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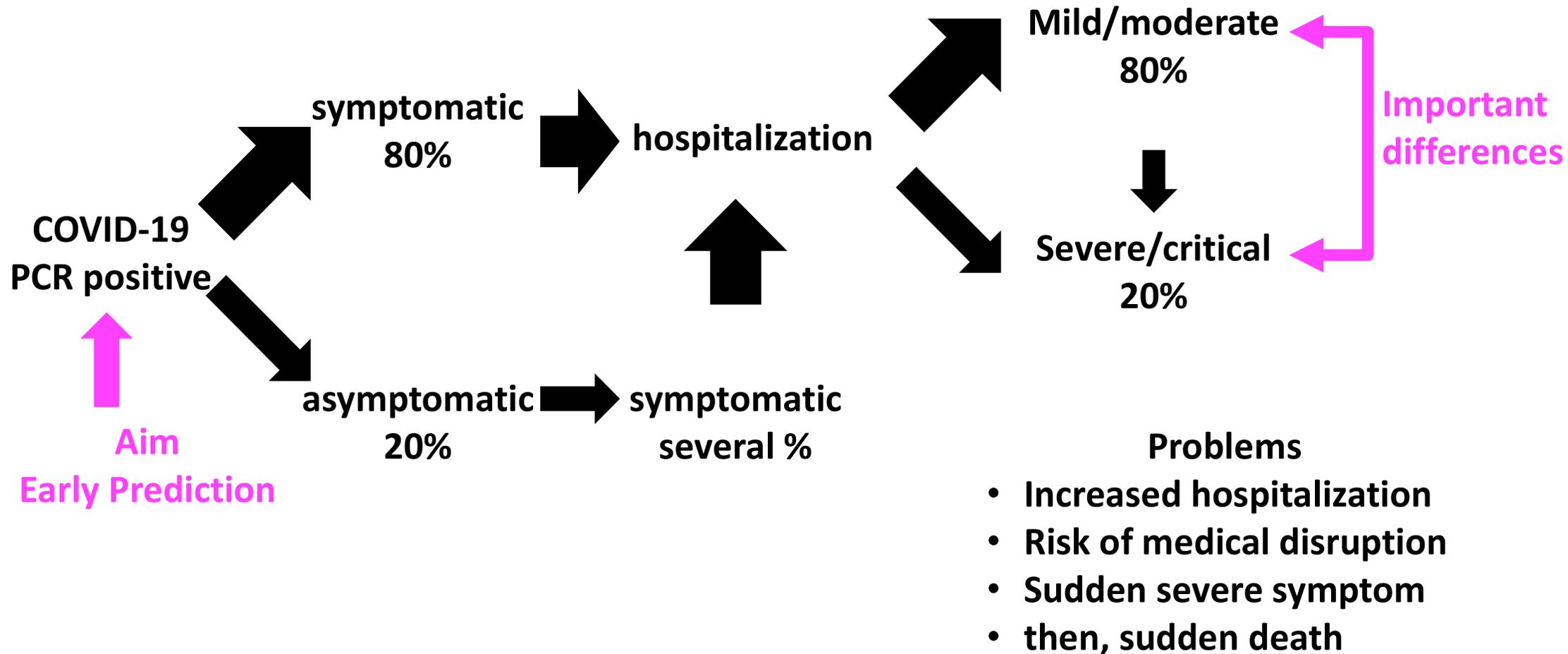
Serum predictive factors for severe symptoms of COVID-19

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Background/ aim



Aim

- **Comprehensive analysis of humoral factors**
- **Quick and easy diagnosis by blood test**
- **Observation of changes leading to severe diseases**

NIH Guidelines: Defining a COVID-19 Severity Spectrum

Stage	Characteristics
Asymptomatic or presymptomatic infection	<ul style="list-style-type: none">▪ Positive test for SARS-CoV-2 but no symptoms
Mild illness	<ul style="list-style-type: none">▪ Varied symptoms (eg, fever, cough, sore throat, malaise, headache, muscle pain) but no shortness of breath, dyspnea, abnormal imaging
Moderate illness	<ul style="list-style-type: none">▪ SpO₂ ≥ 94% and lower respiratory disease evidenced by clinical assessment or imaging
Severe illness	<ul style="list-style-type: none">▪ SpO₂ < 94%, PaO₂/FiO₂ < 300, respiratory rate > 30 breaths/min, or lung infiltrates > 50%
Critical illness	<ul style="list-style-type: none">▪ Respiratory failure, septic shock, and/or multiorgan dysfunction

Humoral factors

- | | | | | |
|---------|----------|--------------------|------------------|------------------|
| • CCL1 | • CX3CL1 | • IFN- α 2 | • IL-1 α | • FGF2 |
| • CCL2 | • CXCL1 | • IFN- γ | • IL-1 β | • G-CSF |
| • CCL3 | • CXCL2 | • IFN- λ 1 | • IL-2 | • GM-CSF |
| • CCL4 | • CXCL5 | • IFN- λ 2 | • IL-3 | • HGF |
| • CCL5 | • CXCL6 | • IFN- λ 3 | • IL-4 | • LIF |
| • CCL7 | • CXCL9 | | • IL-5 | • M-CSF |
| • CCL8 | • CXCL10 | | • IL-6 | • MIF |
| • CCL11 | • CXCL11 | | • IL-7 | • PDGF-BB |
| • CCL13 | • CXCL12 | | • IL-8 | • SCF |
| • CCL15 | • CXCL13 | | • IL-9 | • SCGF- β |
| • CCL17 | • CXCL16 | | • IL-10 | • TNF- α |
| • CCL19 | | | • IL-12 (p40) | • TNF- β |
| • CCL20 | | | • IL-12 (p70) | • TRAIL/ TNFSF10 |
| • CCL21 | | | • IL-13 | • VEGF |
| • CCL22 | | | • IL-15 | • β -NGF |
| • CCL23 | | | • IL-16 | |
| • CCL24 | | | • IL-17 | |
| • CCL25 | | | • IL-18 | |
| • CCL26 | | | • IL-1Ra | |
| • CCL27 | | | • IL-2R α | |
-

Screening cohort clinical background

(a first point of their hospitalization)

Variable	Mild/Moderate n=16	Severe/Critical n=12	p value
Age (y) (IQR)	46.5 (41.0, 55.8)	59.5 (48.0, 72.3)	0.15
Sex M/F (n)	12/4	9/3	1.0
Temperature (°C)	37.6±1.0	37.9±0.8	0.33
Pulse (bpm)	82.2±16.6	83.8±9.2	0.76
Respiratory rate (breaths/min)	17.8±2.1	20.3±5.6	0.11
SpO ₂ (%)	96.0±2.1	95.4±1.6	0.42
ALB (g/dL)	4.2±0.3	3.2±0.6	<0.001
AST (U/L)	29.4±16.5	45.8±28.2	0.09
ALT (U/L)	32.8±29.3	48.2±44.5	0.28
LDH (U/L)	194.7±97.2	352.8±185.6	0.01
Creatinine (mg/dL)	0.9±0.2	1.2±1.4	0.45
CRP (mg/dL)	1.6±2.9	9.1±4.3	<0.001
WBC (10 ³ /μL)	5.1±1.9	6.0±2.6	0.37
Neutrophils (%)	64.3±15.0	79.4±8.8	0.016
Lymphocytes (%)	26.0±12.4	24.0±21.3	0.77
PLT (10 ⁴ /μL)	20.1±4.7	16.8±8.9	0.21
HIV + (%)	12.5	0	1.0
HBV + (%)	6.3	8.3	1.0
CKD (%)	0	16.7	0.48
HT (%)	18.8	58.3	0.03
T2DM (%)	12.5	25.0	0.39
DL (%)	25.0	25.0	1.0
Smoker (%)	25.0	25.0	1.0
COPD (%)	0	16.7	0.48
CABG (%)	0	8.3	0.24
HA (%)	12.5	8.3	0.72
ACEi/ARB (%)	18.8	8.3	0.44
βB (%)	0	8.3	0.24

Mild : no pneumonia

Moderate : weak pneumonia

Severe : oxygen supplement and severe pneumonia

Critical : respiratory failure

Results

Serum factors associated with COVID-19 severe symptom

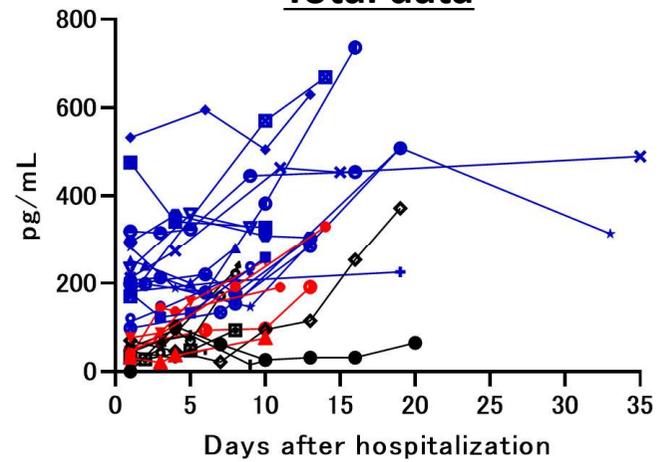
Category A	Category B
CCL17	IFN- λ 3
	IP-10
	CXCL9
	IL-6

Category A: Predictive Factor at an early phase of infection

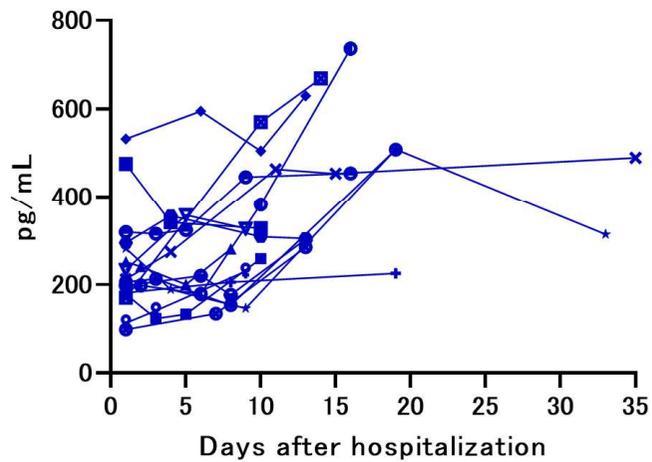
Category B: Predictive factors of severe disease onset

CCL17 (Cat-A)

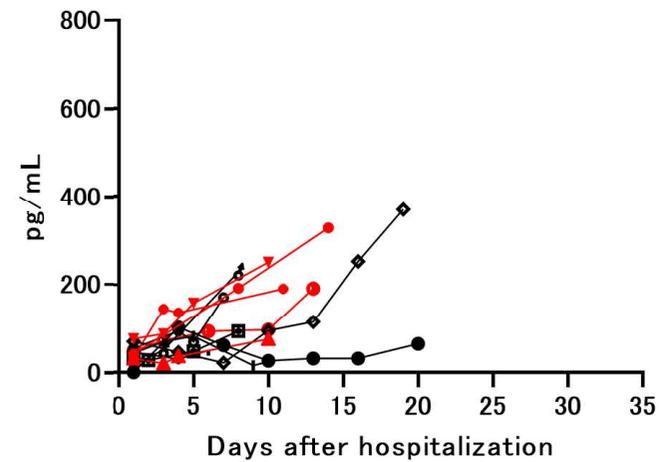
Total data



Mild/moderate

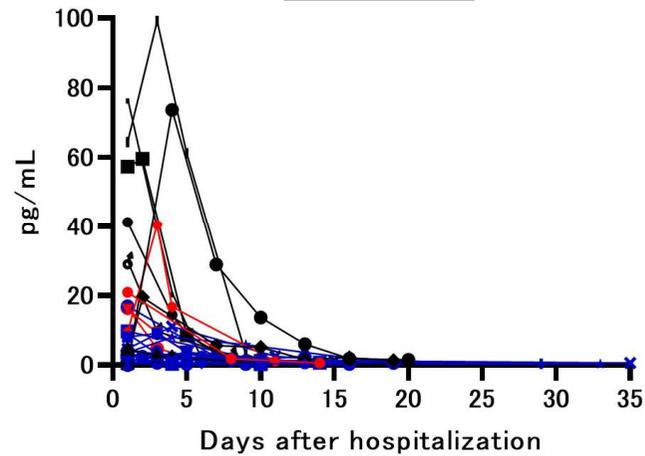


Severe/critical

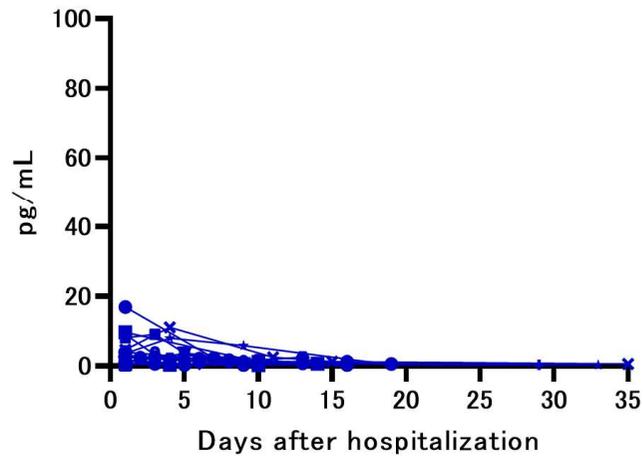


IFN-λ3 (Cat-B)

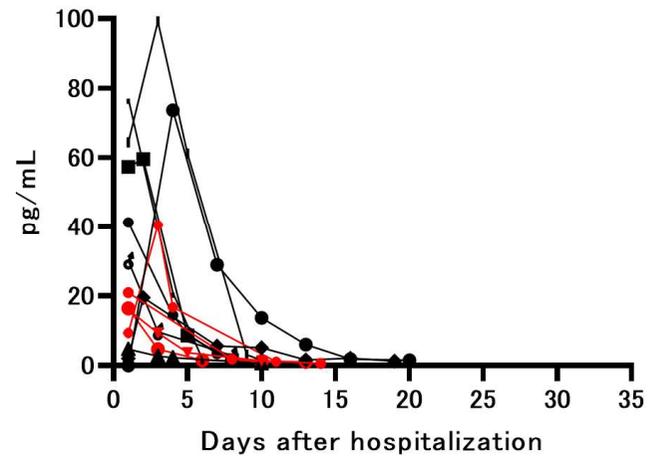
Total data



Mild/moderate



Severe/critical



CCL17 level and severe/critical symptom

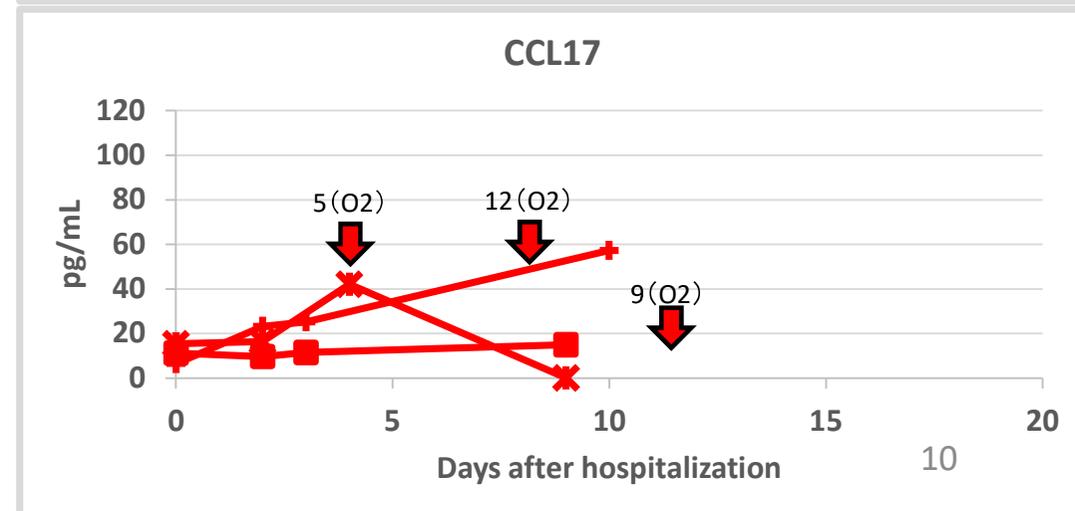
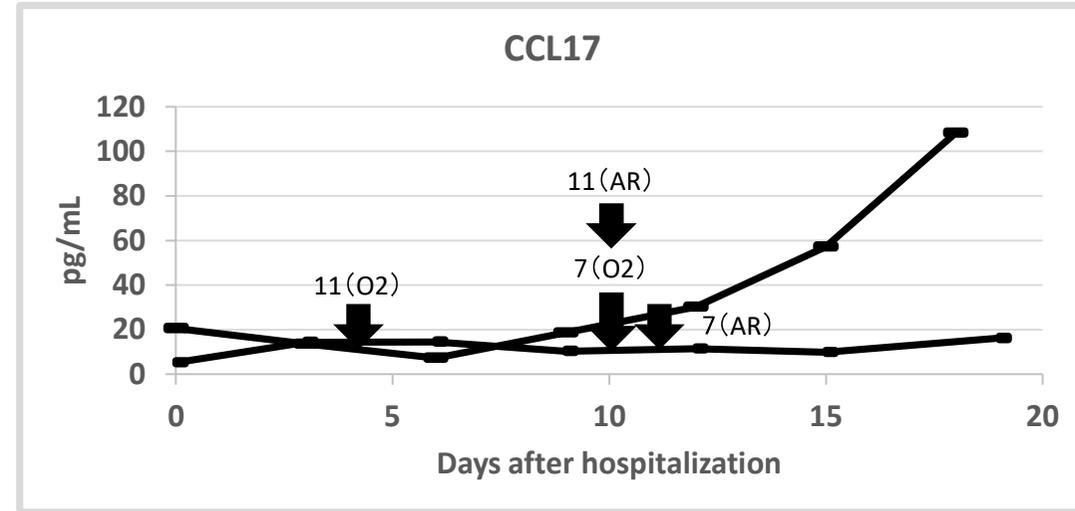
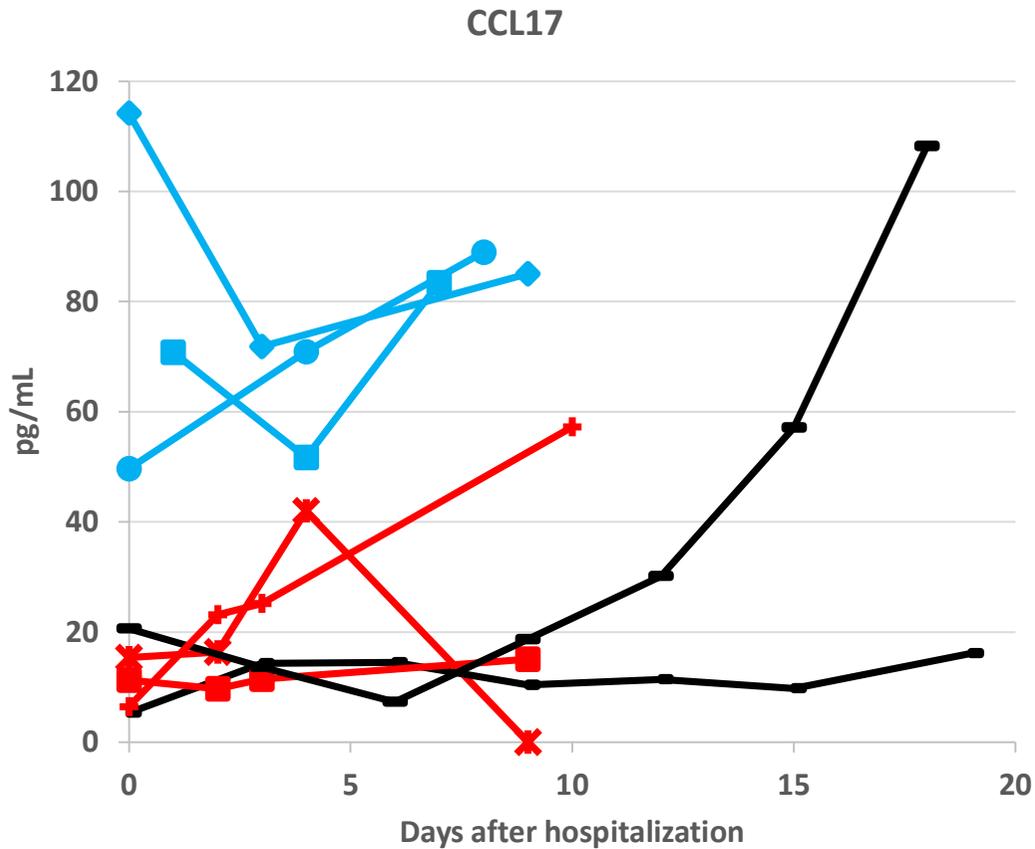
Mild : no pneumonia

Moderate : weak pneumonia

Severe : oxygen supplement and severe pneumonia

Critical : respiratory failure

AR: artificial respirator



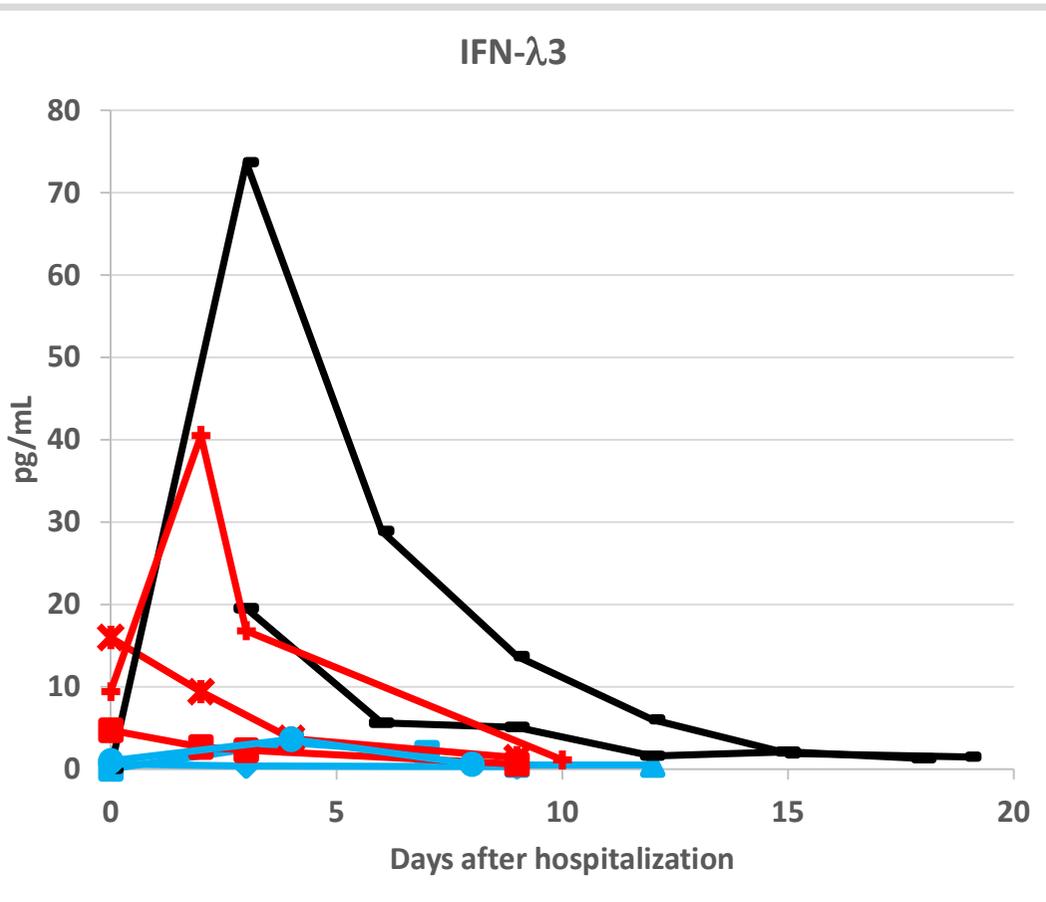
IFN-λ3 level and severe/critical symptom

Mild : no pneumonia

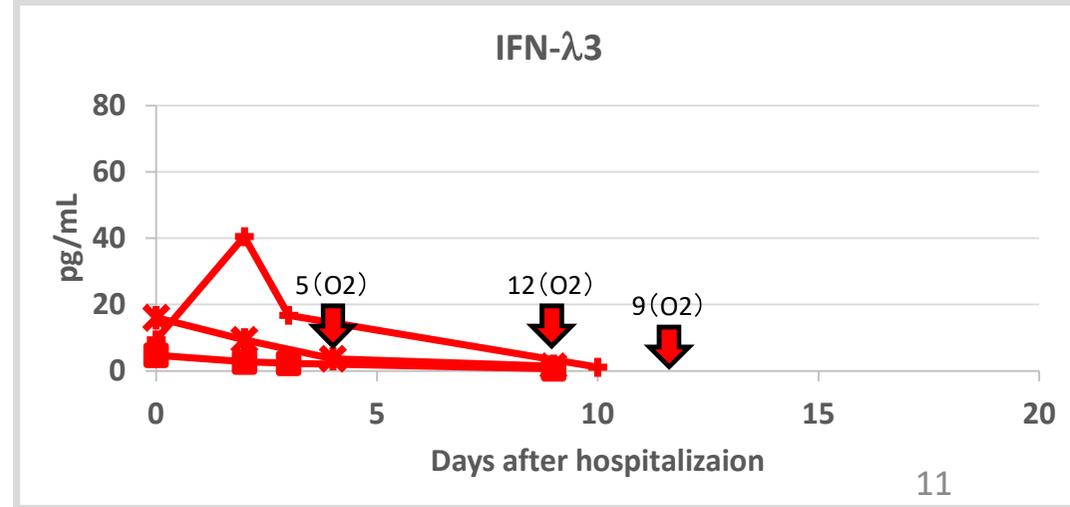
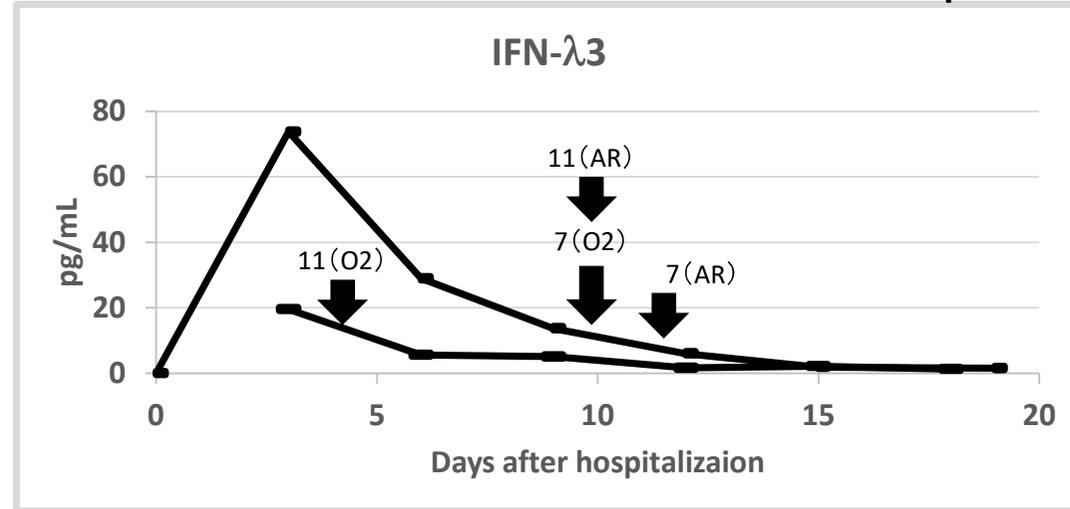
Moderate : weak pneumonia

Severe : oxygen supplement and severe pneumonia

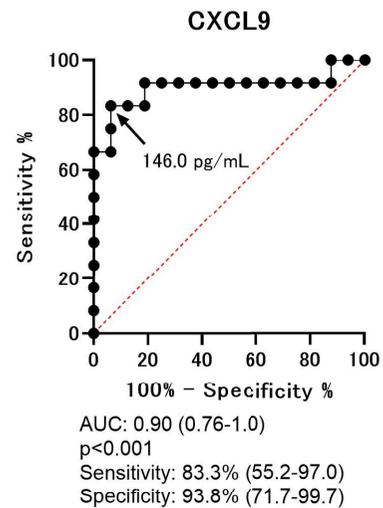
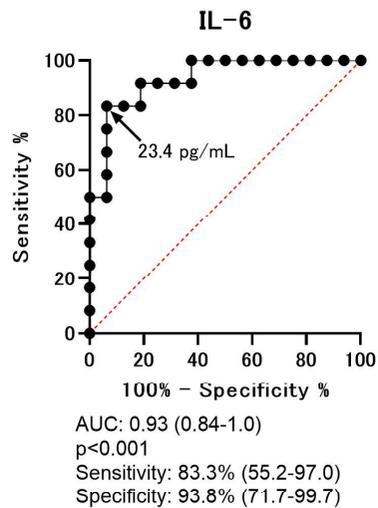
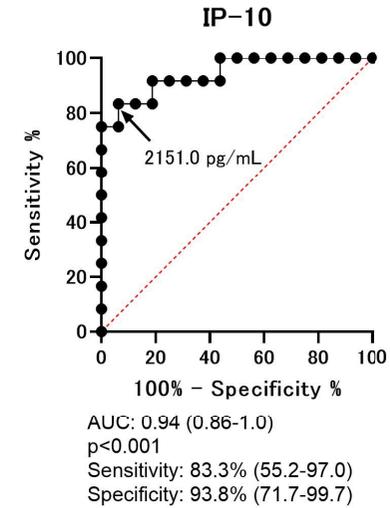
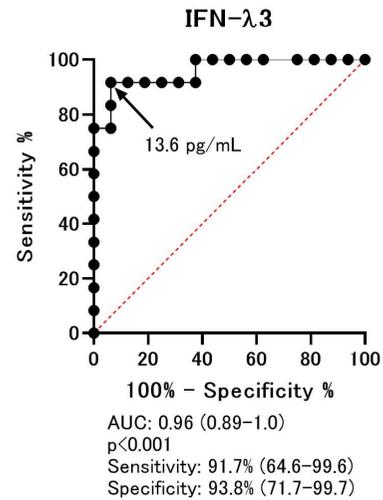
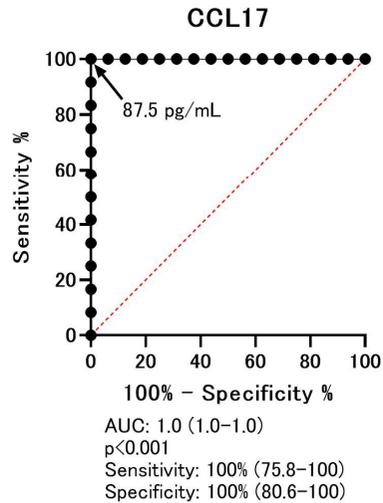
Critical : respiratory failure



AR: artificial respirator



ROC analysis

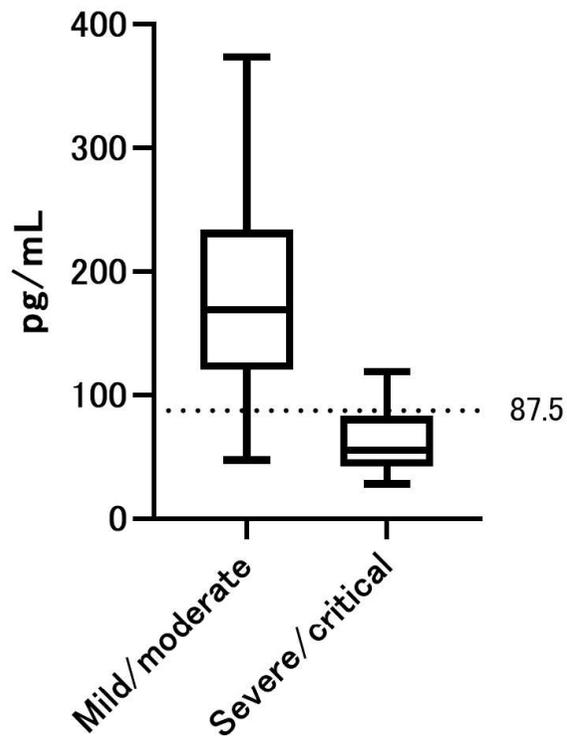


Validation cohort

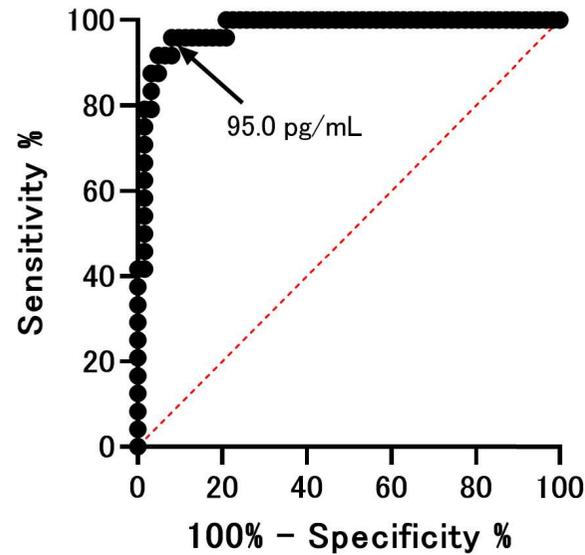
	Mild/Moderate	Severe/Critical	Univariate	Multivariate
Variable	n=46	n=12	p value	p value
Age (y) (IQR)	43.5 (29.3, 59.3)	67.5 (50.3, 73.3)	0.003	
Sex M/F (n)	30/16	10/2	0.23	
Temperature (°C)	37.4±0.9	38.3±1.0	0.005	
Pulse (bpm)	83.1±13.8	79.4±18.6	0.44	
Respiratory rate (breaths/min)	17.7±2.3	20.0±3.9	0.02	
SpO ₂ (%)	97.1±1.2	96.6±1.1	0.22	
ALB (g/dL)	3.9±0.6	3.4±0.4	0.004	
AST (U/L)	35.4±21.6	66.8±38.1	<0.001	
ALT (U/L)	39.2±30.9	51.1±39.6	0.27	
LDH (U/L)	241.5±79.6	403.2±111.0	<0.001	
Creatinine (mg/dL)	0.9±1.2	0.9±0.2	0.95	
CRP (mg/dL)	3.6±4.4	10.0±3.9	<0.001	
WBC (10 ³ /μL)	6.0±3.7	5.5±2.3	0.69	
Neutrophils (%)	69.6±11.1	79.6±7.9	0.005	
Lymphocytes (%)	22.0±9.1	14.3±5.4	0.006	
PLT (10 ⁴ /μL)	23.3±9.5	18.8±5.7	0.12	
CCL17 (pg/mL)	185.7±84.3	64.2±26.3	<0.001	0.009

CCL17 validation

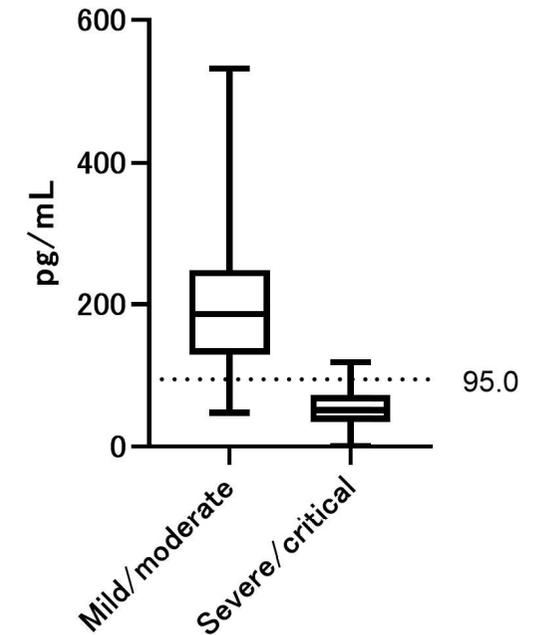
Validation cohort



Combined data



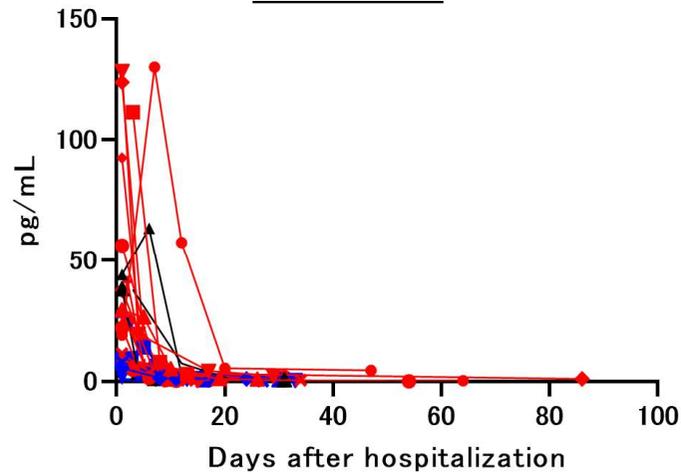
AUC: 0.98 (0.95–1.0)
p<0.001



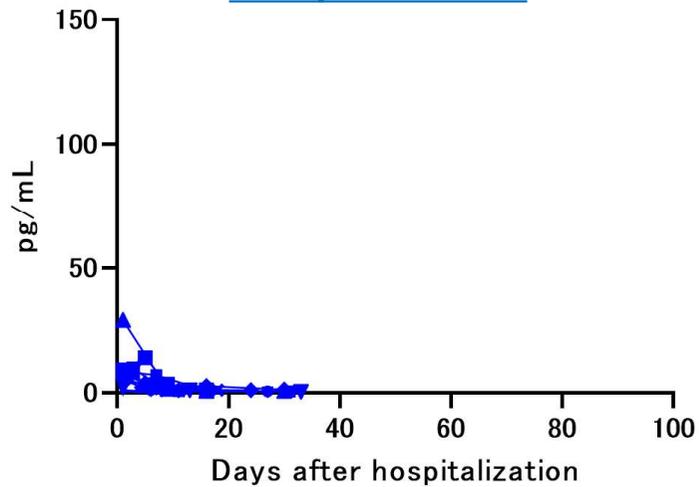
Cut-off value: 95.0 pg/mL
Sensitivity: 91.9% (95%CI 82.2–97.3)
Specificity: 95.8% (95%CI 78.9–99.9)
PPV: 98.3% (95%CI 90.8–100)
NPV: 82.1% (95%CI 63.1–93.9)

IFN-λ3 validation

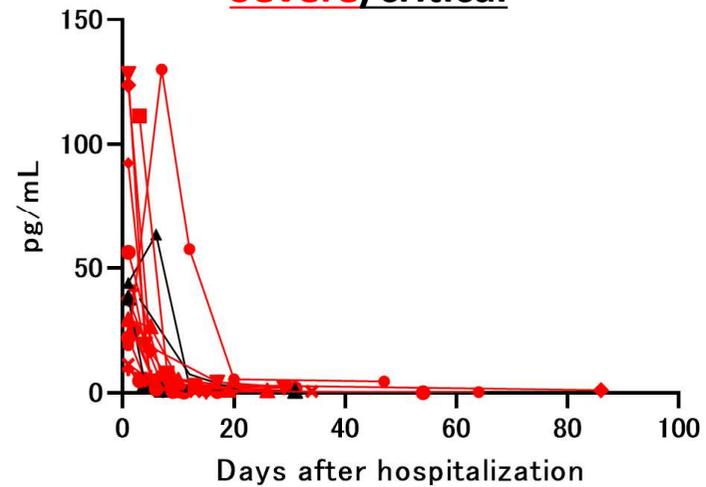
Total data



Mild/moderate

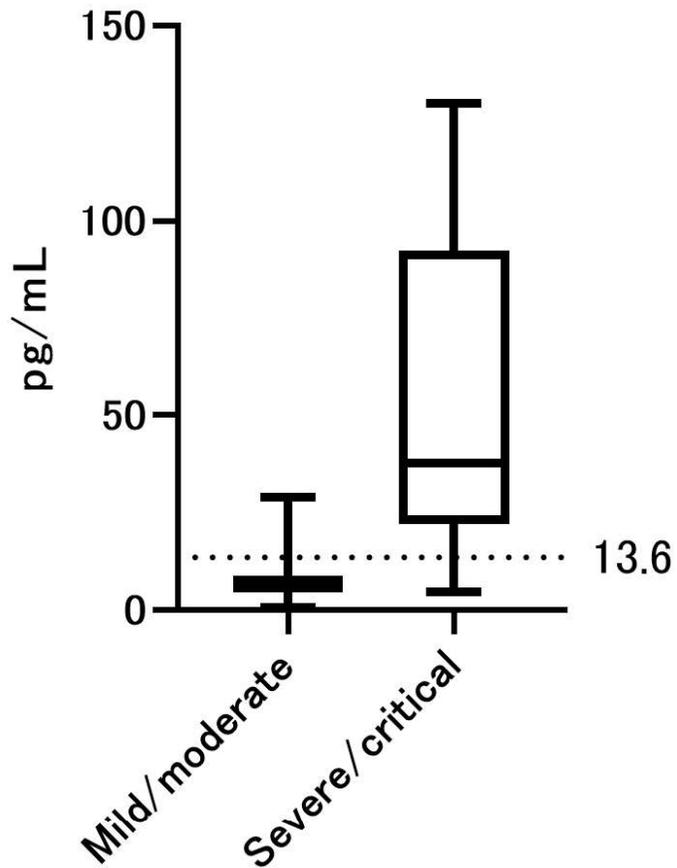


Severe/critical

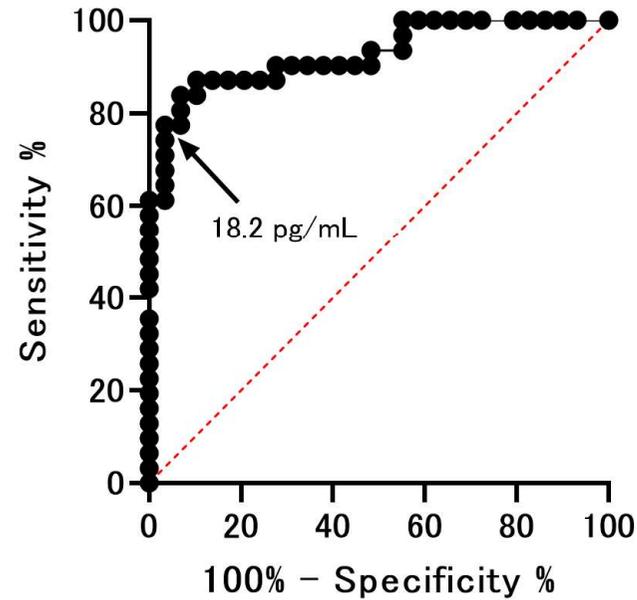


IFN-λ3 validation

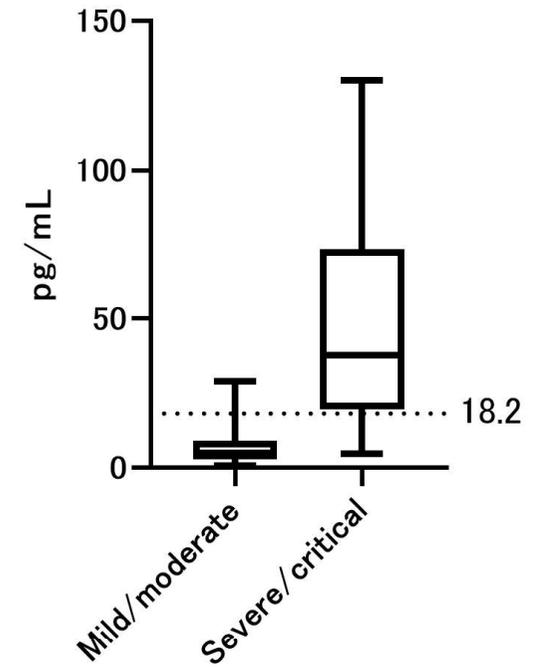
Validation cohort



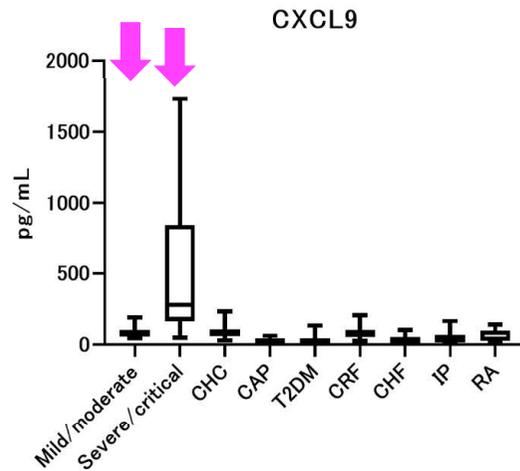
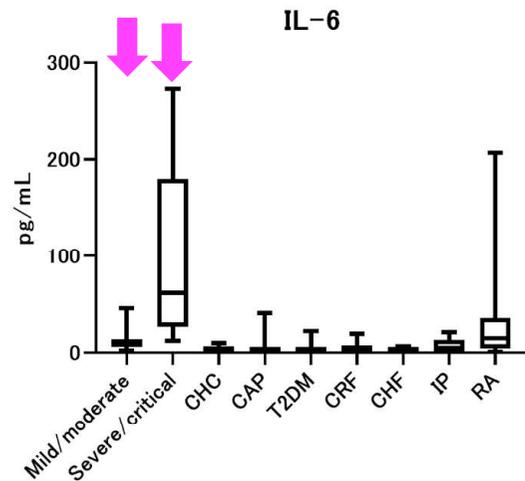
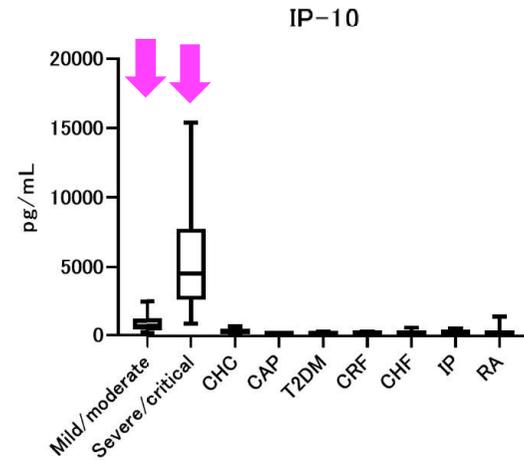
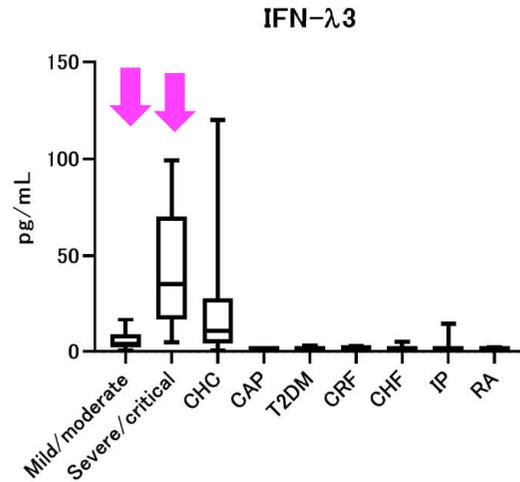
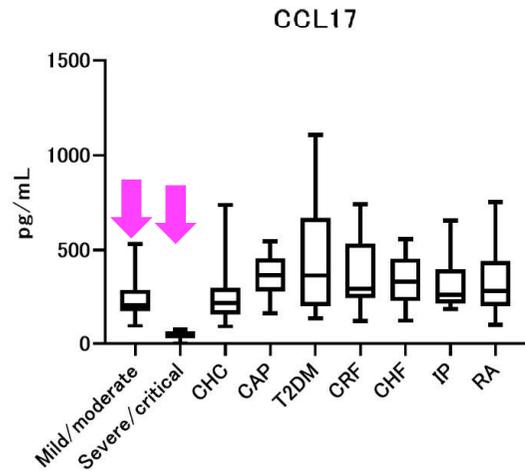
Combined data



AUC: 0.93 (0.86 – 0.99)
p < 0.001
Sensitivity: 77.4% (60.2 – 88.6)
Specificity: 96.6% (82.8 – 99.8)



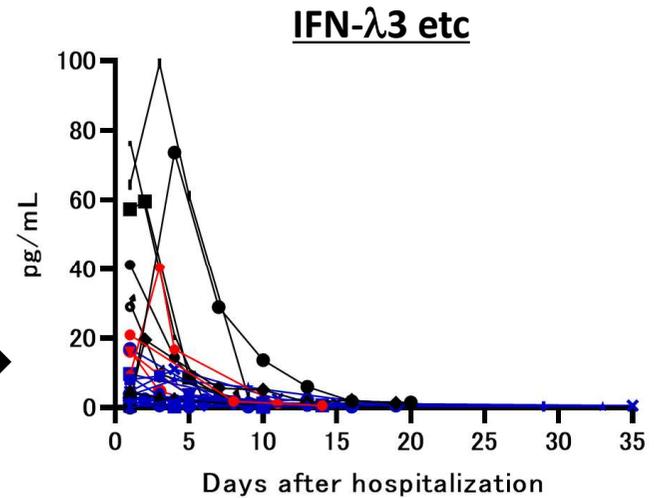
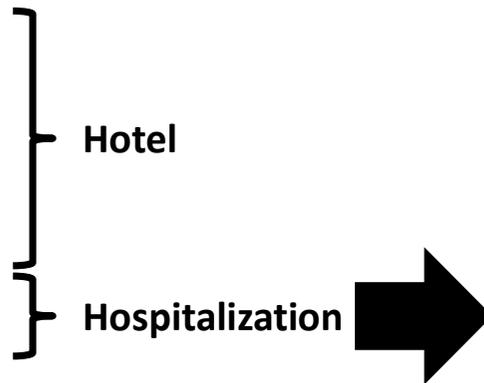
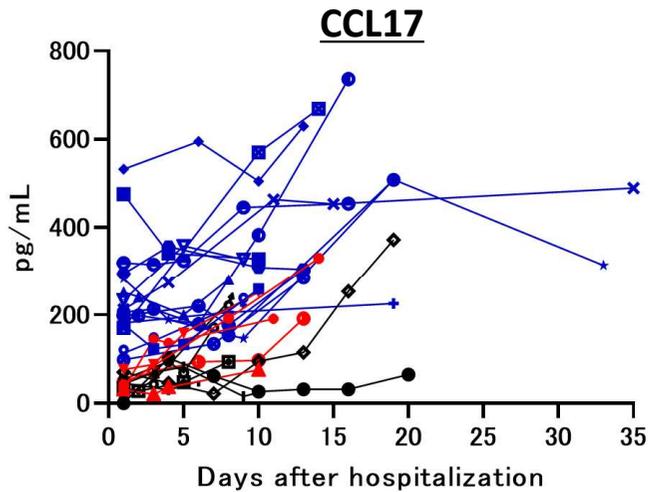
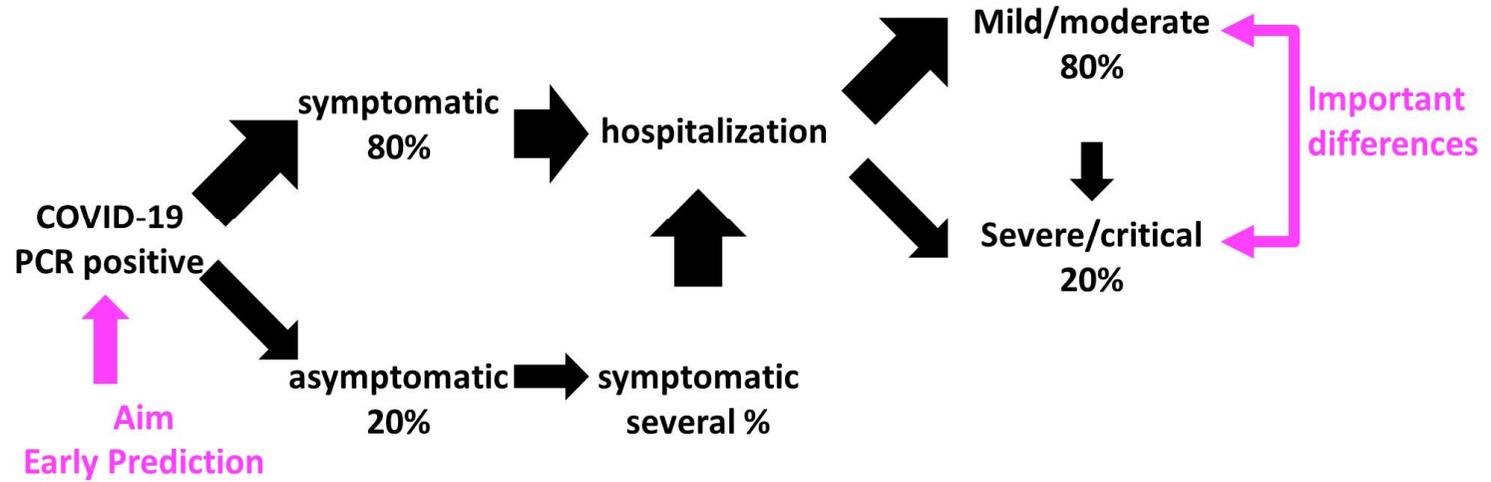
Exclusiveness of each predictive marker



CHC: chronic hepatitis C
 CAP: Child and Adolescent Psychiatry
 T2DM: type 2 diabetes mellitus
 CRF: chronic renal failure
 CHF: chronic heart failure
 IP: interstitial pneumonia
 RA: rheumatoid arthritis

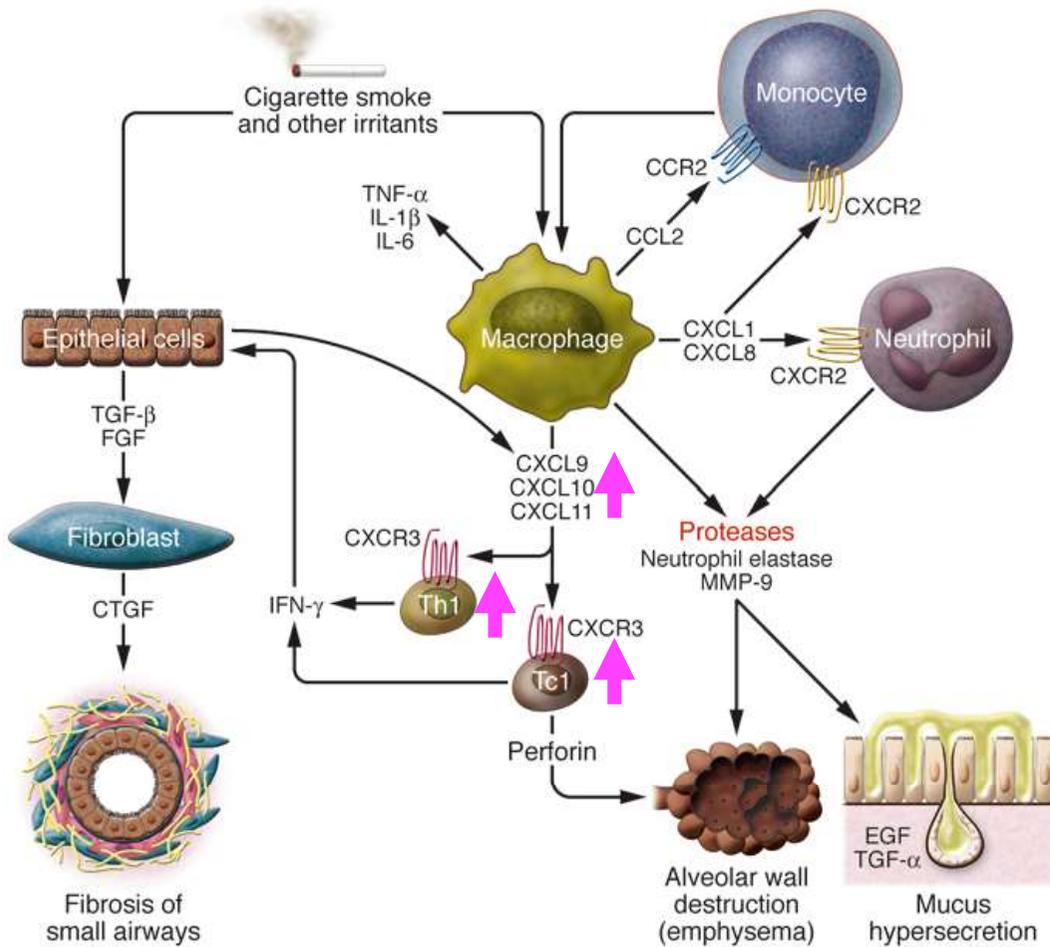
Diagnosis plan

Category A	Category B
CCL17	IFN- λ 3
	IP-10
	CXCL9
	IL-6

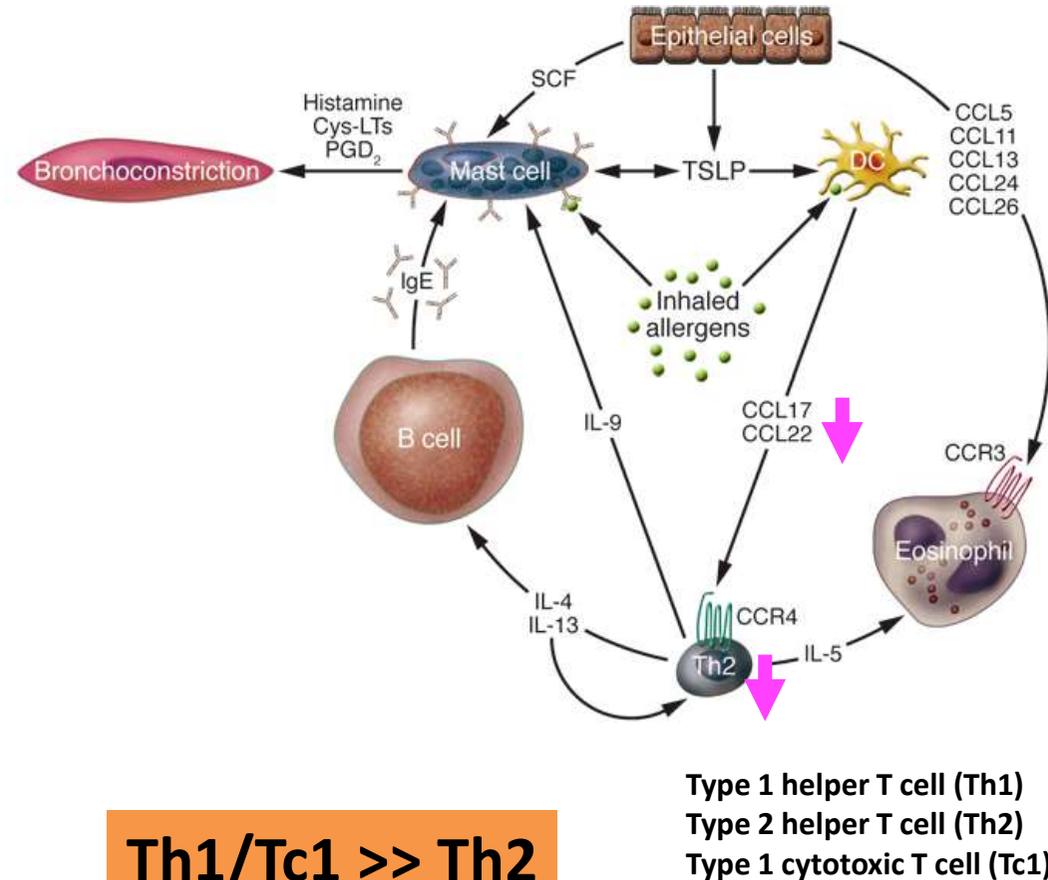


Th1 and Th2 imbalance?

Cytokines involved in COPD



Cytokines involved in asthma



Type 1 helper T cell (Th1)
 Type 2 helper T cell (Th2)
 Type 1 cytotoxic T cell (Tc1)

**Th1/Tc1 >> Th2
 Imbalance**

Future plans

Serum test

- **Patent application for test markers**
- **Multi-center prospective study**
→ **25 leading hospitals**

Drug discovery

- **Animal model**
- **Basic experiment for CCL17 down-regulation and upregulation of IFN- λ 3, IL-6, CXCL9 and CXCL10**