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Latent Class Analysis: An innovative approach for identification of clinical and laboratory markers of disease severity among COVID-19 patients admitted to the Intensive Care Unit.

Lovemore N. Sigwadhi , Jacques L. Tamuzi , Annalise E. Zemlin , Zivanai C. Chapanduka , Brian W Allwood , Coenraad F Koegelenberg , Elvis M. Irusen , Usha Lalla , Veranyuy D. Ngah , Anteneh Yalew , Perseverence Savieri , Isaac Fwemba , Thumeka P Jalavu , Rajiv T. Erasmus , Tandi E. Matsha , Alimuddin Zumla , Peter S. Nyasulu , COVID-19 Research Response Collaboration

PII: S2772-7076(22)00130-8
DOI: https://doi.org/10.1016/j.ijregi.2022.10.004
Reference: IJREGI 175

To appear in: *IJID Regions*

Received date: 15 September 2022
Revised date: 13 October 2022
Accepted date: 17 October 2022

Please cite this article as: Lovemore N. Sigwadhi , Jacques L. Tamuzi , Annalise E. Zemlin , Zivanai C. Chapanduka , Brian W Allwood , Coenraad F Koegelenberg , Elvis M. Irusen , Usha Lalla , Veranyuy D. Ngah , Anteneh Yalew , Perseverence Savieri , Isaac Fwemba , Thumeka P Jalavu , Rajiv T. Erasmus , Tandi E. Matsha , Alimuddin Zumla , Peter S. Nyasulu , COVID-19 Research Response Collaboration, Latent Class Analysis: An innovative approach for identification of clinical and laboratory markers of disease severity among COVID-19 patients admitted to the Intensive Care Unit., *IJID Regions* (2022), doi: https://doi.org/10.1016/j.ijregi.2022.10.004

Highlights

- The Scarcity of COVID-19 phenotype and sub-phenotype in Africa.
- Use of latent class analysis model for COVID-19 patients admitted to the ICU.
- COVID-19 phenotypes and sub-phenotypes as prognostic markers in the ICU.
- Pathophysiological mechanisms of COVID-19 phenotypes in ICU patients

Latent Class Analysis: An innovative approach for identification of clinical and laboratory markers of disease severity among COVID-19 patients admitted to the Intensive Care Unit.

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Abstract

Objective: To identify clinical and laboratory phenotype distribution patterns and their usefulness as prognostic markers in COVID-19 patients admitted to the intensive care unit (ICU) in Tygerberg Hospital, Cape Town.

Methods and Results: We used a latent class analysis (LCA) model in a prospective, observational cohort study. Data from 343 COVID-19 patients was analysed. Two distinct phenotypes 1 and 2, comprising 68.46 % and 31.54% patients respectively, were identified. The phenotype 2 patients were characterised by increased coagulopathy markers (D-dimer, median value 1.73 ng/L vs 0.94 ng/L, p <0.001), end-organ dysfunction (creatinine, median 79 µmol/L vs 69.5 µmol/L , p <0.003), under-perfusion marker (lactate, median value 1.60 mmol/L, vs 1.20 mmol/L, p <0.001), abnormal cardiac function markers (median N-terminal pro-brain natriuretic peptide (NT-proBNP) 314 pg/ml vs 63.5 pg/ml, p <0.001 and median high-sensitivity cardiac troponin (Hs-TropT) 39 ng/l vs 12 ng/ l, p<0.001) and acute inflammatory syndrome (median neutrophil-to-lymphocyte ratio 15.08 vs value 8.68, p <0.001 and monocyte, median value 0.68 × 10^9/L vs 0.45 × 10^9/L, p <0.001).

Conclusion: The identification of COVID-19 phenotypes and sub-phenotypes in ICU patients could help as prognostic markers in day-to-day management of COVID-19 patients admitted to the ICU.

Key words: Latent class analysis, phenotype, sub-phenotype, COVID-19, ICU, prognostic marker

1. Introduction

The clinical spectrum of COVID-19 ranges from asymptomatic infection to severe pneumonia with respiratory failure and death (WHO, 2022). Many studies have reported on the clinical and laboratory characteristics of COVID-19 (Bastug et al., 2020; Parohan et al., 2020; Williamson et al, 2020; Kim et al., 2021; Zemlin et al., 2022). In 2020, the critical emphasis was on identifying and assessing distinct risk factors associated with COVID-19 mortality (Parohan et al., 2020; Williamson et al., 2020). A retrospective study conducted in the United States of America showed that patients who were admitted with COVID-19 could be grouped according to two distinct phenotypes that associated with mortality (Teng et al., 2021). In the first group, patients were older, with several comorbidities and a higher mortality rate. In the second group, patients were younger, more likely to be obese, and male, with higher levels of the inflammatory markers specifically C-reactive protein (CRP) and alanine aminotransferase (Teng et al., 2021). In contrast, a study found that SARS-CoV-2 infected females had lower levels of CRP, serum creatinine, and D-dimer markers in a phenotype (Lusczek et al., 2021). Another retrospective study conducted in Spain reported that patients with COVID-19 could be categorised to three distinct phenotypes (Gutiérrez-Gutiérrez et al., 2021). The first group comprised of young patients who were less frequently male, with moderate viral symptoms, and normal inflammatory parameters (Azoulay et al., 2020). The second group comprised patients with obesity, lymphocytopenia and inflammatory parameters that were not excessively elevated. The third group had older patients with several comorbidities and higher inflammatory parameters than the second group (Gutiérrez-Gutiérrez et al., 2021). Similar phenotypes were reported in France among

the patients who were admitted to the intensive care unit (ICU) (Azoulay et al., 2020). An extensive literature review did not find any evidence of research conducted in Africa on the COVID-19 phenotype. African studies assessing COVID-19 outcomes showed that gender, age, inflammatory proteins, cardiac function, and coagulation parameters may constitute potential COVID-19 phenotypes (Nachega et al., 2020; Dalal et al., 2021; Allwood et al., 2022; Zemlin et al., 2022). However, it remains unclear if similar phenotypes exist in South Africa, due to different mortality rates and comorbidities.

Among COVID-19 deaths, characteristics and underlying pathophysiology of each phenotypic group appear to be distinct (Teng et al., 2021). Hence, the identification of different phenotypes and sub-phenotypes of COVID-19 may provide guidance to basic, clinical, and translational research in sub-Saharan Africa. Due to the diversity of the populations across the world, a broad understanding could assist clinicians and researchers to enhance customised therapy that may result in reduced mortality rates among severe COVID-19 patients (Teng et al., 2021). To our knowledge, there is little evidence about the phenotypic profile of COVID-19 ICU patients in sub-Saharan Africa (Goswami et al., 2021). We performed a study to identify clinical and laboratory phenotype distribution patterns and their usefulness as prognostic markers in COVID-19 patients admitted to the ICU in South Africa.

2. Methods

2.1. Study design

This prospective cohort study was conducted at Tygerberg Hospital (TBH) during the first two waves of the COVID-19 pandemic between 27 March 2020 and 10 February

2021. The TBH is a 1380-bed hospital that serves as the main teaching hospital for Stellenbosch University Faculty of Medicine and Health Sciences. TBH was designated as a centre for COVID-19 management with additional critical care service. It provides tertiary service to around 3.5 million people.

Study population and sample size

The study included data of 343 adult patients admitted with severe COVID19 pneumonia to the designated ICU during the above-mentioned dates waves. The diagnosis was confirmed with a positive SARS-CoV-2 polymerase chain reaction (PCR) (figure 1). Three hundred or more cases was desirable to uncover classes with low memberships without poor functioning fit indices and convergence failures (Nylund-Gibson et al., 2022). Details regarding admission criteria to ICU are documented in the Western Cape Government's provincial guidelines (Critical Care Society of Southern Africa, 2020).

2.2. Data collection

Clinical data were extracted from ICU clinical notes and entered onto a REDCap® (Research Electronic Data Capture, Stellenbosch, South Africa) database, a secure web application. Laboratory data were imported from the National Health Laboratory Service (NHLS) Laboratory Information System (TrakCare® Lab Enterprise) onto the REDCap database. Data quality assurance were undertaken by the research assistants and later verified by the supervisor of the research team to ensure data quality prior to

analysis. Detailed information about the clinical parameters is defined in the previously published articles (Zemlin et al., 2022).

2.3. Statistical analysis

Class-defining variables for latent class identification included baseline demographic features as well as clinical and laboratory data. Continuous variables were reported as median and IQR (non-normal) and categorical variables as percentages. All variables with missing data were excluded from further analysis.

A multivariate mixture model was used to identify two distinct latent classes based on the variables of interest. We used a binomial response distribution for binary categorical variables and a Gaussian response distribution for continuous variables. These variables were centred and scaled to unit variance prior to model inference. We log-transformed continuous variables that were skewed prior to analysis.

Data were allocated to the latent class based on the posterior model probability (probability of class assignment >50%). The Wilcoxon rank sum was used to compare the median differences between the two identified classes for continuous variables. Pearson Chi-squared test was used for categorical variables.

Two-sided p-value < 0.05 was considered statistically significant. The two-class model was compared to a model including an additional latent class based on model selection criteria (Akaike Information Criteria, AIC, and Bayesian Information Criteria BIC), likelihood ratio test, the size of the smallest class, the probability of class assignment,

and qualitative evaluation of the defining class characteristics. We used BIC for parameterized Gaussian mixture models fitted by an Expectation-maximization (EM) algorithm initialized by model-based hierarchical clustering to get the number of latent classes. Integrated Complete-data Likelihood (ICL) was used to confirm the number of classes obtained using the BIC criteria. The standardised mean of continuous class-defining variables was compared to understand the clinical and biological characteristics that distinguished the two classes (Figures. 1A, B, C), and raw data compared by class. A sub-analysis was done to assess if there were any notable differences among the patients who died and discharged. Stata (V.16, Stata Corp, College Station, Texas, USA) was used for data cleaning, manipulation, Wilcoxon, and Pearson-Chi square test. R (V, 4.1.0, R Core Team) with R Studio (V.1.4.1, R Studio Team) was used for analysis to obtain the required number of classes.

3. Results

Baseline characteristics of the cohort (n = 343) are presented in Table 1. The cohort had a slightly higher proportion of females (n=184, 53.6%) and ICU mortality was high (n=216, 63%). We identified two latent classes representing 75.8% (Class 1, n = 260) and 24.2% (Class 2, n = 83) of the cohort, respectively. Class 2 was notable primarily by increased coagulopathy markers (D-dimer, median value 1.73 ng/L, IQR (0.61- 5.70) in Class 2 vs 0.94 ng/L, IQR (0.41- 4.13) in Class 1 ($p < 0.001$), underperfusion (increased lactate, median value 1.60 mmol/L, IQR (1.10- 2.10) in Class 2 vs 1.20 mmol/L, IQR (1.00- 1.40) in Class 1 ($p < 0.001$), end-organ dysfunction (creatinine

(median value 79 µmol/L, IQR (65- 110) in Class 2 vs 69.5 µmol/L, IQR (56.5- 83) in Class 1 (p <0.003), Table 2), cardiac function markers NT-proBNP (median value 314 pg/ml, IQR (72- 1346) in Class 2 vs value 63.5 pg/ml, IQR (32.5- 193.5) in Class 1 (p <0.001), Hs-TropT (median value 39 ng/L, IQR (13-102) in Class 2 vs value 12 ng/L, IQR (8-22) in Class 1 (p <0.001) Table 2), neutrophil-to-lymphocyte ratio (NLR) , median value 15.08, IQR (8.75-24.41) in Class 2 vs value 8.68, IQR (5.71-14.21) in Class 1 (p <0.001) and monocyte, median value 0.55 × 10^9/L, IQR (0.36-1.11) in class 2 vs 0.45 × 10^9/L, IQR (0.31-0.72) in class 1, p = 0.011), Table 2). In addition, females in class 2 had lower mean haemoglobin than in class 1 (11.88 g/dL (1.75) vs 12.67 (1.37), p = 0.014). In the same line, the median pH value was lower in class 2 than in class 1 (7.36, IQR (7.30-7.43) vs 7.47 (7.45-7.50) (p <0.001)), and the median value HCO_3std was also lower in class 2 than class 1 (23.05 mmol/L, IQR (19.70-25.60) vs 27.30 mmol/L, IQR (25.10-29.10) (p <0.001)). In contrast, the median value $PaCO_2$ was higher in class 2 than in class 1 (5.70 kPa, IQR (4.30-6.90) vs 4.80 kPa, IQR (4.30-5.30) (p <0.001)).

When comparing class 2 patients to class 1 patients, the mortality risk was 1.44 (95% CI: 1.22-1.67, p<0.001).

An optimum of two latent classes was obtained (Table 3) among the patients who died. Class 2 was notable primarily by increased acute inflammatory syndrome (C-reactive protein, median value 194, IQR (133-307) in Class 2 vs 153, IQR (100-247) in Class 1 (p=0.015), NLR 12.53, IQR (7.34-22.56) in class 2 vs 9.50, IQR (6.21-15.16) in class 1, p = 0.013 and monocyte, median value 0.68 × 10^9/L (0.39-2.70) in class 2 vs 0.45 × 10^9/L (0.30-0.68) in class 1, p <0.001), under-perfusion (increased lactate, median

value 1.60 mmol/L, IQR (1.60- 2.60) in Class 2 vs 1.40 mmol/L, IQR (1.10- 1.90) in Class 1 (p =0.021)), end-organ dysfunction(creatinine (median value 107 µmol/L , IQR (77- 157) in Class 2 vs 72 µmol/L, IQR (57- 86) in Class 1 (p <0.001), Table 3), cardiac function markers NT-proBNP (median value 784 pg/ml, IQR (217- 2377) in Class 2 vs value 337 pg/ml, IQR (125- 307) in Class 1 (p <0.001), Hs-TropT (median value 25 ng/L, IQR (12-62) in Class 2 vs value 14 ng/L, IQR (9-28) in Class 1 (p <0.001) Table 3). In class 2, females had lower mean (SE) haemoglobin than in class 1 ((11.96 g/dL (1.60) vs 12.60 g/dL (1.43), p = 0.040)). Furthermore, the median value pH and HCO_3std was lower in class 2 than class 1 (7.37, IQR (7.30-7.44) vs 7.47 (7.44 -7.50) (p <0.001)) and 23.55 mmol/L, IQR (20.00-25.90) vs 27.10 mmol/L, IQR (25.00-29.20) (p <0.001), respectively) (Table 3). In contrast, the median value $PaCO_2$ was higher in class 2 than class 1 (5.70 kPa, IQR (4.50-6.70) vs 4.90 kPa, IQR (4.30-5.30) in class 1 (p <0.001) (Table 3). Platelets/Lymphocyte ratio was found as borderline between Class 2 and Class 1 (247.31 (70.59-475.51) vs 296.64 (206.58-451.59), p = 0.054) (Table 3). The standardised mean of continuous class-defining variables was compared, to understand the clinical and biological characteristics that distinguished the two classes among the patients who died (Fig 1: B). Notable differences were observed between fig 1 A and B.

Figure 1 C showed a different trend seen in Figure 1 A suggesting that what we observed overall was not what was observed among discharged patients (Fig 1 A vs C). Comparing raw data by class among discharged patients. Class 2 was notable, primarily by increased coagulopathy markers (D-dimer, median value 1.93 ng/L, IQR

(0.55- 5.12) in Class 2 vs value 0.40 ng/L, IQR (0.25- 0.60) in Class 1 (p <0.001), under-perfusion (increased lactate, median value 1.60 mmol/L, IQR (1.10- 2.10) in Class 2 vs 1.20 mmol/L, IQR (1.00- 1.40) in Class 1 (p <0.001)) end-organ dysfunction (creatinine (median value 79 µmol/L, IQR (65- 110) in Class 2 vs 69.5 µmol/L, IQR (56.5- 83) in Class 1 (p <0.003), Table 4), cardiac function markers NT-proBNP (median value 314 pg/ml, IQR (72- 1346) in Class 2 vs value 63.5 pg/ml, IQR (32.5- 193.5) in Class 1 (p <0.001), Hs-TropT (median value 13 ng/L, IQR (9-36) in Class 2 vs value 6 ng/L, IQR (5-10.5) in Class 1 (p <0.001) ,Table 4), the median value NLR was 12.39, IQR (6.48-20.29) in Class 2 vs value 6.67, IQR (4.51-9.00) in Class 1 (p <0.001), Table 4). Furthermore, the median value $PaCO_2$ was higher in Class 2 than in Class 1 (5.70 kPa, IQR (4.50-6.70) vs 4.90 kPa, IQR (4.30-5.30) (p <0.001). In contrast, the median value HCO_3std was lower in Class 2 than Class 1 (23.55 mmol/L, IQR (20.00-25.90) vs 27.10 mmol/L, IQR (25.00-29.20) (p <0.001)).

4. Discussion

In this first study from Africa to report on clinical phenotypes associated with COVID-19, two distinct latent subclasses were identified based on patients' demographic, clinical, and laboratory profiles. The inflammatory syndrome, coagulopathy markers, under-perfusion markers, end-organ dysfunction, and cardiac function markers were identified as statistically and clinically significant phenotypes. Except for the high HIV prevalence in the death sub phenotype, demographics and comorbidities did not differ between the

deceased and recovering sub phenotypes in each sub-analysis. This implies that distinct COVID-19 progression pathways exist and, are independent of baseline risk factors for disease severity.

Our LCA showed that the class 2 phenotype, which comprised 31.54% (83/343) of the total sample size, was associated with increased coagulopathy markers, end-organ dysfunction, under-perfusion markers, cardiac function markers, and acute inflammatory syndrome. Recent evidence suggests that altered coagulation is an important phenotypic marker in COVID-19-associated ARDS (Ranjeva et al., 2021). Ranjeva et al. demonstrated that the more severe phenotype was distinguished by significantly elevated D-dimer (Ranjeva et al., 2021). A high burden of thromboembolic disease was found among post-mortem patients with severe COVID-19 infection (Nadkarni et al., 2020; Ranjeva et al., 2021). Furthermore, elevated baseline D-dimer among COVID-19 patients has been shown to predict major coagulation-associated complications, critical illness, and death (Al-Samkari et al., 2020; Ranjeva et al., 2021). Several mechanisms have also been proposed to explain the association between NT-proBNP and Hs-TropT COVID-19 outcomes in the ICU. These include progressive inflammation, hypoxaemia, sepsis, myocardial injury, and volume overload states, all of which can increase myocardial stress (Babapoor-Farrokhran et al., 2020; Kazory et al., 2020; Yoo et al., 2021; Bertini et al., 2022). COVID-19 vascular complications, such as pulmonary embolism and acute kidney injury, may aggravate myocardial stress. These mechanisms may characterise a cardiac function phenotype in COVID-19 patients admitted to the ICU (Yoo et al., 2021; Azevedo et al., 2021). This was demonstrated in

our study by the presence of elevated NT-proBNP and Hs-TropT in the class 2 phenotype. The NLR is considered a surrogate marker of systemic hyperinflammation, and an independent predictor of poor outcome associated with COVID-19 (Li et al., 2020). In severe or patients who died with COVID-19, the lymphocyte count decreases progressively, while the neutrophil count gradually increases (Li et al., 2020). On the one hand, neutrophils are generally regarded as pro-inflammatory cells with a range of antimicrobial activities, which can be triggered by virus-related inflammatory factors, such as interleukin-6 and 8 (Li et al., 2020; Mangalmurti and Hunter, 2020). Similarly, a dysregulated monocyte response can be damaging to the host, as is seen in the macrophage activation syndrome induced by severe infections, including in infections with the related virus SARS-CoV-2 (Merad and Martin, 2020). Systematic inflammation triggered by SARS-CoV-2 significantly depresses cellular immunity, leading to a decrease in CD3 + T cells, CD4 + T cells and CD8 + T cells (Li et al., 2020). As the pathophysiology is further clarified below, this results in hypoinflammatory and hyperinflammatory states in class 2 and 1, respectively. Emphasis is also placed on moderate anaemia in the Class 2. Wang et al. reported lower haemoglobin levels in patients with more severe COVID-19 (Wang et al., 2020). This anaemia in Class 2 is probably due to hyperinflammatory processes associated with SARS-CoV-2 infection and normal mean Hb in Class 1 may be due to a hypoinflammatory state.

In the sub-analyses that included COVID-19 mortality in the ICU, the Class 2 phenotype had higher levels of acute-phase proteins in inflammation, end-organ dysfunction, under-perfusion, and cardiac function markers than the Class 1 phenotype.

Furthermore, HIV-positive status was more prevalent in the Class 1 phenotype than in the Class 2 phenotype. Recent LCA involving COVID-19 patients revealed a hyperinflammatory syndrome in the sub-phenotypes (da Silva et al., 2020; Wang et al., 2021), with markedly elevated CRP defining these sub-phenotypes, as demonstrated by our findings. In contrast, 62.5% (135/216) of patients who died in the Class 1 phenotype were hypoinflammatory. The plausible explanation may be the viral cytotoxicity as a primary driver of mortality in the hypo-inflammatory sub phenotype, whereas excessive inflammation is a primary driver of mortality in the hyperinflammatory sub phenotype, as evidenced by higher levels of pro-inflammatory markers and an increased prevalence of multiorgan failure (Sinha et al., 2021). Hypo-inflammatory factors may explain mortality in the Class 1 sub phenotype in our study. In addition, the Class 1 phenotype for those who died had a high HIV prevalence of 18.5% (24/135). Indeed, SARS-CoV-2 and HIV may both decrease CD4 count and lymphocytes (Tamuzi et al., 2020), which could explain the hypo-inflammatory phenotype in Class 1. Our findings also revealed that sub-phenotype 2 was characterized by elevated lactate and creatinine levels. Two LCAs revealed that the renal morbidity and high morbidity phenotypes had more in-hospital complications than the low-morbidity phenotype (da Silva et al., 2020; Ranjeva et al., 2021). Phenotypes were associated with an increased risk of myocardial infarction, heart failure, and acute kidney injury (Benítez et al., 2022). This could account for the high prevalence of acute coronary syndromes in sub phenotype 2. Another study found that a subclass with kidney dysfunction and hyperinflammatory response, defined by renal failure (elevated serum creatinine), lymphopenia, and elevated CRP, had the highest likelihood of ICU transfer or in-hospital mortality when compared to other

subclasses (Wang et al., 2021). Higher creatinine and lower platelets indicate that clinical worsening within the severe baseline stratum is caused by cell death, macrophage activation, and overt organ dysfunction with disseminated intravascular coagulation (Webb et al., 2020; Su et al., 2021).

Class 2 including COVID-19 discharged patients 59.05% (75/127) represented elevated coagulopathy markers, end-organ dysfunction, cardiac function markers, and elevated lymphocytes and platelet counts in the sub-analyses. D-dimer was found to be positively associated with CRP, serum ferritin, procalcitonin (PCT), and interleukin (IL)-2R in this study (Long et al., 2020). Thus, we hypothesise that moderately elevated D-dimer may induce an adequate inflammatory syndrome in the sub-phenotype Class 2 of survived patients, potentially improving the prognosis of COVID-19 patients. Furthermore, moderately elevated D-dimer may be less likely to be associated with a cytokine storm, as observed in the mortality sub-analyses. D-dimer does not directly stimulate IL-6, the key factor inducing a cytokine storm which is associated with mortality caused by sepsis or sepsis shock (Eljilany et al., 2020). We also hypothesise that because D-dimer was moderately elevated, thromboembolism prophylaxis may have been more effective in the survived sub-phenotype classes 1 and 2. End-organ failure has been reported following hospital discharge with COVID-19 (Ayoubkhani et al., 2021). COVID-19 pathogenesis and multiple organ injury include direct virus-induced cytotoxicity in angiotensin converting enzyme 2 (ACE2)-expressing cells, renin-angiotensin-aldosterone system (RAAS) dysregulation due to virus-mediated ACE2 downregulation, immune response dysregulation, endothelial cell injury, and thrombo-inflammation, and

tissue fibrosis (Gupta et al., 2020; Lopes-Pacheco et al., 2021). We can reasonably speculate that the high survival rate in sub phenotype 2 was due to a milder phenotype of COVID-19 organ failure. This is also true for sub phenotype 2's elevated cardiac function markers and acute coronary syndrome. Even though sub phenotype 1 was characterised by organ failure, D-dimer was <1.0 µg/mL on admission, which has been associated with a lower risk of fatality (Chen et al., 2020; Eljilany et al., 2020; Guan et al., 2020; Tang et al., 2020; Zhou et al., 2020; Lopes-Pacheco et al., 2021).

To the best of our knowledge, this is Africa's first LCA to report the underlying phenotypes of COVID-19 patients admitted to the ICU. This LCA demonstrates the benefit of evaluating prognostic markers within sub phenotypes. These analyses assisted in identifying groups of COVID-19 ICU patients who are at the highest risk of death and may benefit from additional clinical attention. Another advantage of this study is that it presents a phenotyping schema that divides COVID-19 ICU patients into less heterogeneous subgroups. Furthermore, inconsistency in case reporting, with 30% (147/490) of patients admitted to the ICU having missing data, may have impacted data completeness. LCA using missing co-morbidities, clinical symptoms, CD4 count among HIV-infected patients, and therapies may also be important, as more informative profiles may strengthen our phenotypes model.

5. Conclusion

In summary, our analysis has identified two different phenotypes in COVID-19 patients admitted to the ICU. These two phenotypes were markedly different, characterised by increased coagulopathy markers, end-organ dysfunction, acute inflammatory syndrome, cardiac function markers, and underperfusion markers in phenotype 2. In the sub-analysis, the two sub phenotypes were also different with increased acute inflammatory syndrome, end-organ dysfunction, cardiac function markers, and under-perfusion in sub phenotype 2. Among those who died, HIV-positive status had a higher proportion in sub phenotype 1. Among those who survived, the two sub phenotypes were again different with elevated coagulopathy markers, end-organ dysfunction, cardiac function markers, and acute coronary syndromes in sub phenotype 2. Given the different COVID-19 sub phenotypes identified, this could help clinicians in day-to-day decision-making, such as the prognosis and management of COVID-19 patients admitted to the ICU.

6. Abbreviations

ACE 2: angiotensin-converting enzyme 2; CAC: COVID-19 associated coagulopathy; COVID-19: Coronavirus Disease 2019; Hb: haemoglobin; ICU: intensive unit care; NHLS: National Health Laboratory Service; PCR: Polymerase Chain Reaction; REDCap: Research Electronic Data Capture; SARS-COV-2: severe acute respiratory syndrome coronavirus 2; TBH: Tygerberg Hospital.

7. Footnotes

Author Contributions: Data curation: LNS, VND, and AY. Formal analysis: LNS. Investigation: ZCC, AZ, and PSN. Methodology: LNS, VND, PSN. Supervision: PSN,

ZCC, and AZ. Writing – review & editing: LNS, JLT**,** ZCC, IA, BA, CFK, EI, UL, AEZ, TEM, RTE, TPJ, VDN, AY, NB, PS, IF, MMA, AKB, VKM, CN, AA, MWM, MM, MSS, PS, AZ, and PSN.

Research funding: This work was carried out under the Stellenbosch University Special Vice-Rector (RIPS) Fund and the COVID-19 Africa Rapid Grant Fund supported under the auspices of the Science Granting Councils Initiative in Sub-Saharan Africa (SGCI) and administered by South Africa's National Research Foundation (NRF) in collaboration with Canada's International Development Research Centre (IDRC), the Swedish International Development Cooperation Agency (SIDA), South Africa's Department of Science and Innovation (DSI), the Fonds de Recherche du Québec (FRQ), the United Kingdom's Department of International Development (DFID), United Kingdom Research and Innovation (UKRI) through the Newton Fund, and the SGCI participating councils across 15 countries in sub-Saharan Africa.

Informed Consent Statement: The Investigators obtained ethical approval and waiver of consent from the Health Research Ethics Committee of the Faculty of Medicine and Health Sciences, Stellenbosch University and Research Committee of the Tygerberg Hospital.

Ethics: This study was approved by the Health Research Ethics Committee of Stellenbosch University, approval number: N20/04/002_COVID-19. Patient confidentiality was ensured by labelling data with a unique episode number. The research project followed the laid down guidelines in the ethical conduct of studies involving human participants.

Availability of data and materials: Data are available upon reasonable request from the corresponding author.

Conflicts of Interest: all authors declare no conflicts of interest.

Acknowledgements: Sir Prof Alimuddin Zumla, is co-Principal Investigator of the (PANDORA-ID-NET), the Pan-African Network for Rapid Research, Response, Relief and Preparedness for Infectious Disease Epidemics, supported by the EDCTP. He is in receipt of a UK National Institutes of Health Research, Senior Investigator Award and is a Mahathir Foundation Science Award laureate.

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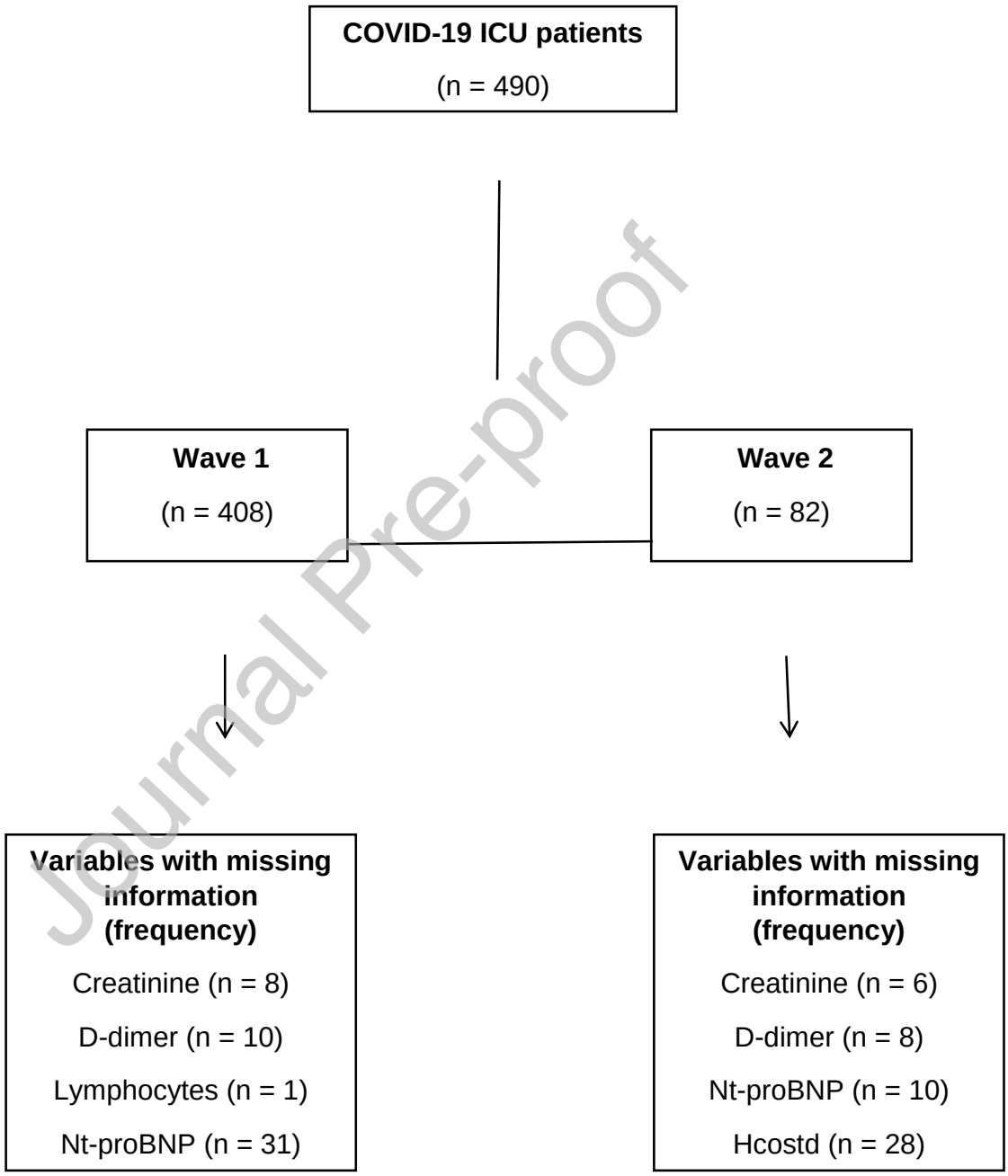

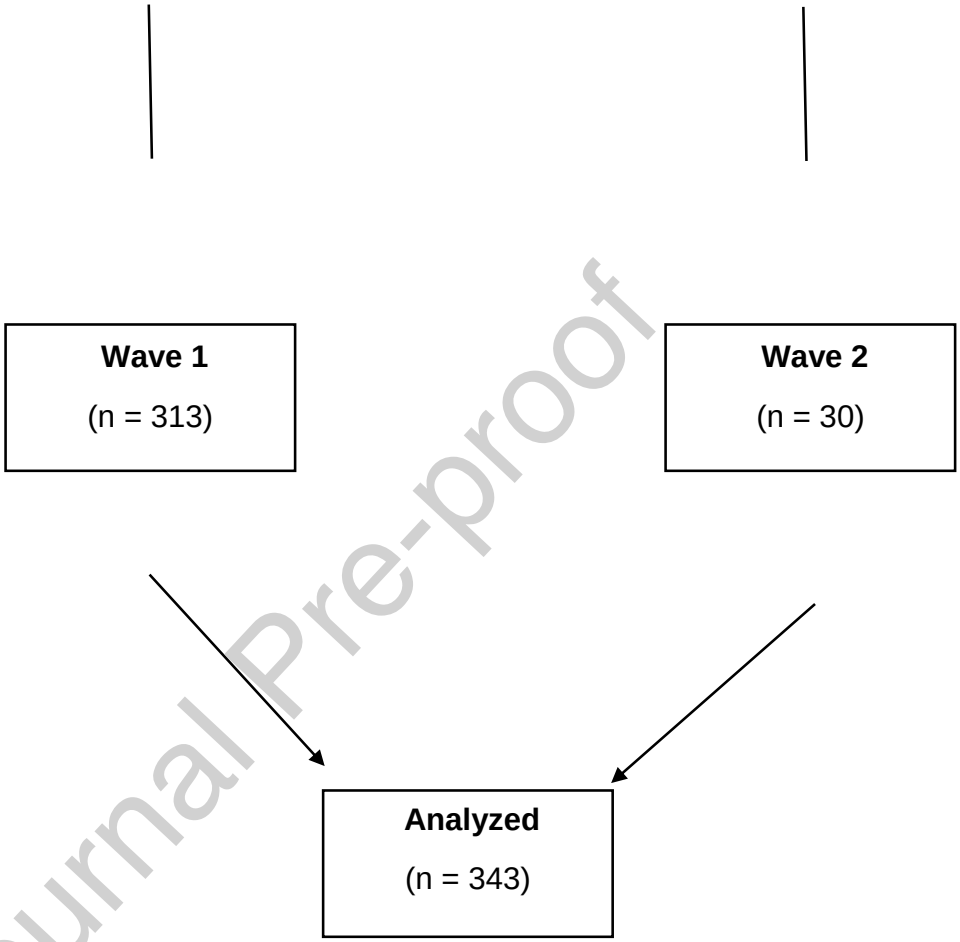

Figure 1: Consort diagram

Fig. 1. A: Differences in the mean standardized values of continuous class-defining variables by latent class among the ICU patients.

Fig. 1. B: Differences in the mean standardized values of continuous class-defining variables by latent class among the patients who died.

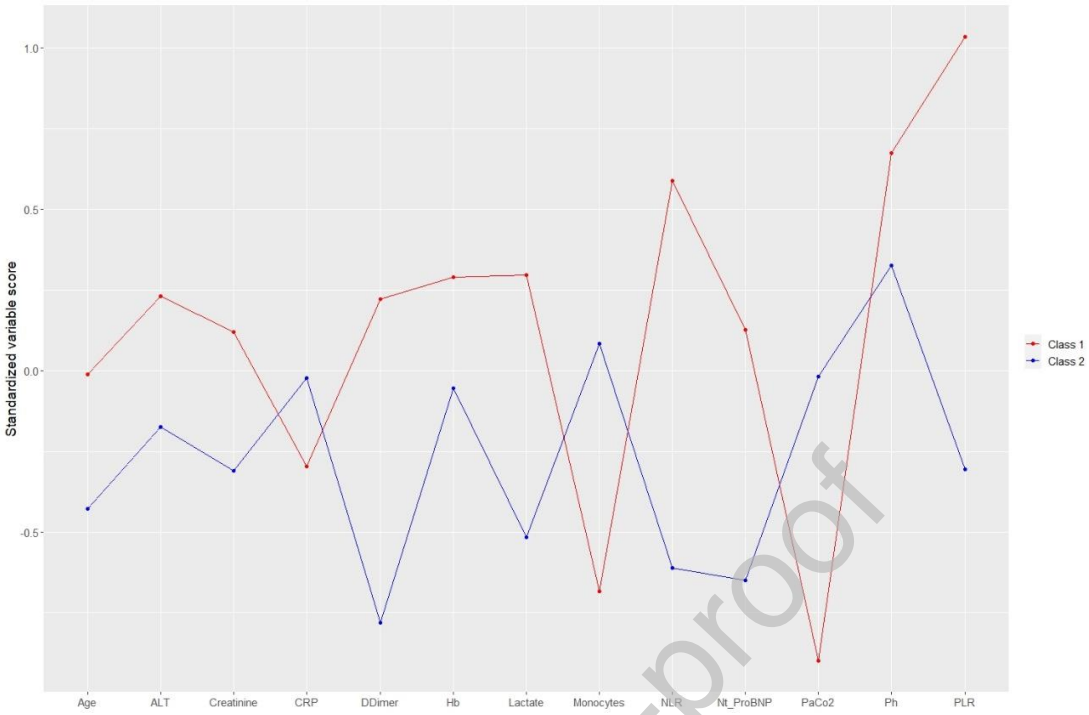

Fig. 1. C: Differences in the mean standardized values of continuous class-defining variables by latent class among the patients who were discharged.

Table 1: Baseline clinical variables among the COVID-19 ICU study cohort.

Characteristic	N	Study cohort, median (IQR) or n (%)
Age (yrs)	343	55.56 (46.14-62.28)
Gender: Female	343	184 (53.6%)
Diabetes Mellitus	338	169 (50.0%)
Hypertension	338	204 (60.4%)
HIV Positive	300	39 (13.0%)
ICU mortality	343	216 (63.0%)
Ventilation: Non-invasive	343	294 (85.7%)
pH	343	7.46 (7.41-7.50)
$PaCO_2$	343	4.90 (4.30-5.60)
C-reactive protein	343	176.00 (109.00-270.00)
Neutrophils	343	9.92 (7.34-14.93)
Platelets	343	298.00 (231.00-373.00)
Nt-proBNP	343	303.00 (89.00-976.00)
Haemoglobin	343	13.20 (11.90-14.10)
Monocytes	343	0.48 (0.32-0.80)
D dimer	343	1.09 (0.45-4.40)
Creatinine	343	77.00 (63.00-102.00)
Alanine aminotransferase (ALT)	343	33.00 (20.00-50.00)
Platelets/Lymphocyte ratio	343	9.48 (6.15-16.71)
Neutrophil/Lymphocyte ratio	343	285.06 (177.59-474.47)
Lactate	343	1.40 (1.10-1.90)
Hs-Troponin-T	343	13.00 (8.00-30.00)

Abbreviations: HIV: human immunodeficiency virus; PaCO2: Partial Pressure of Carbon Dioxide, pH: potential of hydrogen, Nt-proBNP: N-terminal pro-brain natriuretic peptide

Table 2: Difference in clinical and laboratory characteristics between latent subclasses among COVID-19 ICU patients

Characteristic	Reference intervals	Latent subclasses		p-value
		Class 1 (n=260)	Class 2 (n=83)	
Age(yrs)		55.87 (46.27-62.80)	53.98 (43.83-60.79)	0.28
Gender				0.25
Female		116 (44.6%)	43 (51.8%)	
Male		144 (55.4%)	40 (48.2%)	
Diabetes mellitus				0.37
		125 (48.6%)	44 (54.3%)	
Hypertension				0.98
Yes		155 (60.3%)	49 (60.5%)	
No		102 (39.7%)	32 (39.5%)	
HIV status				0.096
Positive		34 (14.8%)	5 (7.1%)	
Creatinine	49–90 µmol/L	72.00 (58.00-87.00)	112.00 (79.00-169.00)	<0.001
D Dimer	0.00-0.25mg/L	0.94 (0.41-4.13)	1.73 (0.61-5.70)	0.012
	Male: 13.0-17.0g/dL	13.69 (1.66)	13.24 (2.22)	0.301
Haemoglobin	Female: 12.0-15.0g/dL	12.67 (1.37)	11.88 (1.75)	0.014
Monocytes	0.30-0.80x 10^9/L	0.45 (0.31-0.72)	0.55 (0.36-1.11)	0.011
NT-proBNP	< 125 pg/mL	219.00 (64.50-600.00)	1661.00 (362.00-4694.00)	<0.001
Hs-TropT	< 100 ng/l	12.00 (8.00-22.00)	39.00 (13.00-102.00)	<0.001
C-reactive protein	< 10 mg/L	176.00 (107.00-268.50)	200.00 (116.00-273.00)	0.39
pH	7.35 - 7.45	7.47 (7.45-7.50)	7.36 (7.30-7.43)	<0.001
PaCO2	4.26 – 6.38 kPa	4.80 (4.30-5.30)	5.70 (4.30-6.90)	<0.001
Lactate	0.5 – 2.2 mmol/L	1.40 (1.00-1.80)	1.70 (1.20-3.30)	<0.001
Alanine aminotransferase	7 – 40 U/L	31.00 (21.00-48.00)	37.00 (17.00-63.00)	0.33
HCO3std	19 – 24 mmol/L	27.30 (25.10-29.10)	23.05 (19.70-25.60)	<0.001
Neutrophil/ Lymphocyte ratio		8.68 (5.71-14.21)	15.08 (8.75-24.41)	<0.001
Platelets/		284.98	287.76 (154.87-	0.58

Lymphocyte ratio		(179.11-451.85)	584.13)	

Table 3: Difference in clinical and laboratory characteristics between latent subclasses for COVID-19 ICU death patients

Characteristic		**Latent subclasses**		**p-value**
		Class 1 (n=135)	**Class 2 (n=81)**	
Age(years)		57.77 (49.22-63.66)	57.07 (47.25-62.57)	0.52
Gender				0.48
Female		65 (48.1%)	43 (53.1%)	
Male		70 (51.9%)	38 (46.9%)	
Diabetes mellitus				0.27
Yes		68 (50.4%)	46 (58.2%)	
No		67 (49.6%)	33 (41.8%)	
Hypertension				0.65
Yes		83 (61.5%)	51 (64.6%)	
No		52 (38.5%)	28 (35.4%)	
HIV status				0.016
Positive		24 (18.5%)	3 (5.2%)	
Negative		106 (81.5%)	55 (94.8%)	
Creatinine	49–90 µmol/L	72.00 (57.00-86.00)	107.00 (77.00-157.00)	<0.001
D-dimer	0.00-0.25mg/L	1.43 (0.48-6.72)	1.33 (0.52-4.46)	0.52
Haemoglobin	Male:13.0-17.0g/dL; Female:12.0-15.0g/dL	13.73 (1.52) 12.60 (1.43)	13.44 (1.94) 11.96 (1.60)	0.469 0.040
Monocytes	0.30-0.80x 10^9/L	0.45 (0.30-0.68)	0.68 (0.39-2.70)	<0.001
NT-proBNP	< 125 pg/mL	337.00 (125.00-799.00)	784.00 (217.00-2377.00)	<0.001
Hs-TropT	< 100 ng/l	14.00 (9.00-28.00)	25.00 (12.00-62.00)	<0.001
C-reactive protein	< 10 mg/L	194.00 (133.00-307.00)	153.00 (100.00-247.00)	0.015
pH	7.35 - 7.45	7.47 (7.44-7.50)	7.37 (7.30-7.44)	<0.001
PaCO2	4.26 – 6.38 kPa	4.90 (4.30-5.30)	5.70 (4.50-6.70)	<0.001
Lactate	0.5 – 2.2 mmol/L	1.40 (1.10-1.90)	1.60 (1.20-2.60)	0.021
Alanine aminotransferase	7 – 40 U/L	31.00 (22.00-47.00)	34.00 (17.00-51.00)	0.91
HCO3std	19 – 24 mmol/L	27.10 (25.00-29.20)	23.55 (20.00-25.90)	<0.001

Neutrophil/Lymphocyte ratio		9.50 (6.21-15.16)	12.53 (7.34-22.56)	0.013
Platelets/Lymphocyte ratio		296.64 (206.58-451.59)	247.31 (70.59-475.51)	0.054

Table 4: Difference in clinical and laboratory characteristics between latent subclasses for COVID-19 ICU discharged patients

Characteristic	**Reference Intervals**	**Latent subclasses**		**p-value**
		Class 1 (n=52)	**Class 2 (n=75)**	
Age(years)		48.72 (39.46-58.43)	52.18 (43.75-60.51)	0.16
Gender				0.06
Female		26 (50%)	25 (33%)	
Male		26 (50%)	50 (67%)	
Diabetes mellitus				0.11
Yes		26 (53%)	29 (39%)	
No		23 (47%)	46 (61%)	
Hypertension				0.32
Yes		25 (51%)	45 (60%)	
No		24 (49%)	30 (40%)	
HIV status				0.55
Positive		5 (13%)	7 (9%)	
Negative		33 (87%)	67 (91%)	
Creatinine	49–90 µmol/L	69.50 (56.50-83.00)	79.00 (65.00-110.00)	0.003
D dimer	0.00-0.25mg/L	0.40 (0.25-0.60)	1.93 (0.55-5.12)	<0.001
Haemoglobin	Male:13.0-17.0g/dL;	13.62 (1.81)	13.49 (2.07)	0.500
	Female:12.0-15.0g/dL	13.08 (0.98)	12.26 (1.78)	0.122
Monocytes	0.30-0.80x 10^9/L	0.48 (0.29-1.94)	0.41 (0.32-0.62)	0.15
NT-proBNP	< 125 pg/mL	63.50 (32.50-193.50)	314.00 (72.00-1346.00)	<0.001
Hs-TropT	< 100 ng/l	6.00 (5.00-10.50)	13.00 (9.00-36.00)	<0.001
C-reactive protein	< 10 mg/L	151.50 (89.00-234.50)	176.00 (93.00-270.00)	0.31
pH	7.35 - 7.45	7.48 (7.46-7.50)	7.47 (7.42-7.51)	0.13
PaCO2	4.26 – 6.38 kPa	4.90 (4.70-5.30)	4.40 (3.90-5.20)	<0.001
Lactate	0.5 – 2.2 mmol/L	1.20 (1.00-1.40)	1.60 (1.10-2.10)	<0.001

Alanine aminotransferase	7 – 40 U/L	32.50 (22.50-47.50)	33.00 (19.00-63.00)	0.46
HCO3std	19 – 24 mmol/L	27.90 (27.10-29.20)	25.80 (23.20-28.00)	<0.001
Neutrophil/Lymphocyte ratio		6.67 (4.51-9.00)	12.39 (6.48-20.29)	<0.001
Platelets/Lymphocyte ratio		223.58 (53.94-368.61)	346.81 (234.65-530.00)	<0.001