enterococci*). Only one gene (*HLA-B*) was differentially expressed. The distribution of these virulent germs was similar across endotypes (12 in EE1 and 6 in EE2, chi-square $P = 0.323$).

Response to surgery

Nine and five patients from EE1 and EE2, respectively, were submitted to surgery for valve replacement. The median time from diagnosis to surgery was 1 (0-4) days, with no relevant differences between clusters (1 [0-3] vs 2 [1-5], $P = 0.147$).

In 13 of these patients (nine in EE1 and four in EE2 cluster), a new transcriptomic profile was obtained the day after surgery. We then compared the gene expression before and after surgery in each cluster. There were 1196 and 4076 differentially expressed genes in EE1 and EE2, respectively. Of these, 795 genes with differential expression were shared between clusters. The GSEA revealed that the enriched categories in EE1 were related to the repression

Table 1
Demographical and clinical data.

	Endotype 1	Endotype 2	*P*-value
Sex	15	11	1
Male	3	3	
Female			
Age (year)	71 (60.75-81)	69 (63.5-74.5)	0.746
Body mass index (kg/m^2)	26.97 (22.31-30.34)	28.57 (25.4-33.48)	0.166
Charlson score	4 (2.25-5)	4 (2.25-4)	0.524
Comorbidities			
Chronic obstructive pulmonary disease	2	0	0.581
Coronary disease	2	3	0.759
Congestive heart failure	5	2	0.628
Diabetes	5	6	0.606
Arterial hypertension	11	7	0.788
Dyslipidemia	8	10	0.243
Chronic kidney failure	1	2	0.819
Chronic liver failure	2	0	0.581
Etiology			
Type			0.155
Prosthetic valve	12	5	
Native valve	6	8	
Intracardiac device	0	1	
Affected valve			0.722
Aortic	8	8	0.188
Mitral	13	6	1
Tricuspid	1	0	0.898
Pulmonic	0	1	0.898
Pacemaker	0	1	
Isolated germs			0.5
None	1	1	
S aureus/lugdunensis	6	3	
S epidermidis	2	3	
E faecalis	4	0	
Streptococci	3	3	
Gram-negative bacilli	0	1	
Other	1	2	
Polymicrobial	1	1	
Clinical data and treatment			
Initial symptoms			1
Fever	14	11	0.411
Cardiological	6	2	1
Neurological	4	3	0.304
Pulmonary	5	1	1
Renal	1	0	0.358
Rheumatic	0	2	
Time since symptom onset			0.695
Less than 2 weeks	10	8	
2-4 weeks	3	2	
1-3 months	5	3	
More than 3 months	0	1	
Surgery	9	5	0.653
EuroSCORE-II–predicted mortality	24 (10.75 - 44.75)	20 (11.25 - 29)	0.53
Data at diagnosis			
Leukocytes (μ/l)	12735 (10550 - 19707.5)	9925 (8582.5 - 14025)	0.16
Neutrophils (μ/l)	11310 (8842.5 - 17495)	8240 (6630 - 11667.5)	0.149
Lymphocytes (μ/l)	880 (655 - 1175)	730 (615 - 1282.5)	0.582
Neutrophil/lymphocyte ratio	13.72 (8.43 - 20.58)	12.98 (6.34 - 19.21)	0.779
Monocytes (μ/l)	825 (610 - 1100)	900 (555 - 1045)	0.97
Hemoglobin (g/dl)	11.25 (9.53 - 12.12)	12.45 (11.22 - 13.17)	0.196
Platelets (μ/l)	154000 (127750 - 240000)	214500 (91000 - 258000)	0.909
Creatinine (mg/dl)	1.16 (0.94 - 1.56)	1.23 (0.92 - 1.48)	0.924
C-reactive protein (IU/l)	13.18 (11.47 - 23.37)	12.3 (6.9 - 17.2)	0.254
Interleukin-6 (pg/ml)	25.45 (11.22-93.97)	25.90 (19.14-71.50)	0.881

of the innate immune response and natural killer cell activity (Supplementary Figure 4). However, changes in EE2 after surgery resembled those observed in EE1 (at diagnosis and after surgery), with downregulation of pathways related to lymphocyte and T-cell activation and RNA processing (Supplementary Figure 5). The UMAP representation of transcriptomes before and after surgery confirms these changes in gene expression and the evolution of the profile in EE2 (Supplementary Figure 6).

To find a differential response to surgery between endotypes, we identified those pathways with enrichment scores with opposite sign in each cluster after surgery (Figure 3a). First, we observed that there were no pathways with positive enriched scores, suggesting that surgery leads to a massive shutdown of gene expression in both endotypes. Differential categories included repression of neutrophil function and cytokine-mediated inflammation in EE1 and downregulation of a large variety of genetic and epigenetic

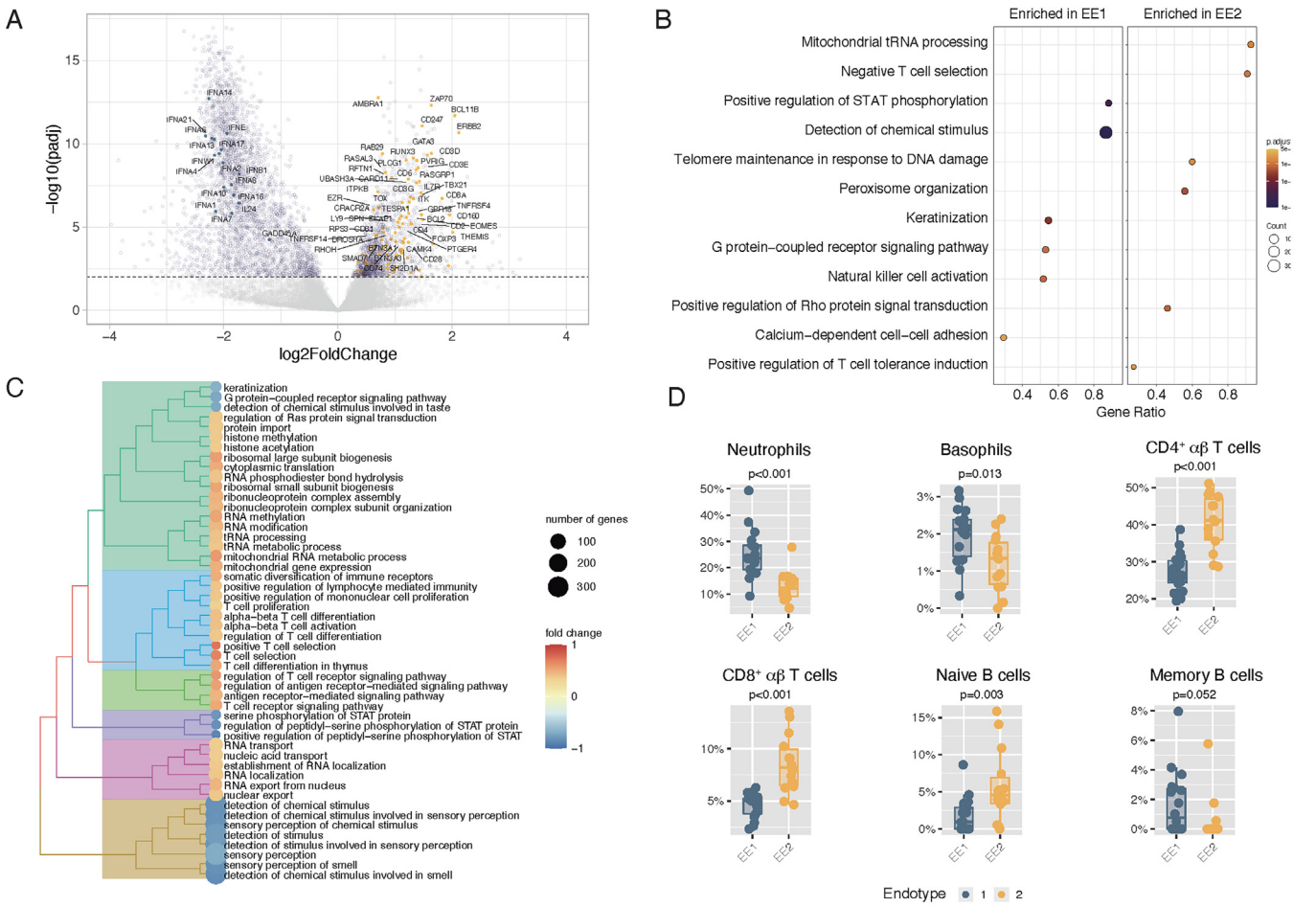

Figure 2. Differences in gene expression between endotypes. (a) Volcano plot illustrating fold change and statistical significance for each gene. Genes corresponding to the interferon pathway (enriched in EE1) and in T-cell proliferation and differentiation (enriched in EE2) are labelled. (b) Top enriched gene ontologies in each endotype. (c) Tree plot showing the pathways with differential enrichment. DC: deconvolution of main transcriptionally active cell populations in peripheral blood in each endotype (see Supplementary Figure 3 for all the identified populations). *P*-values represent the result of a Wilcoxon test.
CD, clusters of differentiation; EE1 and EE2, endocarditis endotypes 1 and 2; STAT, signal transducer and activator of transcription protein family.

mechanisms, including RNA metabolism and histone methylation, in EE2. When circulating cell populations were quantified by RNA deconvolution, all the differences between clusters observed at diagnosis disappeared after surgery (Figure 3b).

Outcomes

Despite no clinical differences at diagnosis, the two identified endotypes showed different trajectories of the disease. Regarding organ failures, there were no differences in the incidence of kidney or liver failure; however, patients assigned to EE1 showed a higher proportion of cardiac failure (Figure 4a). There were no differences in outcomes between patients with and without isolation of a virulent germ (Supplementary Figure 7).

Patients in the EE1 cluster showed a significantly higher in-hospital mortality: 10 of 18 (56%) patients in EE1 died compared with two of 14 (14%) in EE2 (odds ratio 7.0421 [1.075-83.333], Fisher's test $P = 0.027$). In the patients submitted to surgery, mortality was four of nine in EE1 and two of five in EE2. In non-operated patients, mortality was six of nine in EE1 and zero of nine in EE2.

When compared using a competing risks analysis, after adjusting by age, sex, presence of a virulent germ, and involvement of either native or prosthetic valves, assignment to EE1 was associated with higher mortality (Figure 4b), with a hazard ratio of 13.8 (2.7-71.4). Figures 4b and 4c show the probabilities of hospital discharge alive and death, respectively.

Validation in COVID-19 and non–COVID-19 sepsis cohorts

To translate our findings to a different cohort of severe patients, we first computed a transcriptomic score in patients with endocarditis, using the 20 genes with top absolute values of the Wald statistic. After normalization, a cut-off value of −0.5 correctly classified patients in the corresponding endotype (Figure 5a). Then, the same score was calculated in a cohort of 56 patients with severe COVID-19, and the cut-off point was used to classify them into two clusters (Figure 5b). Individual expression of the genes used to compute this score is shown in Supplementary Figure 8. These clusters of patients with COVID-19 showed differences in transcriptionally active neutrophils, clusters of differentiation 4+, and clusters of differentiation 8+ cells similar to those observed in EE1 and EE2 (Supplementary Figure 9). Similarly, the score was calculated in an additional cohort of 19 patients with sepsis admitted to the ICU. The −0.5 cut-off point classified them into two clusters (Figure 5c). The individual expression of the genes used to compute this score is shown in Supplementary Figure 8. The overlap in main gene ontologies, differentially enriched between endotypes and for each condition, is depicted in Figure 5d. Importantly, 432 endotype-specific gene ontologies were shared across the three co-

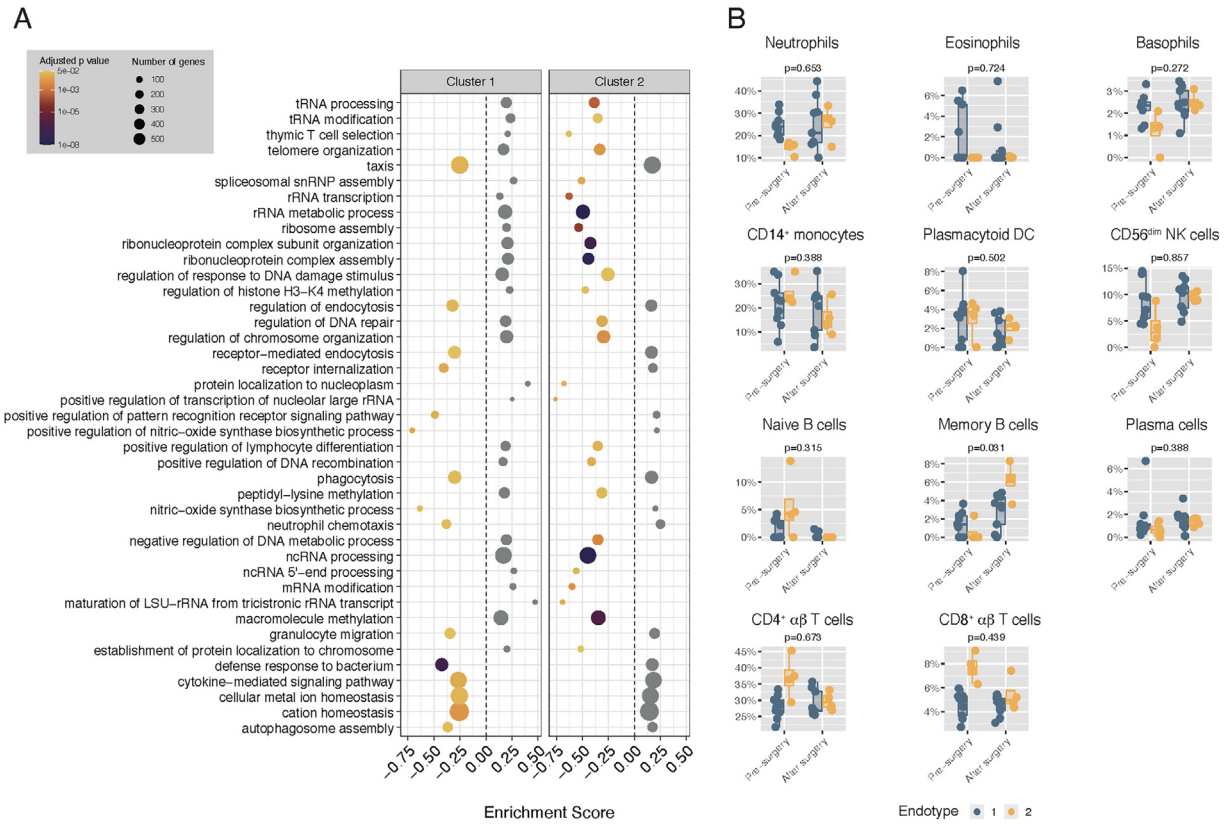

Figure 3. Response to surgery. (a) Pathways with opposite enrichment in each endotype after surgery. (b) Cell populations before and after surgery in each endotype. *P*-values were obtained using an analysis of the covariance to account for regression to the mean.
CD, clusters of differentiation; DC, dendritic cell; NK, natural killer.

horts. In line with the previous results, ICU outcome (Figures 5e and f) was better in patients assigned to cluster 2 in both validation cohorts.

Discussion

Our results illustrate how transcriptomics can help reveal two endotypes in patients with endocarditis, each one with specific pathophysiologic mechanisms, despite no major clinical differences. Moreover, the responses to surgery and outcomes in these two endotypes are different. These findings suggest that risk prediction and therapeutic approaches in endocarditis can be personalized based on peripheral blood gene expression.

Systemic responses in endocarditis

Transcriptomic profiling and pathway analysis allowed us to identify the underlying pathogenetic mechanisms in each endotype. EE1, linked to higher mortality rates, is characterized by the overexpression of interferon genes and the corresponding downstream activation of the signal transducer and activator of transcription protein family pathway, with an increase in functional circulating neutrophils. In contrast, EE2 patients show an increased expression of genes related to T-cell activation. These changes could be interpretated as either the predominance of dysregulated or adaptative responses to the infection.

The systemic response to endocarditis depends on pathogen- and host-related factors. Although specific bacterial strains may precipitate systemic responses due to the release of virulence factors, we did not find differences in isolated bacteria between endotypes. Other studies have identified genomic variants in interleukin-6 and interleukin-1β linked to a more severe systemic response to endocarditis [24]. Because we did not have our patients' genotypes, the link between genome and transcriptome cannot be clarified. However, the switch observed in EE2 patients after surgery suggests that there are environmental, non-genetic mechanisms modulating these responses [25].

Endocarditis treatment includes antibiotics and surgery to eradicate the germs, remove the source of the infection, and restore valvular function and hemodynamics. The timing of surgery is still a matter of debate [4]. A randomized clinical trial [26] showed a benefit in relapse and hospitalization, with no differences in the 6-month survival in patients assigned to early surgery; however, the study population was younger and had less severe cases than our cohort. It is unclear how this strategy can be translated to more severe cases, in which as cardiac surgery can precipitate further deterioration [27]. Interestingly, changes in gene expression after surgery did not alter the phenotype in EE1 but caused a switch in EE2 toward an EE1-like profile. This raises the hypothesis that a second hit or the accumulation of insults to the immune system may lead to the activation of an innate, neutrophil-mediated response that may be pathogenetic [28]. Although the reduced sample size precludes any firm conclusion, it must be noted that all the deaths in EE2 were after surgery and maybe the delay in surgery in this group with adaptative responses can facilitate healing and avoids the accumulation of triggering events. Moreover, a suppression of T-cell–mediated response in the early postoperative period has been described in patients undergoing abdominal surgery [29]. This surgery-induced immune response could have also contributed with the endotype switching observed in our cohort.

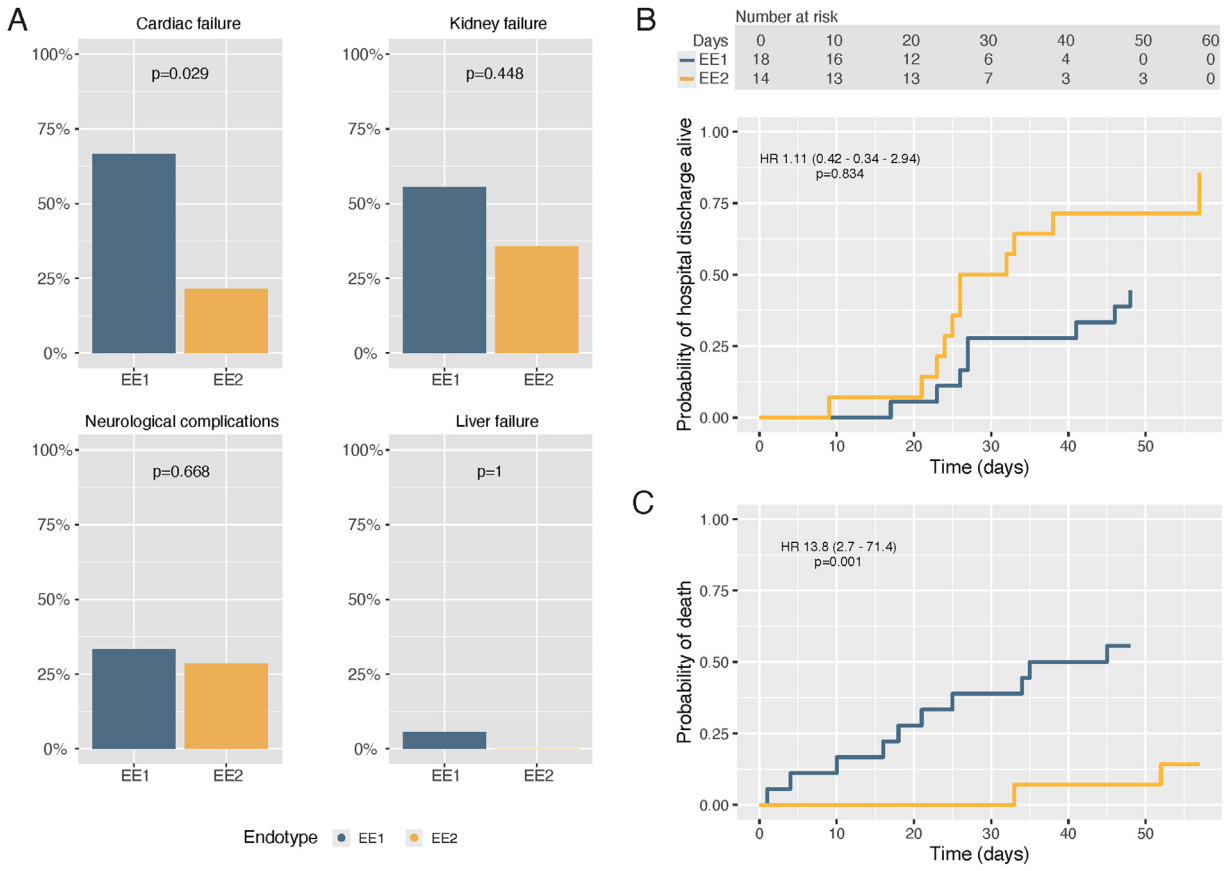

Figure 4. Outcomes. (a) Incidence of organ failure in each endotype. (b) Cumulative incidence of hospital discharge alive in each endotype. (c) Cumulative incidence of death. HR were calculated after adjustment by age, sex, presence of a virulent germ (*Staphylococcus aureus* or *Enterococci*), and involvement of native or prosthetic valve. EE1 and EE2, endocarditis endotypes 1 and 2; HR, hazard ratios.

Other endotypes in critically ill patients

Our findings are in line with studies identifying endotypes/subphenotypes within other critical conditions. It has been shown that patients with sepsis and acute respiratory distress syndrome can be separated into clinically relevant subgroups. However, the identified subphenotypes are mainly driven by differences in underlying diagnoses [30].

Regarding endotypes (i.e. differences in gene expression in patients with the same disease), we described two clusters in patients with severe infection caused by SARS-CoV-2 [10]. The translation of the endocarditis endotypes to this cohort showed similar results, suggesting that endotypes are host-dependent features, common to different diseases. Other endotypes with differences in peripheral blood cell counts and pathogenesis have been identified in sepsis [8,9], reinforcing this hypothesis. Similarly, we have also recapitulated the endocarditis endotypes in an additional cohort of patients with sepsis. Notably, all these clustering analyses show that those patients in which the systemic response is characterized by neutrophilia and the expression of proinflammatory cytokines show higher mortality rates and might benefit from immunomodulatory treatments such as steroids [10]. In contrast, those patients with adaptative responses, characterized by T- and B-cell activation, show better outcomes and could be harmed by steroids. In line with this, host-directed therapies that interfere with endotype-related cell and immune responses promoting an exacerbated inflammation and tissue injury instead of a beneficial and controlled immune response may have the potential to improve these patients prognosis. Such personalized therapies have been tested in previous trials of drugs targeting immune response with negative results in an unselected populations of patients with sepsis [31,32]. However, the same therapeutic approach could yield different results in selected populations according to their biological response to infection.

Limitations and clinical consequences

The limitations of the current study must also be highlighted. First, we performed a single measurement to categorize patients, and different clustering strategies may yield divergent results. Moreover, the sample size is reduced and could have missed more refined endotypes. Multicentric studies could provide with larger sample size and, thus, higher resolution to identify additional subgroups. Another limitation may be the absence of healthy controls. However, the identified groups have significant differences in circulating cell profiles and, more importantly, outcomes. This suggests that the identified endotypes may be clinically relevant. Besides, a healthy population in this setting may not be a reliable comparator given the objective of this study of identifying disease-related endotypes.

An additional limitation is the lack of an external validation cohort that confirms our finding. Because we were unable to identify other published datasets with transcriptomic data in this setting, we translated our findings to a well-characterized cohort of patients with COVID-19 with clinical and transcriptomic data available. This external validation showed similar results and raised the hypothesis that these endotypes are a host-dependent trait, common to different diseases. Additional studies on endotypes are warranted to confirm these results. Finally, treatments were not ran-

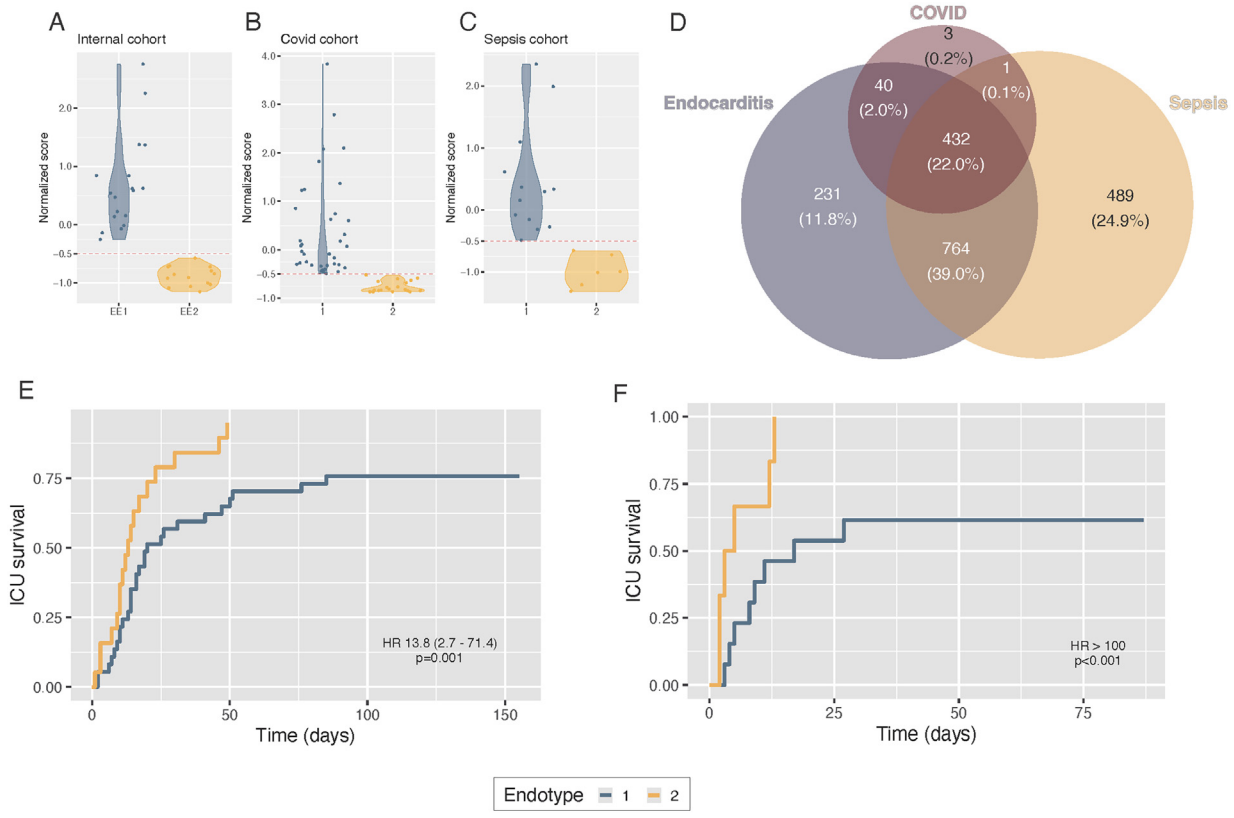

Figure 5. Translation of the endotype signature to a COVID-19 cohort. (a) Transcriptomic score calculated in patients with endocarditis using the normalized expression of the top 20 genes with highest Wald statistic. From these data and after normalizing, a cut-off point of −0.5 (dashed red line) was selected to discriminate between endotypes. (b) A transcriptomic score using the previously defined signature was calculated in a cohort of patients with COVID-19, classified using the same cut-off point into two clusters. (c) Previously defined signature calculated in a cohort of patients with sepsis, classified using the same cut-off point into two clusters. (d) Venn diagram illustrating the overlap of endotype-enriched gene ontologies for patients with endocarditis, COVID-19, and sepsis. (e) Cumulative incidence of ICU discharge alive and spontaneously breathing in each COVID-19 cluster. HR was calculated after adjustment by age and sex. (f) Cumulative incidence of ICU discharge alive and spontaneously breathing in each sepsis cluster. HR was calculated after adjustment by age and sex.
HR, hazard ratio; ICU, intensive care unit.

domized, so there is a risk of indication bias regarding surgery or other unmeasured confounders.

Given these limitations, our findings should be taken with caution. Current guidelines advocate for emergent surgery in endocarditis with systemic embolisms and severe hemodynamic instability [33] and are to be followed until more evidence is available. Similarly, the use of immunomodulatory agents from steroids to specific anti-inflammatory antibodies should only be considered within research studies.

Conclusion

Our results show that clinically similar patients with endocarditis can be clustered in two groups with different systemic responses to the pathogen and surgery that result in different mortality rates. These findings could help to optimize outcome prediction and define a personalized therapeutic approach to this high-risk population.

Declarations of competing interest

The authors have no competing interests to declare.

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Author contributions

Conceptualization: GMA, LAR. Data Curation: RRG, IDDH, GMA. Formal analysis: IDDH, CLM, MCL, LAR, GMA. Funding acquisition: LAR, GMA. Investigation: IDDH, CLM, RRG, DP, PMV, SER, KML, LI, MGI, MFR, MCL, ILA, JG, EC, RGF, BPG, JF, LAR, GMA. Methodology: IDDH, CLM, GMA. Supervision: LAR, GMA. Writing-original draft: GMA. Writing-review and editing: all authors.

Access to data: Transcriptomes have been deposited at Gene Expression Omnibus (accession number GSE240321, https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE240321). All raw data

and codes used for analysis are available at https://github.com/Crit-Lab/endocarditis_endotypes.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2024.107235.

References

[1] Iung B, Duval X. Infective endocarditis: innovations in the management of an old disease. *Nat Rev Cardiol* 2019;**16**:623–35. doi:10.1038/s41569-019-0215-0.

[2] Armiñanzas C, Fariñas-Alvarez C, Zarauza J, Muñoz P, González Ramallo V, Martínez Sellés M, et al. Role of age and comorbidities in mortality of patients with infective endocarditis. *Eur J Intern Med* 2019;**64**:63–71. doi:10.1016/j.ejim.2019.03.006.

[3] Østergaard L, Voldstedlund M, Bruun NE, Bundgaard H, Iversen K, Køber N, et al. Temporal changes, patient characteristics, and mortality, according to microbiological cause of infective endocarditis: a nationwide study. *J Am Heart Assoc* 2022;**11**:e025801. doi:10.1161/JAHA.122.025801.

[4] Wang A, Fosbøl EL. Current recommendations and uncertainties for surgical treatment of infective endocarditis: a comparison of American and European cardiovascular guidelines. *Eur Heart J* 2022;**43**:1617–25. doi:10.1093/eurheartj/ehab898.

[5] Chu VH, Cabell CH, Benjamin DK, Kuniholm EF, Fowler VG, Engemann J, et al. Early predictors of in-hospital death in infective endocarditis. *Circulation* 2004;**109**:1745–9. doi:10.1161/01.CIR.0000124719.61827.7F.

[6] Pericàs JM, Hernández-Meneses M, Muñoz P, Álvarez-Uría A, Pinilla-Llorente B, de Alarcón A, et al. Outcomes and risk factors of septic shock in patients with infective endocarditis: a prospective cohort study. *Open Forum Infect Dis* 2021;**8**:ofab119. doi:10.1093/ofid/ofab119.

[7] Bos LDJ, Ware LB. Acute respiratory distress syndrome: causes, pathophysiology, and phenotypes. *Lancet* 2022;**400**:1145–56. doi:10.1016/S0140-6736(22)01485-4.

[8] Davenport EE, Burnham KL, Radhakrishnan J, Humburg P, Hutton P, Mills TC, et al. Genomic landscape of the individual host response and outcomes in sepsis: a prospective cohort study. *Lancet Respir Med* 2016;**4**:259–71. doi:10.1016/S2213-2600(16)00046-1.

[9] Scicluna BP, van Vught LA, Zwinderman AH, Wiewel MA, Davenport EE, Burnham KL, et al. Classification of patients with sepsis according to blood genomic endotype: a prospective cohort study. *Lancet Respir Med* 2017;**5**:816–26. doi:10.1016/S2213-2600(17)30294-1.

[10] López-Martínez C, Martín-Vicente P, Gómez de Oña J, López-Alonso I, Gil-Peña H, Cuesta-Llavona E, et al. Transcriptomic clustering of critically ill COVID-19 patients. *Eur Respir J* 2023;**61**:2200592. doi:10.1183/13993003.00592-2022.

[11] Li JS, Sexton DJ, Mick N, Nettles R, Fowler VG, Ryan T, et al. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. *Clin Infect Dis* 2000;**30**:633–8. doi:10.1086/313753.

[12] Amado-Rodríguez L, Salgado Del Riego E, Gomez de Ona J, López Alonso I, Gil-Pena H, López-Martínez C, et al. Effects of IFIH1 rs1990760 variants on systemic inflammation and outcome in critically ill COVID-19 patients in an observational translational study. *eLife* 2022;**11**:e73012. doi:10.7554/eLife.73012.

[13] Patro R, Duggal G, Love MI, Irizarry RA, Kingsford C. Salmon provides fast and bias-aware quantification of transcript expression. *Nat Methods* 2017;**14**:417–19. doi:10.1038/nmeth.4197.

[14] Soneson C, Love MI, Robinson MD. Differential analyses for RNA-seq: transcript-level estimates improve gene-level inferences. *F1000Res* 2015;**4**:1521. doi:10.12688/f1000research.7563.2.

[15] Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 2014;**15**:550. doi:10.1186/s13059-014-0550-8.

[16] Wu T, Hu E, Xu S, Chen M, Guo P, Dai Z, et al. clusterProfiler 4.0: a universal enrichment tool for interpreting omics data. *Innovation (Camb)* 2021;**2**:100141. doi:10.1016/j.xinn.2021.100141.

[17] Vallania F, Tam A, Lofgren S, Schaffert S, Azad TD, Bongen E, et al. Leveraging heterogeneity across multiple datasets increases cell-mixture deconvolution accuracy and reduces biological and technical biases. *Nat Commun* 2018;**9**:4735. doi:10.1038/s41467-018-07242-6.

[18] Gunst J, Debaveye Y, Güiza F, Dubois J, De Bruyn A, Dauwe D, et al. Tight blood-glucose control without early parenteral nutrition in the ICU. *N Engl J Med* 2023;**389**:1180–90. doi:10.1056/NEJMoa2304855.

[19] Resche-Rigon M, Azoulay E, Chevret S. Evaluating mortality in intensive care units: contribution of competing risks analyses. *Crit Care* 2006;**10**:R5. doi:10.1186/cc3921.

[20] Muley M, Finamore P, Pedone C, Margiotta DPE, Gilardi E, Sambuco F, et al. Incidence and outcome of pneumomediastinum in non-ICU hospitalized COVID-19 patients. *Crit Care Med* 2023;**51**:47–56. doi:10.1097/CCM.0000000000005680.

[21] Wickham H. *ggplot2: elegant graphics for data analysis*. New York: Springer-Verlag New York; 2016.

[22] Kolde R. *_pheatmap: Pretty Heapmaps. R package version 1.0.12*; 2019 *https://CRAN.R-project.org/package=pheatmap*.

[23] Therneau TM, Grambsch PM. *Modeling survival data: extending the cox model*. New York: Springer; 2000.

[24] Weinstock M, Grimm I, Dreier J, Knabbe C, Vollmer T. Genetic variants in genes of the inflammatory response in association with infective endocarditis. *PLoS One* 2014;**9**:e110151. doi:10.1371/journal.pone.0110151.

[25] Diab M, Tasar R, Sponholz C, Lehmann T, Pletz MW, Bauer M, et al. Changes in inflammatory and vasoactive mediator profiles during valvular surgery with or without infective endocarditis: a case control pilot study. *PLoS One* 2020;**15**:e0228286. doi:10.1371/journal.pone.0228286.

[26] Kang D-H, Kim Y-J, Kim S-H, Sun BJ, Kim D-H, Yun S-C, et al. Early surgery versus conventional treatment for infective endocarditis. *N Engl J Med* 2012;**366**:2466–73. doi:10.1056/NEJMoa1112843.

[27] Wang A, Chu VH, Athan E, Delahaye F, Freiberger T, Lamas C, et al. Association between the timing of surgery for complicated, left-sided infective endocarditis and survival. *Am Heart J* 2019;**210**:108–16. doi:10.1016/j.ahj.2019.01.004.

[28] Margraf A, Ludwig N, Zarbock A, Rossaint J. Systemic inflammatory response syndrome after surgery: mechanisms and protection. *Anesth Analg* 2020;**131**:1693–707. doi:10.1213/ANE.0000000000005175.

[29] Albertsmeier M, Prix NJ, Winter H, Bazhin A, Werner J, Angele MK. Monocyte-dependent suppression of T-cell function in postoperative patients and abdominal sepsis. *Shock* 2017;**48**:651–6. doi:10.1097/SHK.0000000000000924.

[30] Calfee CS, Delucchi K, Parsons PE, Thompson BT, Ware LB, Matthay MA, et al. Subphenotypes in acute respiratory distress syndrome: latent class analysis of data from two randomised controlled trials. *Lancet Respir Med* 2014;**2**:611–20. doi:10.1016/S2213-2600(14)70097-9.

[31] Fisher CJ, Agosti JM, Opal SM, Lowry SF, Balk RA, Sadoff JC, et al. Treatment of septic shock with the tumor necrosis factor receptor:Fc fusion protein. The Soluble TNF Receptor Sepsis Study Group. *N Engl J Med* 1996;**334**:1697–702. doi:10.1056/NEJM199606273342603.

[32] Fisher CJ, Dhainaut JF, Opal SM, Pribble JP, Balk RA, Slotman GJ, et al. Recombinant human interleukin 1 receptor antagonist in the treatment of patients with sepsis syndrome. Results from a randomized, double-blind, placebo-controlled trial. Phase III rhIL-1ra Sepsis Syndrome Study Group. *JAMA* 1994;**271**:1836–43. doi:10.1001/jama.1994.03510470040032.

[33] Habib G, Lancellotti P, Antunes MJ, Bongiorni MG, Casalta J-P, Del Zotti F, et al. 2015 ESC Guidelines for the management of infective endocarditis: the Task Force for the Management of Infective Endocarditis of the European Society of Cardiology (ESC). Endorsed by: European Association for Cardio-Thoracic Surgery (EACTS), the European Association of Nuclear Medicine (EANM). *Eur Heart J* 2015;**36**:3075–128. doi:10.1093/eurheartj/ehv319.