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 sTNFRI 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**

|                     | Italy Cohort | Brazil Cohort |
|---------------------|--------------|---------------|
| 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**

|                     | Italy Cohort | Brazil Cohort |
|---------------------|--------------|---------------|
| 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

A.  

131 Patients Enrolled in Italy

Emergency Department Visit

60 Discharged to Home

1 Died

71 Admitted to Hospital

68 Admitted to Ward

11 Died

9 Transferred to ICU

3 Admitted to ICU

B.  

200 Patients Enrolled in Brazil

Emergency Department Visit

119 Discharged to Home

81 Admitted to Hospital

7 Readmitted

62 Admitted to Ward

3 Died

5 Transferred to ICU

19 Admitted to ICU

12 Died

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 (*$p<0.05$, **$p<0.01$, ***$p<0.001$ ****$p<0.0001$).