UNIVERSITÀ DEGLI STUDI DI MILANO

Sistema Socio Sanitario
Regione Lombardia
ASST Santi Paolo e Carlo

# Immunopathogenesis of long-COVID

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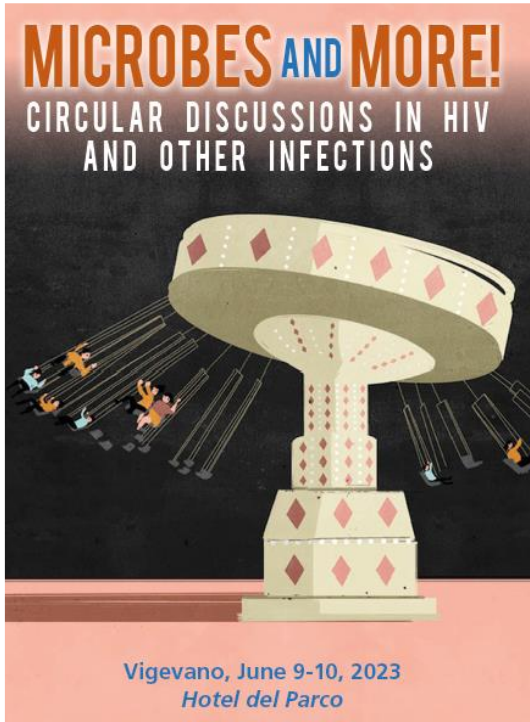

# Presentation outline

1. Background
2. Aim
3. Methods
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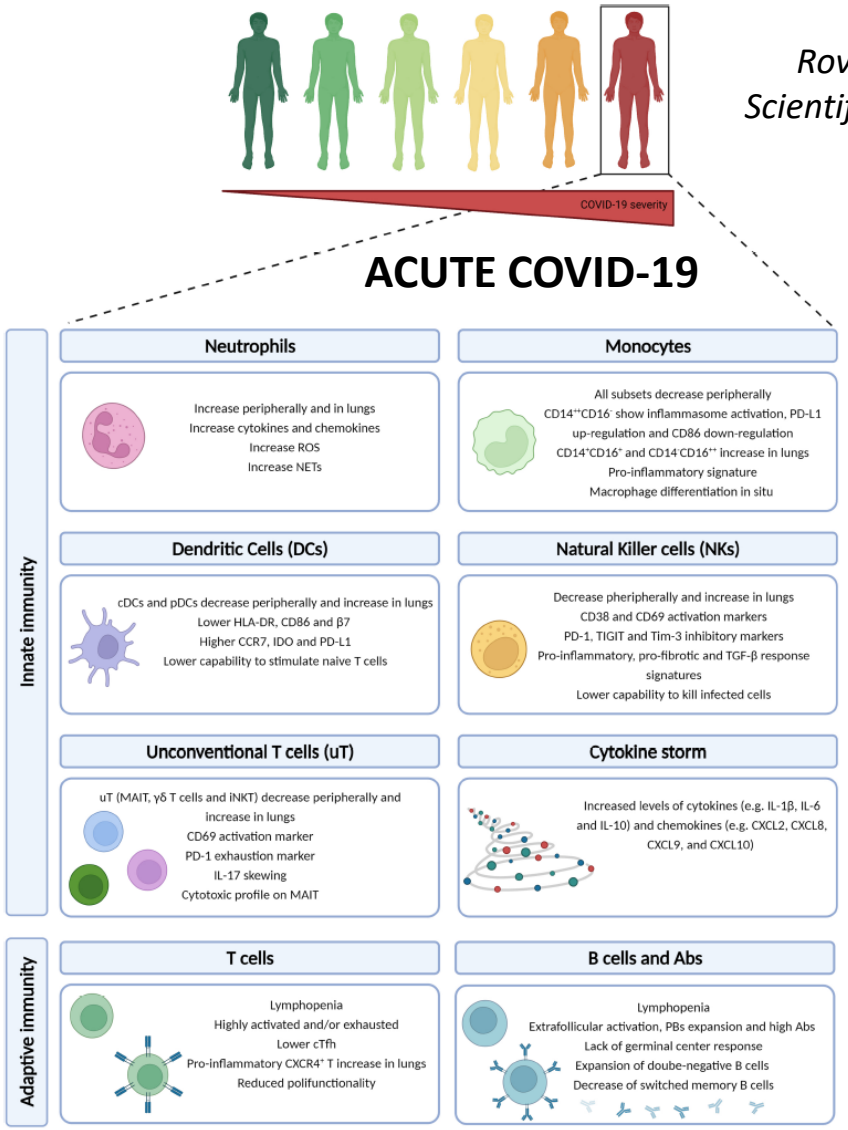
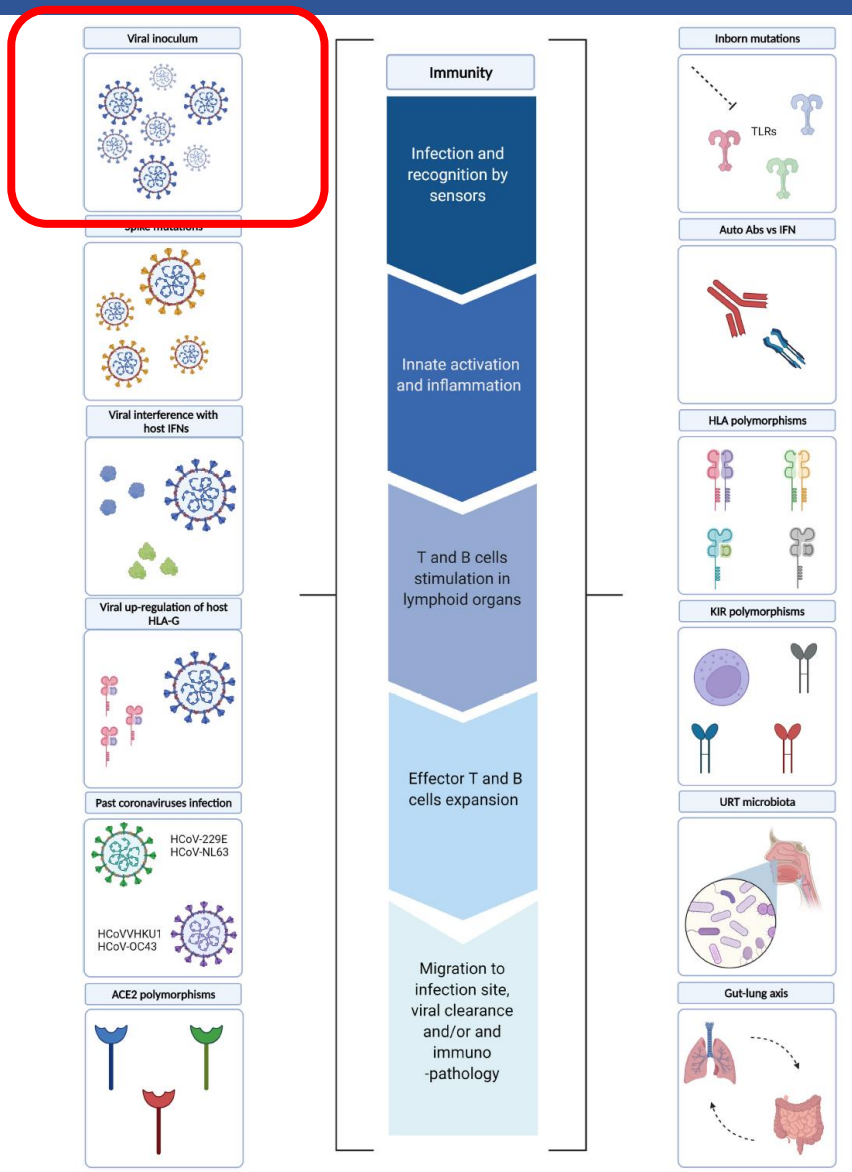
# Background II: acute COVID-19




*Rovito R. et al, Frontiers in Immunology, 2022*

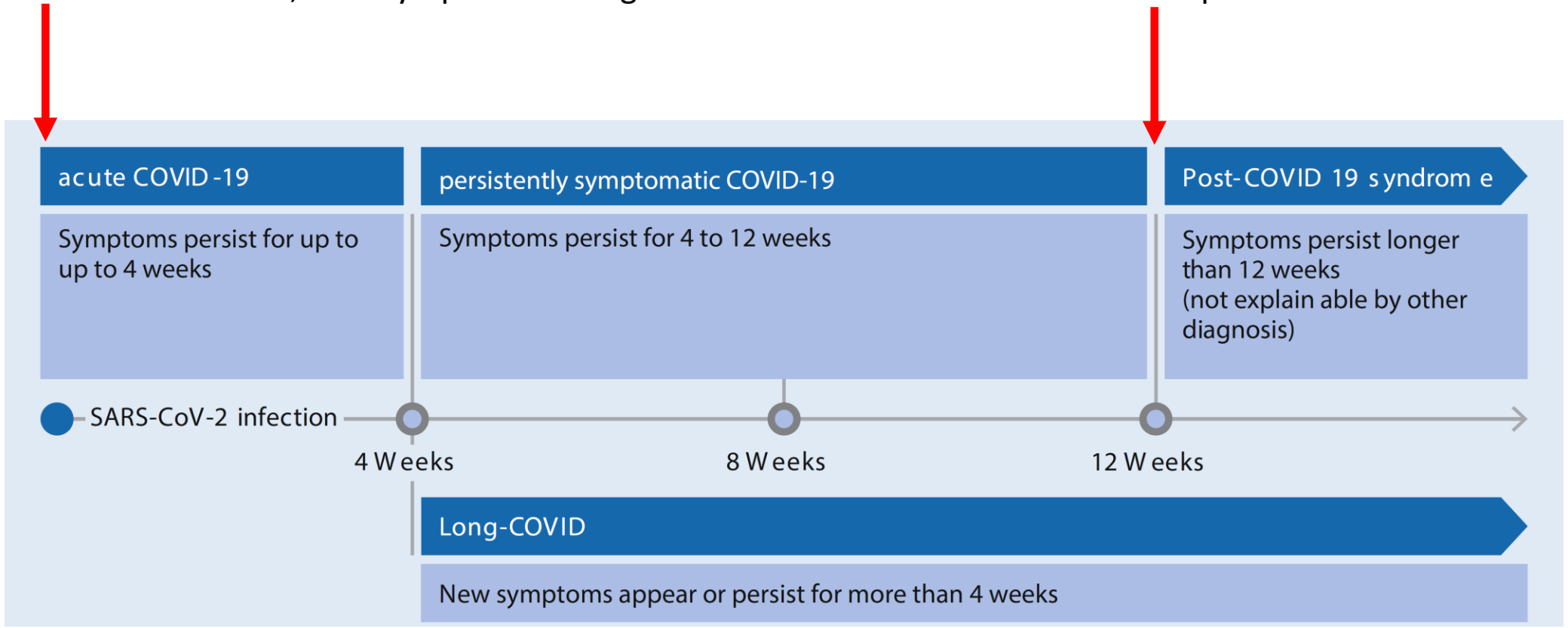
*Rovito R. et al, Scientific Reports, 2022*



# Background II: long-COVID

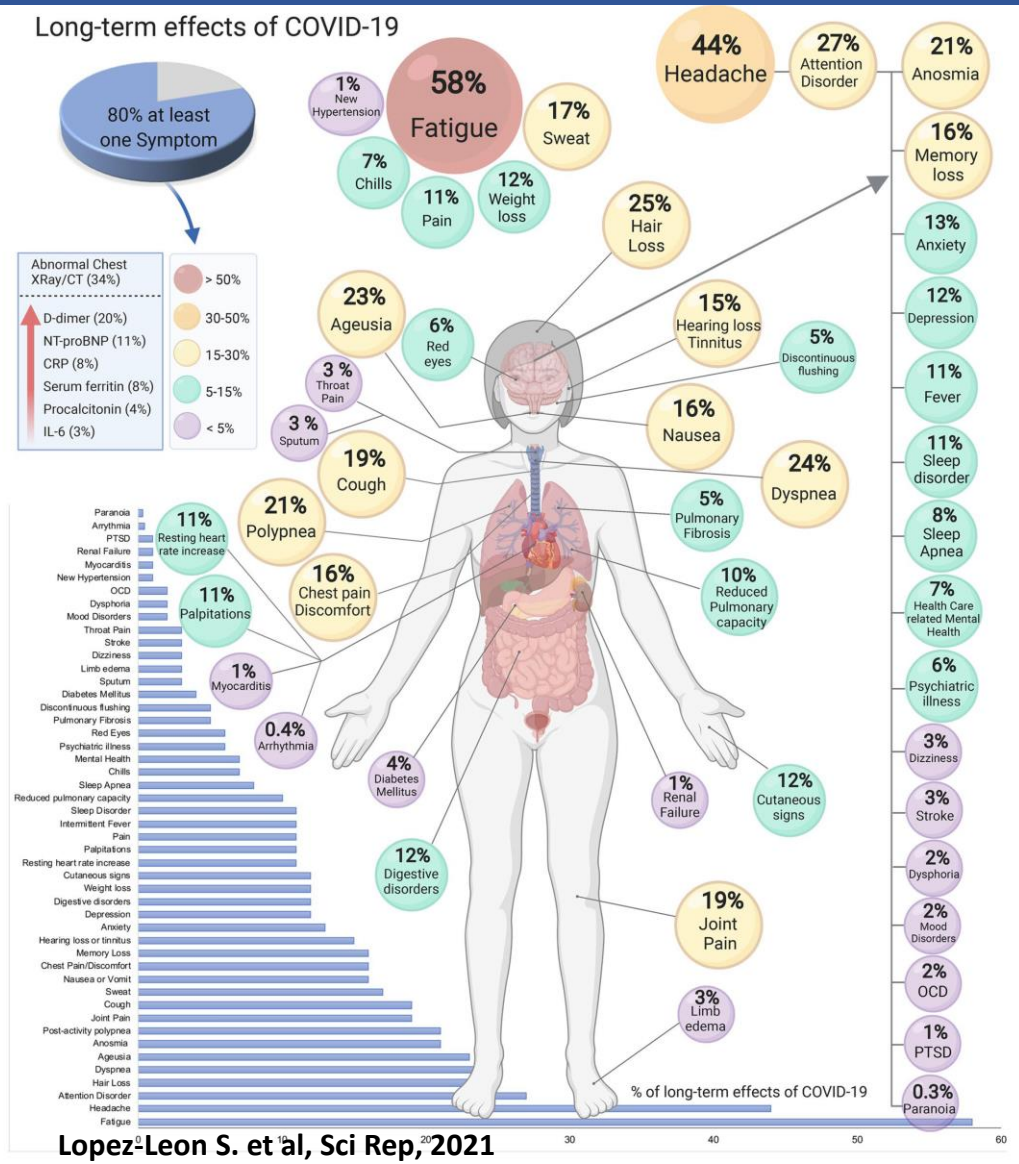
15% of infected individuals → Prolonged symptoms after recovery from acute disease

**Long-COVID definition (WHO):** continuation or development of new symptoms 3 months after the initial SARS-CoV-2 infection, with symptoms lasting for at least 2 months with no other explanation.




*Seifart U., Main Topic, 2023*

# Background III: heterogeneous immundysregulation in long-COVID

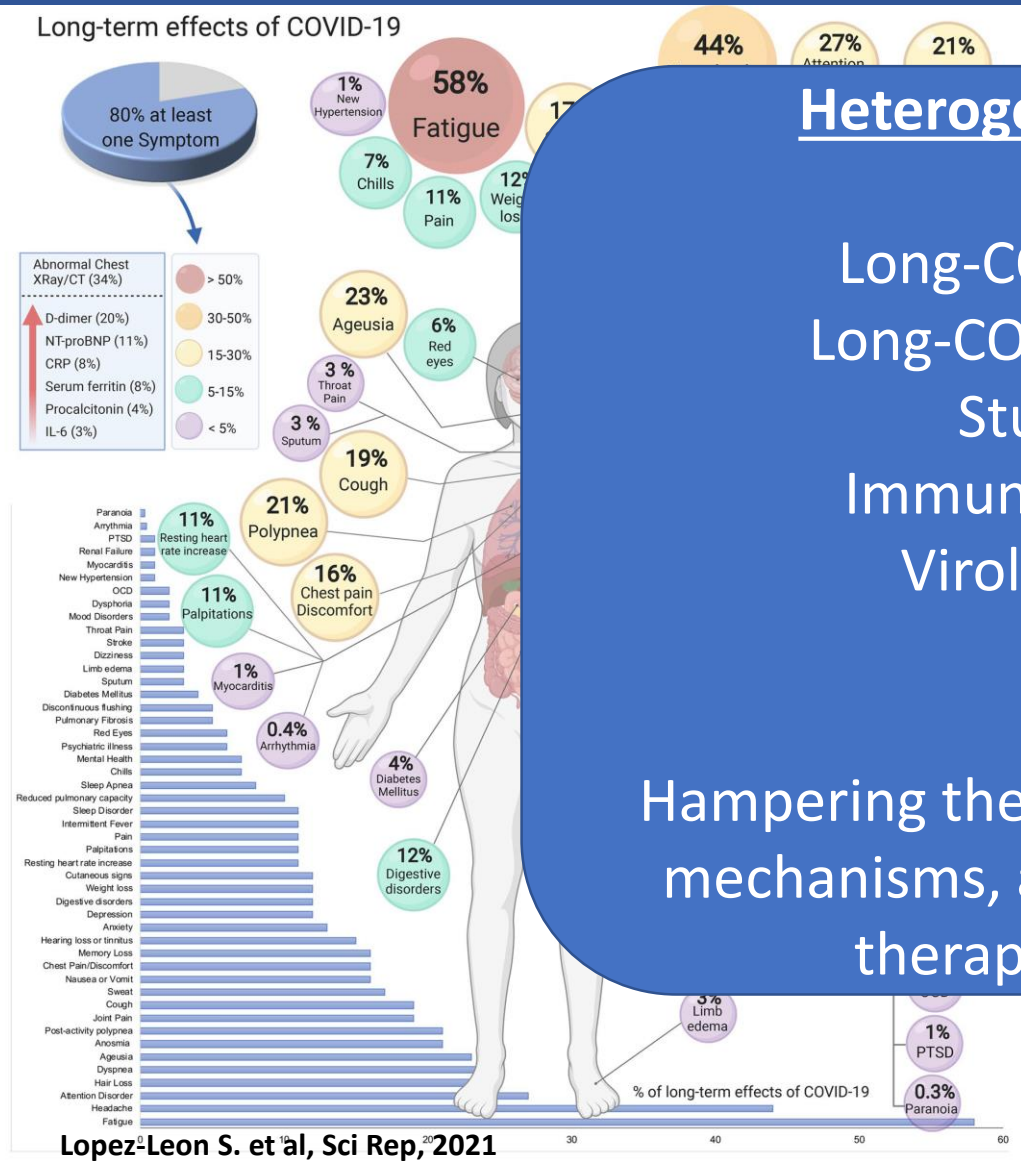



Lopez-Leon S. et al, Sci Rep, 2021

## Heterogeneity of immune dysregulation

- No differences in non-hospitalized COVID-19 patients at 2-11 months (Fang H. et al, Viruses, 2021; Galan M. et al, Front Immunol, 2022);
- Lower magnitude and functionality of SARS-CoV-2-specific humoral responses in hospitalized COVID-19 patients at 12 months (García-Abellán, J et al, Front Immunol, 2022);
- Lower funcitonality of humoral responses and higher cell activation when considering neurologic manifestation at 2-3 months (Spatola M. et al, Brain, 2023);
- Higher IFNs and activated circulating cells, lower lymphocytes functionality in a mixed cohort of hospitalized and non-hospitalized at 6-8 months (Santa-Cruz A. et al, Nat Commun, 2023; Phetsouphanh C. et al, Nat Commun, 2022).

# Background IV: heterogeneous virological parameters in long-COVID




Lopez-Leon S. et al, Sci Rep, 2021

## Heterogeneity is the hallmark

Long-COVID manifestations
Long-COVID time of diagnosis
Study populations
Immunological parameters
Virological parameters

## Downside

Hampering the identification of underlying mechanisms, and consequently of timely therapeutic interventions.

## Heterogeneity of virological parameters

ing the acute phase in a cohort -hospitalized anticipate long- mptoms) at 2-3 months (Y. Su et al,

ed also in urine and stool tients at 1-2 months (Tejerina F. et al,

d Agenemia more likely to be ividuals up to 1 year (V. Craddock et

tected up to 1 year in about individuals who were not e phase (Swank Z. et al, Clin Inf Dis, 2023);

- Discrepancies for treatment and viral strain (MH Chuang et al, Journal of Medical Virology, 2023; P. Bertuccio et al, Infection, 2023; AR Percze et al, Inflammopharmacology, 2023; C. Fernández-de-las-Peñas et al, Viruses, 2022)

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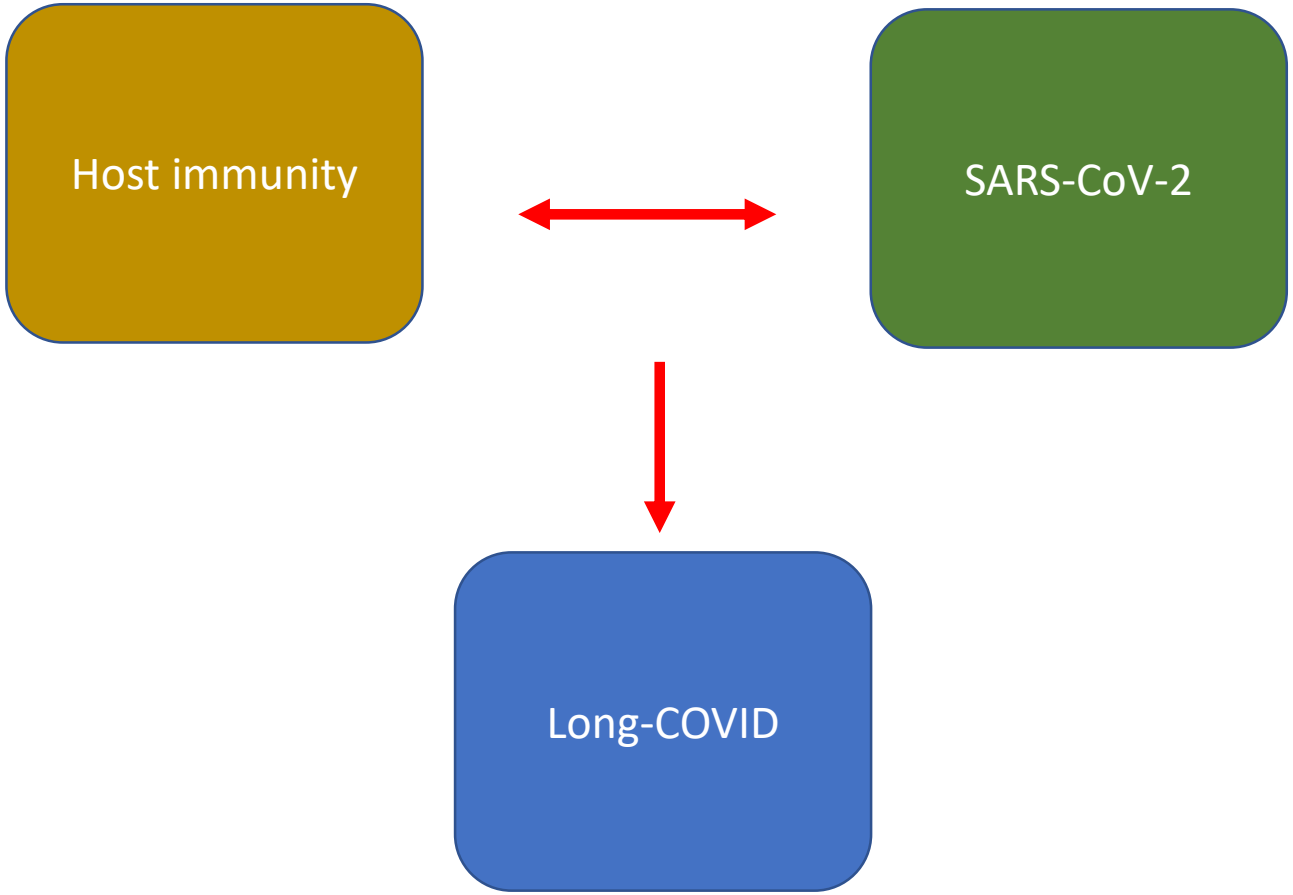
# Aim of the project

Interplay between host immunity and virus on long-COVID development



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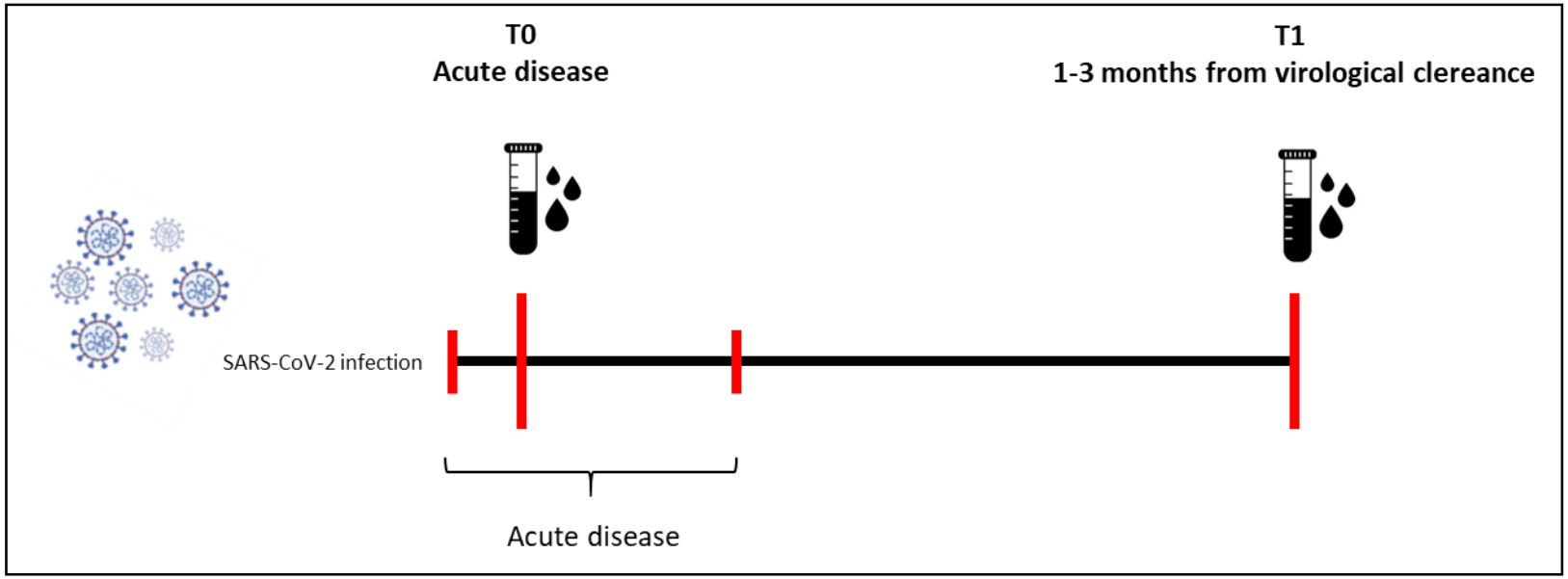
# Methods: study design

1. **Longitudinal study design:** T0, T1




**2. Homogeneous study populations of naive unvaccinated and hospitalized individuals enrolled in 2020:**

- Group 1: individuals developing long-COVID
- Group 2: individuals not developing long-COVID

**3. Long-COVID symptoms:** any phisical symptoms excluding those psicological (fatigue, dyspnea, anosmia/dysgeusia, diarrhea, pain, mnestic disorders)

# Methods: experimental design

| Virologic parameters | Technique | Sample |
|---|---|---|
| Viral load | RT-PCR | Plasma (RNAemia) |

**Statistics:** A) Mann-Whitney test to compare differences between groups at each time point and Wilcoxon test to evaluate dynamics' within each group; B) Selection of significant paramenters associated to long-COVID; C) Spearman's correlations.

| Immunologic parameters | Technique | Sample | Note |
|---|---|---|---|
| RBD-specific total Abs | ELISA (AUC) | Plasma | - |
| Neutralization | Pseudovirus neutralization ($ID_{50}$) | Plasma | - |
| ADCC | FC (%) | Plasma | Antibody-dependent cellular cytotoxicity |
| B cells – RBD-specific B cells | Immunophenotype + Surrogate of Tetramer staining in 1 panel (FC) | PBMCs | B cells: CD3, CD19, CD20/CD38 (PB/PC), CD21/CD27 (Naive CD21+CD27-, classical CD21+CD27+, activated CD21-CD27+), IgM/IgG/IgA/IgD (class-switching), RBD-PE, RBD-PEVio770 (Ag-specificity) |
| T cells + SARS-CoV-2-specific T | Immunophenotype + AIM + ICS in one panel (FC) | PBMCs | CD3, CD4, CD8, CD137/CD69 (AIM), CD45RA/CCR7 (Naïve CD45RA+CCR7+, CM CD45RA-CCR7+, EM CD45RA-CCR7-, EMRA CD45RA+CCR7-), IFN-γ/TNF-α/IL-2 (ICS), CD25/CD127 (Treg), Ki67/PD-1/CD57 (proliferation/exhaustion/senescence), CXCR5 (cTfh), CXCR3/CCR6 (Th1, Th2, Th17 skewing), perforin (degranulaiton) |

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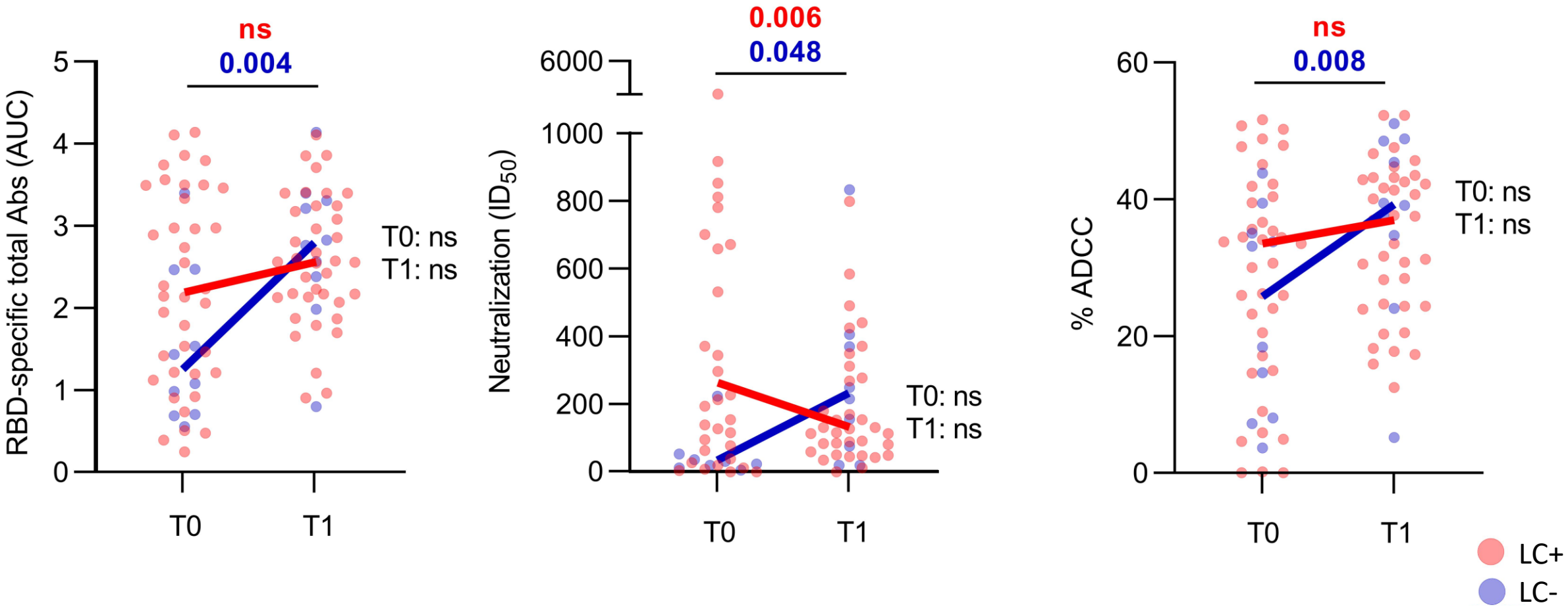
# Results I: study population

| | LC+ (*n*=38) | LC– (*n*=10) | *P* value LC+ *vs* LC–* |
|---|---|---|---|
| **Age**, years [median (IQR)] | 60 (53–66) | 51 (30–63) | 0.1119 |
| **Sex** [*n* (%)] | | | 0.2917 |
| Male | 18 (47.4) | 7 (70) | |
| Female | 20 (52.6) | 3 (30) | |
| **Comorbidities** [*n* (%)] | | | |
| Any comorbidity | 22 (57.9) | 4 (40) | 0.4777 |
| Hypertension | 16 (42.1) | 1 (10) | 0.0744 |
| Chronic heart disease | 4 (10.5) | 1 (10) | >0.9999 |
| Chronic pulmonary disease | 2 (5.3) | 2 (20) | 0.1871 |
| Chronic kidney disease | 1 (2.6) | 0 (0) | >0.9999 |
| Diabetes | 4 (10.5) | 0 (0) | 0.5665 |
| Neurologic disease | 1 (2.6) | 0 (0) | >0.9999 |
| History of cancer | 3 (7.9) | 0 (0) | >0.9999 |
| Obesity (BMI >30) | 10 (26.3) | 3 (30) | >0.9999 |
| **Smoke** [*n* (%)] | | | 0.4587 |
| Yes | 15 (39.5) | 2 (20) | |
| No | 23 (60.5) | 8 (80) | |
| **Symptoms at hospital admission** [*n* (%)] | | | |
| Fever | 35 (92.1) | 10 (100) | >0.9999 |
| Arthromyalgia | 4 (10.5) | 3 (30) | 0.1469 |
| Fatigue | 30 (79) | 8 (80) | >0.9999 |
| Anosmia/dysgeusia | 25 (65.8) | 4 (40) | 0.1639 |
| Sore throat | 1 (2.6) | 2 (20) | 0.1058 |
| Cough | 26 (68.4) | 7 (70) | >0.9999 |
| Chest pain | 2 (5.3) | 2 (20) | 0.1871 |
| Dyspnea | 35 (92.1) | 6 (60) | **0.0266** |
| Vomiting/nausea | 1 (2.6) | 2 (20) | 0.1058 |
| Diarrhea | 6 (15.8) | 2 (20) | 0.6656 |
| **Duration of symptoms before hospital admission**, days [median (IQR)] | 7 (5–10) | 11 (4.5–15.5) | 0.1437 |

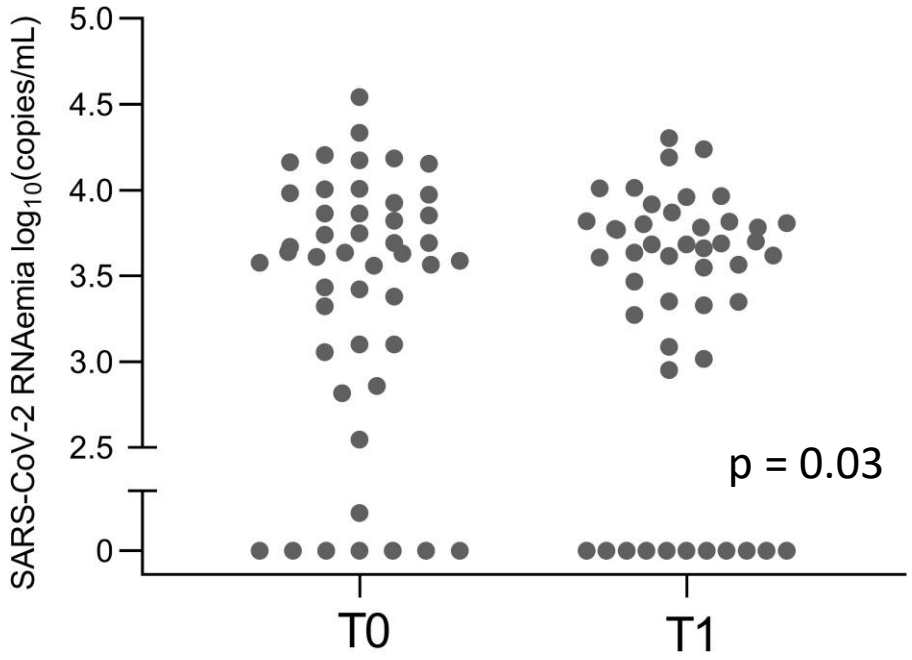
# Results I: study population

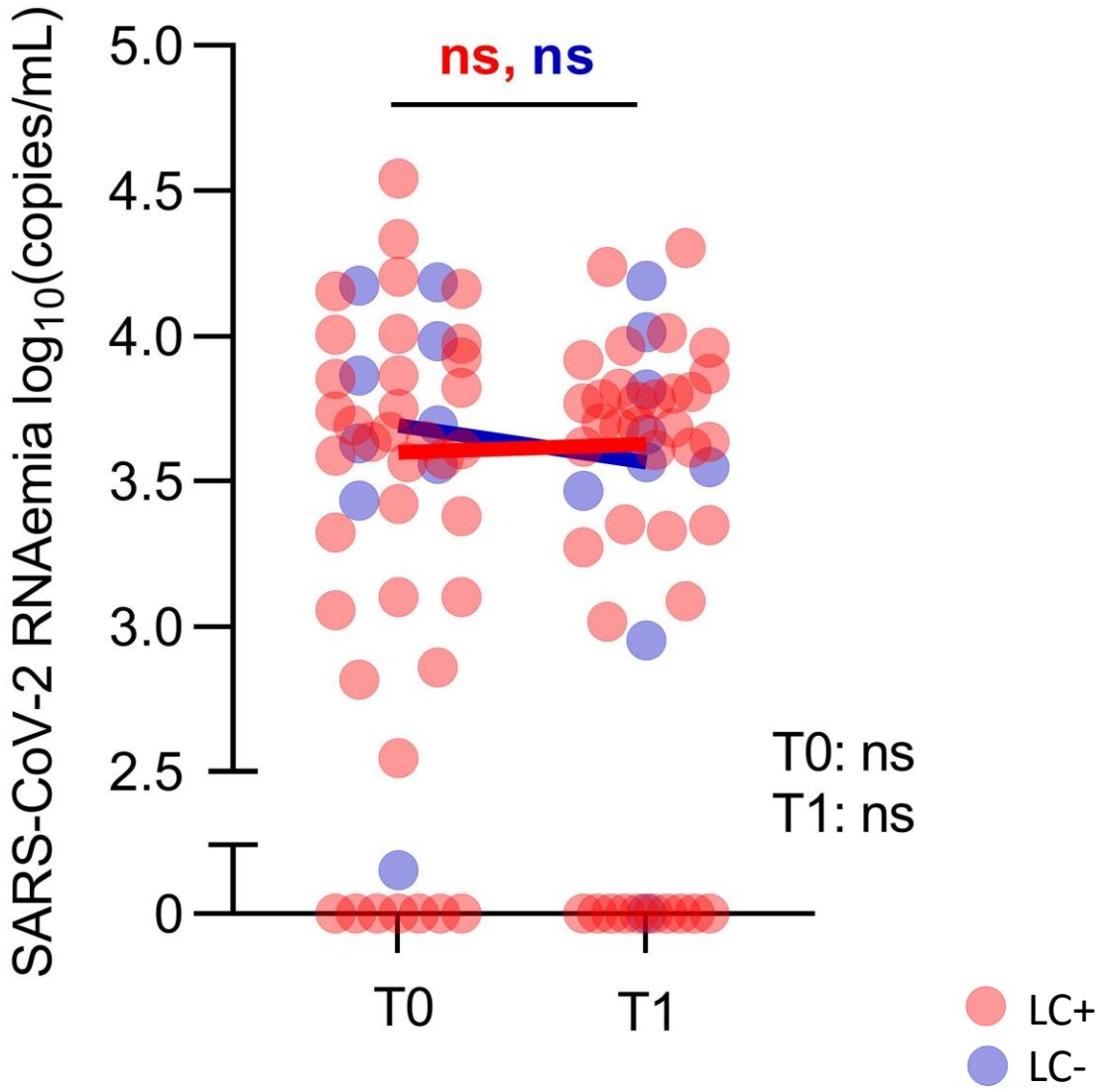
| | LC+ (*n*=38) | LC– (*n*=10) | *P* value LC+ *vs* LC–* |
|---|---|---|---|
| **Radiologically documented pneumonia** [*n* (%)] | 36 (94.7) | 9 (90) | 0.5123 |
| **Maximum oxygen therapy** [*n* (%)] | | | 0.5774 |
| None | 2 (5.3) | 1 (10) | |
| Low-flow systems | 14 (36.8) | 5 (50) | |
| CPAP/NIV/OTI | 22 (57.9) | 4 (40) | |
| **Follow-up symptoms** | | | |
| **Number of symptoms** [*n* (%)] | | - | - |
| 1 | 14 (36.8) | - | - |
| 2 | 12 (31.6) | - | - |
| 3 | 9 (23.7) | - | - |
| 4 | 2 (5.3) | - | - |
| **Fatigue** [*n* (%)] | 27 (71.1) | - | - |
| **Dyspnea** [*n* (%)] | 16 (42.1) | - | - |
| **Anosmia/dysgeusia** [*n* (%)] | 4 (10.5) | - | - |
| **Diarrhea** [*n* (%)] | 3 (7.9) | - | - |
| **Pain** [*n* (%)] | 11 (28.9) | - | - |
| **Mnestic disorders** [*n* (%)] | 12 (31.6) | | |

# Results II: impaired humoral response in long-COVID

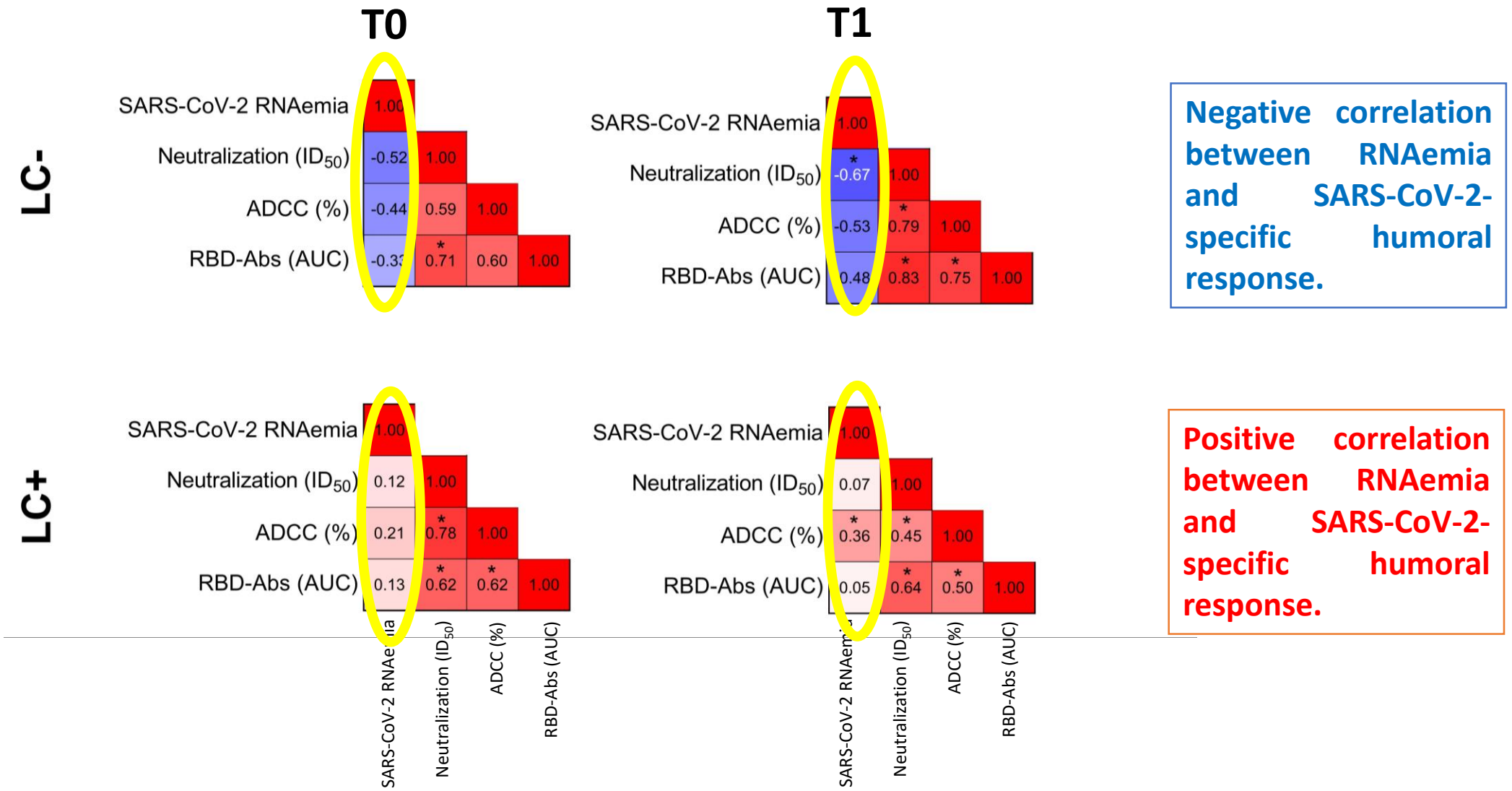


# Results III: comparable RNAemia in LC+ and LC-



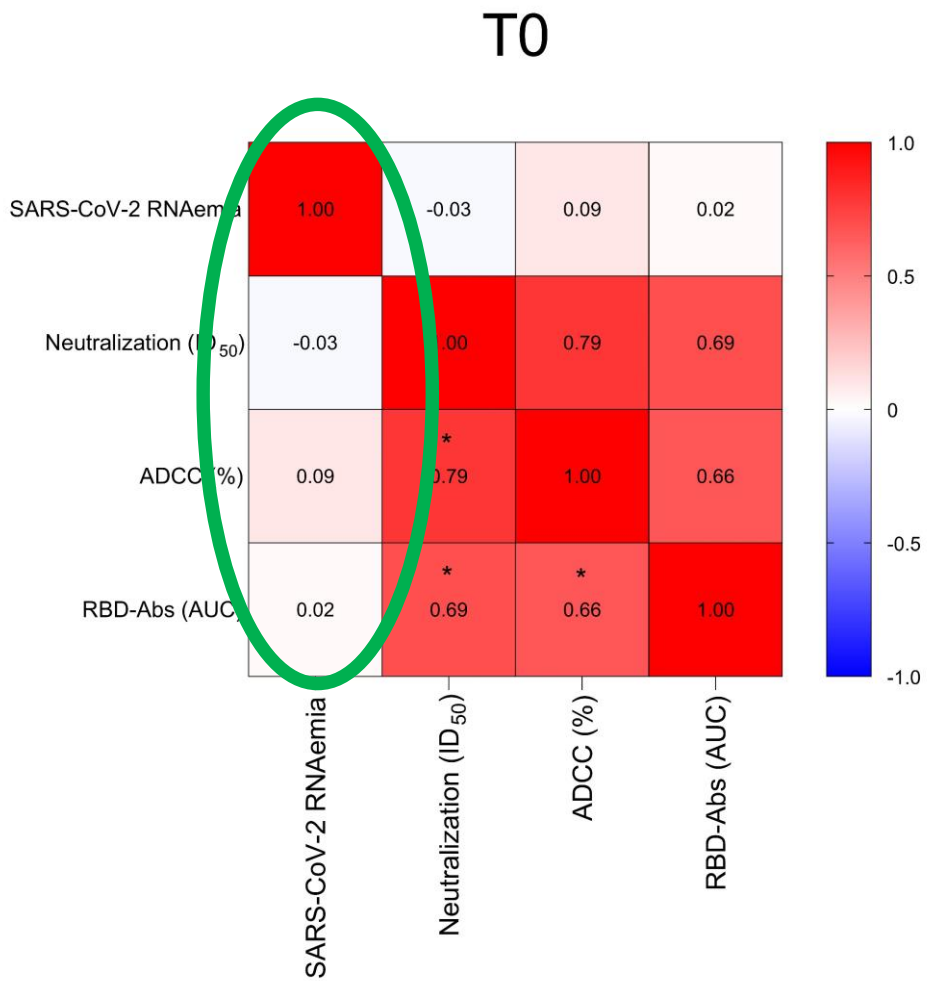
# Results IV: humoral impairment associates to RNAemia

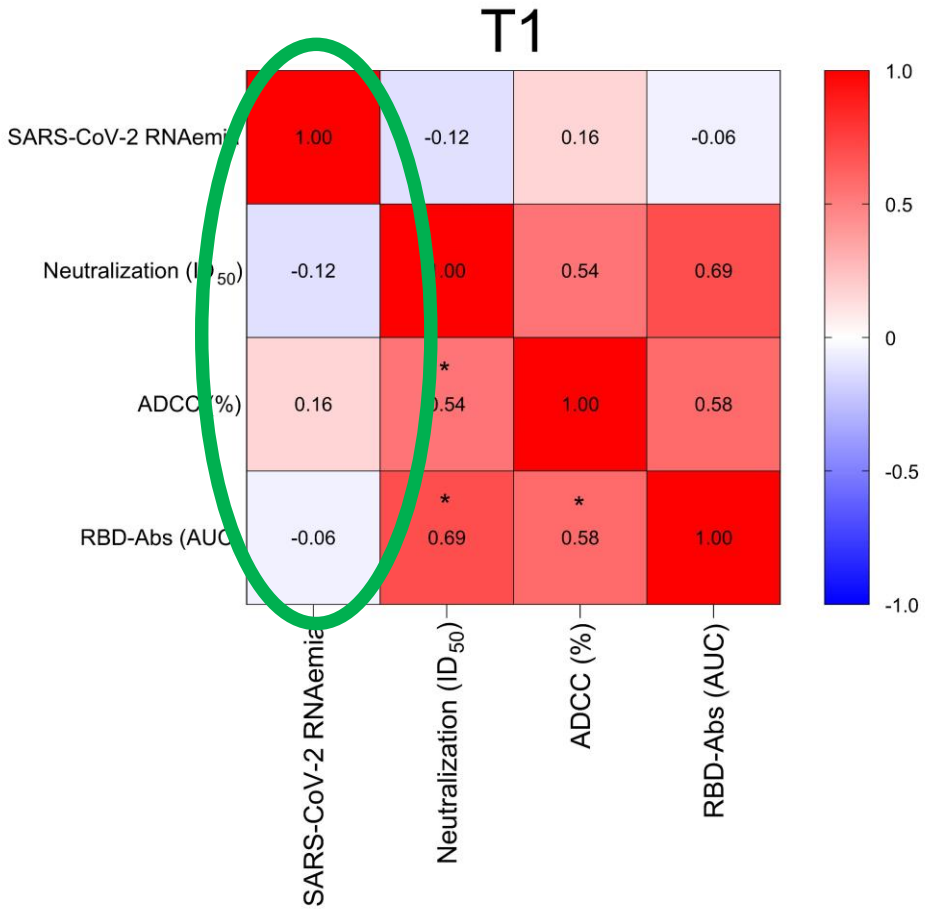



**Negative correlation between RNAemia and SARS-CoV-2-specific humoral response.**

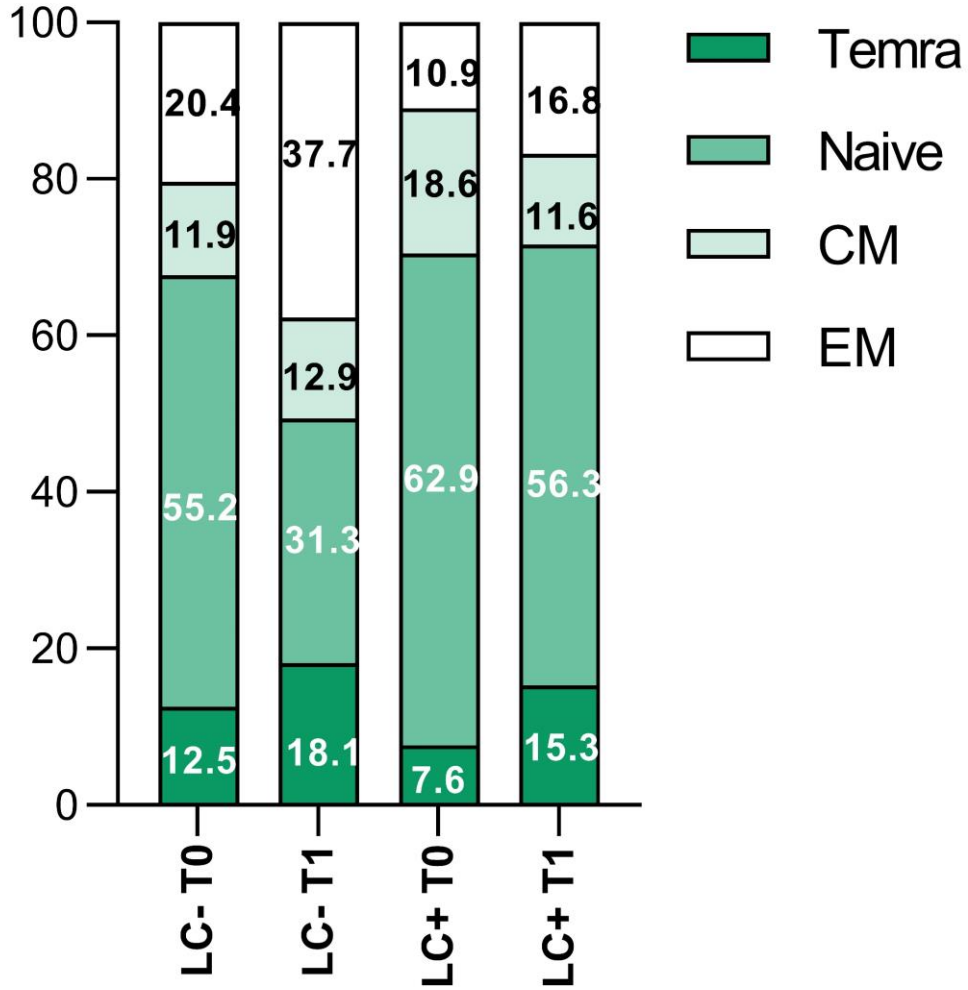
**Positive correlation between RNAemia and SARS-CoV-2-specific humoral response.**

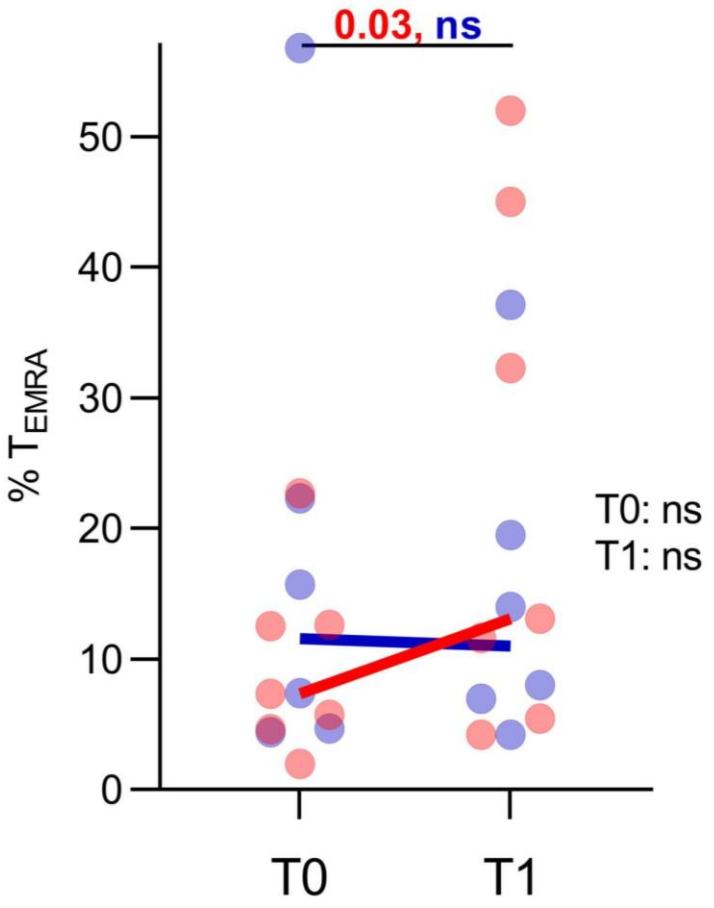
# Results V: overall no correlation between humoral response and RNAemia



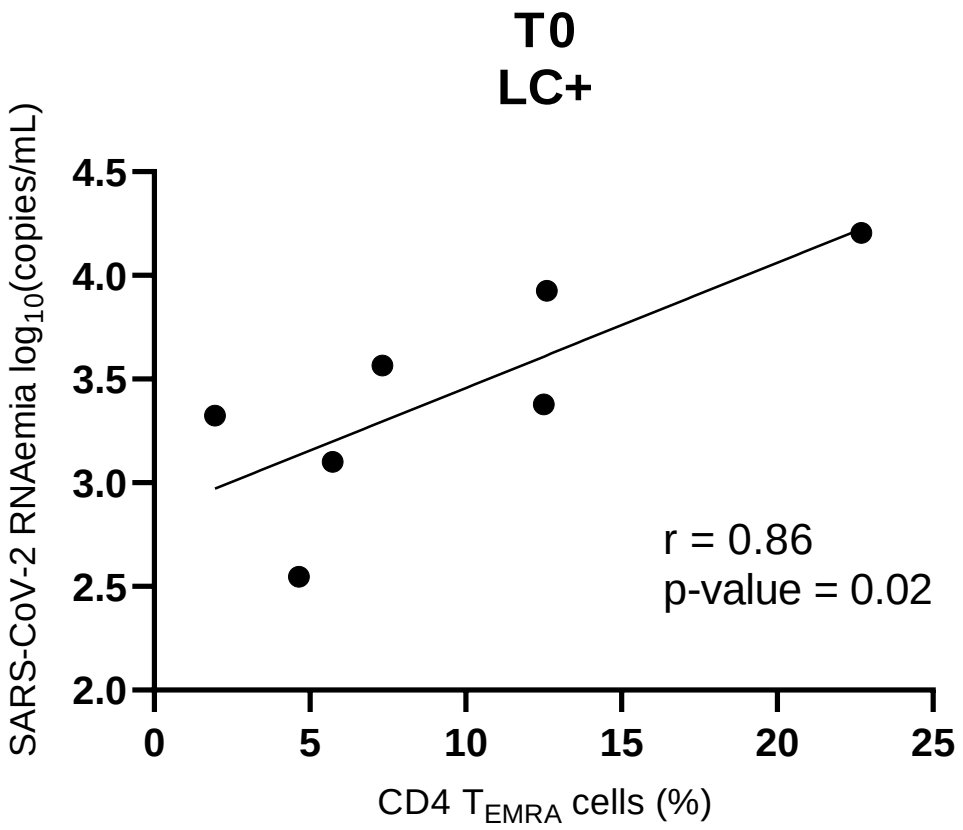
# Results VI: CD4 $T_{EMRA}$ cells associates with long-COVID

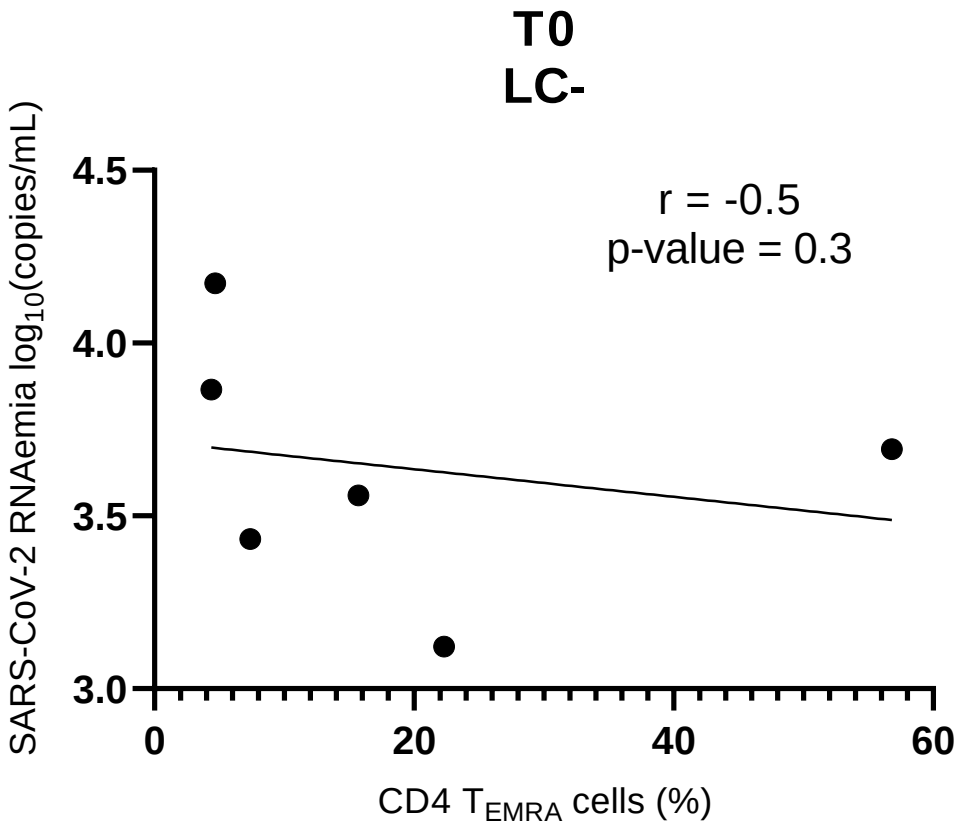


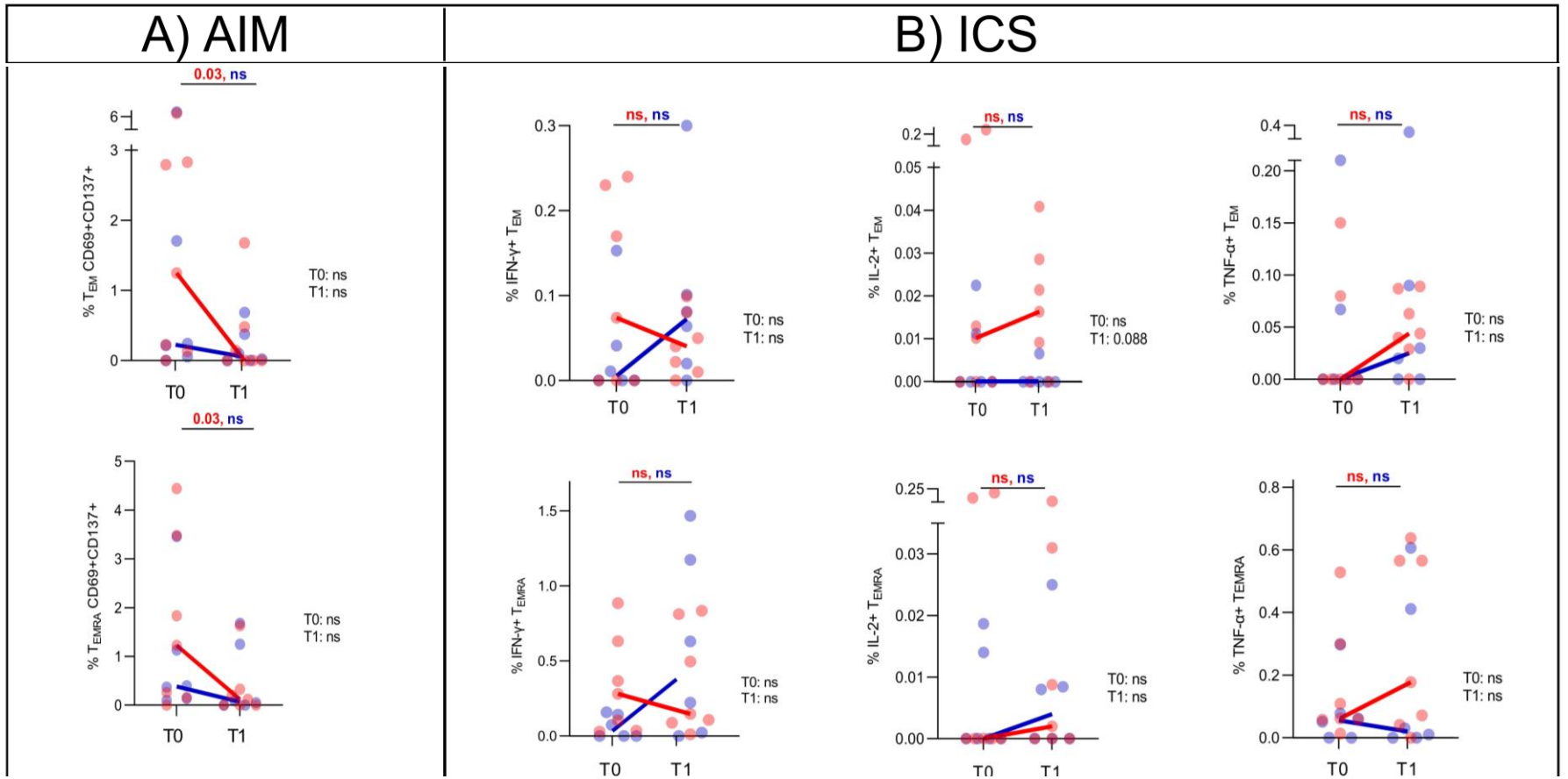

LC+
LC-

# Results VII: … and RNAemia



# Results VIII: impaired SARS-CoV-2-specific CD4 response associates to long-COVID but not RNAemia




LC+

LC-

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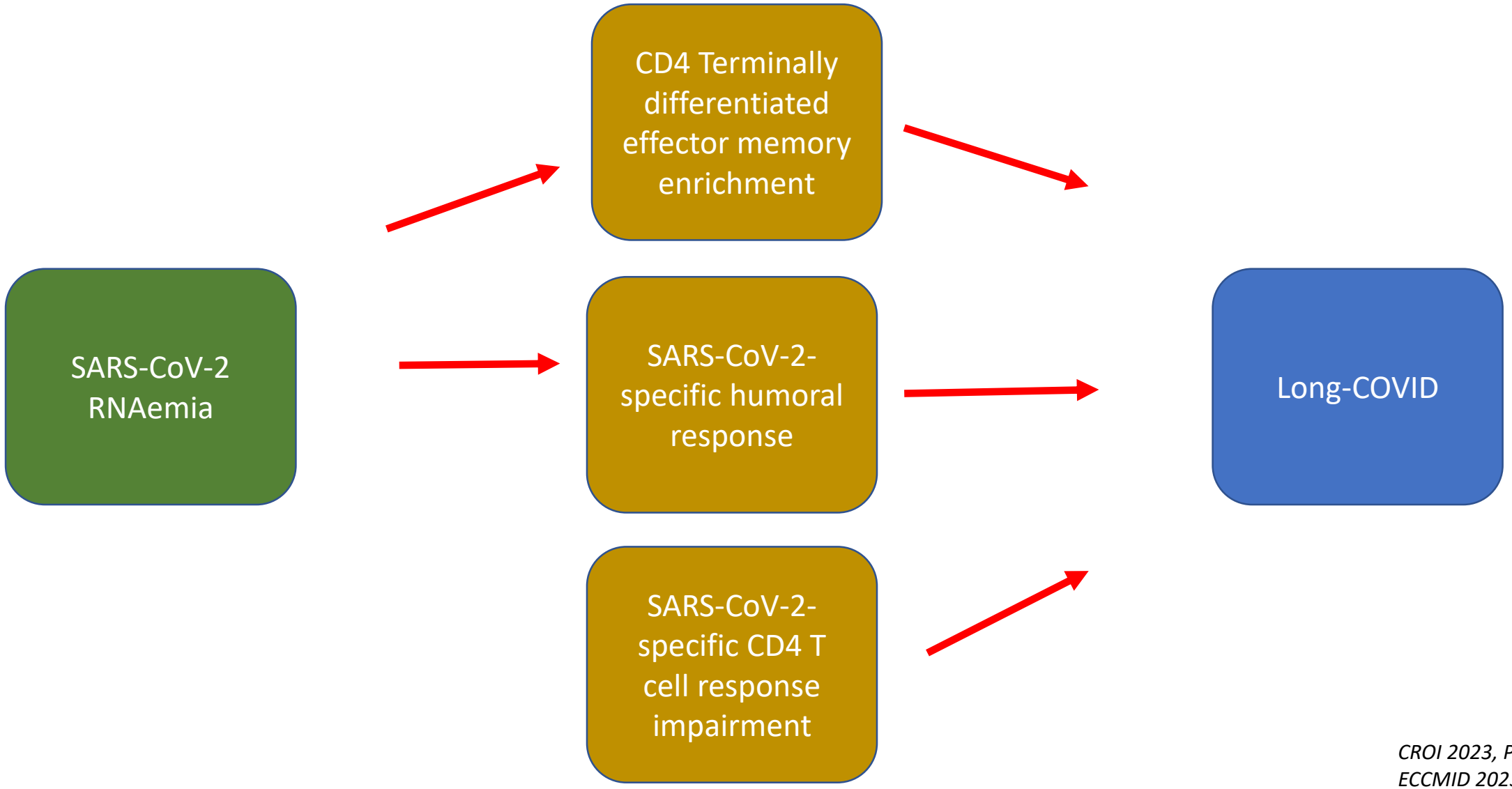


*CROI 2023, Poster presentation*
*ECCMID 2023, Poster presentation*

# Acknowledgements


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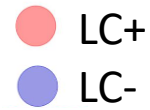
- All clinical staff
- Patients and their families


LC+
LC-