Supplementary Materials For:

A Biomarker Assay to Risk-Stratify Patients with Symptoms of Respiratory Tract Infection

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Supplemental Methods

RALI-Dx Assay: Whole blood samples were collected in the ED and sent to the clinical lab for plasma processing. Samples were stored at -80°C, then thawed overnight at 4°C and diluted 1:1 in assay diluent before testing. 60uL of diluted plasma was loaded on a custom 96-well microtitre plate that contained a standard curve and a high and low positive control derived from the reference standard for each RALI-Dx biomarker. World Health Organization (WHO) reference standards were used for IL-6, IL-8, and IL-10, and a Quantikine ELISA standard (R&D Systems, MN, USA) was used for sTNFR1 and sTREM1. Protein concentrations for each biomarker were determined by quantitative immunofluorescence using the automated sqidlite™ system (SQI Diagnostics, ON, CAN). RALI-Dx analytical validation, including detection capability (i.e., limits of detection and quantification) have been completed (Supplemental Table S3).

Model Development: The Canada cohort was randomly partitioned 80:20 for training (n=248) and testing (n=62); the Italy (n=131) and Brazil (n=200) cohorts were used for external testing. Model performance was assessed using the area under the receiver operating characteristic curve (AUROC) with the null hypothesis that the AUROC was 50%. AUROC significance testing of RALI-Dx model versus the CRB-65 model was determined by Bootstrapping with 10,000 iterations and the null hypothesis that CRB-65 outperforms the RALI-Dx model.

Post-Hoc Model Assessments: The number of patients who developed severe illness (i.e., required ICU care, or died during the 28-day follow-up) were assessed for correctly being predicted to require hospitalization based on the model results from the ED blood sample. Analyses were completed using the optimal probability threshold derived from the Canada cohort (i.e., probability of admission >45%). The RALI-Dx model was evaluated on the COVID-19 positive cohort for model performance (AUROC).

Statistical Analysis: Descriptive statistics of patient enrollment characteristics were evaluated using Chi-squared, Fisher's exact, or Mann-Whitney U tests as appropriate to determine patient factors associated with clinical outcomes. For logarithmic graphs, protein concentrations below the lower limit of detection (LLOD) were assigned a value of 0.5*LLOD in the corresponding figures. Protein distributions followed a non-parametric distribution and were assessed using Mann-Whitney U or Kruskal-Wallis tests where appropriate; multiple comparisons were made using Dunn's correction of the Kruskal-Wallis test. The predictive ability of the individual biomarkers was assessed using AUROC, with the null hypothesis that the AUROC was 50%.

Supplemental Tables

Table S1: Patient characteristics at ED baseline for the external validation cohorts

	Italy Cohort	Brazil Cohort
Number of Patients	131	200
Mean Age (SD) - Years	60 (20)	48 (18)
Male (%)	54 (42%)	94 (47%)
COVID-19+ (%)	56 (43%)	142 (71%)
Respiratory Symptoms		
Cough (%)	56 (43%)	128 (64%)
Fever (%)	75 (57%)	115 (58%)
Sore Throat (%)	11 (8.4%)	86 (43%)
Dyspnea (%)	68 (52%)	107 (54%)
Chest Pain (%)	28 (21%)	72 (36%)
Loss of Taste (%)	23 (18%)	63 (32%)
Loss of Smell (%)	16 (12%)	62 (31%)
Myalgia (%)	··	144 (72%)
Fatigue (%)	··	170 (85%)
RALI-Dx Biomarker Levels		
IL-6 pg/mL (Median [IQR])	6 [0-71]	12 [0-51]
IL-8 pg/mL (Median [IQR])	0 [0-0]	0 [0-0]
IL-10 pg/mL (Median [IQR])	0 [0-0]	0 [0-12]
sTNFR1 pg/mL (Median [IQR])	1064 [704-2173]	838 [610-1386]
sTREM1 pg/mL (Median [IQR])	261 [150-470]	170 [101-306]

Table S2: Outcome severity and RALI-Dx biomarker levels of COVID-19 patients

	Canada COVID-19 Patients	Italy COVID-19 Patients	Brazil COVID-19 Patients	p-value
Number of Patients	47	56	142	-
Hospitalized (%)	27 (57%)	40 (71%)	78 (55%)	0.10
Required ICU Care (%)	4 (9%)	8 (14%)	22 (15%)	0.48
Mechanical Ventilation (any) (%)	3 (6%)	7 (12%)	17 (5%)	0.53
Invasive MV (%)	3 (6%)	1 (2%)	17 (5%)	0.06
Non-invasive MV (%)	··	6 (11%)	··	··
28-Day Mortality (%)	4 (9%)	7 (12%)	18 (13%)	0.73
IL-6 pg/mL (Median [IQR])	13 [0-54]	19 [0-66]	22 [0-66]	0.33
IL-8 pg/mL (Median [IQR])	0 [0-0]	0 [0-0]	0 [0-0]	0.33
IL-10 pg/mL (Median [IQR])	0 [0-0]**	0 [0-6]**	8 [0-14]	<0.0001
sTNFR1 pg/mL (Median [IQR])	1049 [661-1628]	1127 [748-2088]	1006 [702-1553]	0.24
sTREM1 pg/mL (Median [IQR])	287 [123-535]	189 [127-378]	199 [107-338]	0.18

Legend: MV=mechanical ventilation. p-values are reported as Chi square test for hospitalization, ICU care, mechanical ventilation and mortality; Kruskal-Wallis test p-values are reported for protein measurements. Note that (*) indicates a Kruskal-Wallis test multiple comparisons significant difference versus the Brazil cohort (**p<0.01).

Table S3: RALI-Dx performance characteristics

	IL-6	sTNFR1	IL-8	IL-10	sTREM1
ULOQ (pg/mL)	3318	10057	10354	1538	11989
LLOQ (pg/mL)	18.6	118	28	11	238
LOD (pg/mL)	5.1	17	27	7	44

Legend: ULOQ=upper limit of quantification; LLOQ=lower limit of quantification; LOD=lower limit of detection.

Table S4: Univariate logistic regression results for RALI-Dx biomarkers to predict hospitalization following ED presentation

	Canada Cohort		Italy Cohort		Brazil Cohort	
	AUROC	95% CI	AUROC	95% CI	AUROC	95% CI
IL-6	69%	63-74%	80%	72-88%	82%	76-88%
IL-8	57%	53-60%	58%	48-67%	57%	49-65%
IL-10	60%	55-64%	62%	53-72%	67%	59-75%
sTNFR1	77%	72-82%	84%	78-91%	88%	83-93%
sTREM1	70%	64-76%	67%	58-76%	84%	78-89%

Table S5: RALI-Dx biomarker levels (pg/mL) measured in n=20 healthy control subjects

	Median [IQR]
IL-6	0 [0-0]
IL-8	0 [0-0]
IL-10	0 [0-0]
sTNFR1	637 [600-847]
sTREM1	174 [75-288]

Supplemental Figures

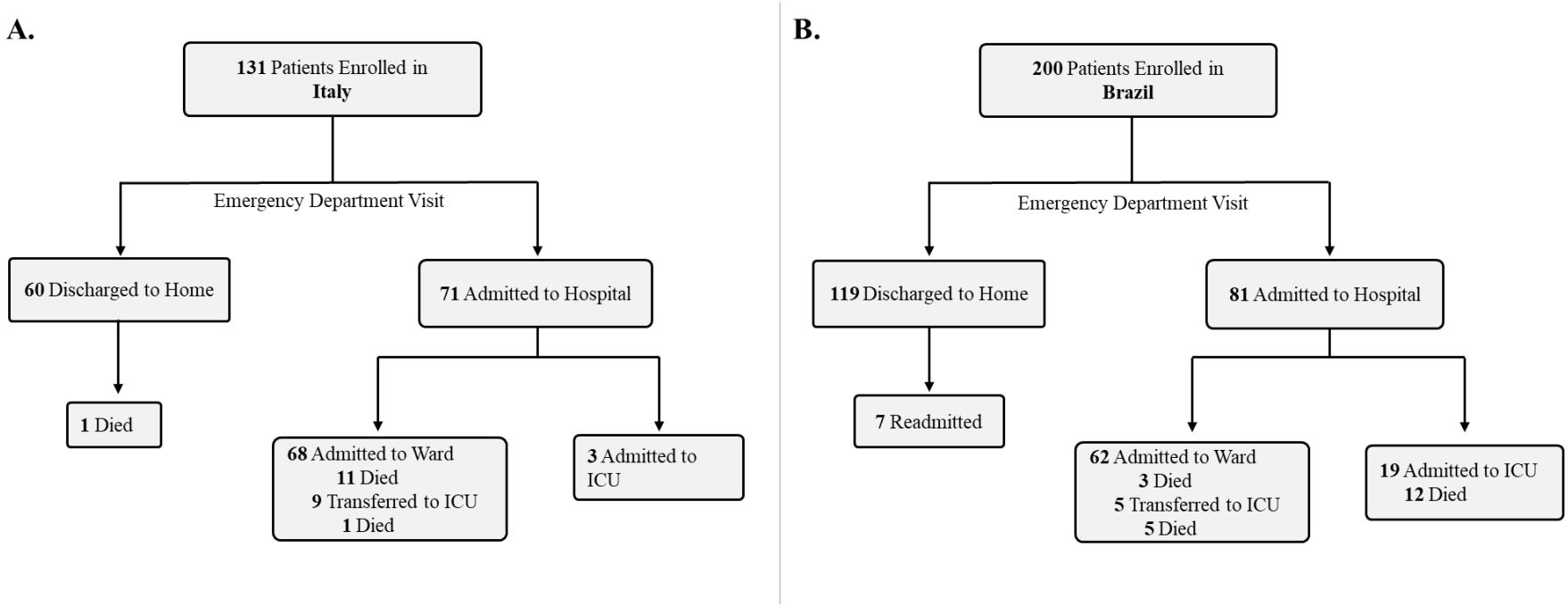

Figure S1: Patient outcomes following ED presentation in **Italy** (A) and **Brazil** (B).

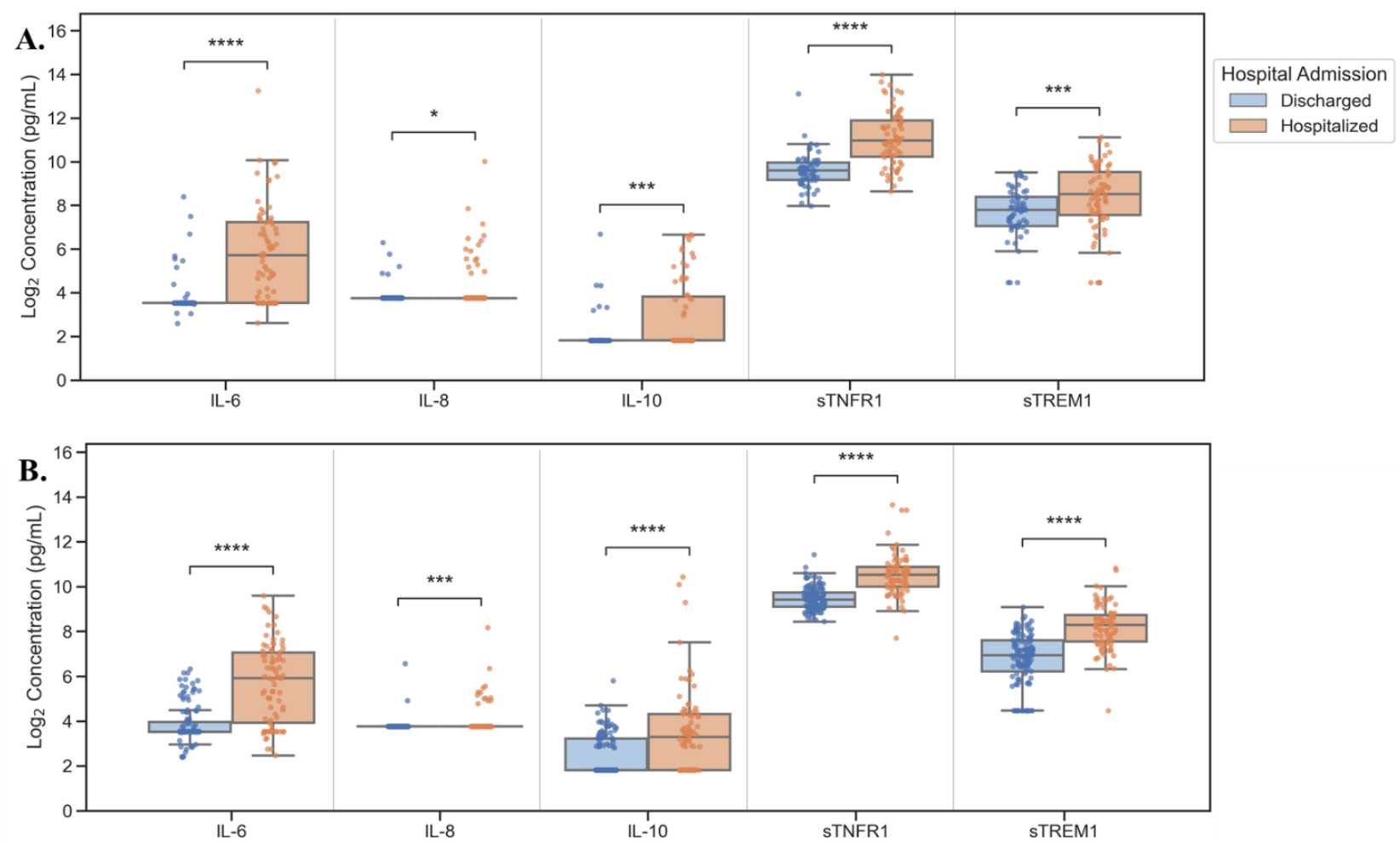

Figure S2: RALI-Dx biomarkers are elevated in hospitalized patients. Box and Whisker plots for patients that were discharged (blue) or hospitalized (orange) following ED presentation for: IL-6, IL-8, IL-10, sTNFR1, and sTREM1 in Italy (A, n=131) and Brazil (B, n=200). Mann-Whitney U test p-values are indicated within each graph (*p<0.05, **p<0.01, ***p<0.001 ****p<0.0001).