CD4+ and CD8+ T-cell count cutoffs as an aid in determining risks of intubation with mechanical ventilation and mortality in hospitalized COVID-19 patients:
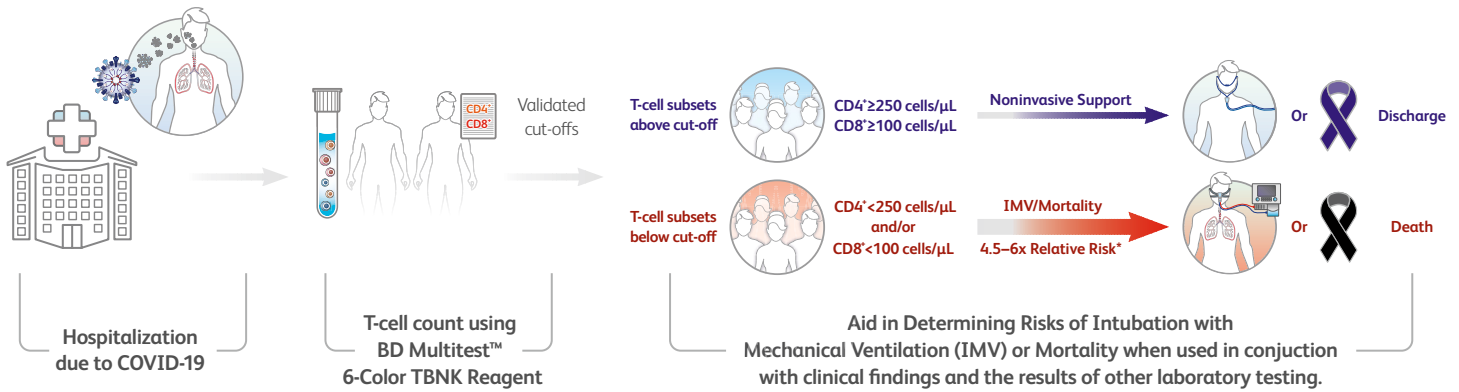
Validation study in an Italian cohort

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Highlights

- Many studies have shown that low CD4+ and CD8+ T-cell counts are associated with disease severity in COVID-19

- Cutoffs for CD4+ (250 cells/μL) and CD8+ (100 cells/μL) T-cell counts were predetermined for the risk of intubation with mechanical ventilation (IMV) and mortality based on analysis of previously published cohorts of COVID-19 patients

- Cutoffs for CD4+ and CD8+ T-cell counts were subsequently validated in a retrospective-prospective study using data from an Italian cohort of 160 COVID-19 patients

- Patients having CD4+ and/or CD8+ T-cell counts below the predetermined cutoffs are at ~5 to 6 times higher relative risk of IMV and at ~4.5 times higher relative risk of mortality

- Classification of patients by the predetermined CD4+ and CD8+ T-cell cutoffs is among the best predictors of IMV and mortality in regression analysis in comparison with routine laboratory tests and clinical parameters; and resulted in higher absolute and relative risk estimates as compared to classification by other published CD4+ T-cell, CD8+ T-cell, or total lymphocyte cutoffs

- Exploratory analyses: 1) CD4+ and CD8+ T-cell cutoffs were explored together with an IL-6 cutoff, demonstrating possibility for additional risk stratification; 2) CD4+ and CD8+ T-cell counts longitudinally assessed during hospitalization tend to decrease in patients that do not survive and increase in patients who are ultimately discharged

- In conclusion, in conjunction with clinical findings and the results of other laboratory testing, validated cutoffs for CD4+ and CD8+ T-cell counts can aid in determining the risks of IMV and mortality for COVID-19 patients

BD

Aid in Determining Risks of Intubation with Mechanical Ventilation (IMV) or Mortality when used in conjuction with clinical findings and the results of other laboratory testing.

Introduction

In winter and spring 2020, results from COVID-19 patient cohorts in China were emerging of low CD4^{+} and CD8^{+} T-cell counts associated with—and being independent predictors of—disease severity and poor outcomes, as summarized in a meta-analysis of 20 studies with over 3,000 patients.[1] We hypothesized that cutoffs for low CD4^{+} and CD8^{+} T-cell counts could be established as an aid in determining the risk of poor outcomes such as Intensive Care Unit (ICU) admission, intubation with mechanical ventilation (IMV), and mortality. Methods and results using published information mainly from Chinese cohorts of COVID-19 patients to develop CD4^{+} and CD8^{+} T-cell cutoffs (250 CD4^{+} and 100 CD8^{+} T-cells/μL), and the validation of these predetermined cutoffs in a retrospective-prospective study in an Italian cohort of 160 COVID-19 patients, are described below.

Determination of cutoffs

To define best estimate CD4^{+} and CD8^{+} T-cell count cutoffs for poor outcomes of COVID-19, we focused on studies from a published meta-analysis[1] that measured CD4^{+} and CD8^{+} T-cell counts by flow cytometry and reported on poor patient outcomes, in particular ICU admission and/or mortality, which are frequently associated with IMV.[2,3,*] We further focused on studies that used BD Biosciences reagents, namely BD Multitest™ 6-Color TBNK Reagent or BD Multitest™ IMK Kit (which use the same antibody clones to identify lymphocyte subsets including CD4^{+} and CD8^{+} T-cells). We also ensured reasonable sample sizes for patients with poor outcomes. This resulted in three analysis approaches to determine best estimate cutoffs:

* In a cohort of 2,215 adults with COVID-19, who were admitted to ICU at 65 sites in the United States, 84% received IMV, including 94% of those who died.[2] In four studies across U.S. and European sites, the percent of patients admitted to ICU, who had IMV was 59.0, 83.3, 88.5, and 89.9%, with high mortality rates; in three additional Chinese sites these were 29.1, 45.5, and 64.0%.[3]

Analysis 1: An analysis of mortality in studies with BD reagents, combining one of the studies from the meta-analysis (Diao et al.,[4] BD Multitest™ IMK with BD LSRFortessa™ Cell Analyzer) with an additional study from an Italian cohort (Lombardi et al.,[5,6] using BD Multitest™ 6-Color TBNK Reagent with BD FACSLyric™ Flow Cytometer) to have reasonable sample size in the mortality group. BD LSRFortessa™ and BD FACSLyric™ Flow Cytometers perform similarly and are not expected to impact the analysis.

Analysis 2: An analysis of all the studies in the meta-analysis that reported mortality (Diao et al., Du et al., Wang et al., and Xu et al.,[4,7–9]), with the caveat that most of these studies have unreported flow cytometry methods and may have other study variables that add noise.

Analysis 3: An analysis of ICU admission, for which the Diao et al. study[4] was the only one that met the criteria.

Using the published information for each of the three analyses, we were able to estimate data distributions as log-normal and to statistically model the underlying data 2,000 times in each analysis. In each modeling run, we found the cutoffs optimizing sensitivity and specificity (Youden's index). The average of all 2,000 runs was considered the best estimate cutoff and is provided along with confidence intervals and other results in Table 1 for both CD4^{+} and CD8^{+} T-cell counts.

The estimated CD4^{+} T-cell cutoffs are similar for mortality (267 cells/μL) or ICU admission (266 cells/μL) in the analyses with BD reagents and the 90% confidence intervals include the estimated CD4^{+} T-cell cutoff derived from all relevant studies from the meta-analysis (analysis 2). Since analysis 2 includes studies with unknown flow cytometry methods, it is given less weight than analyses 1 and 3. Taking all analyses into consideration, a cutoff of 250 CD4^{+} T-cells/μL is determined, which is near the point estimates for analyses 1 and 3 and within the confidence interval of analysis 2. The estimated CD8^{+} T-cell cutoffs are lower than the estimated CD4^{+} T-cell cutoffs, as expected due to generally lower

CD8^{+} T-cell counts, and are very similar for mortality (103 cells/µL) and ICU admission (104 cells/µL) (analyses 2 and 3). Therefore, a cutoff of 100 CD8^{+} T-cells/µL is determined. The results show that a single CD4^{+} and a single CD8^{+} T-cell cutoff can apply to both ICU admission and mortality, consistent with the observation that mortality is a common outcome of ICU admission in COVID-19. IMV is also commonly associated with ICU admission and mortality in COVID-19, as described above, and the same cutoffs will hence be used for IMV.

These determined cutoffs are in agreement with other information not used in analysis 1, 2, or 3, including CD4^{+} and CD8^{+} T-cell measurements of the critical group from a cohort in Shanghai using the BD Multitest™ 6-Color TBNK Reagent with BD Trucount™

Tubes,[10] a CD4^{+} T-cell cutoff of 250 cells/µL established as a guideline in Shanghai,[11] a relationship from a cohort in Shanghai that each 100 cells/µL increase in CD4^{+} T-cell counts halves the odds of ICU admission,[12] and a CD8^{+} T-cell cutoff of 75 cells/µL from a cohort in Wuhan where patients below the cutoff had ~4-fold increased odds of mortality.[7] The determined CD4^{+} T-cell cutoff is also not dissimilar from the CD4^{+} T-cell cutoff of 200 cells/µL used in advanced HIV disease.[13] As the determined cutoffs of 250 CD4^{+} and 100 CD8^{+} T-cells/µL were further corroborated by the additional information as described above, they were subsequently evaluated in a validation cohort of 160 patients from Rome, Italy.

Table 1: Summary of results from CD4^{-} and CD8^{-} T-cell cutoff determination.

Abbreviations used: Positive predictive value (PPV); Negative predictive value (NPV); Confidence interval (CI).

Analysis	Outcome	References (Reagent used)	N	CD4^{+} T-cells					CD8^{+} T-cells				
				Best estimate cutoff cells/µL (90% CI)	Sensitivity (90% CI)	Specificity (90% CI)	PPV (90% CI)	NPV (90% CI)	Best estimate cutoff cells/µL (90% CI)	Sensitivity (90% CI)	Specificity (90% CI)	PPV (90% CI)	NPV (90% CI)
Analysis 1	Mortality	Diao et al. Lombardi et al. (BD Only)	18 died; 257 survived	267 (175, 376)	82% (61%, 100%)	70% (50%, 88%)	17% (11%, 27%)	98% (96%, 99%)	NA for Lombardi et al.				
Analysis 2	Mortality	Diao et al.; Du et al.; Wang et al; Xu et al. (Various)	132 died; 806 survived	195 (141, 256)	69% (57%, 82%)	68% (55%, 80%)	25% (21%, 31%)	93% (92%, 95%)	103 (71, 145)	67% (53%, 80%)	74% (59%, 86%)	29% (23%, 38%)	94% (92%, 95%)
Analysis 3	ICU Admission	Diao et al. (BD only)	20 ICU; 479 non-ICU	266 (143, 433)	72% (45%, 95%)	64% (38%, 87%)	8% (5%, 13%)	98% (97%, 99%)	104 (60, 163)	75% (55%, 95%)	79% (60%, 94%)	15% (8%, 27%)	98% (98%, 99%)

Validation Study Design

The BD Multitest™ 6-Color TBNK Reagent with BD Trucount™ Tubes (TBNK) is a clinical in vitro diagnostic product available for use on the BD FACSCanto™ II and BD FACSLyric™ flow cytometry platforms to determine percentages and absolute counts of mature human lymphocyte subsets (including CD4^{+} and CD8^{+} T-cells, B-cells, and NK cells) in peripheral whole blood. Here, we tested the application of this product with predetermined CD4^{+} and CD8^{+} T-cell cutoffs of 250 and 100 cells/µL, respectively, in predicting the risk of IMV and of mortality in COVID-19 patients. The analysis used data collected under standard care of COVID-19 patients hospitalized in spring 2020 at Policlinico Tor Vergata in Rome, Italy. Data on 160 patients including TBNK measurements at hospital admission and every 1–2 weeks during hospital stay, routine laboratory measurements, demographics, co-morbidities, oxygen supplementation, and outcomes were assembled retrospectively into a database under a protocol sponsored by the site and approved by the local Ethics Committee. The database was anonymized for transfer to BD. BD prospectively defined cutoffs, objectives and endpoints to evaluate prior to receiving the database, hence this may be considered a retrospective-prospective study. The primary objectives tested the predetermined CD4^{+} and CD8^{+} T-cell cutoffs for

determining the risk of IMV; the secondary objective compared these cutoffs with other laboratory measurements for IMV; and additional objectives tested the CD4^{+} and CD8^{+} T-cell cutoffs for the risk of mortality and ICU admission, compared them to other cutoffs, and tested repeat longitudinal CD4^{+} and CD8^{+} T-cell measurements.

Validation Cohort Subject Characteristics

Hospitalized patients at Policlinico Tor Vergata in Rome, Italy during spring 2020 were enrolled in the study if older than 18 years of age, of either sex, with SARS-CoV-2 infection diagnosed by molecular examination of nasopharyngeal swab and/or bronchial lavage and/or broncho-aspirate, with presence of clinical symptoms, and with TBNK and other laboratory measurements at hospital admission. The subject demographics and co-morbidities of all 160 subjects are described for the whole dataset, by CD4^{+} T-cell cutoff (250 cells/µL), CD8^{+} T-cell cutoff (100 cells/µL) and outcomes of IMV and mortality, in Table 2. For analysis of IMV, subjects who died without undergoing IMV, which can be due to limited ventilator supplies and reduced usage in advanced age patients, were considered non-evaluable, resulting in 141 patients that were further analyzed.

Table 2: Subject demographics and co-morbidities.

Subject demographics and co-morbidities by CD4⁺ and CD8⁺ T-cell counts above or below cutoffs, by IMV outcome and by mortality outcome.

	All (n = 160)	CD4⁺ and CD8⁺ T-cell Absolute Count Cutoffs				IMV Outcome			Mortality Outcome	
		CD4≥250 cells/µL (n = 131)	CD4<250 cells/µL (n = 29)	CD8≥100 cells/µL (n = 134)	CD8<100 cells/µL (n = 26)	All (excl death before IMV) (n = 141)	IMV (n = 19)	No IMV (n = 122)	Death (n = 34)	Discharge (n = 126)
Age, gender										
Median age (25–75% Quartiles)	62 (51, 79)	61 (50, 77)	72 (55, 87)	60 (49, 76)	78 (57, 87)	59 (50, 75)	71 (55, 77)	57 (49, 73)	80 (72, 86)	57 (49, 73)
Number of males (%)	98 (61.3%)	76 (58%)	22 (75.9%)	80 (59.7%)	18 (69.2%)	88 (62.4%)	17 (89.5%)	71 (58.2%)	24 (70.6%)	74 (58.7%)
Co-morbidities number of patients, (%)										
Obesity	25 (15.6%)	22 (16.8%)	3 (10.3%)	21 (15.7%)	4 (15.4%)	24 (17.0%)	4 (21.1%)	20 (16.4%)	4 (11.8%)	21 (16.7%)
Cardiovascular diseases	81 (50.6%)	60 (45.8%)	21 (72.4%)	64 (47.8%)	17 (65.4%)	67 (47.5%)	14 (73.7%)	53 (43.4%)	27 (79.4%)	54 (42.9%)
Diabetes	27 (16.9%)	23 (17.6%)	4 (13.8%)	19 (14.2%)	8 (30.8%)	21 (14.9%)	4 (21.1%)	17 (13.9%)	10 (29.4%)	17 (13.5%)
Endocrine diseases	16 (10.0%)	16 (12.2%)	0 (0.0%)	14 (10.4%)	2 (7.7%)	14 (9.9%)	0 (0.0%)	14 (11.5%)	2 (5.9%)	14 (11.1%)
Cerebrovascular diseases	10 (6.2%)	8 (6.1%)	2 (6.9%)	8 (6.0%)	2 (7.7%)	8 (5.7%)	1 (5.3%)	7 (5.7%)	3 (8.8%)	7 (5.6%)
Hepatitis	3 (1.9%)	2 (1.5%)	1 (3.4%)	2 (1.5%)	1 (3.8%)	3 (2.1%)	1 (5.3%)	2 (1.6%)	1 (2.9%)	2 (1.6%)
Pulmonary diseases	17 (10.6%)	14 (10.7%)	3 (10.3%)	12 (9.0%)	5 (19.2%)	12 (8.5%)	4 (21.1%)	8 (6.6%)	8 (23.5%)	9 (7.1%)
Renal diseases	18 (11.2%)	11 (8.4%)	7 (24.1%)	9 (6.7%)	9 (34.6%)	11 (7.8%)	4 (21.1%)	7 (5.7%)	11 (32.4%)	7 (5.6%)
Solid tumor	18 (11.2%)	13 (9.9%)	5 (17.2%)	13 (9.7%)	5 (19.2%)	14 (9.9%)	2 (10.5%)	12 (9.8%)	6 (17.6%)	12 (9.5%)
Hematological malignancy	13 (8.1%)	7 (5.3%)	6 (20.7%)	10 (7.5%)	3 (11.5%)	12 (8.5%)	4 (21.1%)	8 (6.6%)	5 (14.7%)	8 (6.3%)
Psychiatric and/or neurological diseases	34 (21.2%)	27 (20.6%)	7 (24.1%)	28 (20.9%)	6 (23.1%)	24 (17.0%)	3 (15.8%)	21 (17.2%)	12 (35.3%)	22 (17.5%)
Immunologic or rheumatic diseases	7 (4.4%)	7 (5.3%)	0 (0.0%)	6 (4.5%)	1 (3.8%)	7 (5.0%)	0 (0.0%)	7 (5.7%)	0 (0.0%)	7 (5.6%)
Other	53 (33.1%)	40 (30.5%)	13 (44.8%)	43 (32.1%)	10 (38.5%)	44 (31.2%)	5 (26.3%)	39 (32.0%)	13 (38.2%)	40 (31.7%)

Results

Absolute risk, relative risk and time-to-event/survival analysis (Kaplan-Meier)

The CD4⁺ and CD8⁺ T-cell cutoffs were first examined to determine whether they could classify patients at increased risk of IMV or mortality and with faster time-to-event. For subjects below the CD4⁺ and/or CD8⁺ T-cell cutoffs at hospital admission, the study found significant and substantially increased absolute and relative risk of IMV and mortality and decrease in time-to-IMV and in survival in Kaplan-Meier analysis, with *P* values below 0.001 in all cases (Figures 1 and 2). In particular, the relative risk of IMV was more than 5 times higher for patients with CD4⁺ T-cell counts below the cutoff and more than 6 times higher for patients with

CD8⁺ T-cell counts below the cutoff (Figure 1A). Similarly, the relative risk of mortality was ~4.5 times higher for patients with either CD4⁺ or CD8⁺ T-cell counts below the cutoffs (Figure 2A). Subjects with both CD4⁺ and CD8⁺ T-cell counts below the cutoffs had the highest absolute risk of IMV and mortality (Figures 1A and 2A) and fastest time-to-event in Kaplan-Meier analysis (Figures 1B and 2B). Subjects below the CD4⁺ and CD8⁺ T-cell cutoffs were also at increased risk for ICU admission, with *P* values below or equal to 0.001 (data not shown).

Figure 1: Analysis of IMV outcome based on CD4$^+$ and CD8$^+$ T-cell count cutoffs.

Contingency table with absolute and relative risk of IMV (**A**), time-to-IMV analysis (Kaplan-Meier) (**B**), and additional clinical evaluation statistics (**C**) for patients above and below the CD4$^+$ and CD8$^+$ T-cell cutoffs at hospital admission.

Abbreviations used: Absolute risk (Abs Risk); Confidence interval (CI); Relative risk (RR); Positive predictive value (PPV); Negative predictive value (NPV). For Kaplan-Meier analysis, discharged patients are censored.

A

Cutoff (cells/µL)	No IMV	IMV	Total	Abs Risk	Abs Risk CI	P value	RR	RR CI
CD4$^+$ T-cells								
CD4≥250	110	10	120	8.3%	4.1%, 14.8%	<0.001	5.14	2.38, 11.13
CD4<250	12	9	21	42.9%	21.8%, 66.0%			
CD8$^+$ T-cells								
CD8≥100	113	10	123	8.1%	4.0%, 14.4%	<0.001	6.15	2.9, 13.05
CD8<100	9	9	18	50.0%	26.0%, 74.0%			
CD4$^+$ & CD8$^+$ T-cells combined								
CD4≥250 or CD8≥100	118	13	131	9.9%	5.4%, 16.4%	<0.001	6.05	2.94, 12.45
CD4<250 and CD8<100	4	6	10	60.0%	26.2%, 87.8%			
Total	**122**	**19**	**141**	**13.5%**				

Cutoff (cells/µL)	No IMV	IMV	Total
CD4$^+$ & CD8$^+$ T-cells combined			
CD4≥250 and CD8≥100	105 (93.8%)	7 (6.2%)	**112**
CD4<250 and CD8≥100	8 (72.7%)	3 (27.3%)	**11**
CD4≥250 and CD8<100	5 (62.5%)	3 (37.5%)	**8**
CD4<250 and CD8<100	4 (40.0%)	6 (60.0%)	**10**

B
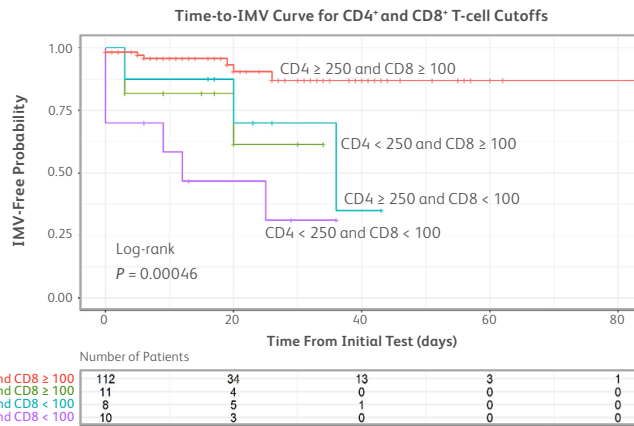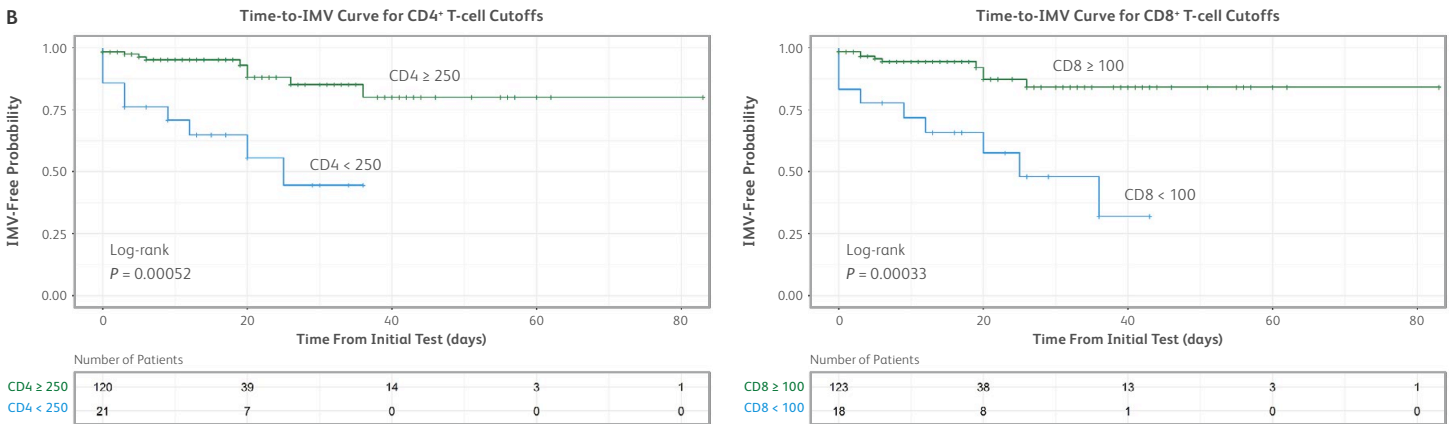
C

	CD4$^+$ T-cell cutoff		CD8$^+$ T-cell cutoff		CD4$^+$ CD8$^+$ T-cell cutoffs (below both cutoffs)	
	Estimate	CI	Estimate	CI	Estimate	CI
Sensitivity	47.4%	24.4%, 71.1%	47.4%	24.4%, 71.1%	31.6%	12.6%, 56.6%
Specificity	90.2%	83.4%, 94.8%	92.6%	86.5%, 96.6%	96.7%	91.8%, 99.1%
PPV	42.9%	21.8%, 66.0%	50.0%	26.0%, 74.0%	60.0%	26.2%, 87.8%
NPV	91.7%	85.2%, 95.9%	91.9%	85.6%, 96.0%	90.1%	83.6%, 94.6%

Figure 2: Analysis of mortality outcome based on CD4+ and CD8+ T-cell count cutoffs.

Contingency tables with absolute and relative risk of mortality (**A**), survival analysis (Kaplan-Meier) (**B**), and additional clinical evaluation statistics (**C**) for patients above and below the CD4+ and CD8+ T-cell cutoffs at hospital admission.

Abbreviations used: Absolute risk (Abs Risk); Confidence interval (CI); Relative risk (RR); Positive predictive value (PPV); Negative predictive value (NPV). For Kaplan-Meier analysis, discharged patients are censored.
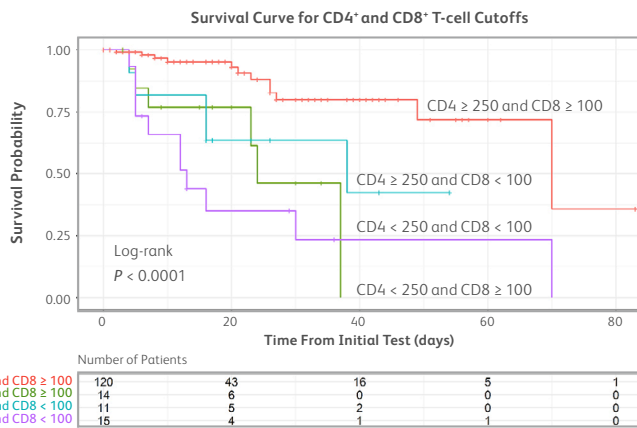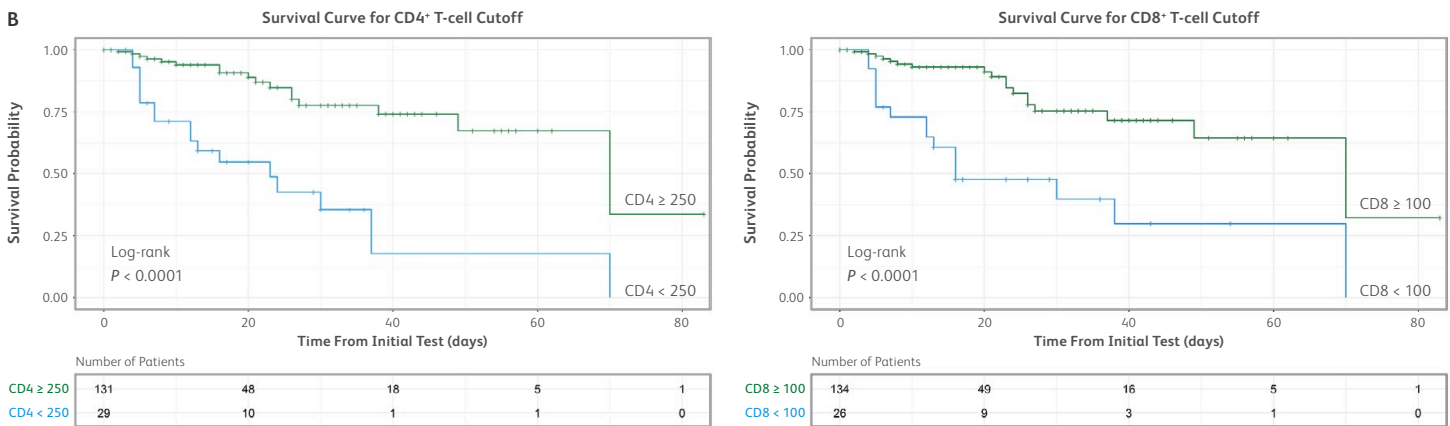
A

Cutoff (cells/µL)	Discharge	Death	Total	Abs Risk	Abs Risk CI	P value	RR	RR CI
CD4+ T-cells								
CD4≥250	114	17	131	13.0%	7.7%, 20.0%	<0.001	4.52	2.64, 7.74
CD4<250	12	17	29	58.6%	38.9%, 76.5%			
CD8+ T-cells								
CD8≥100	116	18	134	13.4%	8.2%, 20.4%	<0.001	4.58	2.71, 7.76
CD8<100	10	16	26	61.5%	40.6%, 79.8%			
CD4+ & CD8+ T-cells combined								
CD4≥250 or CD8≥100	122	23	145	15.9%	10.3%, 22.8%	<0.001	4.62	2.85, 7.5
CD4<250 and CD8<100	4	11	15	73.3%	44.9%, 92.2%			
Total	**126**	**34**	**160**	**21.2%**				

Cutoff (cells/µL)	Discharge	Death	Total
CD4+ & CD8+ T-cells combined			
CD4≥250 and CD8≥100	108 (90.0%)	12 (10.0%)	**120**
CD4<250 and CD8≥100	8 (57.1%)	6 (42.9%)	**14**
CD4≥250 and CD8<100	6 (54.5%)	5 (45.5%)	**11**
CD4<250 and CD8<100	4 (26.7%)	11 (73.3%)	**15**

B

Survival Curve for CD4+ T-cell Cutoff

Number of Patients

CD4 ≥ 250	131	48	18	5	1
CD4 < 250	29	10	1	1	0

Survival Curve for CD8+ T-cell Cutoff

Number of Patients

CD8 ≥ 100	134	49	16	5	1
CD8 < 100	26	9	3	1	0

Survival Curve for CD4+ and CD8+ T-cell Cutoffs

Number of Patients

CD4 ≥ 250 and CD8 ≥ 100	120	43	16	5	1
CD4 < 250 and CD8 ≥ 100	14	6	0	0	0
CD4 ≥ 250 and CD8 < 100	11	5	2	0	0
CD4 < 250 and CD8 < 100	15	4	1	1	0

C

	CD4+ T-cell cutoff		CD8+ T-cell cutoff		CD4+ CD8+ T-cell cutoffs (below both cutoffs)	
	Estimate	CI	Estimate	CI	Estimate	CI
Sensitivity	50.0%	32.4%, 67.6%	47.1%	29.8%, 64.9%	32.4%	17.4%, 50.5%
Specificity	90.5%	84.0%, 95.0%	92.1%	85.9%, 96.1%	96.8%	92.1%, 99.1%
PPV	58.6%	38.9%, 76.5%	61.5%	40.6%, 79.8%	73.3%	44.9%, 92.2%
NPV	87.0%	80.0%, 92.3%	86.6%	79.6%, 91.8%	84.1%	77.2%, 89.7%

Statistical regression analyses, Akaike's Information Criterion model selection and Cox proportional hazard analysis

The CD4^{+} and CD8^{+} T-cell cutoffs were next compared along with many other laboratory measurements (all at hospital admission), co-morbidities, and demographic parameters as predictors of IMV and mortality. Univariate logistic regression analysis showed that classification of patients by the CD4^{+} or CD8^{+} T-cell cutoffs were among the best predictors, as assessed by P value, of IMV (Table 3) and mortality (Table 4). Figure 3 shows univariate logistic regression modelling of all subject CD4^{+} and CD8^{+} T-cell counts against probability of IMV (A) or mortality (B). Derived odds ratios indicate that the odds of IMV and mortality increase by estimated 1.9-fold and 2.8-fold, respectively, for every 250 cells/µL decrease in CD4^{+} T-cell count; and by estimated 2.0-fold and 1.9-fold, respectively, for every 100 cells/µL decrease in CD8^{+} T-cell count. Additional flow cytometric measurements related to CD4^{+} and CD8^{+} T-cells from the TBNK assay were also significant in the univariate logistic regression analysis.

Bivariate logistic regression analysis was next used to assess the predictive value of CD4^{+} or CD8^{+} T-cell cutoffs while controlling for the effect of each of the other laboratory, co-morbidity, or demographic parameters (Tables 3 and 4). The statistical significance (P < 0.05) of CD4^{+} and CD8^{+} T-cell cutoffs in this analysis held against each other and against all other parameters. In contrast, some well-established parameters lost statistical significance while controlling for the effect of CD4^{+} or CD8^{+} T-cell cutoffs, including total lymphocyte count and neutrophil-to-lymphocyte ratio (NLR) for IMV, and total lymphocyte count and C-reactive protein (CRP) for mortality.

Regarding flow cytometry data, the CD4^{+} and CD8^{+} T-cell cutoffs both retained statistical significance when controlled for each other or any other flow cytometry data; conversely, whereas most other flow cytometry data retained statistical significance when controlled for the CD4^{+} or CD8^{+} T-cell cutoffs in the case of mortality, they did not in the case of IMV.

CD4^{+} and CD8^{+} T-cell cutoffs were also tested together with a third parameter in trivariate logistic regression analysis and the CD4^{+} and/or CD8^{+} T-cell cutoffs retained significance (P < 0.05) in all cases. Some well-established parameters lost significance, including NLR, total lymphocyte count, CRP, ferritin, and IL-6 for both IMV and mortality, plus d-dimer and sex for mortality, and number of co-morbidities, age, and neutrophil count for IMV. Akaike's Information Criterion (AIC) was then used to identify the best fit model with lowest number of parameters for predicting IMV and mortality. The CD8^{+} T-cell cutoff was selected in the best model for IMV, and the CD4^{+} T-cell cutoff was selected in the best model for mortality. Finally, Cox proportional hazards analysis was used to determine the relationship of all laboratory, co-morbidity, and demographic parameters for time-to-event for IMV and mortality. Again, CD4^{+} and CD8^{+} T-cell cutoffs were among the best predictors with P values below or equal to 0.0003 in all cases.

Altogether, these results indicate that patient classification by the predetermined CD4^{+} and CD8^{+} T-cell cutoffs are among the best predictors of IMV and mortality within the validation cohort.

Figure 3: Relationship between CD4^{+} and CD8^{+} T-cell counts and probability of IMV and mortality.

Fitted Probability of IMV (**A**) and mortality (**B**) against CD4^{+} or CD8^{+} T-cell count across all patient data (univariate logistic regression model).
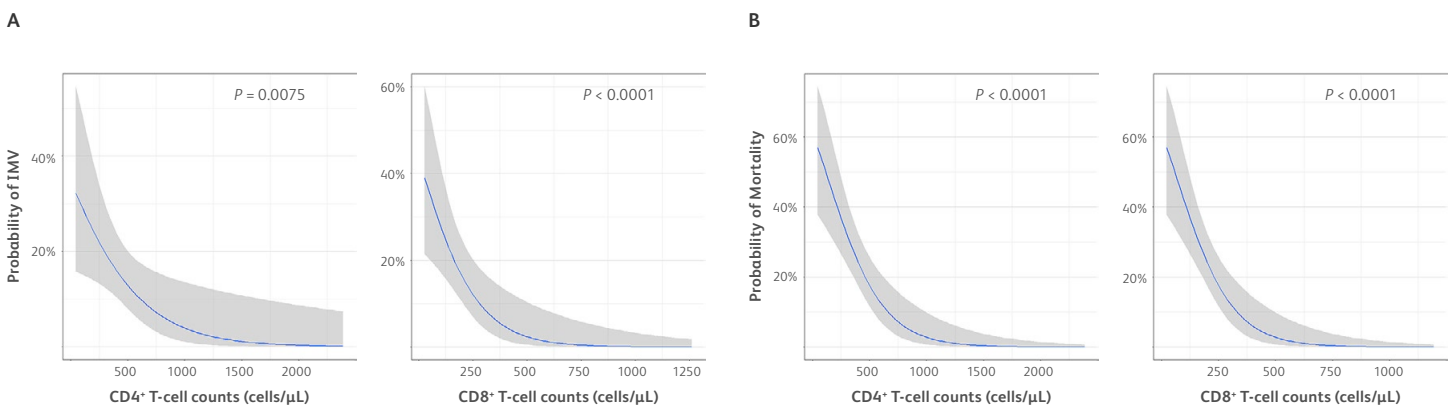
Table 3: IMV outcome: univariate and bivariate logistic regression analysis of laboratory, co-morbidity, and demographic parameters.

Bivariate analysis is performed with each parameter in comparison to the CD4⁺ or CD8⁺ T-cell cutoff. *P* values below 0.05 are indicated in red.

	P value Obtained in a Logistic Regression Analysis for IMV Outcome				
		Bivariate			
	Univariate	Parameter	CD4⁺ T-cell cutoff (above/below)	Parameter	CD8⁺ T-cell cutoff (above/below)
Parameter					
CD8⁺ T-cell cutoff (above/below)	<0.0001	0.0041	0.0261	NA	NA
Lactate dehydrogenase (LDH or LD)	0.0001	0.0152	0.012	0.0062	0.0007
D-dimer	0.0002	0.0134	0.0007	0.0251	0.0012
Complete Blood Count: Total lymphocyte	0.0002	0.1105	0.0437	0.1423	0.0167
CD4⁺ T-cell cutoff (above/below)	0.0002	NA	NA	0.0261	0.0041
Neutrophil to CD8⁺ T-cell ratio (N8R)	0.0005	0.0621	0.0127	0.3437	0.0117
Neutrophil to lymphocyte ratio (NLR)	0.0008	0.1238	0.0157	0.4134	0.0073
Creatinine	0.0012	0.1136	0.0048	0.0191	0.0002
Sex	0.0045	0.0323	0.0004	0.0226	<0.0001
Complete Blood Count: Neutrophils	0.0053	0.0389	0.001	0.1869	0.0006
Cardiovascular diseases	0.0128	0.1131	0.001	0.0381	<0.0001
Aspartate transaminase (AST)	0.0209	0.024	0.0002	0.0122	<0.0001
Complete Blood Count: Hemoglobin (Hb or Hgb)	0.0301	0.0358	0.0002	0.1201	<0.0001
Ferritin	0.0327	0.5264	0.0066	0.034	0.004
Endocrine disease	0.0384	0.9925	0.0003	0.9926	<0.0001
Complete Blood Count: Total lymphocyte cutoff (above/below)	0.0411	0.7014	0.0021	0.9474	0.0007
Complete Blood Count: White blood cells	0.0426	0.1139	0.0003	0.335	0.0001
Renal diseases	0.0435	0.071	0.0002	0.382	0.0001
Number of co-morbidities	0.0465	0.1358	0.0003	0.174	<0.0001
Interleukin 6 (IL-6) cutoff (above/below)	0.0591	0.233	0.0004	0.098	<0.0001
Pulmonary diseases	0.0626	0.0904	0.0002	0.1214	<0.0001
Hematological malignancy	0.0626	0.4477	0.0006	0.0486	<0.0001
Age	0.0805	0.1391	0.0002	0.3523	<0.0001
Conjugated/direct bilirubin	0.1024	0.1331	0.0002	0.2453	<0.0001
C-Reactive Protein (CRP)	0.1172	0.6041	0.0007	0.9391	0.0002
Immunologic or rheumatic diseases	0.149	0.9919	0.0002	0.9907	<0.0001
Alanine transaminase (ALT)/alanine aminotransferase (ALAT)	0.1641	0.092	0.0001	0.115	<0.0001
Creatine kinase (CK)	0.179	0.4109	0.0008	0.0841	0.0001
Prothrombin time (in percent)	0.2019	0.8136	0.0003	0.2886	<0.0001
Fibrinogen	0.2129	0.3869	0.0002	0.3162	<0.0001
Interleukin 6 (IL-6)	0.3118	0.7449	0.0002	0.3045	<0.0001
Procalcitonin (PCT)	0.3459	0.5381	0.0002	0.5545	0.0003
Hepatitis	0.3739	0.5199	0.0002	0.5887	<0.0001
Diabetes	0.4366	0.1327	<0.0001	0.6522	<0.0001
Tumor necrosis factor alpha (TNF-α)	0.5317	0.6631	<0.0001	0.8192	<0.0001
Other co-morbidities	0.6162	0.5395	0.0001	0.6937	<0.0001
Complete Blood Count: Platelets	0.6211	0.4869	0.0001	0.7185	<0.0001
Obesity	0.6236	0.4926	0.0001	0.793	<0.0001
Prothrombin time (in seconds)	0.6984	0.7622	0.0001	0.5965	<0.0001
Psychiatric and/or neurological diseases	0.8769	0.6218	0.0001	0.661	<0.0001
Solid tumor	0.926	0.8639	0.0001	0.9826	<0.0001
Cerebrovascular diseases	0.9331	0.9889	0.0001	0.9355	<0.0001
Total bilirubin	0.9544	0.7729	0.0001	0.8771	<0.0001
International Normalized Ratio (INR)	0.999	0.5413	<0.0001	0.9944	<0.0001
Parameter - flow cytometry (TBNK)					
CD8⁺ T-cell cutoff (above/below)	<0.0001	0.0041	0.0261	NA	NA
CD8⁺ T-cell absolute count	<0.0001	0.039	0.0257	0.1354	0.025
CD4⁺ T-cell cutoff (above/below)	0.0002	NA	NA	0.0261	0.0041
T-cell absolute count	0.0005	0.1746	0.0444	0.236	0.0105
CD4⁻ CD8⁻ double negative T-cell absolute count	0.0009	0.0612	0.0022	0.0862	0.0008
Total lymphocyte absolute count	0.0012	0.2303	0.0149	0.3327	0.006
CD4⁺ CD8⁺ double positive T-cell absolute count	0.0027	0.148	0.0044	0.31	0.0021
CD4⁺ T-cell absolute count	0.0075	0.7206	0.0089	0.5662	0.0015
Ratio of CD4⁺ to CD8⁺ T-cell absolute count	0.0091	0.001	<0.0001	0.1739	0.0004
B-cell absolute count	0.0653	0.6233	0.0005	0.6954	0.0002
NK cell absolute count	0.6359	0.6834	0.0002	0.525	<0.0001

Table 4: Mortality outcome: univariate and bivariate logistic regression analysis of laboratory, co-morbidity, and demographic parameters.

Bivariate analysis is performed with each parameter in comparison to the CD4$^+$ or CD8$^+$ T-cell cutoff. *P* values below 0.05 are indicated in red.

| | *P* value Obtained in a Logistic Regression Analysis for Mortality Outcome | | | | |
| | | Bivariate | | | |
	Univariate	Parameter	CD4$^+$ T-cell cutoff (above/below)	Parameter	CD8$^+$ T-cell cutoff (above/below)
Parameter					
Age	<0.0001	<0.0001	<0.0001	<0.0001	0.0001
Neutrophil to lymphocyte ratio (NLR)	<0.0001	0.0025	0.0036	0.0075	0.0141
CD8$^+$ T-cell cutoff (above/below)	<0.0001	0.0011	0.0011	NA	NA
CD4$^+$ T-cell cutoff (above/below)	<0.0001	NA	NA	0.0011	0.0011
Neutrophil to CD8$^+$ T-cell ratio (N8R)	<0.0001	0.0059	0.0007	0.0546	0.0139
Lactate dehydrogenase (LDH or LD)	<0.0001	0.0014	0.0002	0.0011	0.0001
Creatinine	<0.0001	0.0039	0.0002	0.0052	<0.0001
Number of co-morbidities	<0.0001	<0.0001	<0.0001	0.0004	<0.0001
Complete Blood Count: Neutrophils	<0.0001	0.0001	<0.0001	0.0004	<0.0001
Complete Blood Count: Total lymphocyte	<0.0001	0.0624	0.0022	0.0603	0.0041
Renal diseases	<0.0001	0.0009	<0.0001	0.0077	<0.0001
Cardiovascular diseases	0.0001	0.0036	<0.0001	0.0012	<0.0001
Interleukin 6 (IL-6) cutoff (above/below)	0.0002	0.0087	<0.0001	0.0022	<0.0001
Complete Blood Count: White blood cells	0.0003	0.0008	<0.0001	0.002	<0.0001
D-dimer	0.0005	0.025	<0.0001	0.0514	<0.0001
Complete Blood Count: Total lymphocyte cutoff (above/below)	0.0005	0.1703	0.0002	0.184	0.0002
C-Reactive Protein (CRP)	0.0043	0.0757	<0.0001	0.2419	0.0001
Complete Blood Count: Hemoglobin (Hb or Hgb)	0.005	0.0203	<0.0001	0.0213	<0.0001
Aspartate transaminase (AST)	0.0097	0.0134	<0.0001	0.0065	<0.0001
Pulmonary diseases	0.0114	0.0036	<0.0001	0.0307	<0.0001
Conjugated/direct bilirubin	0.0218	0.016	<0.0001	0.0265	<0.0001
Psychiatric and/or neurological diseases	0.0307	0.0258	<0.0001	0.0206	<0.0001
Diabetes	0.0369	0.0091	<0.0001	0.1623	<0.0001
Prothrombin time (in percent)	0.0374	0.4176	<0.0001	0.0675	<0.0001
Ferritin	0.047	0.5964	0.0047	0.054	0.0097
Immunologic or rheumatic diseases	0.064	0.9916	<0.0001	0.9904	<0.0001
Interleukin 6 (IL-6)	0.0698	0.4428	<0.0001	0.1597	<0.0001
Creatine kinase (CK)	0.0872	0.2303	<0.0001	0.0525	<0.0001
Procalcitonin (PCT)	0.108	0.2981	<0.0001	0.2392	<0.0001
Hematological malignancy	0.1385	0.6774	<0.0001	0.1716	<0.0001
Total bilirubin	0.1892	0.5245	<0.0001	0.0963	<0.0001
Sex	0.2016	0.5754	<0.0001	0.3354	<0.0001
Solid tumor	0.2049	0.3673	<0.0001	0.4415	<0.0001
Prothrombin time (in seconds)	0.2105	0.6589	<0.0001	0.1877	<0.0001
International Normalized Ratio (INR)	0.2998	0.66	<0.0001	0.2899	<0.0001
Endocrine disease	0.3406	0.9517	<0.0001	0.4243	<0.0001
Fibrinogen	0.4444	0.6926	<0.0001	0.8654	<0.0001
Complete Blood Count: Platelets	0.4475	0.7938	<0.0001	0.3815	<0.0001
Obesity	0.4726	0.7102	<0.0001	0.4505	<0.0001
Other comorbidities	0.4793	0.932	<0.0001	0.6275	<0.0001
Tumor necrosis factor alpha (TNF-α)	0.487	0.6358	<0.0001	0.8261	<0.0001
Cerebrovascular diseases	0.502	0.4911	<0.0001	0.5458	<0.0001
Alanine transaminase (ALT)/alanine aminotransferase (ALAT)	0.6035	0.3529	<0.0001	0.4415	<0.0001
Hepatitis	0.6248	0.8197	<0.0001	0.8639	<0.0001
Parameter - flow cytometry (TBNK)					
T-cell absolute count	<0.0001	0.0196	0.0282	0.0151	0.0189
CD4$^+$ CD8$^+$ double positive T-cell absolute count	<0.0001	0.0078	0.001	0.0231	0.005
CD8$^+$ T-cell cutoff (above/below)	<0.0001	0.0011	0.0011	NA	NA
CD4$^+$ T-cell cutoff (above/below)	<0.0001	NA	NA	0.0011	0.0011
CD4$^-$ CD8$^-$ double negative T-cell absolute count	<0.0001	0.0074	0.0002	0.009	0.0003
Total lymphocyte absolute count	<0.0001	0.0572	0.004	0.0467	0.0061
CD4$^+$ T-cell absolute count	<0.0001	0.0893	0.0128	0.0241	0.0028
CD8$^+$ T-cell absolute count	<0.0001	0.0176	0.0013	0.062	0.0064
Ratio of CD4$^+$ to CD8$^+$ T-cell absolute count	0.0225	0.0014	<0.0001	0.6614	<0.0001
B-cell absolute count	0.0663	0.4873	<0.0001	0.7138	<0.0001
NK cell absolute count	0.347	0.801	<0.0001	0.7007	<0.0001

Alternative CD4^{+} and CD8^{+} T-cell cutoffs and total lymphocyte count

Approximately 10 studies with over 3,000 patients, primarily in Chinese and European cohorts, including our own cutoff determination study described above, have determined cutoffs for low CD4^{+} and/or CD8^{+} T-cell counts as indicative of risk of COVID-19 disease severity or mortality.[9,14–22] Cutoffs for critical outcomes (mortality, IMV, ICU admission) tend to be lower than for disease severity (including non-invasive oxygen supplementation), consistent with the widespread observation that lower CD4^{+} and CD8^{+} T-cell counts portend worse outcomes.[1] The cutoffs of 250 CD4^{+} and 100 CD8^{+} T-cells/µL predetermined and validated in this report are close to the median of all the cutoffs in the critical outcome group. For comparative purposes, we also examined another set of cutoffs (325 CD4^{+} and 150 CD8^{+} T-cells/µL) because they were developed in a well-designed study measuring CD4^{+} and CD8^{+} T-cell counts at hospital admission using the BD Multitest™ 6-Color TBNK Reagent with BD Trucount™ Tubes on BD FACSCanto™ II Flow Cytometer in a relatively large Chinese cohort (739 patients, 51 fatal) monitored for outcome of mortality.[20]

These alternative cutoffs were tested in our Italian validation cohort and also demonstrated statistically significant increased risk of IMV and mortality, albeit with reduced risk estimates as compared to our predetermined cutoffs (absolute risk, relative risk, and *P* value for IMV: CD4^{+} T-cell cutoff 29.7%, 3.86, 0.002; CD8^{+} T-cell cutoff 33.3%, 5.67, <0.001, and for mortality: CD4^{+} T-cell cutoff 45.8%, 4.28, <0.001; CD8^{+} T-cell cutoff 42.9%, 3.66, <0.001). We also analyzed the optimum cutoffs with confidence intervals for the validation cohort by maximizing sensitivity and specificity using Youden's index, and the predetermined cutoffs of 250 CD4^{+} and 100 CD8^{+} T-cells/µL fell within the confidence intervals for both IMV and mortality (IMV: CD4^{+} T-cell cutoff CI 157 to 293 cells/µL, CD8^{+} T-cell cutoff CI 74 to 216 cells/µL; mortality: CD4^{+} T-cell cutoff CI 250 to 368 cells/µL, CD8^{+} T-cell cutoff CI 86 to 271 cells/µL).

Since CD4^{+} and CD8^{+} T-cells are included in the common total lymphocyte measurement available by complete blood count, we also tested a total lymphocyte cutoff, 1,100 cells/µL, derived from a meta-analysis.[23] While this cutoff also demonstrated elevated risk of IMV and mortality, the risk estimates were lower than for the CD4^{+} and CD8^{+} T-cell cutoffs, with marginal significance for IMV (absolute risk, relative risk, and *P* value for IMV: 19.7%, 2.46, 0.05; and for mortality, 32.1%, 3.17, <0.001).

Taken as a whole, the literature establishes the use of low CD4^{+} and CD8^{+} T-cell cutoffs to aid in determining the risk of critical outcomes in COVID-19, and the validation data from this study support the use of the predetermined cutoffs of 250 CD4^{+} and 100 CD8^{+} T-cells/µL.

Exploratory Analysis: Use of CD4^{+} and CD8^{+} T-cell cutoffs together with an IL-6 cutoff

Although the study was not originally designed or powered for this analysis, we wanted to explore the absolute and relative risk of IMV and mortality if CD4^{+} and CD8^{+} T-cell cutoffs were used in combination with an IL-6 cutoff, since 1) T-cells and IL-6 measure distinct aspects of the immune response, 2) at least two studies have demonstrated additive predictive value for combined use of T-cell and IL-6 cutoffs,[14,20] 3) a cutoff for IL-6 (35 pg/mL) has already been established for assessing the risk of IMV[24] and 4) the U.S. FDA has granted emergency use authorizations for use of this cutoff with Roche, Beckman Coulter, and Siemens IL-6 assays. A Siemens IL-6 assay was used at hospital admission as standard care in the validation cohort. Patients were analyzed for risk of IMV and mortality based on whether they were positive (meaning below CD4^{+} and/or CD8^{+} T-cell cutoffs or above IL-6 cutoff), for none, 1, 2, or all 3 of the cutoffs. The absolute and relative risk of both IMV and morality increased for patients positive for an increasing number of cutoffs (Figures 4A and 5A), with poorer outcomes of time-to-IMV and survival (Figures 4B and 5B). For patients positive for all three cutoffs the absolute risks of IMV and mortality climbed to 83.3% and 90.9%, respectively, and in comparison to patients negative for all three cutoffs *P* values were <0.001 and relative risks climbed to 18.3 and 15.7, respectively. These results suggest that clinical utility may potentially be improved by using CD4^{+} and CD8^{+} T-cell cutoffs together with an IL-6 cutoff; the results should be further validated in other cohorts.

Figure 4: Analysis of IMV outcome based on CD4+ and CD8+ T-cell count cutoffs together with an IL-6 cutoff.

Contingency table with absolute and relative risk of IMV (**A**) and time-to-IMV analysis (Kaplan-Meier) (**B**) for patients positive for none, 1, 2, or all 3 of CD4+ T-cell, CD8+ T-cell, and IL-6 cutoffs at hospital admission. Abbreviations used: Absolute risk (Abs Risk); Confidence interval (CI); Relative risk (RR). For Kaplan-Meier analysis, discharged patients are censored.

A

Cutoff	No IMV	IMV	Total	Abs Risk	Abs Risk CI	P value	RR	RR CI
CD4+, CD8+ and IL-6 combined								
Negative for all cutoffs	63	3	66	4.5%	0.9%, 12.7%			
Positive for one cutoff	41	6	47	12.8%	4.8%, 25.7%	0.16	2.81	0.74, 10.67
Positive for two cutoffs	10	4	14	28.6%	8.4%, 58.1%	0.0158	6.29	1.58, 25.02
Positive for all cutoffs	1	5	6	83.3%	35.9%, 99.6%	<0.001	18.33	5.74, 58.6
Total	**115**	**18**	**133**	**13.5%**				

B


Time-to-IMV Curve for CD4+, CD8+ T-cells and IL-6 Cutoffs
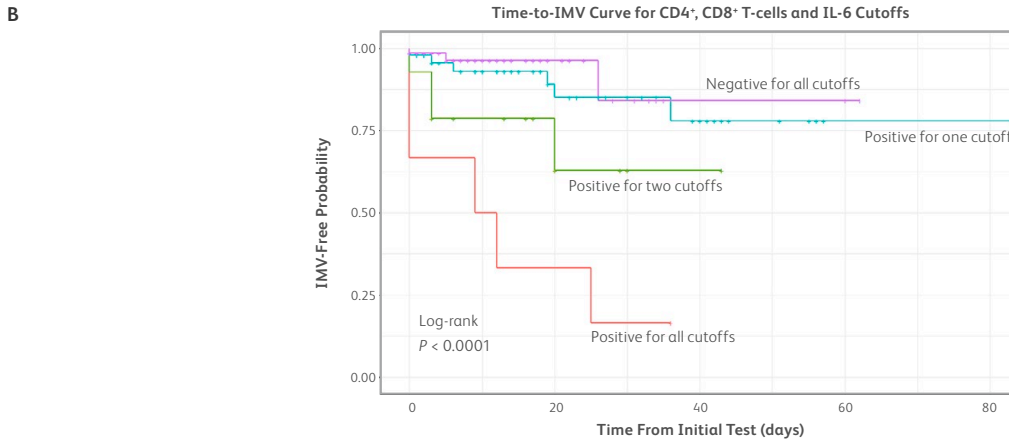
Figure 5: Analysis of mortality outcome based on CD4+ and CD8+ T-cell count cutoffs together with an IL-6 cutoff.

Contingency table with absolute and relative risk of mortality (**A**) and survival analysis (Kaplan-Meier) (**B**) for patients positive for none, 1, 2, or all 3 of CD4+ T-cell, CD8+ T-cell, and IL-6 cutoffs at hospital admission. Abbreviations used: Absolute risk (Abs Risk); Confidence interval (CI); Relative risk (RR). For Kaplan-Meier analysis, discharged patients are censored.

A

Cutoff	Discharge	Death	Total	Abs Risk	Abs Risk CI	P value	RR	RR CI
CD4+, CD8+ and IL-6 combined								
Negative for all cutoffs	65	4	69	5.8%	1.6%, 14.2%			
Positive for one cutoff	43	9	52	17.3%	8.2%, 30.3%	0.0722	2.99	0.97, 9.16
Positive for two cutoffs	10	8	18	44.4%	21.5%, 69.2%	<0.001	7.67	2.6, 22.63
Positive for all cutoffs	1	10	11	90.9%	58.7%, 99.8%	<0.001	15.68	5.95, 41.34
Total	**119**	**31**	**150**	**20.7%**				

B


Survival Curve for CD4+, CD8+ T-cells and IL-6 Cutoffs
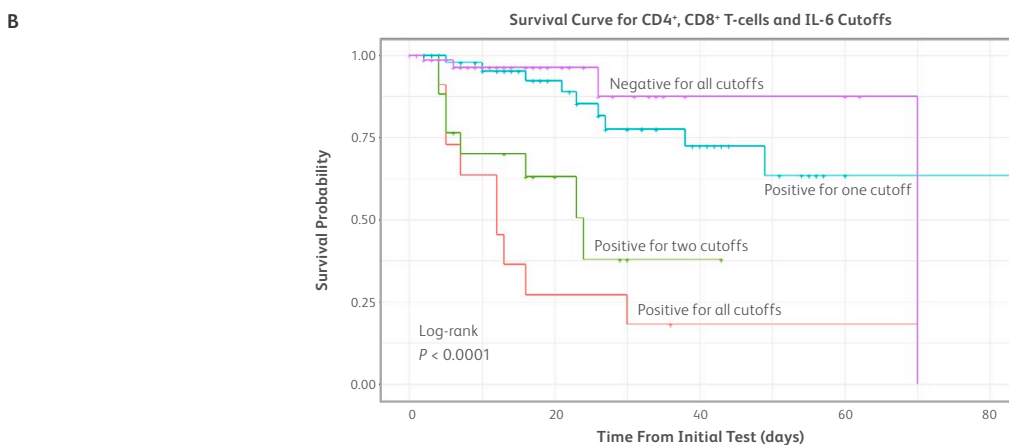
Exploratory analysis: Longitudinal evaluation

For patients who remained in hospital and had subsequent TBNK measurements (every 1 to 2 weeks, 77 patients in total), the measurement at hospital admission was compared with the last available measurement. This demonstrated that patients who are ultimately discharged are associated with higher and increasing CD4^{+} and CD8^{+} T-cell counts over time, whereas patients who died are associated with lower and decreasing CD4^{+} and CD8^{+} T-cell counts over time (Figure 6A). Patients were also evaluated for absolute and relative risk of IMV and mortality based on the lowest measured CD4^{+} and CD8^{+} T-cell count per patient over time (as opposed to the initial measurement at hospital admission reported in sections above). This produced similar results to the measurement at hospital admission, with P values below 0.001, with lower absolute risks, and similar or higher relative risks (Figure 6B). These findings are generally consistent with other reports showing relative stability of CD4^{+} and CD8^{+} T-cell counts in hospitalized patients over time,[10] with decreases correlating with poor outcomes and mortality,[1] and increases correlating with recovery.[25,26] Altogether, these findings are consistent with a role for CD4^{+} and CD8^{+} T-cell cutoffs for ongoing immunological assessment of COVID-19 patients during hospitalization.

Figure 6: Analysis of CD4^{+} and CD8^{+} T-cell counts over time and outcomes of IMV and mortality.

Assessment of CD4^{+} and CD8^{+} T-cell absolute counts over time (**A**) and absolute and relative risk of IMV and mortality considering the lowest CD4^{+} and CD8^{+} T-cell counts measured over time (**B**). Abbreviations used: Absolute risk (Abs Risk), Confidence interval (CI), Relative risk (RR)
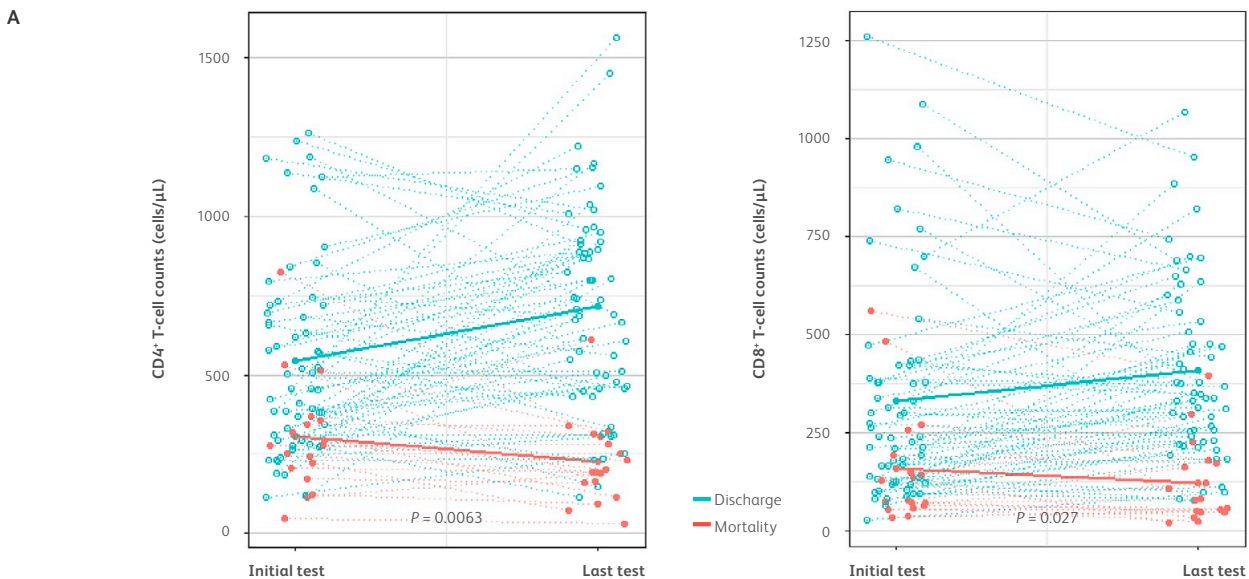
Cutoff (cells/µL)	IMV			Mortality		
	Abs risk (above, below cutoff)	*P* value	RR	Abs risk (above, below cutoff)	*P* value	RR
CD4^{+} T-cells						
CD4<250	6.5%, 35.3%	<0.001	5.39	10.5%, 47.8%	<0.001	4.54
CD8^{+} T-cells						
CD4<100	6.0%, 48.0%	<0.001	7.95	11.9%, 55.9%	<0.001	4.69

Conclusion

The results of this study evaluating predetermined cutoffs for CD4$^+$ and CD8$^+$ T-cells (250 and 100 cells/µL, respectively) in hospitalized COVID-19 patients support the use of these cutoffs as an aid in determining the risks of IMV and mortality. These cutoffs classified patients at ~5- to ~6-fold increased risk of IMV and ~4.5-fold increased risk of mortality, with significantly worsened time-to-IMV and survival by Kaplan-Meier analyses. In comparison to other parameters, the CD4$^+$ and CD8$^+$ T-cell cutoffs were among the best predictors of IMV and mortality using logistic regression analysis and AIC model selection; some commonly used laboratory tests such as total lymphocyte count, neutrophil-to-lymphocyte ratio, CRP, and ferritin lost significance in various analyses in comparison to CD4$^+$ and CD8$^+$ T-cell cutoffs. The cutoffs of 250 CD4$^+$ and 100 CD8$^+$ T-cells/µL resulted in higher absolute and relative risk estimates as compared to other CD4$^+$ and CD8$^+$ T-cell cutoffs and a cutoff for total lymphocyte count. In exploratory analysis, CD4$^+$ and CD8$^+$ T-cell cutoffs used together with an IL-6 cutoff demonstrated that patients who were positive for all three cutoffs (CD4$^+$ and CD8$^+$ T-cells below cutoffs and IL-6 above cutoff) had further increased absolute and relative risks of IMV and mortality, with worsened time-to-IMV and survival by Kaplan-Meier analyses. In another exploratory analysis, longitudinal CD4$^+$ and CD8$^+$ T-cell measurements demonstrated that patients who were ultimately discharged were associated with higher initial CD4$^+$ and CD8$^+$ T-cell counts that increased over time, whereas patients who died were associated with lower initial CD4$^+$ and CD8$^+$ T-cell counts that further decreased over time. In summary, this study validates predetermined cutoffs for CD4$^+$ and CD8$^+$ T-cells in hospitalized COVID-19 patients and supports their use as an aid in determining the risk of IMV and mortality. CD4$^+$ and CD8$^+$ T-cell counts alone are not indicative of the need for IMV or impending mortality and should be used in conjunction with clinical findings and the results of other laboratory testing.

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