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Clinical Phenotyping for Prognosis and Immunotherapy Guidance in Bacterial Sepsis and COVID-19

OBJECTIVES: It is suggested that sepsis may be classified into four clinical phenotypes, using an algorithm employing 29 admission parameters. We applied a simplified phenotyping algorithm among patients with bacterial sepsis and severe COVID-19 and assessed characteristics and outcomes of the derived phenotypes.

DESIGN: Retrospective analysis of data from prospective clinical studies.

SETTING: Greek ICUs and Internal Medicine departments.

PATIENTS AND INTERVENTIONS: We analyzed 1498 patients, 620 with bacterial sepsis and 878 with severe COVID-19. We implemented a six-parameter algorithm (creatinine, lactate, aspartate transaminase, bilirubin, C-reactive protein, and international normalized ratio) to classify patients with bacterial sepsis intro previously defined phenotypes. Patients with severe COVID-19, included in two open-label immunotherapy trials were subsequently classified. Heterogeneity of treatment effect of anakinra was assessed. The primary outcome was 28-day mortality.

MEASUREMENTS AND MAIN RESULTS: The algorithm validated the presence of the four phenotypes across the cohort of bacterial sepsis and the individual studies included in this cohort. Phenotype α represented younger patients with low risk of death, β was associated with high comorbidity burden, and δ with the highest mortality. Phenotype assignment was independently associated with outcome, even after adjustment for Charlson Comorbidity Index. Phenotype distribution and outcomes in severe COVID-19 followed a similar pattern.

CONCLUSIONS: A simplified algorithm successfully identified previously derived phenotypes of bacterial sepsis, which were predictive of outcome. This classification may apply to patients with severe COVID-19 with prognostic implications.

KEYWORDS: COVID-19; immunotherapy; mortality; phenotypes; sepsis

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DOI: 10.1097/CCE.0000000000001153

Since the beginning of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, also known as COVID-19, millions of deaths have been recorded (1). Mortality is mainly attributed to acute hypoxemic respiratory failure (AHRF) and multiple organ failure. AHRF is associated with severe immune alterations and coagulopathy (2, 3), a pattern similar to sepsis of bacterial origin (4). Thus, several immune-modifying treatments have been explored for severe COVID-19, as has previously been the case for bacterial sepsis. However, results from clinical trials are characterized by substantial heterogeneity (5), highlighting the need to identify patients that might display benefit or harm from specific interventions. To that end, efficient biomarkers or classifiers, easy to use by the bedside, either prognostic or with the potential to guide therapeutic decisions, are lacking.

In a precision medicine approach, a large observational cohort study clustered over 60,000 patients with bacterial sepsis (SENECA cohort) into four

KEY POINTS

Question: Can a simplified clinical algorithm identify sepsis phenotypes in patients with sepsis and COVID-19?

Findings: From an original classification system of 29 variables, a simplified algorithm of six variables was able to classify patients with sepsis into four phenotypes with similar characteristics and outcomes as the original classification, independently of initial comorbidities. Similar phenotypes were identified in COVID-19.

Meaning: We describe an easy-to-use prognostic tool, applicable by the bedside, for patients with sepsis.

distinct clinical phenotypes (α, β, γ, and δ), based on 29 clinical and biological characteristics upon hospital admission (6). Those phenotypes differed in terms of 28-day mortality and may be used for enrichment in clinical studies. The present study primarily aimed to evaluate phenotypes derived through a simplified version of the suggested classification algorithm to make this more readily usable at the bedside; secondarily, the usefulness of this simplified algorithm in identifying similar phenotypes in viral sepsis, among patients with severe COVID-19 was evaluated. Finally, we assessed potential heterogeneity of treatment effect of immunotherapy across phenotypes in COVID-19.

MATERIALS AND METHODS

This is a retrospective analysis of prospectively collected data from a composite cohort of patients with bacterial sepsis and with severe COVID-19. All procedures were followed in accordance with the ethical standards of the Helsinki Declaration of 1975 and the International Council for Harmonization Guidance for Good Clinical Practice. The institutional review boards approved the respective protocols and written consent was obtained from the subjects or their legal representatives. Further details on study design, definitions and data collection are provided in **eMETHODS** (http://links.lww.com/CCX/B400).

Bacterial Sepsis Cohort

All patients receiving placebo from three multicenter, randomized, controlled clinical trials (A06-269 with NCT01223690; ACA-GREC with NCT00297674; INtravenous CLArithromycin for Sepsis and multiple organ dysfunction Syndrome (INCLASS) with NCT03345992) and one prospective observational study (PROMPT, with NCT03295825) were included; patients were recruited in nine ICUs, four medical wards, and one emergency department in Greece from June 2004 to June 2019. The studies were approved by the National Ethics Committee of Greece (IS 4297/20-6-2007; 2004-01; 64/17), the National Organization of Medicines of Greece (IS 7305/10-02-2007; 0-15/2nd/1-06-2004; 60/17), the Ethics Committee of ATTIKON University Hospital, Athens, Greece (16th/31/10/2017), and the respective local Ethics Committees of the participating institutions.

Eligibility criteria were: 1) adults (≥ 18 yr), 2) written informed consent provided by the patients or legal representatives, 3) sepsis diagnosis by Sepsis-3 definitions, and 4) a confirmed infection (community-acquired pneumonia, healthcare-associated pneumonia, hospital-acquired pneumonia, or ventilator-associated pneumonia); intra-abdominal infection; acute pyelonephritis; primary bacteremia; or other (7–10). All included patients received standard of care (SoC) treatment according to Surviving Sepsis Campaign guidelines (11).

Severe COVID-19 Cohort

Patients enrolled in two multicenter, open-label immunotherapy trials (SAVE: NCT04357366 and ESCAPE: NCT04339712) were studied; recruitment took place in 16 ICUs and 29 COVID-specific medical wards in Greece, from April 2020 to December 2020. Both trials were approved by the National Ethics Committee of Greece (IS 38/20, 30/20) and the National Organization for Medicines of Greece (IS 28-20, 021-20). Eligibility criteria were: 1) adults (≥ 18 yr), 2) written informed consent provided by the patients or legal representatives, 3) molecular detection of SARS-CoV-2 by real-time polymerase chain reaction in a respiratory sample (nasopharyngeal swab or bronchoalveolar lavage), and d) severe COVID-19, according to the World Health Organization (WHO) (12). All patients with critical

COVID-19 were mechanically ventilated. Patients would receive anakinra or tocilizumab, in addition to SoC; IV dexamethasone was added to SoC from mid-July 2020 (13). Baricitinib was not yet available. Both trials studied patients from the early pandemic period as non-immunotherapy receiving comparators (14, 15).

Data Collection

Data allowing baseline phenotype identification were collected for each patient, at the time of enrollment in the respective study. Further details are provided in eMETHODS (http://links.lww.com/CCX/B400).

Phenotype Classification

A simplified algorithm was used for phenotype assignment, employing six of 29 parameters of the original publication (creatinine, lactate, aspartate transaminase, bilirubin, C-reactive protein [CRP], international normalized ratio); those were selected and prioritized by order of importance in the clustering process, according to the rank of the mean of standardized differences of each value across all phenotype pairs (variables that tended to be more different among phenotypes ranked higher than more similar ones) (6). We set the mean values of those variables in Sepsis ENdotyping in Emergency CAre (SENECA) cohorts as centroids for each phenotype and calculated the Euclidean distances from the respective parameters of patients enrolled in the present study. All variables were normalized to their natural logarithm for the purposes of standardization and comparability.

We first assessed whether this algorithm would identify sepsis phenotypes, particularly regarding 28-day -outcome, in the cohort of bacterial sepsis. This analysis was performed for the composite and each of the four individual bacterial sepsis cohorts. We further compared the results of the simplified six-variable algorithm to a full model using all of the 29 variables included in the original publication by Seymour et al (6).

In a second step, we implemented the simplified algorithm among non-immunotherapy receiving patients with severe COVID-19, admitted between April 2020 and June 2020 (before WHO treatment recommendations regarding dexamethasone were made), to explore similar prognostic patterns. This was based on the assumption that dexamethasone alters the natural course of severe COVID-19 and reduces mortality (16), particularly in the δ phenotype (17). Thus, patients admitted after June 2020 were included in a separate analysis, since starting July 2020, dexamethasone was added to SoC, becoming the new "baseline." Finally, we attempted to analyze immunotherapy outcomes by phenotype classification in both time periods. More details regarding phenotype development are provided in eMETHODS (http://links.lww.com/CCX/B400).

Statistical Analysis

Phenotype assignment was performed as described above. Missing data were not imputed and the remaining values were used to find the closest Euclidean distance. Survival by phenotype at 28 days was compared by log-rank test (overall for the interaction of phenotype membership and mortality) and confirmed through univariate and stepwise multivariable Cox regression analysis (with α phenotype as the reference for between-phenotype comparisons). Additional comparisons of each phenotype with δ (the extreme phenotype) were provided and expressed as hazard ratio and 95% CI. Charlson Comorbidity Index was included in the multivariable model as a predetermined variable; Sequential organ failure assessment and Acute Physiology and Chronic Health Evaluation II scores were not, as they were possibly on the causal pathway due to overlap with two of six phenotype-defining variables (creatinine and bilirubin). Patients with severe COVID-19 were analyzed separately for the periods before (April 2020 to June 2020) and following (July 2020 to December 2020) the introduction of dexamethasone as SoC. The heterogeneity of treatment effect by phenotype was tested in a "full" stepwise Cox regression model, including the Charlson Comorbidity Index, mechanical ventilation at baseline, phenotype, treatment, and interaction between phenotype and treatment and in a "nested" model without interaction terms. The *p* values of the interaction terms in the full regression model are provided. Any two-sided *p* value of less than 0.05 was considered significant.

RESULTS

Phenotype Classification in Bacterial Sepsis

The study flow is shown in **Figure 1**. From a total of 633 patients with bacterial sepsis, 13 patients were excluded due to the absence of all six parameters used for phenotyping, resulting in a composite cohort of

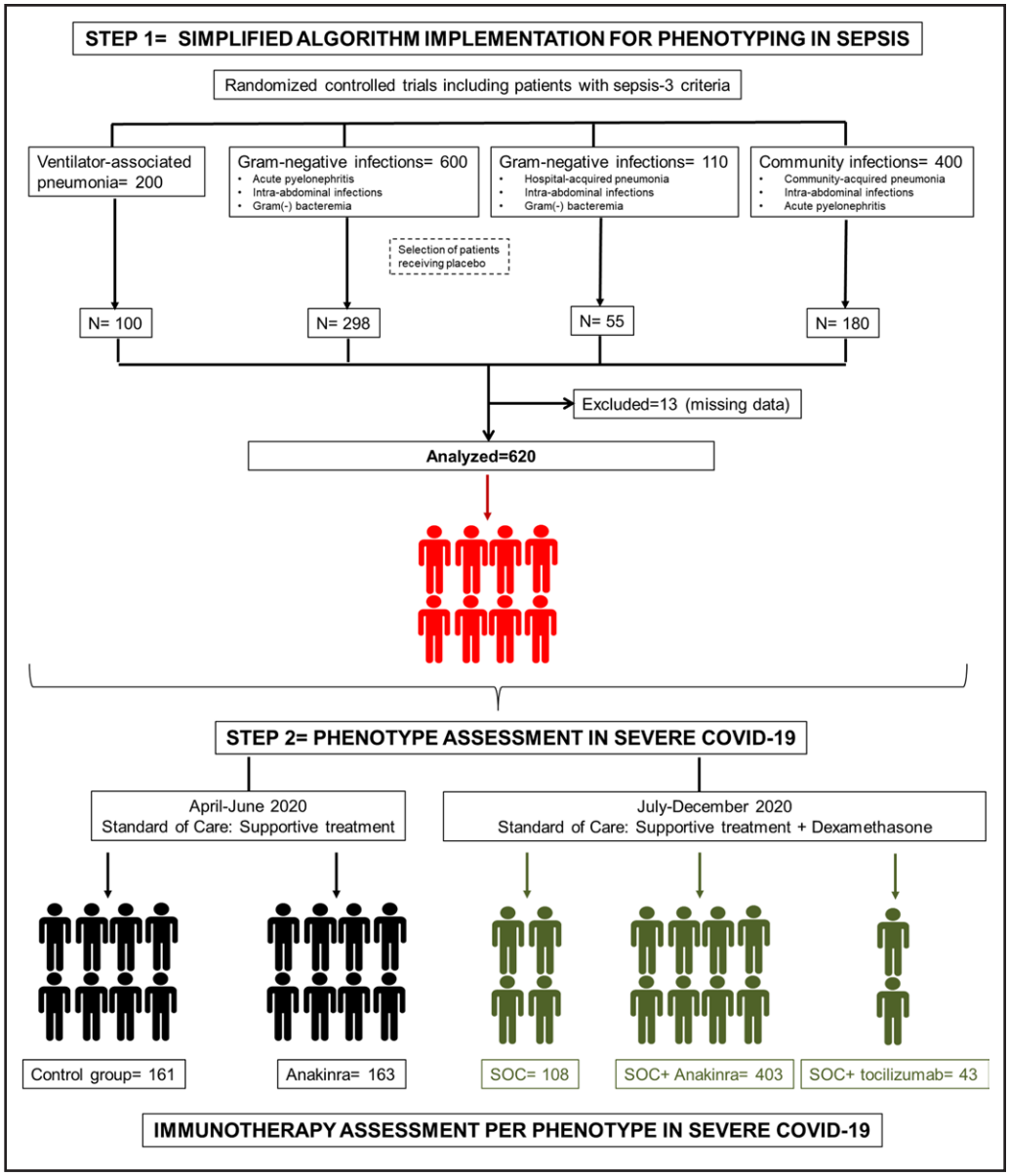


Figure 1. Study flow chart. Initially, patients with bacterial sepsis, included in the placebo arm of three randomized clinical trials and one observational prospective study, were assigned into derivation and validation cohorts to investigate phenotype classification and compare 28-d prognosis. As a second step, patients with severe COVID-19 were classified into phenotypes to assess differential response to immunotherapy by phenotype with regard to 28-d mortality. SOC = standard of care.

620 patients with bacterial sepsis. The initial individual cohorts (ACA-GREC, A06-269, INCLASS, and PROMPT) were highly heterogeneous, as presented in **Table 1**. Overall, 50% of patients were male, with median age was 58.2 and 28.9% was recruited in the ICU. After implementing the classification algorithm using data collected a median of 0 days (0–2 d) from hospital admission, the presence of all four phenotypes was confirmed in the overall cohort. Phenotypes α and γ were the most prevalent, but significant differences in phenotype distribution among the individual cohorts were observed ($p < 0.001$). Only 7.7% of patients had all six variables calculated (with lactate being the most frequently missing variable), another 74.7% had four or five variables and 14.8% had three variables available for phenotyping. Mortality by day 28 was confirmedfor all patients since they were part of prospective interventional trials with the same key endpoint.

The main phenotype features, as reported in the original publication by Seymour et al (6), were preserved (**Table 2**). Phenotype α was associated with younger age, lower comorbidity burden, and associated 28-day mortality of 13.9%, ranging between 9.8% and 25% in the individual cohorts (5–9% in the original publication); phenotype β included older patients with higher comorbidity burden, increased frequency of acute renal injury, and 28-day mortality of 28.2%, ranging between 25.7% and 41.7% in the individual cohorts (11–13% in the original publication); patients with phenotype γ displayed higher inflammatory markers and 28-day mortality of 18%, ranging between 12.9% and 50.0% in the four cohorts (9–24% in the original publication); finally, phenotype δ was more prone to hepatobiliary dysfunction, hemodynamic instability, and ICU admission and associated with higher 28-day mortality (42.5%, ranging between 30% and 69% in the individual cohorts and

TABLE 1.
Baseline Characteristics of the Cohorts of Patients With Bacterial and Viral Sepsis

Variable	Bacterial Cohort (*n* = 620)				Viral Cohort (*n* = 878)	
	ACA-GREC (*n* = 99)	A06-269 (*n* = 290)	INtravenous CLArithromycin for Sepsis and multiple organ dysfunction Syndrome (*n* = 55)	PROMPT (*n* = 176)	SAVE (*n* = 654)	ESCAPE (*n* = 224)
Age (yr), mean (SD)	59.8 (18)	66.9 (19)	69.1 (14)	74.8 (16)[a]	62.3 (14)	64.1 (13)[a]
Male sex, *n* (%)	74 (74.7)[a]	117 (40.3)	33 (60.0)	82 (46.6)	417 (63.8)	175 (78.1)[a]
Caucasian race, *n* (%)	99 (100.0)	290 (100.0)	55 (100.0)	176 (100.0)	653 (100)	224 (100)
Charlson Comorbidiy Index, mean (SD)	3 (2)	4 (2)	6 (3)[a]	5 (3)	3 (2)	3 (2)
Acute Physiology and Chronic Health Evaluation II, mean (SD)	18 (6)[a]	13 (7)	22 (7)[a]	12 (5)	6 (4)	10 (5)[a]
Sequential Organ Failure Assessment, mean (SD)	8 (4)[a]	5 (4)	11 (3)[a]	3 (2)	2 (1)	6 (3)[a]
Site of infection, *n* (%)						
Lower respiratory tract infection	99 (100.0)[a]	0 (0.0)[a]	38 (69.1)	73 (41.5)	654 (100.0)	224 (100.0)[a]
Intra-abdominal infection	0 (0.0)	112 (38.6)[a]	13 (23.6)	21 (11.9)	0 (0.0)	0 (0.0)
Primary bacteremia	0 (0.0)	45 (15.5)[a]	4 (7.3)	4 (2.3)	0 (0.0)	0 (0.0)
Acute pyelonephritis	0 (0.0)	133 (45.9)[a]	0 (0.0)	35 (19.9)	0 (0.0)	0 (0.0)
Other	0 (0.0)	0 (0.0)	0 (0.0)	44 (25.0)[a]	0 (0.0)	0 (0.0)
Ward of enrollment, *n* (%)						
ICU	99 (100.0)[a]	37 (12.8)	43 (78.2)	0 (0.0)[a]	6 (0.9)	160 (100.0)[a]
Ward	0 (0.0)	253 (87.2)[a]	12 (21.8)	0 (0.0)	648 (100.0)[a]	64 (28.6)
Emergency department	0 (0.0)	0 (0.0)	0 (0.0)	176 (100)[a]	0 (0.0)	0 (0.0)
Days to enrollment, median (Q1–Q3)	6 (5–10)[a]	1 (0–3)	9 (4–17)[a]	0 (0–0)	1 (1–3)	4 (2–7)[a]
Phenotype, *n* (%)						
α	57 (57.6)	92 (31.7)	16 (29.1)	94 (53.4)	395 (60.4)	118 (52.7)
β	30 (30.3)	74 (25.5)	12 (21.8)	33 (18.8)	72 (11.0)	16 (7.1)
γ	0 (0.0)	94 (32.4)	14 (25.5)	31 (17.6)	169 (25.8)	79 (35.3)
δ	12 (12.1)	30 (10.3)	13 (23.6)	18 (10.2)	18 (2.8)	11 (4.9)

[a]$p < 0.05$ in between-bacterial and between-viral cohort comparisons by Pearson χ^2 test, one-way analysis of variance test, or Kruskal-Wallis test.

29–40% in the original publication). Largely similar phenotype characteristics were displayed among each of the four individual sepsis cohorts (**eTables 1–4**, http://links.lww.com/CCX/B400). Phenotype classification, and particularly assignment to phenotype δ, was independently associated with the final outcome in the composite and individual cohorts, even after adjustment for baseline comorbidities, expressed by the Charlson Comorbidity Index (**Fig. 2**; and **eFigs. 1–4**, http://links.lww.com/CCX/B400).

To test the validity of the simplified six-variable algorithm, we assessed phenotype assignment compared with

TABLE 2.
Baseline Characteristics of Clinical Phenotypes in the Composite Cohort of Patients With Bacterial Sepsis

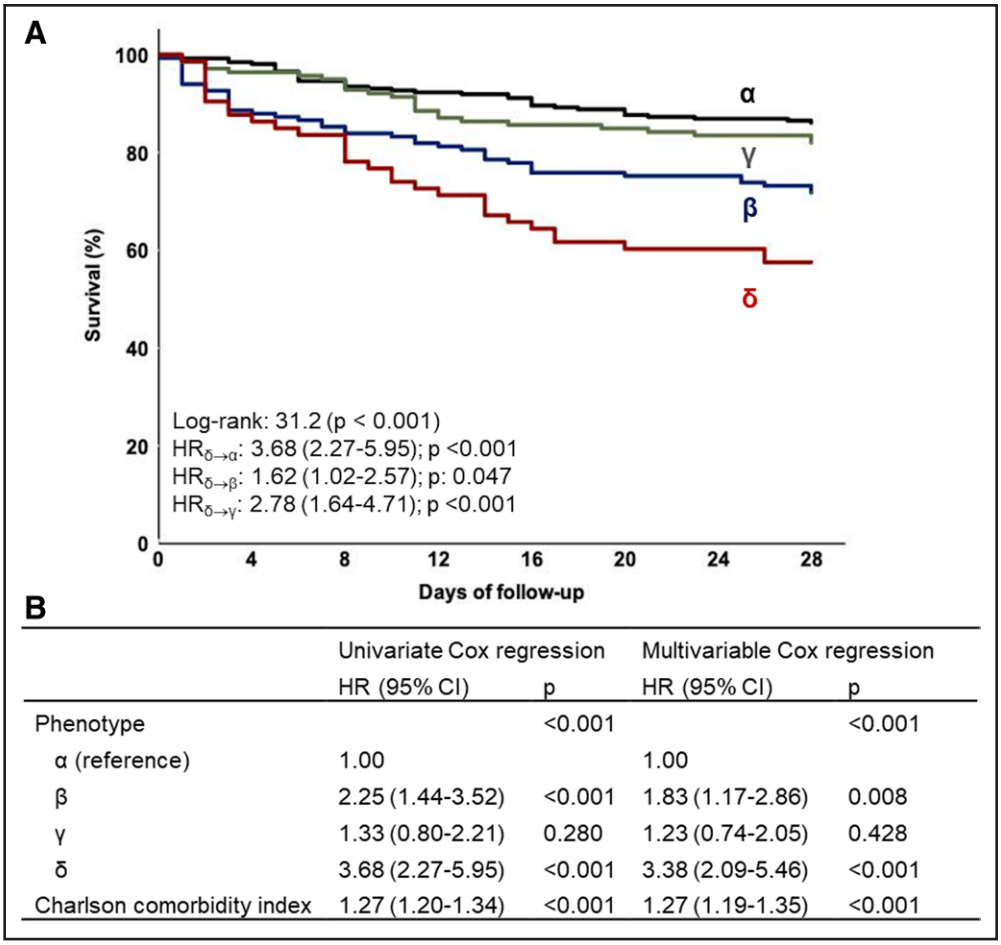
Phenotype (*n*)	Overall (*n* = 620)	α (*n* = 259)	β (*n* = 149)	γ (*n* = 139)	δ (*n* = 73)	*p*
Age (yr), mean (SD)	58.2 (18)	66.8 (18)	71.1 (17)	67.7 (18)	68.4 (20)	0.082
Male sex, *n* (%)	306 (49.9)	126 (48.6)	74 (49.7)	62 (44.6)	44 (60.3)	0.187
Charlson Comorbidiy Index, mean (SD)	4 (3)	4 (3)	5 (3)	4 (3)	5 (2)	< 0.001
Acute Physiology and Chronic Health Evaluation II, mean (SD)	14 (7)	13 (7)	17 (7)	12 (7)	18 (8)	< 0.001
Sequential Organ Failure Assessment, mean (SD)	6 (4)	5 (4)	7 (4)	5 (4)	8 (5)	< 0.001
WBCs, 10^3/uL, mean (SD)	14.2 (9.4)	13.0 (6.3)	14.7 (8.5)	15.3 (14.0)	15.2 (8.6)	0.038
Pao_2/Fio_2, mean (SD)	265 (126)	272 (125)	252 (121)	283 (141)	242 (113)	0.159
Organ failure, *n* (%)						
Acute hypoxemic respiratory failure	256 (41.3)	106 (40.9)	71 (47.7)	39 (28.1)	40 (54.8)	< 0.001
Shock	138 (22.3)	43 (16.6)	47 (31.5)	20 (14.4)	28 (34.4)	< 0.001
Acute kidney injury	254 (41.0)	74 (28.6)	95 (63.8)	45 (32.4)	40 (54.8)	< 0.001
Site of infection, *n* (%)						
Lower respiratory tract infection	209 (33.7)	98 (37.8)	62 (41.6)	21 (15.1)	29 (39.7)	< 0.001
Intra-abdominal infection	146 (23.5)	64 (24.7)	24 (16.1)	42 (30.2)	16 (21.9)	0.040
Primary bacteremia	53 (8.5)	7 (5.8)	7 (9.4)	10 (12.9)	2 (8.2)	0.106
Acute pyelonephritis	168 (27.1)	54 (20.8)	44 (29.5)	51 (36.7)	19 (26.0)	0.007
Other	44 (7.1)b	28 (10.8)	6 (4.0)	7 (5.0)	3 (4.1)	0.024
Main pathogens, *n* (% identified)						
Acinetobacter baumannii	65 (22.3)	28 (25.0)	19 (25.7)	8 (12.5)	10 (24.4)	0.203
Klebsiella pneumoniae	56 (19.2)	17 (15.2)	14 (18.9)	14 (21.9)	11 (26.8)	0.392

p values for comparisons by Pearson χ^2 test, one-way analysis of variance test, or Kruskal-Wallis test are provided.

an extended classifying model, including the original 29 variables. We were able to retrieve data for 24 variables, erythrocyte sedimentation rate, albumin, troponin, chloride, and neutrophil bands not being part of the systematic data recording in our cohorts. Concordant classification between the two methods was 67.1% (**eTable 5**, http://links.lww.com/CCX/B400) and phenotype association with survival was similar to the six-parameter model (**eFig. 5**, http://links.lww.com/CCX/B400).

Phenotype Classification in Viral Sepsis

Taking the above into account, we further assessed phenotype association with 28-day mortality in patients with severe COVID-19. A total of 878 patients were included in this analysis, of which 654 were enrolled in SAVE and 224 in ESCAPE trials (Table 1). Overall, patients were predominantly (67.4%) male, with a median age of 63 years and 18.9% were recruited in the ICU. Phenotype was determined using data obtained 2 days (1–3) after admission. Of 324 patients admitted between April 2020 and June 2020, 163 received anakinra, whereas another 161 did not receive any immunotherapy since no recommendation was made at the time for dexamethasone, tocilizumab, or anakinra (historical controls) (Fig. 1). In the study period from July 2020 to December 2020, after dexamethasone was recommended as SoC, 108 patients received dexamethasone, 403 received dexamethasone and anakinra, and 43 patients received dexamethasone and tocilizumab. Patient characteristics are summarized in



B

	Univariate Cox regression		Multivariable Cox regression	
	HR (95% CI)	p	HR (95% CI)	p
Phenotype		<0.001		<0.001
α (reference)	1.00		1.00	
β	2.25 (1.44-3.52)	<0.001	1.83 (1.17-2.86)	0.008
γ	1.33 (0.80-2.21)	0.280	1.23 (0.74-2.05)	0.428
δ	3.68 (2.27-5.95)	<0.001	3.38 (2.09-5.46)	<0.001
Charlson comorbidity index	1.27 (1.20-1.34)	<0.001	1.27 (1.19-1.35)	<0.001

Figure 2. Phenotype association with 28-day survival in bacterial sepsis. Survival curves by phenotype in the composite cohort of patients with bacterial sepsis ($n = 620$). Overall differences, as assessed by the log-rank test and univariate Cox regression analysis of phenotype comparisons with δ phenotype as a reference, are provided (**A**). Univariat and multivariable Cox regression analysis for 28-day mortality, using α phenotype as reference for between-phenotype comparisons (**B**). HR = hazard ratio.

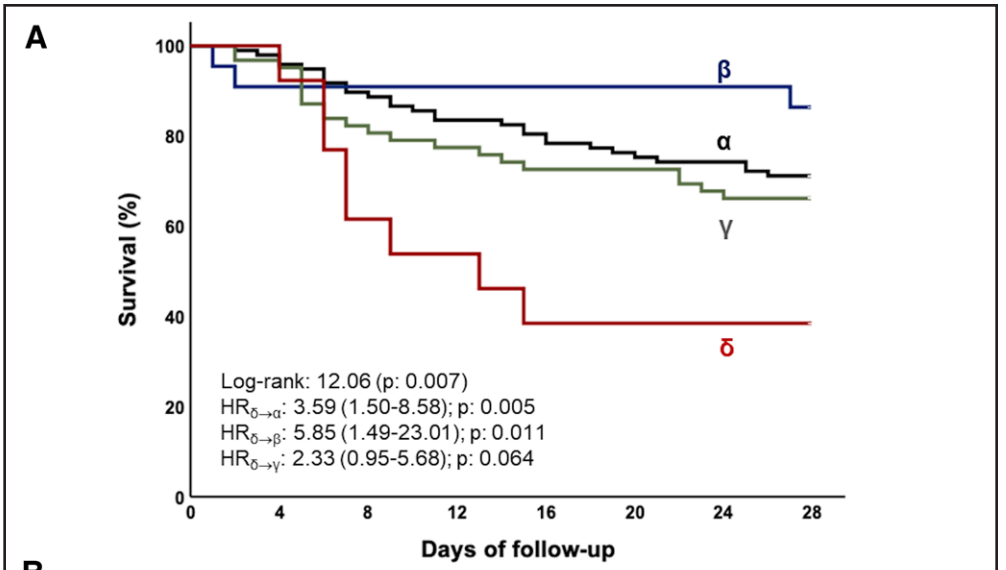
eTable 6 (http://links.lww.com/CCX/B400). Phenotype classification of historical controls displayed similar patterns of 28-day mortality as in bacterial sepsis and remained significant even after adjustment for comorbidities (**Fig. 3**). Similar results were observed in the dexamethasone-only treated patients of the late study period (**eFig. 6**, http://links.lww.com/CCX/B400). Interestingly, baseline serum levels of ferritin, interleukin (IL)-6, and CRP did not differ significantly between phenotypes (**eFig. 7**, http://links.lww.com/CCX/B400).

Treatment Response to Immunotherapy by Phenotype Classification

Immunotherapy response by phenotype was assessed separately for the initial period (April 2020 to July 2020) and the period from July 2020 to December 2020, when all patients were treated with dexamethasone as SoC. Overall, anakinra was associated with survival benefit independently of phenotype classification and without heterogeneity observed across phenotypes in both time periods (**eTable 7**, http://links.lww.com/CCX/B400). We were not able to repeat the analysis for the efficacy of tocilizumab since only 43 tocilizumab-treated patients were included.

DISCUSSION

This was one of the few efforts to use the sepsis paradigm to sequentially explore phenotypic heterogeneity in severe COVID-19. The suggested algorithm was able to classify patients with bacterial sepsis into four previously described clinical phenotypes. Phenotype features remained consistent with the original publication by Seymour et al (6) in the composite and each of the individual bacterial sepsis cohorts, despite high between-cohort heterogeneity, with differences in disease severity, trial designs, or location and timing of phenotype assessment. Phenotype assignment (particularly the δ phenotype) remained independently associated with 28-day mortality across all cohorts (composite and individual), even after adjustment for comorbidities, despite the risk of type II error in the individual cohorts. Importantly, this classification also applies to severe COVID-19. Our findings support the generalizability of the clinical phenotypes, as suggested in the original publication, which included the SENECA derivation cohort (enrollment in the ED, within 6 hr from admission) and several very different validation cohorts (A Controlled Comparison of Eritoran in Severe Sepsis or Recombinant Human



B

	Univariate Cox regression		Multivariable Cox regression	
	HR (95% CI)	p	HR (95% CI)	p
Phenotype		0.015		0.026
α (reference)	1.00		1.00	
β	0.58 (0.17-1.98)	0.387	0.71 (0.21-2.43)	0.590
γ	1.48 (0.76-2.88)	0.249	1.43 (0.73-2.78)	0.294
δ	3.59 (1.50-8.58)	0.005	3.52 (1.47-8.41)	0.005
Charlson comorbidity index	1.11 (0.98-1.26)	0.114	1.13 (0.99-1.29)	0.072
Mechanical ventilation	2.82 (1.57-5.06)	0.001	2.70 (1.50-4.87)	0.001

Figure 3. Phenotype association with 28-day survival in immunotherapy-naive patients with severe COVID-19. Survival curves by phenotype in the cohort of immunotherapy-naive patients with severe COVID-19 ($n = 161$). Patients were admitted between April 2020 and June 2020, before the introduction of dexamethasone to the standard of care. Overall differences, as assessed by the log-rank test and univariate Cox regression analysis of phenotype comparisons with δ phenotype as a reference, are provided (**A**). Univariate and multivariable Cox regression analysis for 28-day mortality, using α phenotype as reference for between-phenotype comparisons (**B**). HR = hazard ratio.

Activated Protein C Worldwide Evaluation in Severe Sepsis randomized controlled trials [RCT], enrolling patients in an ICU setting, with different timing of phenotype assessment) (6).

The benefit of a simplified six-parameter algorithm is the ability of a user-friendly, ready-to-use tool to stratify patient risk at the bedside prospectively. The six-parameter algorithm was 67.1% concordant with the extended 24-parameter model and resulted in similar prognostic patterns. This is a fair trade-off for simplicity and rapidity, which are required for point-of-care testing. Another benefit of the simplified model was the low missingness of the included parameters in our cohorts, compared with the original analysis by Seymour et al (6) (60–90% reported missingness in the most instrumental variables), allowing higher certainty in phenotype assignment. However, this algorithm was not meant to replace the original phenotypes but to validate their presence and prognosis and to potentially offer a tool to prospectively guide immunotherapy.

Sepsis is characterized by a heterogeneous dysregulated host response. Therapeutic trials of the last decades have focused on restraining or modifying this response and using biomarker combinations to identify patient populations that may display benefit or harm from different interventions is crucial (18–20). Severe COVID-19 may be seen as sepsis of viral origin, as it shares similar clinical features of organ failure (5, 21). Thus, phenotypes identified in bacterial sepsis were expected to be present in severe COVID-19. Indeed, the authors of the original publication identified sepsis phenotypes in COVID-19 using the same clustering approach as for bacterial sepsis (17); our findings support this hypothesis.

Phenotyping in COVID-19 has been attempted early in the pandemic; clinical classification according to the type of respiratory failure/acute respiratory distress syndrome (by high or low compliance) resulted in different modes of ventilation (22). On a subclinical level, COVID-19 was found to be expressed as a double immune response: either hyperinflammatory, as a macrophage activation-like syndrome driven by IL-1β, or as a more complex immune dysregulation, with a possible key role of IL-6 (3). It was even possible that both responses were compartmentalized and concomitant in the same patient (23). It became evident that a simple classification by severity was

unable to capture the complexity of clinical expression and to suggest the appropriate type of therapeutic intervention for each patient type, especially the most severely ill.

Similar to the present work, a recent study on 56,000 critically ill patients with COVID-19 and other types of sepsis, confirmed the presence of the four published sepsis phenotypes using hierarchical clustering, during the first 24 hours of ICU admission (17). The main phenotype characteristics were similar to our cohort, with α and γ phenotypes being the most prevalent, the least associated with older age and comorbidity and bearing the most favorable outcome; β and δ phenotypes were the least represented, whereas δ phenotype was associated with the worst prognosis. Minor differences in phenotype relative abundance among the original work (6), our study and the work by Bruse et al (17) may be attributed to differences in study designs and healthcare settings, the latter being a retrospective registry study, exclusively in ICU patients within 24 hours of admission, followed-up for 90 days, whereas the two others focused on all hospitalized patients, followed-up for 30 days. However, the core characteristics remained similar, independently of the origin of sepsis, implying generalizability of sepsis phenotypes across different settings. Other authors also classified 85 ICU patients with COVID-19 in three clusters, close to the concept of the original work (6): one with lower age, comorbidity, and lower mortality; one with higher prevalence of acute kidney injury, intermediate inflammation, and mortality; and one with more severe organ failure, higher inflammation, and mortality (24). The main limitation of these approaches, including our current study, is their lack of a pathogenetic biological basis.

Interestingly, inflammatory biomarkers, traditionally used for prognostication, severity stratification, and selection of immunotherapy (ferritin, CRP, and IL-6) (25, 26) did not differ significantly among phenotypes. To date, none of those biomarkers has been registered as companion diagnostics for immunotherapy in sepsis or COVID-19. Thus, phenotype classification is independent of usual biomarkers and has additive prognostic and, possibly, unproven therapeutic potential. More work needs to be done in biomarker discovery to identify biomarkers or biomarker sets with a common biological base, that may integrate the variability of the immune responses over time, to find the missing link with clinical phenotypes and effectively guide immunotherapy (27).

Our study was unable to identify differential treatment effect of immunotherapy among phenotypes. Available immunotherapy options include dexamethasone or blockade of IL-1, IL-6, and Janus kinase/ signal transducers and activators of transcription pathway pathways (28). Since the results of RECOVERY study, dexamethasone is the cornerstone of treatment for severe COVID-19 (16). The δ phenotype has been reported to respond favorably to dexamethasone, a finding which was not observed in our study (17). However, it remains unclear which patients benefit the most from adjunctive immunotherapies, such as tocilizumab or anakinra. A retrospective analysis of 4620 patients with COVID-19 identified six subphenotypes through latent class analysis within 24 hours of admission; among those, one hyperinflammatory subphenotype with multiple organ dysfunction and one hyperinflammatory with renal dysfunction, possibly overlapping with the δ and β phenotypes, respectively, showed a ten-fold decrease in relative risk of death after tocilizumab administration (29). Another study assessing subphenotypes derived from vital signs trajectories within the first 8 hours from admission in COVID-19 patients identified differential treatment effects of tocilizumab across phenotypes; benefit was shown among normotensive patients with lower temperature, heart rate, and respiratory rate (subphenotype C), whereas those with high temperature, respiratory rate, and heart rate (subphenotypes A and B) displayed harm from tocilizumab administration (30). A large recent RCT showed a protective effect of anakinra against worse clinical status, including 28- and 90-day mortality (31); this effect was seen for patients with Soluble urokinase plasminogen activator receptor (suPAR) greater than or equal to 6 and before progression to severe respiratory failure. However, timely recognition of those patients is hindered when access to suPAR is limited, and every effort aiming to replace a unique biomarker as a selection criterion for anakinra treatment answers an important clinical question (32).

The current study has certain limitations pertaining to the retrospective nature of the analysis, the low prevalence of several phenotypes, and the inclusion of patients and data dating from 2004.

Furthermore, the use of mean values from the original publication as centroids may not have been optimal, especially if the data are skewed, and may not have resulted in the exact same model as the one published by Seymour et al (6). Finally, the present work did not aim to propose a novel clustering approach that might have been the best fit for the included data, but rather to accept an already existing one and to focus on global generalizability of the findings. Thus, this article was not powered to address whether four is the optimal number of clusters in COVID-19 and additional work is warranted to identify the best fit for this population. A prospective trial, including randomization and enrichment by phenotype, would validate the prognostic model and inform the best treatment toward a precision medicine approach.

CONCLUSIONS

This work confirms that sepsis and severe COVID-19 may be classified into four clinical phenotypes with the use of a simple operational algorithm that may prospectively be used as a prognostic and selection tool for appropriate immunotherapy.

ACKNOWLEDGMENTS

We thank the patients, their families, and the clinical, laboratory, and research staff of each site who contributed to the trial.

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Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website (http://journals.lww.com/ccejournal).

Dr. Giamarellos-Bourboulis conceptualized the study design, participated in data analysis and drafting of the article, had full access to all of the study data, and took responsibility for the integrity of the data and the accuracy of the data analysis. Dr. Karakike participated in data analysis and drafted the article, had full access to all of the study data, and took responsibility for the integrity of the data and the accuracy of the data analysis. Drs. Giamarellos-Bourboulis and Karakike verified the underlying data. Drs. Metallidis, Poulakou, Kosmidou, Gatselis, Petrakis, Rovina, Gkeka, Sympardi, Papanikolaou, Koutsodimitropoulos, Tzavara, Adamis, Tsiakos, Koulouras, Mouloudi, Antoniadou, Vlachogianni, Anisoglou, Markou, Koutsoukou, Panagopoulos, Milionis, and Dalekos enrolled patients, collected clinical data, and critically revised the article for important intellectual content. Mr. Kyprianou provided statistical advice, elaborated the phenotyping algorithm, and critically revised the article for important intellectual content. All authors approved the final version of the article to be submitted for publication.

The study was supported by the Hellenic Institute for the Study of Sepsis and by the Horizon 2020 grant "European Sepsis Academy" Marie Skłodowska-Curie International Training Network (grant number 676129- granted to the National and Kapodistrian University of Athens).

Some of the results were presented as a poster and oral presentation at the 40th International Symposium of Intensive Care and Emergency Medicine (ISICEM), Brussels, Belgium August 31, 2021, to September 3, 2021.

Dr. Karakike has received funding by the Horizon2020 Marie Skłodowska-Curie International Training Network "the European Sepsis Academy" (grant number 676129- granted to the National and Kapodistrian University of Athens). Dr. Poulakou reports receiving grant funding and/or speaker's honoraria from Gilead,

Menarini, Merck Sharp & Dohme (MSD), Pfizer, Roche, and Sobi. Dr. Panagopoulos has received honoraria from GILEAD Sciences, Janssen, and MSD. Dr. Milionis reports receiving honoraria, consulting fees, and nonfinancial support from healthcare companies, including Amgen, Angelini, Bayer, Mylan, MSD, Pfizer, and Servier. Dr. Dalekos has acted as advisor/lecturer for Abbvie, Bristol-Myers Squibb, Gilead, Novartis, Roche, Amgen, MSD, Janssen, Ipsen, Genkyotex, Sobi, and Pfizer; he has received grant support from Bristol-Myers Squib, Gilead, Roche, Janssen, Abbvie, and Bayer; and he was or is currently principal investigator in national and international protocols sponsored by Abbvie, Bristol-Myers Squibb, Novartis, Gilead, Novo Nordisk, Genkyotex, Regulus Therapeutics, Tiziana Life Sciences, Bayer, Astellas, Ipsen, Pfizer, Amyndas Pharamaceuticals, CymaBay Therapeutics, and Roche. Dr. Giamarellos-Bourboulis has received honoraria from Abbott Products Operations, bioMérieux, Brahms GmbH, GlaxoSmithKline, InflaRx GmbH, Pfizer, and Swedish Orphan BioVitrum; he received independent educational grants from Abbott Products Operations, bioMérieux, Johnson & Johnson, MSD, Union Chimique Belge, and Swedish Orphan BioVitrum; and he received funding from the Horizon 2020 European grants ImmunoSep and Optimal use of hospital resources and intervention using suPAR for improving prognosis and care for patients with COVID-19 and the Horizon Health grant Equine Polyclonal antibodies Immunotherapy against COVID-19/SARS-CoV2–VOC (granted to the Hellenic Institute for the Study of Sepsis). The remaining authors have disclosed that they do not have any potential conflicts of interest.

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Requests for de-identified and protected health information (PHI)-stripped patient data can be made to the corresponding author with specific data needs, analysis, and dissemination plans. Previous Institutional Review Board/Independent Ethics Committee approval will be required, if applicable. Dates will be time-shifted to eliminate PHI, as needed. The above requests will be reviewed by the study sponsor for release, upon publication. Contact: egiamarel@med.uoa.gr.

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