CHEST Online Supplement

Identification of Distinct Immunophenotypes in Critically Ill Coronavirus Disease 2019 Patients

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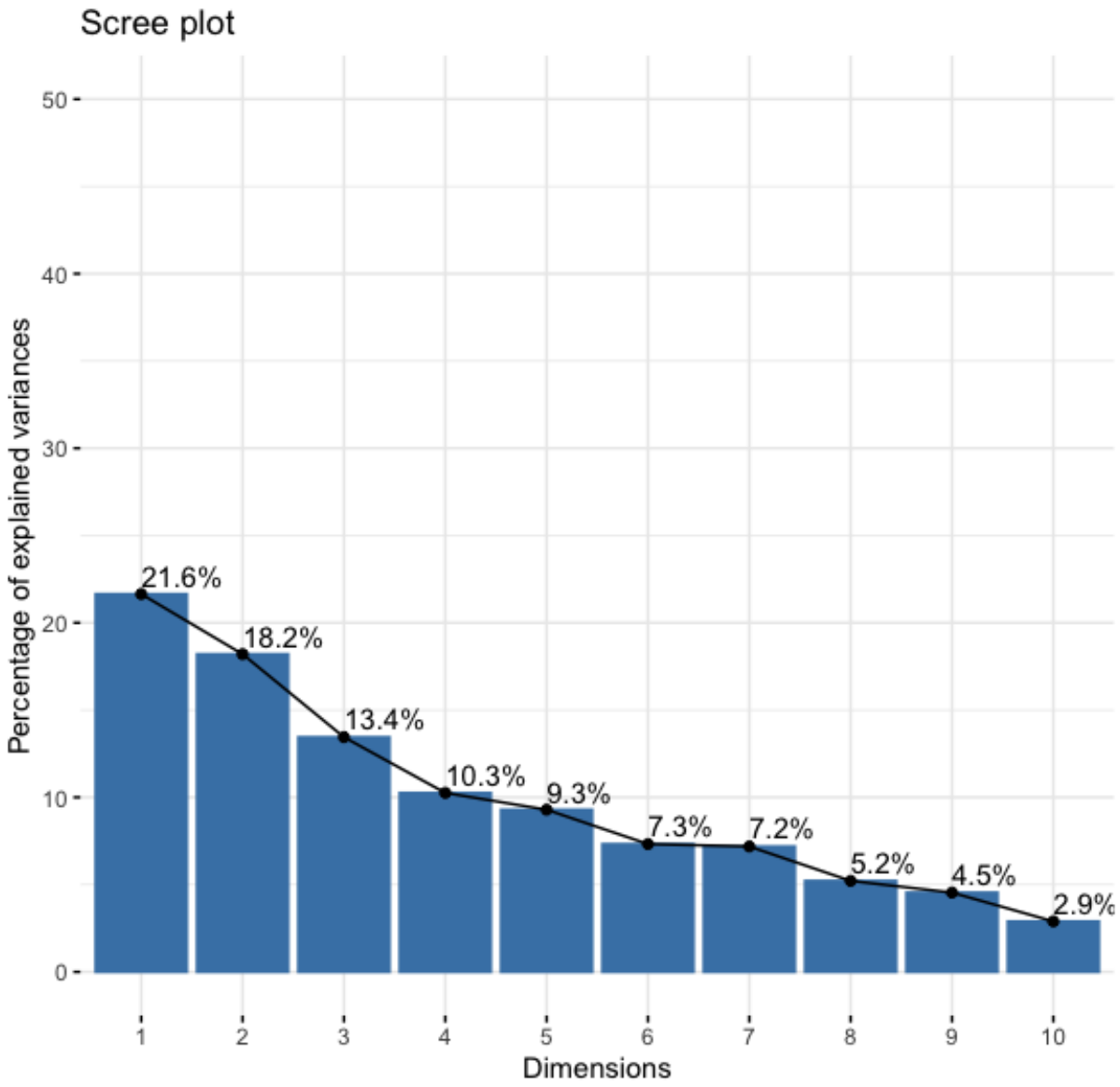
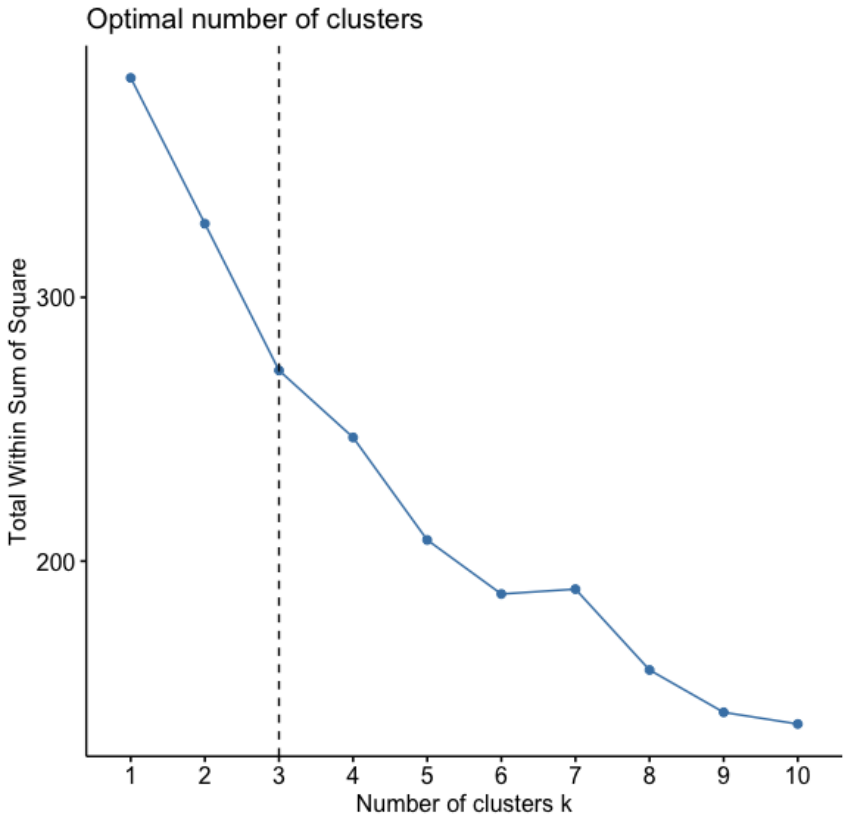
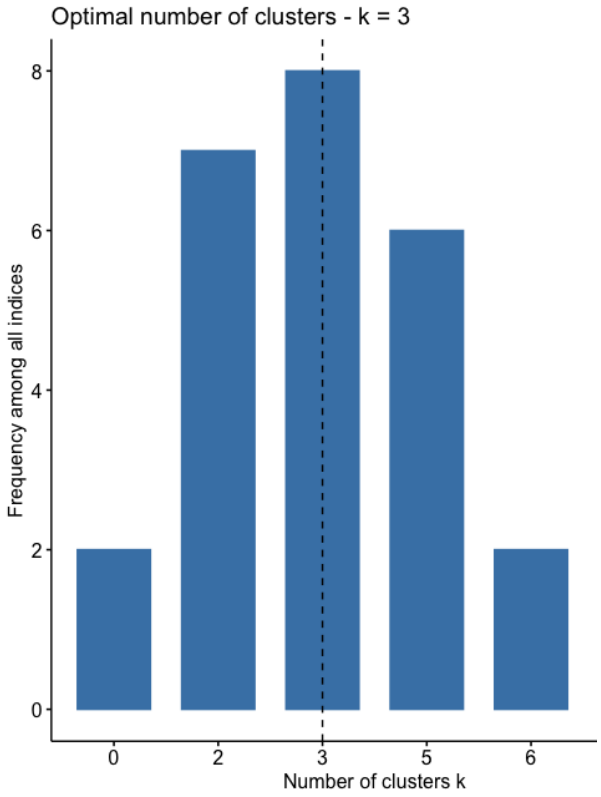


e-Figure 1. Scree plot evaluating the amount of variance explained by the number of PCA dimensions

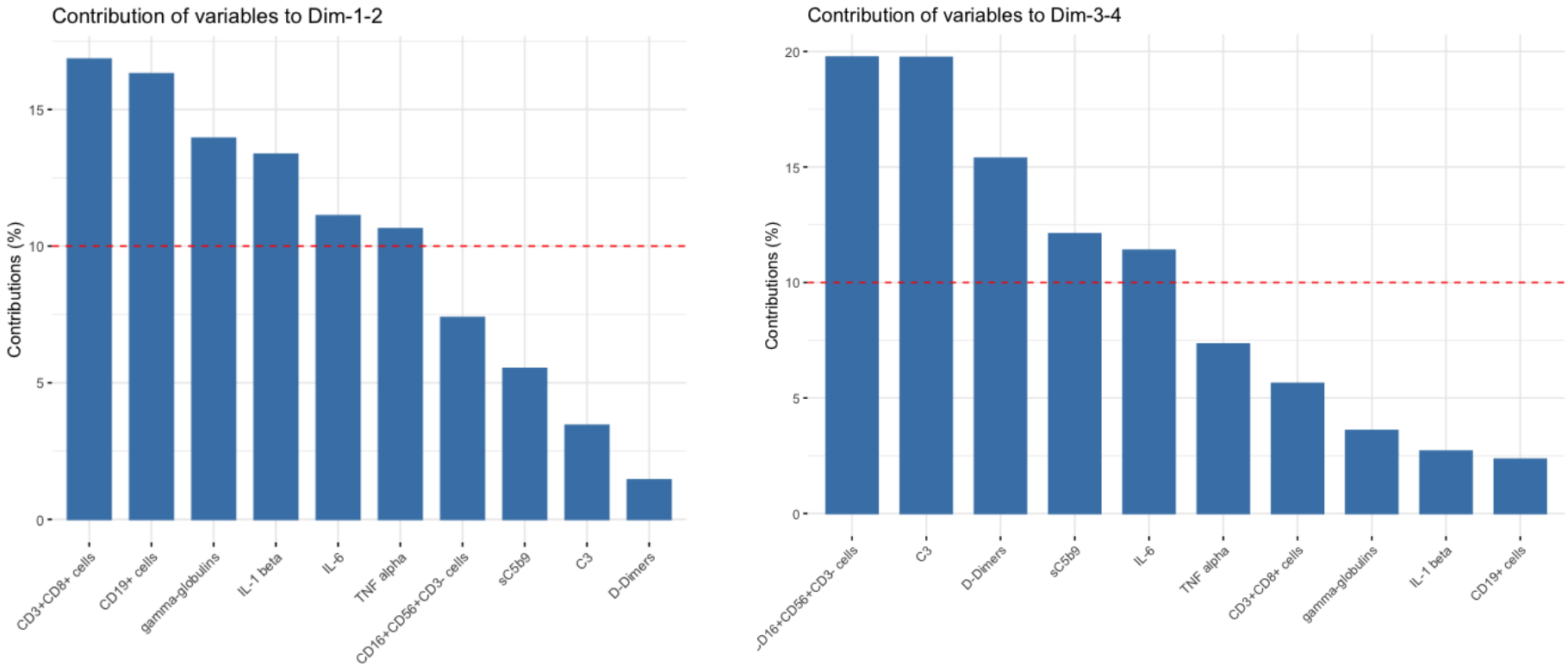
The inertia of the first dimensions shows if there are strong relationships between variables and suggests the number of dimensions that should be studied.
The first two dimensions of the analysis express 39.85% of the total dataset inertia. This value is greater than the reference value that equals 31.19%, the variability explained by this plane is thus significant (the reference value is the 0.95-quantile of the inertia percentages distribution obtained by simulating 740 data tables of equivalent size on the basis of a normal distribution).



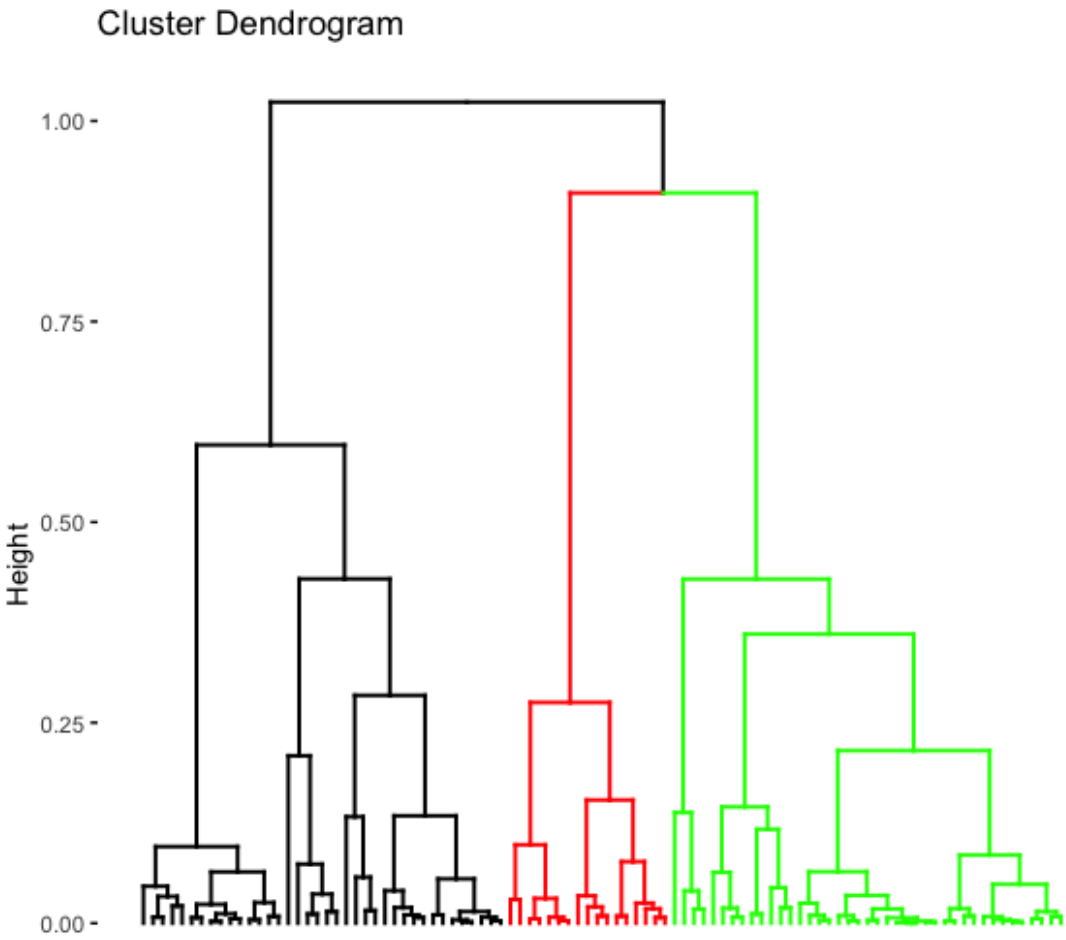


e-Figure 2. Total within Sum of Squares (TWSS) as a function of the number of clusters chosen with a horizontal line at k = 3, the number of clusters chosen for final analysis (panel A).
Frequency of proposed number of cluster according to 30 indices for determining the number of clusters (panel B).

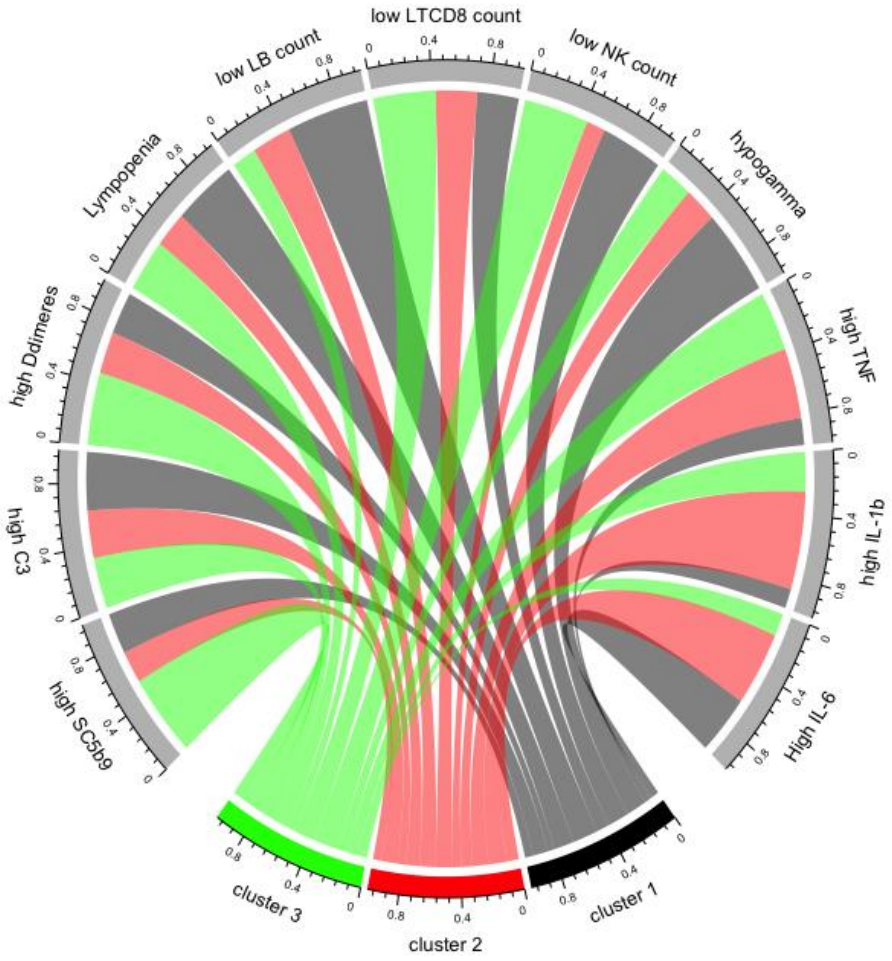
The best clustering scheme from the different results was obtained by varying all combinations of number of clusters, distance measures, and clustering methods.



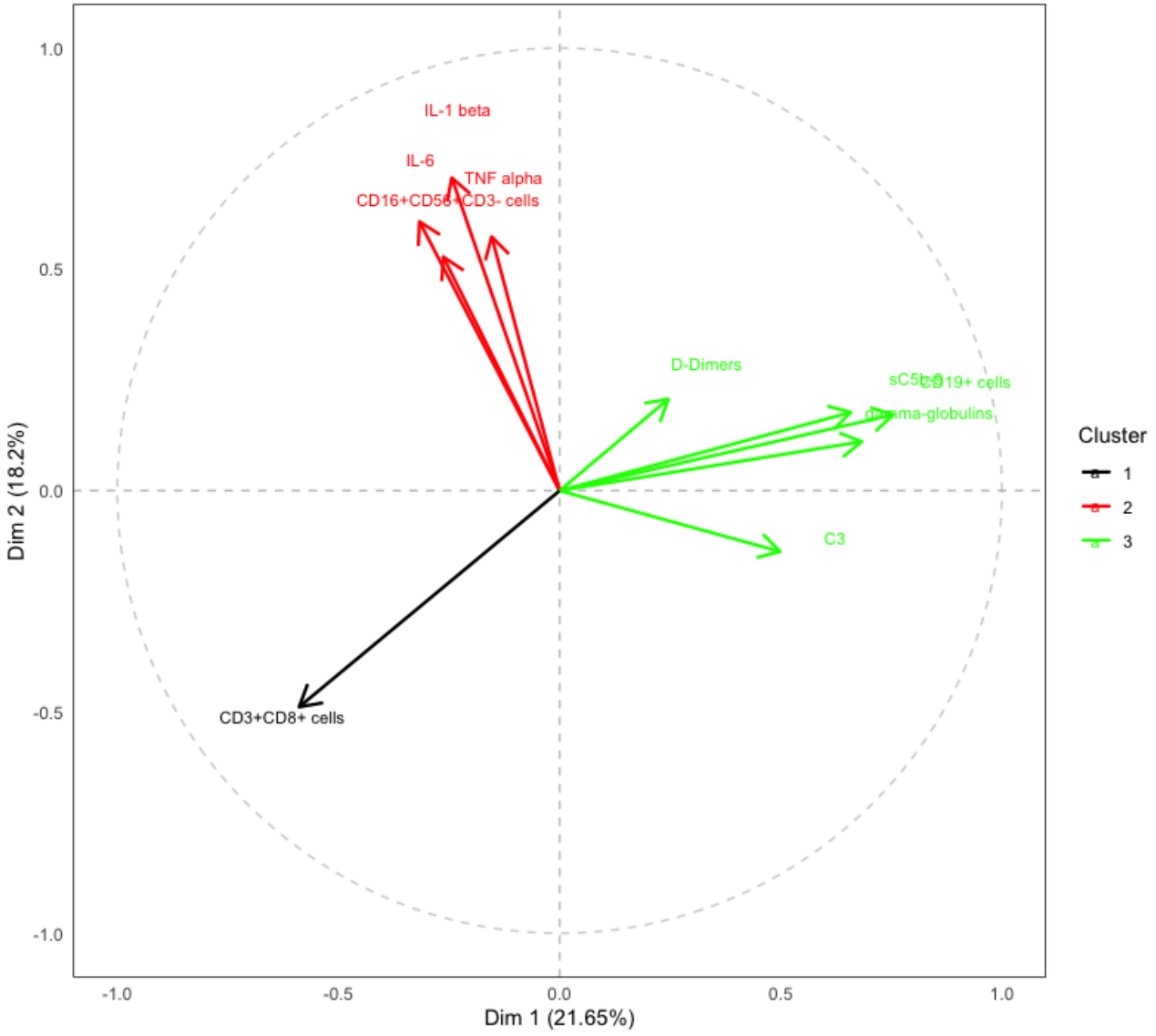
e-Figure 3. Contribution of each variables to the two first dimensions



e-Figure 4: Dendrogram obtained after hierarchical clustering on principal components.



e-Figure 5: Chord map representing the relative abundance of immunologic variables across clusters. High and low values are determined according to conventional laboratory values defining elevated or decreased levels of immunologic variable. High IL-6 > 8.8 pg/mL; High IL-1b > 0 pg/mL; High TNF alpha > 7.8 pg/mL; Hypogammaglobulinemia < 9 g/L; Low NK cells count < 56 cells/ mm^3; Low T CD8 cells count <192 cells/mm^3 ; Low B lymphocyte count < 70 cells/mm^3 ; Low total lymphocyte count < 1400 cells/mm^3; High D-Dimers > 500 ng/mL; High sC5b9: > 380 ng/mL; High C3 > 1250 mg/L;



e-Figure 6: Principal Components Analysis (PCA) on the subset of patients excluding 16 immunocompromised patients (n = 80), showing that cluster affectation seem poorly affected by immunologic status.

Cut-off value for Training dataset /Test dataset	N. patients Training dataset/Test dataset	Model accuracy* on training data set	Model accuracy on test dataset
partition			
80%	79/17	100%	88.24%
70%	69/27	100%	88.89 %
60%	59/37	100%	94.59 %
50%	49/47	100%	91.49 %

***Model accuracy was defined as**: (True Positives + True Negatives)/Total Sample
from the confusion matrix between predicted and observed individual classification in the three clusters.

e-Table 1: Summary of model accuracy statistics using cross-validation with a multinomial model. Obtained after fitting a multinomial model with cluster categories as the response variable and the set of covariates used for cluster analysis as dependent covariates. Predicted values from this model were then computed and applied to our data set, which was previously partitioned in a training and a test datasets. The classification table was then built from predicted and observed values of original cluster to derive the accuracy measurement. Procedure was repeated under various cut-off values between the training and test dataset partition.

	Cluster 1 (n=18)	Cluster 2 (n=19)	Cluster 3 (n=43)	p-value
T lymphocytes (cells/mm^3)	568.5 [355.5;770]	285 [206.5;460.8]	646 [469.5;806.5]	0.004
CD8+ T lymphocytes (cells/mm^3)	225.5 [178.2;327.5]	94 [65;154.2]	184 [141;245.5]	0.003
CD4+ T lymphocytes (cells/mm^3)	349 [190.2;414.2]	182 [150;359.2]	419 [297.5;549]	0.002
NK cells (cells/mm^3)	107.5 [86.5;131.2]	134 [92;256]	105 [73;165.5]	0.16
B lymphocytes (cells/mm^3)	52 [14;70]	100 [65;130]	176 [110.5;276.5]	<0.0001
IL-6 (pg/mL)	62.25 [35.28;90.88]	201 [104;375]	70.4 [33.4;100.8]	<0.0001
D-dimers (µg/L)	855 [582.5;1592]	2135 [1195;3192]	1300 [830;2705]	0.083
C3 (mg/L)	1160 [1110;1290]	1220 [1105;1290]	1480 [1260;1632]	0.0001
sC5b-9 (ng/mL)	238 [185;270]	361 [324.5;396.5]	391 [351;496]	<0.0001
TNFα (pg/mL)	19.45 [17.73;24.25]	29.7 [23.8;37.2]	22 [17.52;25.5]	0.0007
IL-1β (pg/mL)	0.44 [0.3575;0.545]	0.98 [0.71;1.25]	0.365 [0.32;0.5275]	<0.0001
Gamma globulins (g/L)	8.2 [6.8;9.075]	8.75 [7.35;12.25]	10.65 [9.125;11.85]	0.004

e-Table 2: Cluster composition on our subset of patients, excluding immunocompromised patients given as a sensitivity analysis.