Metabolomics and Integrative omics: from data production to analysis

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BARI

Metabolomics approach to identify antiviral defense mechanisms in leukocytes showing in vitro igG memory from SARS-CoV-2-infected patients

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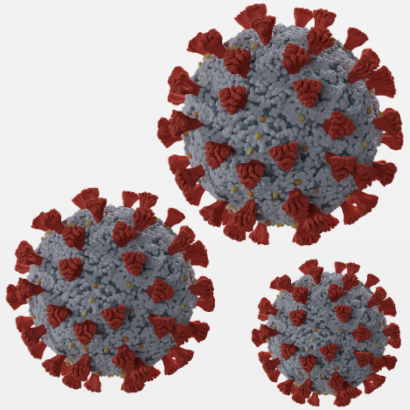

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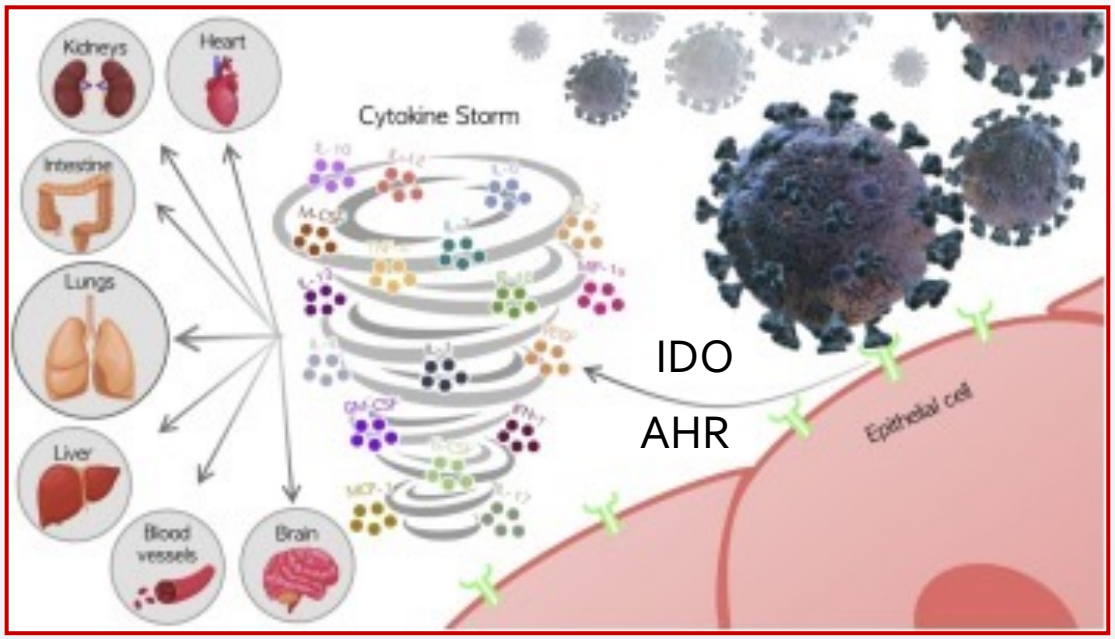
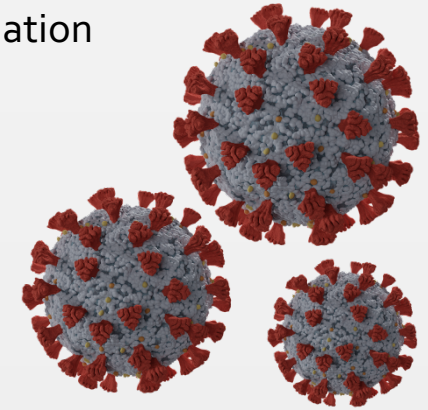
Coronavirus disease 2019 (COVID-19), the highly contagious viral illness caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has had a catastrophic effect on the world's demographics resulting in more than 3.8 million deaths worldwide, emerging as the most consequential global health crisis since the era of the influenza pandemic of 1918



*The viral infection stimulates immune responses, also directed against the spike protein (S1 protein) present on the surface of SARS-Cov-2, being a ligand that binds to ACE2 receptor in host cells

*The attachment of SARS-CoV-2 spike glycoprotein with angiotensin-converting enzyme 2 (ACE2), as its cellular receptor, triggers complex molecular events that leads to hyperinflammation



In severe conditions, excessive and uncontrolled production of pro-inflammatory cytokines including IL-6, IL-1, and TNF-α results in convergence of immune cells and leads to systemic inflammatory response, known as ***cytokine storm***

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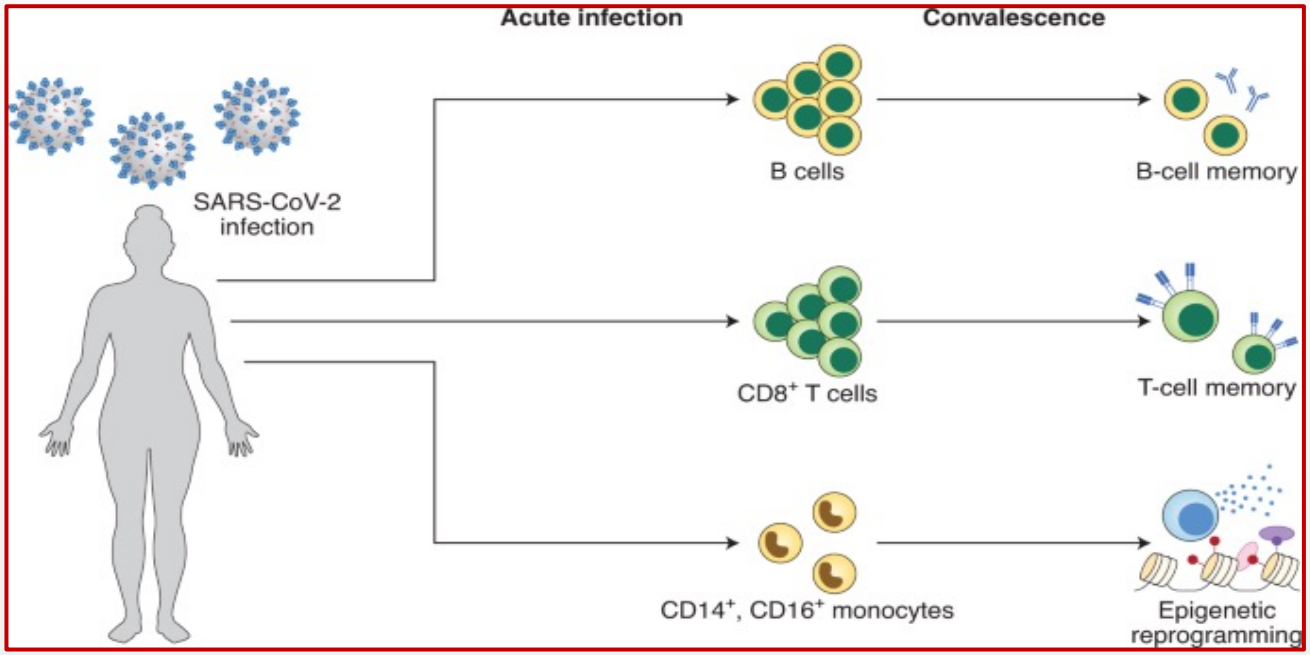
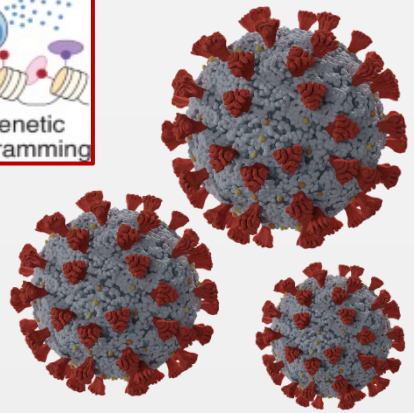
Secretion of such cytokines and chemokines attracts immune cells, notably monocytes and T lymphocytes, from the blood into the infected site. In the respiratory tract, a recruitment of immune cells and lymphocytes from the blood might explain the lymphopenia and increased neutrophil–lymphocyte ratio seen in around 80% of patients with SARS-CoV-2 infection, and after contact with viral antigens most of the effector T cells undergo apoptosis.



A pool of memory T cells is formed to fight reinfection. In the case of a subsequent infection, CD4+ memory T cells become restimulated, activate B cells and other immune cells by producing cytokines, with the help of CD8+ memory T cells that kill virus-infected cells.



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Among the host responses, the metabolic changes and difference in leukocyte composition can be found during various stages of SARSCoV-2 infection, suggesting that monocytes, neutrophils and T-lymphocytes are associated with the onset and progress of COVID-19 infection.

Because immune responses are tightly connected to metabolic programs multiple approaches are currently used to identify key pathways involved in SARS-CoV-2 infection.

- > 70 metabolites including amino acids, lipids, polyamines, sugars, have been discovered to be altered in the plasma of Covid-19 patients with severe symptoms
- cytosine (reflecting viral load), kynurenine (reflecting host inflammatory response), nicotinuric acid, and multiple short chain acylcarnitines (energy metabolism) were altered

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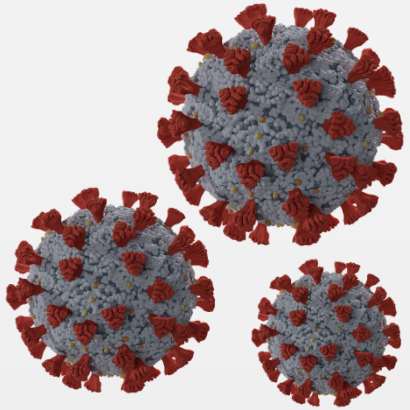
Despite an increasing knowledge on metabolic changes in Covid-19 patients, little attention has been paid to post-infection stages (>60 days) where immune memory becomes responsible to protect against possible SARS-CoV-2 reinfection.



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To better elucidate the underlying biochemistry of leukocytes response, we focused our analysis on the possible relationships between SARS-CoV-2 post-infection stages and distinct metabolic pathways.

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To this regard we performed metabolomics profiling from 41 cell cultures of peripheral blood mononuclear cells (PBMC), 17 of which displayed an *in vitro* IgG memory for spike-S1 antigen 60-90 days after infection determined by a cell-ELISA assay.

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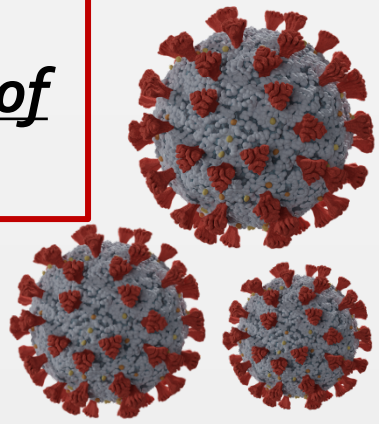
The aim of this preliminary study was to investigate possible relationships between leukocytes displaying an IgG antibody memory to SARS-Cov-2 and their metabolic profile

detect possible metabolites involved in late stages of antiviral defences



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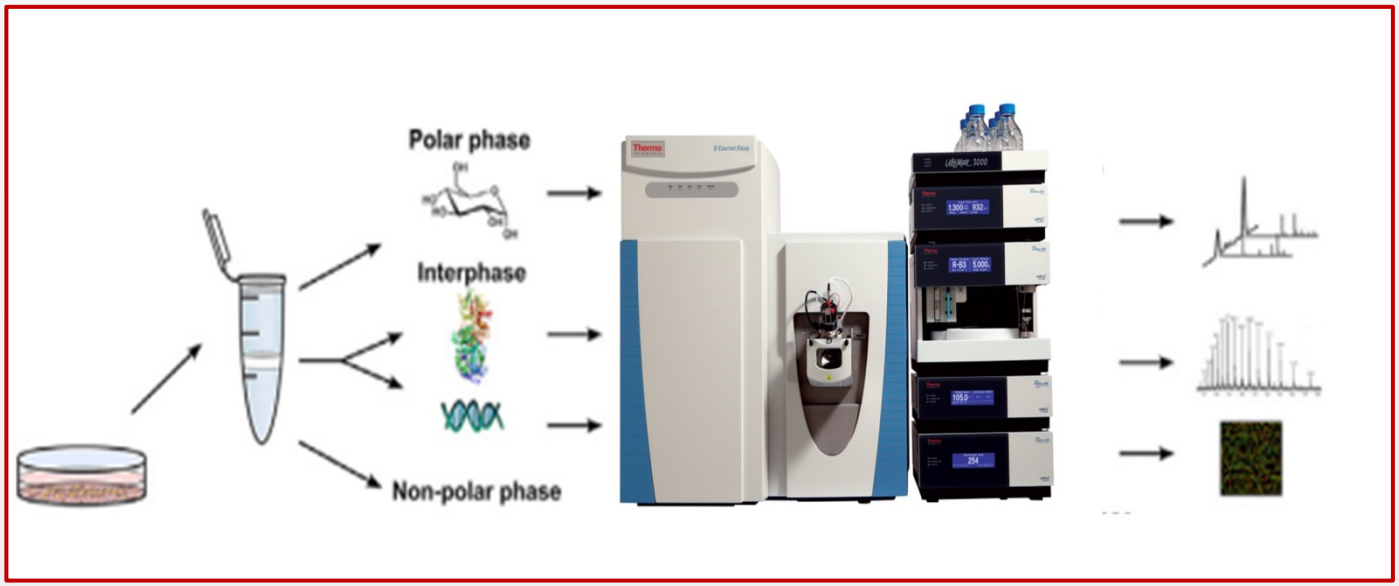
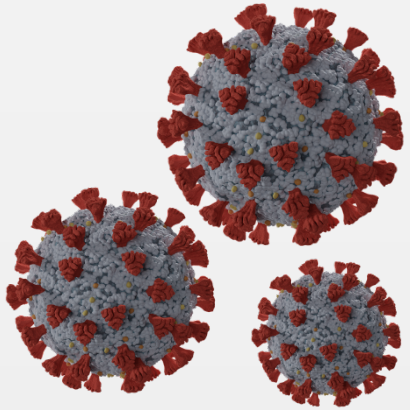
41 subjects of different ages (ranging from 30 to 81 years) undergoing SARS-Cov-2 serological analysis (Centro Polispecialistico Giovanni Paolo I, Viterbo, I) were enrolled in this study from October 2020 to March 2021.

- Determination of in vitro IgG B cell memory for spike-S1 virus protein and of specific IgG were performed by Cell-ELISA assay in PBMC
- 17 resulted positive to *in vitro* specific IgG secretion (IgG^+) and 24 were negative (IgG^-).
- Parallel cultures of PBMC employed in Cell-ELISA were incubated then centrifuged and pelleted cells immediately employed for ***metabolomic analysis.***





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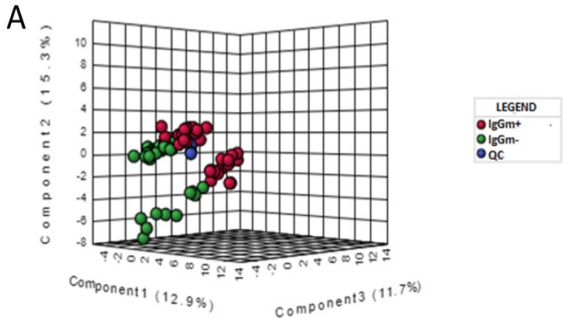

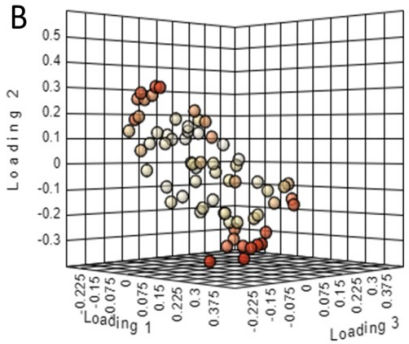

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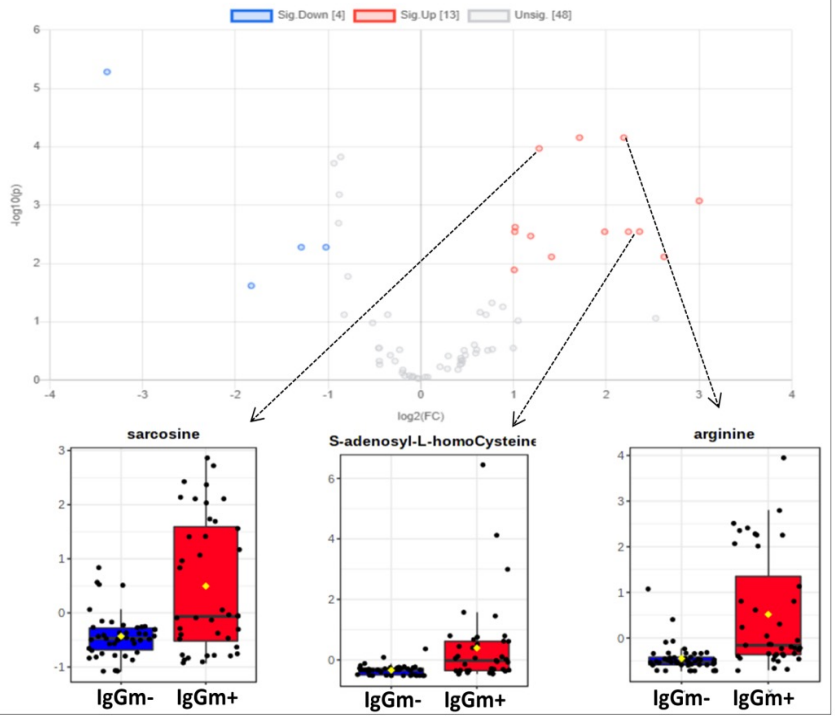
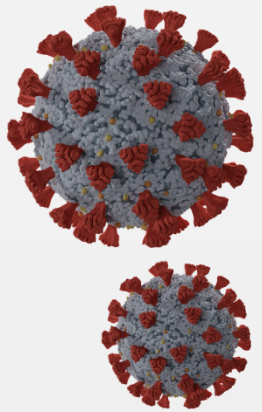
FC	log2(FC)	p.ajusted	-LOG10(p)
0.095896	-3.3824	5.21E-06	5.2834
0.28175	-1.8275	0.024007	1.6197
0.4093	-1.2888	0.005252	2.2797
0.49251	-1.0218	0.005252	2.2797
2.0117	1.0084	0.012858	1.8908
2.0189	1.0136	0.00285	2.5451
2.0253	1.0181	0.002384	2.6226
2.2742	1.1854	0.003381	2.471
2.4232	1.2769	0.000107	3.9718
2.6576	1.4101	0.007692	2.114
3.2763	1.7121	6.96E-05	4.1577
3.9551	1.9837	0.00285	2.5451
4.5615	2.1895	6.96E-05	4.1577
4.7237	2.2399	0.00285	2.5451
5.1347	2.3603	0.002837	2.5472
6.1674	2.6247	0.007692	2.114
8.018	3.0032	0.000844	3.0738



We use MetaboAnalyst 5.0 platforms to perform untargeted metabolomics analysis to identify in IgG⁺-PBMC possible alterations of relevant metabolites, on the basis that the two groups resulted well clustered by the supervised PLS-DA



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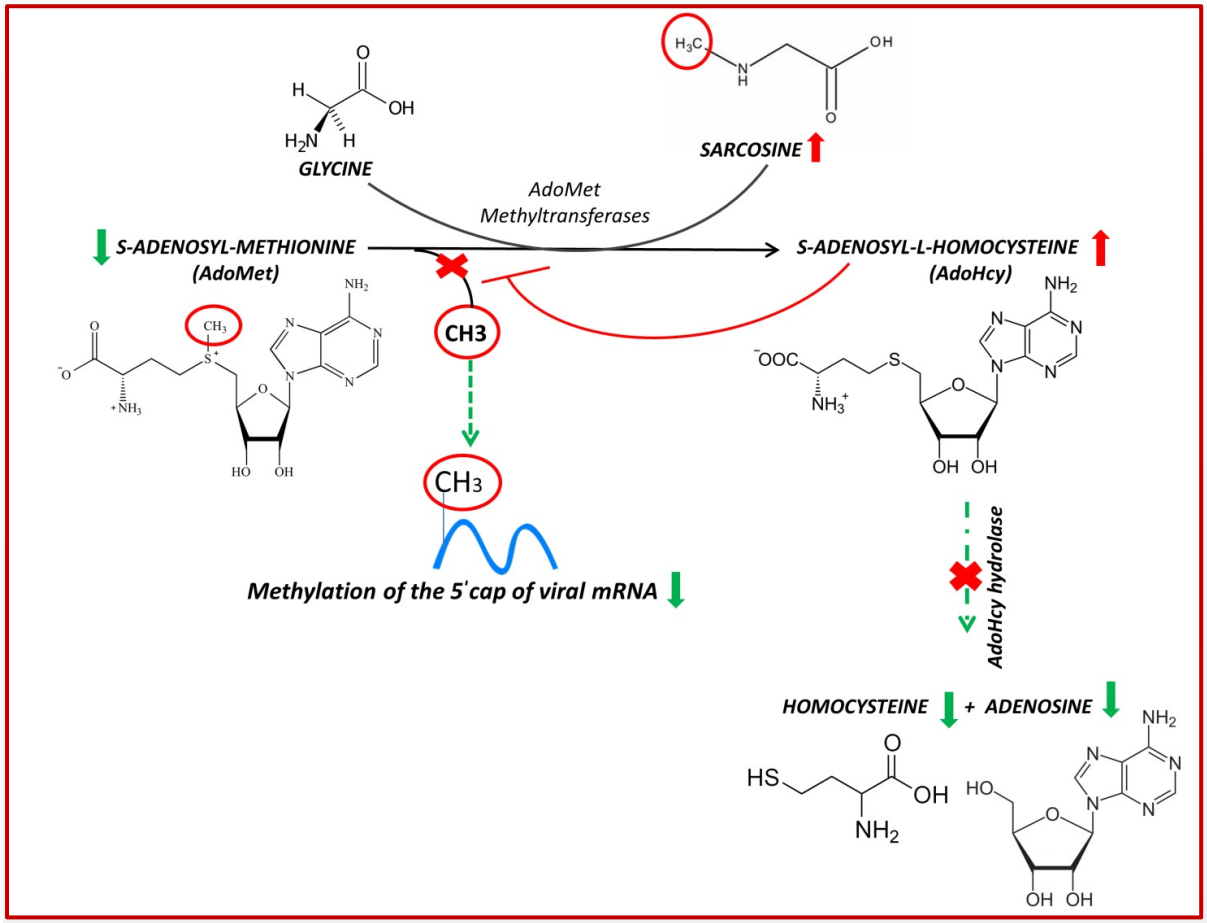
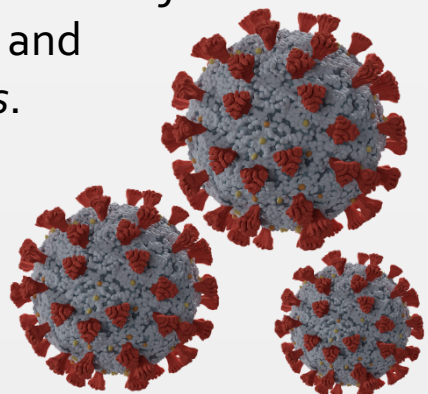
The ratio of AdoMet to AdoHcy is considered as a metabolic gauge controlling in vivo methylation reactions, where a decrease in this ratio predicts reduced methylation capacity.



The strong increase of AdoHcy (which is a potent inhibitor of methyl reaction) allowed us to hypothesize an inhibition of s-adenosyl-L homocysteine hydrolase, and consequently leukocytes may proceed to virus elimination.

The inhibition of this latter mechanism is a target in antiviral strategies employed to fight both *African swine fever virus* and *Ebola virus*.



Blocking capping viral mRNAs could be a potential target for antiviral therapies, due to the translation of viral proteins

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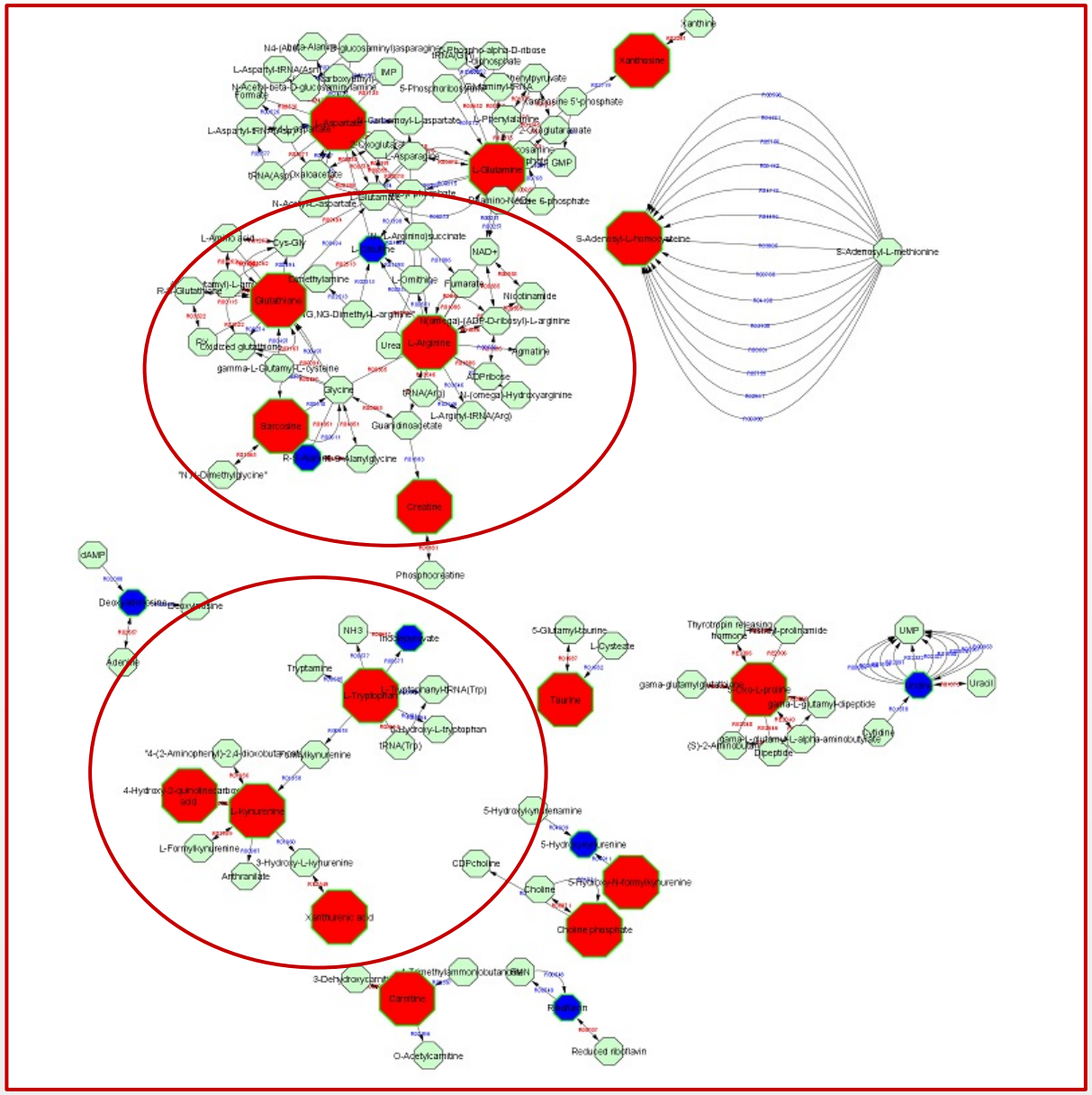
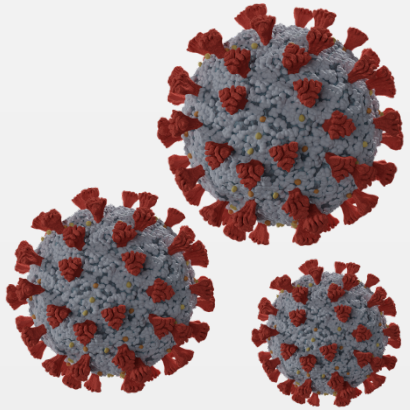


MetScape software running on Cytoscape was used to visualize and interpret metabolites in the context of a global metabolic network.
The network reflects a complexity of effects of the pathology and provides further evidence for the involvement of tryptophan metabolism, urea cycle and metabolism of arginine.



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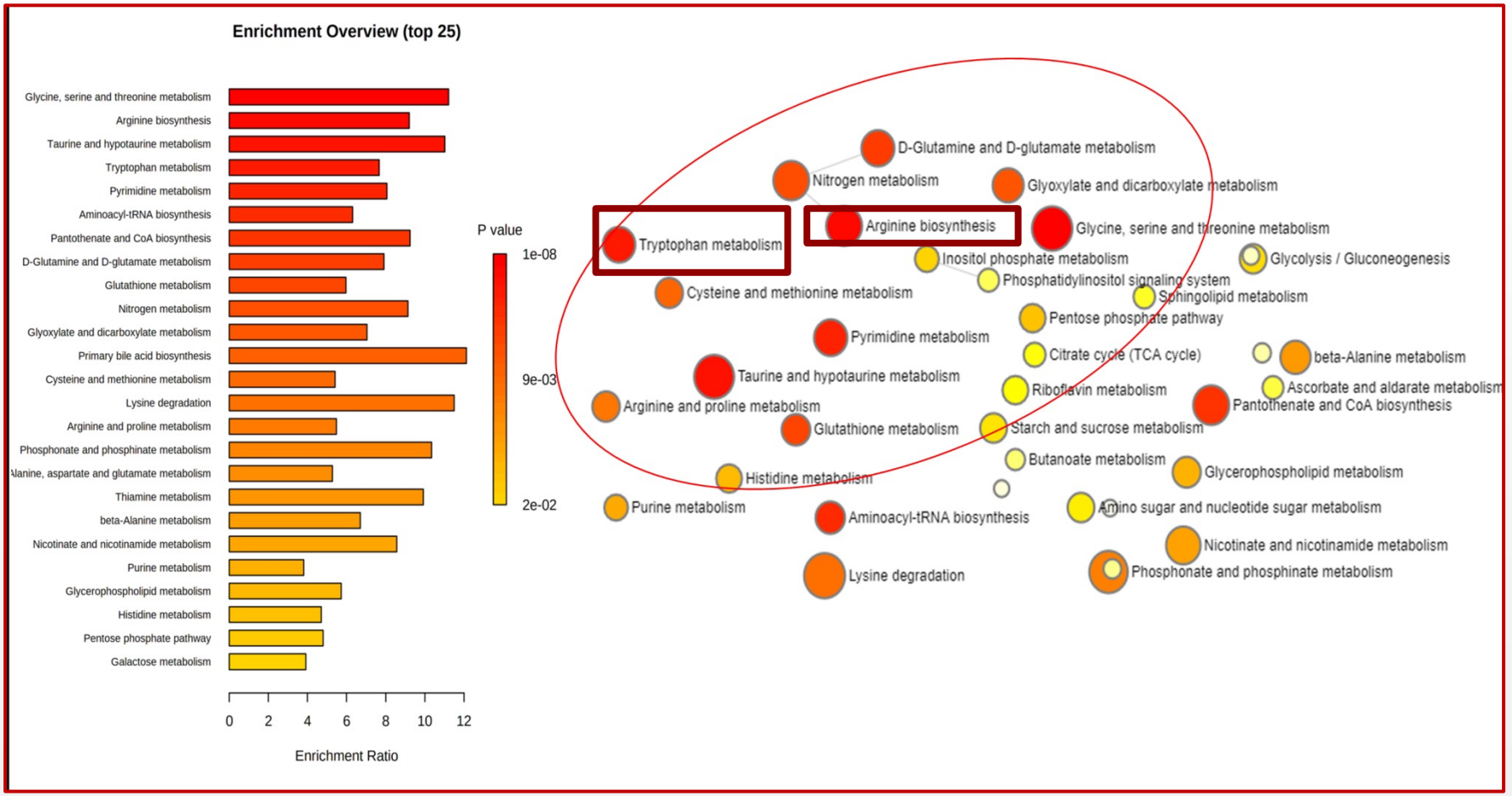

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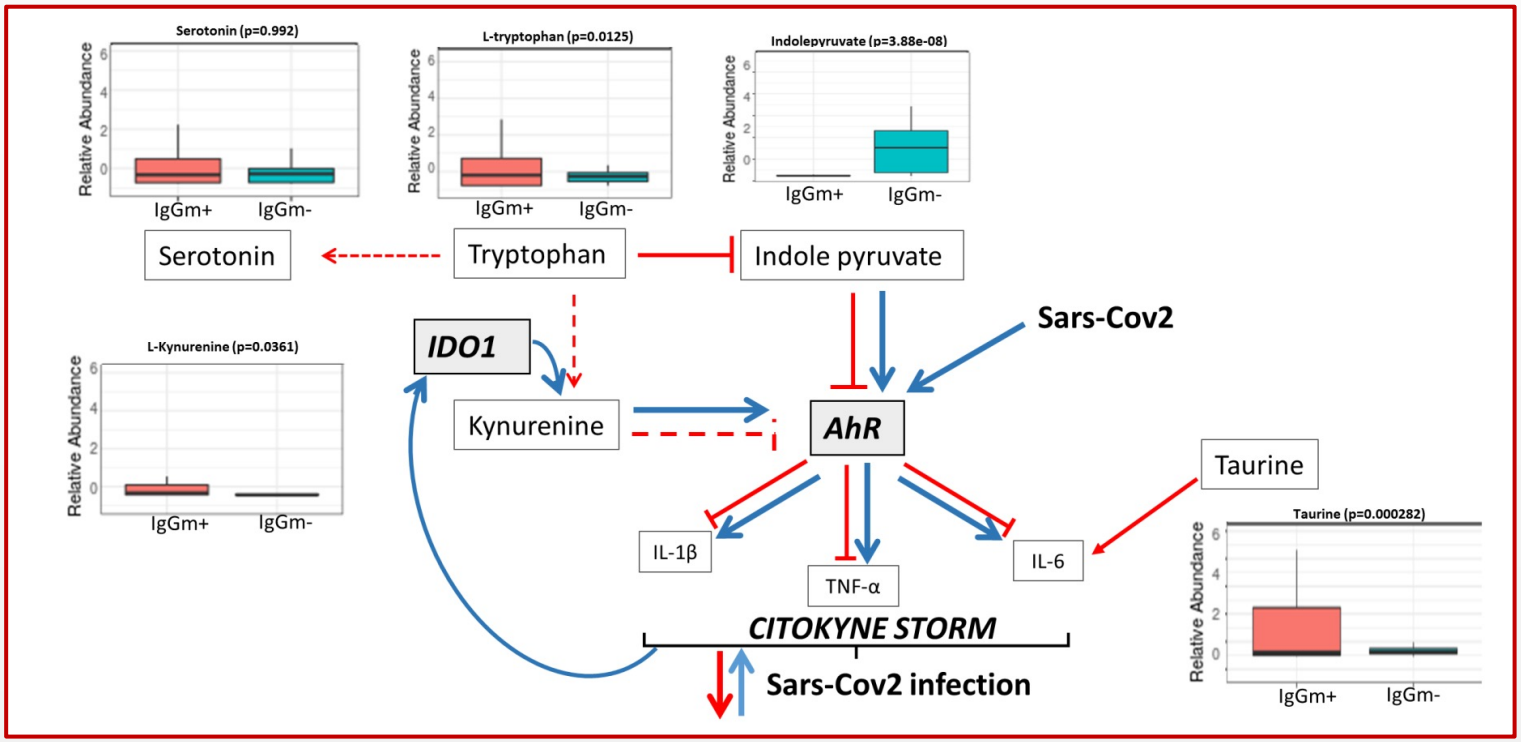
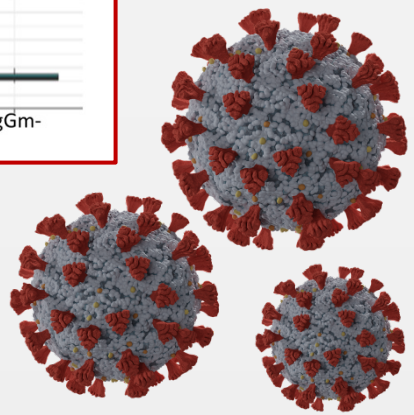
Tryptophan increased in the IgGm+ and serotonin did not undergo significant change compared with controls. At the same time, the reduction in the level of indolepyruvate and the increase in the level of kynurenine, knowing to be rich source for aryl hydrocarbon receptor (AHR) ligands, suggested a restoration of the kynurenine pathway to decrease the cytokines storm.



The potential relevance of these observations confirm the impact of Trp and its metabolites against the severity of COVID-19, and our results highlighted for the first time to our knowledgement the biochemical mechanism adopted by leukocytes to counteract viral infection by reactivating the tryptophan metabolism.



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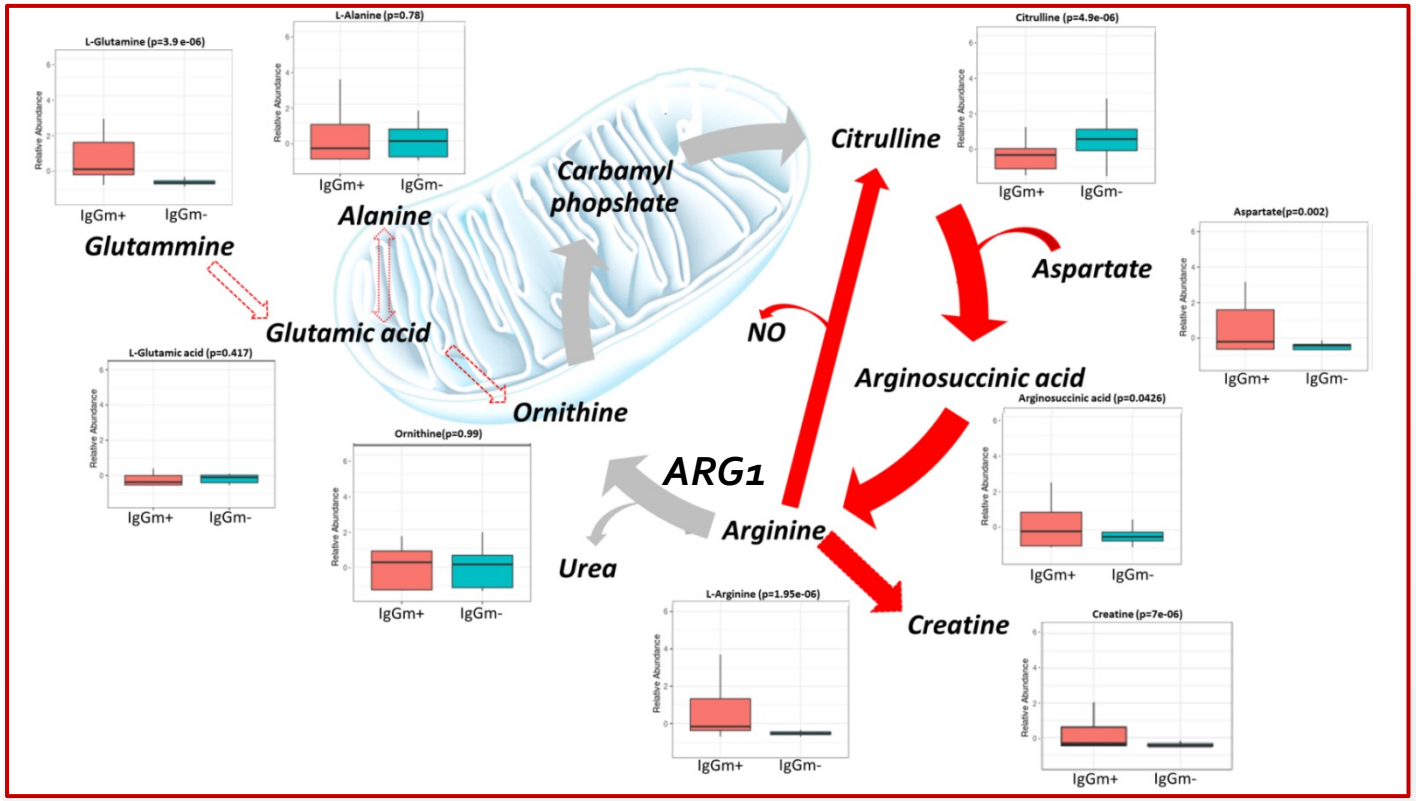
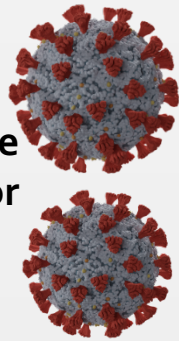
Increase in arginine concentration may play a role in the regulation of immune cell reactivity through the proliferation of T lymphocyte subpopulations and differentiation of naive T cells.



NO produced in macrophages and neutrophils is necessary to kill invasive microorganisms and activate the immune cells in defense mechanisms.
Arginine is a substrate for nitric oxide (NO) production, which can induce antiviral activity against RNA viruses, such as SARS-CoV-2.



Since Arg1 can limit the bioavailability of L-arginine, the inhibition of Arg1 can drive the recycling of L-citrulline to generate L-arginine for the production of NO, paving the way for developing antiviral immunity in IgGm+.

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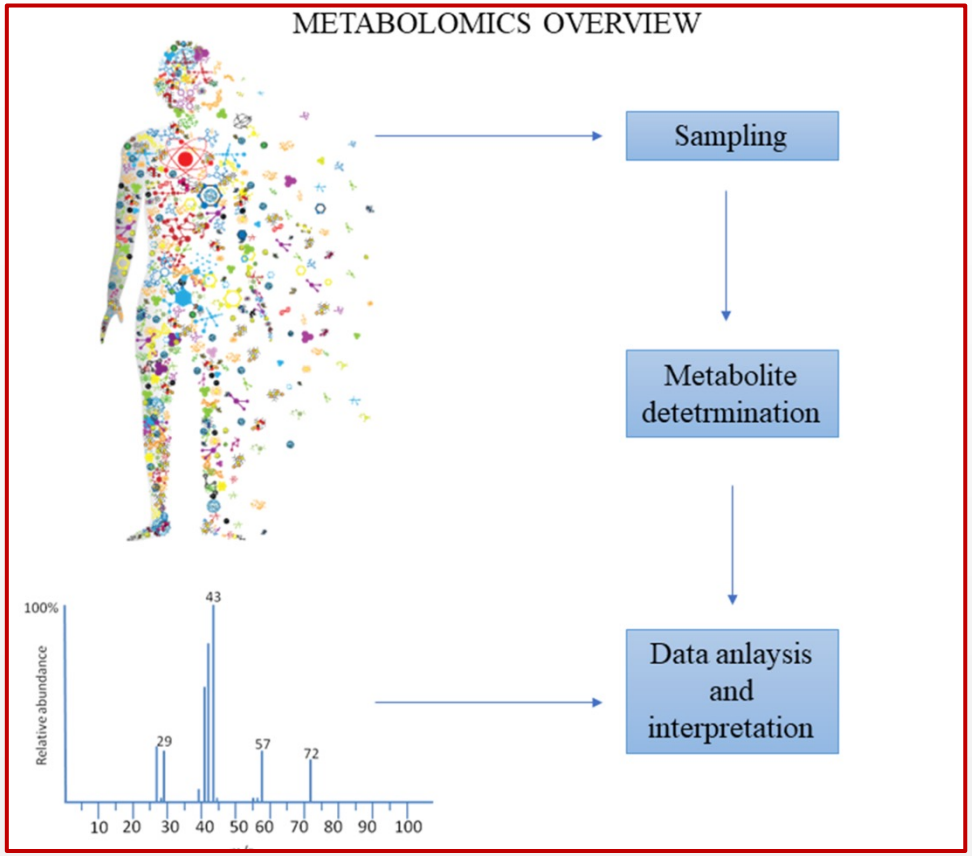
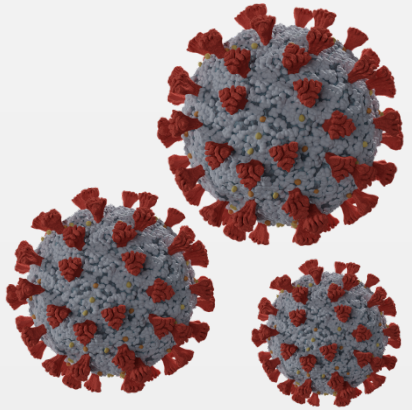


We provide:

-novel insights into later stages of immune defense against SARS-Cov-2 infection, namely when circulating antibodies may be absent, but an antibody memory is present

-novel methods for monitoring protection against the risk of SARS-CoV-2 reinfection and for examining whether the risk of reinfection changes over time.

After viral pathogenesis, metabolomics analysis reveals a reprogrammed of leukocyte metabolism through activation of specific amino acid pathways related to protective immunity against SARS-CoV-2



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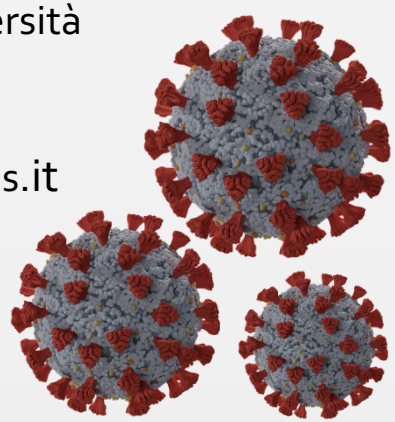

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