ACCADEMIA NAZIONALE DEI LINCEI

Long Covid (PASC)

Statement by the Lincei Committee on Covid-19

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Abstract.

The terms "LONG COVID", Post-COVID- Syndrome (PCS) and Post-Acute Sequelae of SARS-CoV- (PASC), identify a wide variety of symptoms occurring for several weeks or months following Covid-19 infection. The Syndrome mainly includes the following symptoms: cognitive impairment; fatigue; pain syndrome; cardio-pulmonary symptoms; anosmia-dysgeusia; and headache.
Symptoms, related pathological findings, pathogenetic data and prognostic prospects mainly involve Lung, Cardio-vascular System, Neuro-muscular System and Brain. Metabolic imbalances and diabetes have also been reported.

Abnormalities in **lung** function of recovering patients may persist for long periods and fatigue or weakness were the most prevalent physical effects (up to 40%) in PASC patients. Abnormalities in alveolar diffusion capacity, revealed by diffusing lung capacity for carbon monoxide (DPCO) tests persist for long periods and are likely related to interstitial pneumonia, Dyspnea progressively improves over time even if a subgroup of patients may experience persistent dyspnea up to 1 year after COVID-19.

Myocardial injury, known to occurs frequently in patients with acute COVID-19 infection, may persist, possibly related to ongoing viral reservoirs in the heart or to an autoimmune response to cardiac antigens. Most cardiac abnormalities were seen to alleviate with time, but some of them, especially diastolic dysfunction, may persist, raising the presumption of chronic alteration.

Muscle weakness, fatigue and exercise intolerance are among the most frequent symptoms of the PASC. It is not known whether these symptoms, which are similar to the "*Chronic Fatigue Syndrome*"

that may occur following different viral infections, are due to viral persistence or to **immunological factors.** Peripheral neuropathy with features like *Guillain-Barré Syndrome*, probably caused by autoimmunity, has been described in some patients.

Many cognitive and sensory **brain** functions are affected for a long time after COVID-19 infection, in patients both with severe and mild symptomology. The most prevalent symptoms are anosmia-dysgeusia and dysfunction concerning memory, affecting up to 73% of patients. A loss of grey and white matter of brain areas mediating these cognitive and sensory functions is observed after COVID-19 infection, particularly in patient older than 60 years.

Metabolic dysfunctions, such as obesity and insulin resistance, and metabolic diseases, such as **diabetes**, were recognized as predisposing risk factors for severe acute COVID-19. Recent epidemiological studies have now shown that patients with SARS-CoV-2 infection, even those with mild disease and non-hospitalized, have an increased risk of diabetes compared with control groups. Screening for hyperglycemia should therefore be considered not only during the acute phase of COVID-19 but also during the follow-up.

There is evidence that, in some PASC patients, SARS-CoV-2 is capable of **persisting** for months after acute infection in several tissues including, in addition to the respiratory tract, the cardiac and renal systems, the GI tract, muscles, as well as in the brain and lymph nodes. Persistence of SARS- CoV-2 is not unexpected, since other coronaviruses are known to establish persistent infections *in vitro* and *in vivo*. The molecular mechanisms responsible for the establishment of persistent infections remain elusive, although SARS-CoV-2 persistence has been associated with immunosuppression and virus-induced alterations of the host-cell metabolism.

The current understanding of molecular and cellular mechanisms of PASC is limited. The **pathogenesis** of PASC is complex and multifactorial, including SARS-CoV-2 virus dissemination and persistence; activation and response to unrelated viruses such as EBV; anti-viral immune responses and autoimmunity. Inflammation represents a common denominator of PASC in its different presentations. Biomarkers of PASC risk and disease include viral RNAemia, antiviral antibodies, autoantibodies, cytokines, chemokines, PTX3, Interferon Lamda. Type 2 diabetes, itself a risk factor for Covid disease, may also be favored by PASC. Host genetics and microbiome may impact on PASC.

It is reasonable to assume that prevention via vaccination and early treatment of the acute phase of COVID-19 represent invaluable assets to address the challenge of PASC at the level of individuals

and society. Early evidence has been obtained in Israel that childhood vaccination against COVID-19 protects against both, the direct acute and the long-term effects of COVID-19 disease.

Gaps of knowledge-open questions

Understanding of PASC pathogenesis is in its infancy. No information is presently available on the incidence of PASC in patients affected by the Omicron variant. Contribution of SARS-CoV-2 persistence to PASC pathogenesis is not currently understood, but it has been hypothesized that viral RNA and/or selected viral proteins might act as constant stimuli that maintain an inflammatory condition until viral clearance is achieved.

The relative importance of the virus (and its variants) and of immune responses against the virus itself or to self-components remains to be determined. The mechanisms responsible for the triggering of autoimmunity remain undefined. Host genetics plays an important role in susceptibility to Covid-19 but its significance in PASC remains to be defined. The relative importance of different components of pathogenesis in the different manifestations of PASC remain to be defined.

Identifying patients at high risk of developing PASC and the development of preventive strategies represent formidable challenges.

OPEN QUESTIONS ON LONG COVID / PASC

- SARS-CoV-2 virus persistence in Lung, Heart, Brain.
- Auto-immune mechanisms.
- Impact of virus variants (e.g. Omicron).
- Mechanism and time persistence of brain imbalances.
- Impact on PASC of host genetics and microbiome.
- Impact of vaccination on preventing PASC in people who get breakthrough infection

Riassunto.

L'ampia e variegata sintomatologia, osservata a distanza di settimane e mesi dopo la fase acuta dell'infezione da COVID-19, viene ora compresa nei termini, relativamente vaghi, di "Long Covid" o di PASC (Post-Acute Sequele of SARS-CoV). Tale Sindrome comprende difficoltà mnemoniche,

affaticamento, sindrome dolorosa, sintomi cardio-polmonari, anosmia e disgeusia, cefalea.
Risultano quindi interessati vari organi ed apparati, in particolare i sistemi respiratorio, cardio-vascolare, neuro-muscolare e nervoso. Sono state inoltre segnalate affezioni metaboliche, in particolare il diabete.

Anomalie delle funzioni polmonari e senso di affaticamento sono state segnalate con significativa frequenza (sino al 40%) a seguito della fase acuta dell'infezione e possono persistere anche per mesi, talché in alcuni soggetti una dispnea persistente è stata osservata, anche a distanza di un anno. In molti pazienti test di laboratorio hanno rivelato anomalie nella capacità di diffusione alveolare, ascrivibile forse a forme di polmonite interstiziale.

È ben noto che alterazioni riferibili a miocardite sono osservabili nella fase acuta in pazienti affetti da COVID-19. Tali alterazioni possono persistere nel tempo, riferibili forse a persistenza del virus in sede cardiaca o a fenomeni auto-immuni contro antigeni cardiaci. Nella gran maggioranza dei casi, la sintomatologia scompare col tempo, ma in alcuni casi tachicardia e disfunzione diastolica persistono per lungo tempo, tanto da sollevare il dubbio di alterazioni croniche.
Debolezza muscolare, spossatezza e intolleranza all'esercizio fisico sono tra i sintomi più frequenti come sequele al COVID-19. Questi sintomi presentano analogie con la "Sindrome di affaticamento cronico" che fa seguito a diverse infezioni virali, ma non si sa se essi siano dovuti a persistenza virale o a fattori immunologici. In alcuni pazienti, è stata osservata una neuropatia periferica, con aspetti simili alla Sindrome di Guillain-Barré.
Molte capacità cognitive e sensoriali legate alla funzionalità del Sistema nervoso centrale possono presentare per lungo tempo alterazioni in seguito all'infezione da COVID-19, in particolare sono stati rilevati sintomi quali anosmia-disgeusia e deficit di memoria, che possono colpire fino al 73% dei pazienti. In pazienti di età superiore ai 60 anni è stata rivelata, con tecniche di risonanza magnetica, una riduzione selettiva di sostanza cerebrale in aree deputate a funzioni cognitive e sensoriali, ma non è noto quanto tali alterazioni siano persistenti nel tempo.
È ben noto che tra i fattori di rischio che predispongono a forme acute gravi di COVID-19 figurano alterazioni metaboliche quali obesità e resistenza insulinica e malattie metaboliche come il diabete. Recenti studi epidemiologici hanno inoltre rivelato che i pazienti affetti da SARS-CoV-2, anche quelli con malattia in forma lieve e non ospedalizzati hanno un aumentato rischio di insorgenza post-Covid di diabete, rispetto ai gruppi di controllo. Questi dati suggeriscono l'opportunità di sottoporre a screening per iperglicemia non solo pazienti in fase acuta, ma anche durante il follow- up.

Il virus SARS-CoV-2 può persistere per mesi, a seguito dell'infezione acuta, in vari organi: oltre al polmone, anche nei sistemi cardiaco e renale, nel tratto gastro-intestinale, nei muscoli, nel cervello e nei linfonodi. Tale persistenza non appare sorprendente, dal momento che dati ottenuti *in vivo* e *in vitro* indicano che altri tipi di coronavirus possono dar luogo a infezioni persistenti. Il meccanismo molecolare alla base di tali infezioni persistenti non è ancora noto, per quanto si abbiano indicazioni circa la correlazione con stati di immuno-soppressione e con alterazioni indotte dal virus nel metabolismo della cellula ospite.

Non si ha al momento una chiara comprensione dei meccanismi molecolari e cellulari alla base di PASC. La sua patogenesi appare complessa e multifattoriale e potenzialmente include disseminazione e persistenza del virus SARS-CoV-2, attivazione e risposta ad altri virus non correlati, quali EBV, risposte antivirali immuni e auto-immuni. L'infiammazione costituisce un comun denominatore di PASC nelle sue differenti manifestazioni. Biomarcatori indicativi di rischio o di incidenza di PASC sono la RNAemia virale, anticorpi antivirali, autoanticorpi, citochine, chemochine, PTX3 e interferon lambda. Il Diabete tipo 2 e, forse, le caratteristiche geniche ed il microbioma dell'ospite hanno influenza sull'incidenza di PASC.

Pur essendo ben definiti e accertati gli aspetti protettivi della vaccinazione sull'insorgenza e la gravità della malattia acuta, non sono al momento chiari i rapporti tra la vaccinazione anti-COVID e la insorgenza di PASC in soggetti che, pur vaccinati, si ammalino soprattutto per infezione da variante Omicron. Dati preliminari, ottenuti in Israele in bambini vaccinati indicano tuttavia un effetto protettivo della vaccinazione, sia sull'infezione acuta che nelle sequele a lungo termine della malattia.

Questioni aperte (nell'attuale fase delle conoscenze)

La comprensione dei meccanismi patogenetici alla base del PASC-Long Covid è in una fase iniziale ed incerta, né abbiamo informazioni sull'incidenza di tale sindrome in soggetti che si ammalino per infezione da variante Omicron. Non si hanno certezze relativamente all'effetto di un'eventuale persistenza del virus in vari organi sull'insorgenza di PASC, ma è stato ipotizzato che l'RNA virale e/o specifiche proteine virali potrebbero agire come uno stimolo costante atto a mantenere uno stato infiammatorio sino alla completa eliminazione del virus. Rimane dunque da definire l'importanza relativa del virus o di risposte immuni contro il virus o contro antigeni dell'ospite, così come pure eventuali meccanismi in grado di scatenare fenomeni auto-immuni. Le caratteristiche geniche del

paziente sono importanti nel determinare la suscettibilità alla malattia acuta, ma non sappiamo se lo siano anche nel determinare l'insorgenza di sequele. Rimane dunque incerto l'impatto di diversi fattori patogenetici nell'incidenza delle differenti manifestazioni della Sindrome. Oggetto di studio saranno sia la possibile identificazione e selezione dei pazienti a maggior rischio di insorgenza di PASC-Long Covid, sia lo sviluppo di strategie preventive.

Problematiche aperte circa origine ed evoluzione di PASC-Long Covid

- Persistenza del virus SARS-CoV-2 in Polmone, Cuore, Cervello….
- Meccanismi autoimmuni
- Impatto di nuove varianti (Omicron)
- Meccanismo e persistenza di alterazioni cerebrali
- Importanza di caratteristiche geniche e microbioma dell'ospite sull'insorgenza di PASC
- Effetto della vaccinazione nel prevenire l'insorgenza di PASC in soggetti vaccinati

1) Definition and prevalence

The colloquial terms "LONG COVID" and Post-COVID- Syndrome (PCS) have extensively been used to identify a wide variety of symptoms occurring for several weeks or months following the start of diagnosis of COVID-19 or symptoms that were consistent with Covid-19 infection. The Syndrome, now formally known as Post-Acute Sequelae of SARS-CoV- (PASC), mainly includes the following symptoms: cognitive impairment; fatigue; pain syndrome; cardio-pulmonary symptoms; anosmia-dysgeusia; and headache. (Visco et al., 2022) The British National Institute for Health and Care Excellence (NICE) defines PASC as "signs and symptoms that develop during or after an infection consistent with Covid-19, continue for more than 12 weeks and are not explained by an alternative diagnosis". The WHO has established the following clinical case definition of PASC: "it occurs in individuals with a history of probable or confirmed SARS-CoV-2 infection, usually 3 months from the onset of COVID-19 with symptoms and that last for at least 2 months and cannot be explained by an alternative diagnosis" (Soriano et al., 2021). The UK's Office for National Statistics assessed that one in five COVID-19 patients report symptoms beyond 5 weeks, while 10% have symptoms persevering over 12 weeks. (Michelen et al., 2021).

Emerging studies indicate that COVID-19 pandemic affected male and female populations in different ways. Women seem to experience less severe short-term complications but suffer worse long-term COVID complications, including depression, reduced physical activity, and deteriorating lifestyle habits, all of which may impact cardio-vascular risk (Bucciarelli et al., 2022). The impact of COVID-related long-time imbalances affecting the general population should not be under- estimated. The UK Office for National Statistics (ONS) estimates that the number of people in the UK that are self-reporting symptoms lasting more than 4 weeks currently stands at 1.1 million (1.7% of the population), while the real-time assessment of community transmission (REACT) study in England estimated the overall number of people who reported at least one symptom lasting for 12 or more weeks as more than 2 million by February 2021 (Routen et al., 2022).

An ~~very~~ extensive metanalysis on prevalence data collected in UK showed that common residual symptoms among COVID-19 survivors at one-year post infection included fatigue/weakness (28%), dyspnoea (18%), arthromyalgia (26%), depression (23%), anxiety (22%), memory loss (19%), concentration difficulties (18%), and insomnia (12%). A higher risk of diabetes has also been observed (Xie and Al-Aly, 2022). The qualitative review of evidence on risk factors suggested that females and those with severe/critical COVID-19 infection were at higher risk of experiencing long-

term post-COVID symptoms. (Han et al., 2022). In a large population study in Southern Germany (EPILOC cohort, age 18-65), the three most frequent clusters of symptoms were fatigue, neurocognitive and chest/cardiorespiratory, with at least moderate impairment (≥20%) of general health and working capacity in 26% of the subjects (age and sex standardized), including young and middle-aged subjects (Peter et al, 2022).

Symptoms, related pathological findings, pathogenetic data and prognostic prospects are different in different organs, which implies separate analytical reports of patterns involving Lung, Cardio-vascular System, Neuro-muscolar System and Brain. Other organs can also be affected. Besides the already mentioned higher frequency of diabetes, kidney imbalances have been reported. Adult patients who survived COVID-19 beyond the first 30 days of infection exhibited increased risk (and burden) of AKI (acute kidney insufficiency), eGFR (estimated Glomerular Filtration Rate) decline, ESKD (End Stage Kidney Disease), and MAKE (major adverse kidney events) (Bowe et al., 2021). The risks (and burdens) of kidney outcomes increased according to the severity of the acute infection. A separate report has already been dealing with patterns occurring in children, where the multisystem inflammatory syndrome is a matter of great concern (Sacco et al., 2022).

Specific interest will focus on the pathogenesis of the different patterns, either directly or indirectly related to the viral infection and to the influence of vaccination. In fact, it is widely accepted that vaccines reduce the risk of PASC by lowering the chances of contracting COVID-19 in the first place. As discussed below, it is unclear to what extent vaccinated people who get breakthrough infection are protected against PASC (Antonelli et al., 2022; Huang et al, 2021). This point is discussed in Section 4 (Concluding Remarks) in the light of the current prevalent variant omicron.

2a. Lung

Persistent dyspnoea, frequently associated with fatigue, chest pain and cough are affecting up to 20% of patients 3 months after the acute COVID infection (Cares-Marambio et al, 2021). The severity of symptoms is related to the severity of disease, but the persistence of dyspnoea does not seem to be closely related to the initial severity of COVID-19 (Montani et al., 2022). In most cases, dyspnoea progressively improves over time even if a subgroup of patients experiences persistent dyspnoea up to 1 year after COVID-19. Interestingly, among Survivors of Covid-19 experiencing long-term symptoms, increased breathlessness, and reduced quality of life were observed in young, previously healthy working age adults and frequently younger females (Sigfisd et al., 2021).

In survivors of hospital admission after COVID-19 infection, fatigue, dyspnoea, chest pain, and cough are the most prevalent respiratory symptoms found in 52%, 37%, 16% and 14% of patients between 3 weeks and 3 months. The biological mechanisms underlying the persistence of respiratory symptoms are not clear but are possibly related to the pathological processes triggered in the acute phase and a persistent endotheliopathy resulting in a pro-coagulant state (Fogarty et al., 2021); an inflammatory cytokine production could also be involved (Cares-Marambio et al, 2021; Lerum et al., 2021). Abnormalities in alveolar diffusion capacity, revealed by diffusing lung capacity for carbon monoxide (DPCO) tests persist for long periods and are likely related to interstitial pneumonia, which might evolve into pulmonary fibrosis. The risk of developing pulmonary fibrosis is related to the cellular mechanisms that occur in response to acute lung injury and can lead to abnormal and persistent inflammatory response and excessive proliferation of fibroblasts, related to the pathological processes triggered in the acute phase.

Radiological data are important in the management of COVID-19 infected patients and in the follow up after the acute phase. McGroder and coworkers (2021) report that twenty percent of non-mechanically ventilated and 72% of mechanically ventilated patients had fibrotic-like abnormalities (reticulations, traction bronchiectasis or honeycombing) at high-resolution chest CT scan four months after infection. These abnormalities were correlated with decrements in lung function, cough and frailty but not necessarily with dyspnea. Furthermore, this study identified severity of initial illness, duration of mechanical ventilation, the lactate dehydrogenase levels on admission and leukocyte telomere length as independent risk factors for the development of fibrotic-like abnormalities. In a prospective study reporting respiratory outcomes at 12 months after discharge in people recovered from severe COVID-19 who did not require mechanical ventilation, 24% of patients had radiological abnormalities including interstitial thickening and reticular opacity, potential signs of evolving fibrosis (Wu et al., 2021).

Studies on a series of autopsies on patients who died several weeks (up to 65 days) after the diagnosis of COVID-19 infection report lung tissue damage with diffuse alveolar damage in the proliferative phase, showing an excessive collagen deposition. Additional conditions, such as concomitant bacterial pulmonary superinfection, lung aspergillosis, thromboembolic phenomena, and hemorrhages can further worsen tissue damage (Maccio et al., 2022).

A study on immuno-fibrotic drivers of impaired lung function in PASC reports that circulating factors associated with acute neutrophil activation, fibrosis signaling, and alveolar epithelial repair remain elevated in survivors of acute COVID-19 infection and may predict the impairment of

pulmonary function (Chun et al., 2021)

A meta-analysis including a total of 4,478 COVID-19 patients from 16 cohort studies reports that fatigue or weakness (47%) were the most prevalent physical effects of post-acute COVID-19 syndrome. Abnormalities in lung function of recovering patients, i.e., diffusion capacity for carbon Monoxide (DLCO<80%) persisted for long periods. Severe patients were more likely to present joint pain and decreased lung functions compared with non-severe patients (Long et al., 2021).

2b) Cardio-vascular system

Myocardial injury associated or not with the Multisystemic Inflammatory Syndrome (Li et al. 2021) occurs frequently in patients with acute COVID-19 infection (as revealed also by high serum Troponin levels) and is associated with increased mortality during hospitalization. In the general population, an incidence of COVID-19-associated myocarditis of approximately 150 cases per 100,000 was observed (Boehmer et al., 2021) In patients who survive, the incremental mortality at 6 months and 1 year was seen to be low. (Weber et al., 2022).

Some evidence indicates that males between 12 and 17 years of age most likely developed myocarditis within 3 months of SARS-CoV-2 infection (Szarpak et al., 2022).

An accurate statistical analysis to estimate the risks and 12-month burdens of pre-specified incident cardiovascular outcomes confirms that the risk and 12-month burden of incident cardiovascular disease are substantial and span several cardiovascular disease categories (ischemic and non-ischemic heart disease, dysrhythmias, and others). Symptoms may include chest pain, shortness of breath, fatigue, and autonomic manifestations such as postural orthostatic tachycardia are common and associated with significant disability, heightened anxiety, and public awareness (Crea et al., 2022; Martinez-Salazar et al., 2022; Raman et al., 2022). The risks and burdens of cardiovascular disease were evident even among patients who did not necessitate hospitalization for acute COVID-19 disease. (Xie et al, 2022). Inappropriate sinus tachycardia (IST) is a common observation in patients with post-COVID-19 syndrome and cardiac autonomic nervous system imbalance with decreased parasympathetic activity may explain this phenomenon (Araniò et al, 2022). Most cardiac abnormalities were seen to alleviate with time, but some of them, especially diastolic dysfunction, may persist, raising the presumption of chronic alteration. (Tudoran et al., 2022).

The pathogenesis for post-acute cardiac damage is still poorly understood. One possible explanation

is a chronic inflammatory response evoked by persistent viral reservoirs in the heart following the acute infection, with underlying mechanisms suggested for post-acute COVID disease affecting other organs (see below in Item 3b). A second mechanism for delayed damage is an autoimmune response to cardiac antigens through molecular mimicry, and some evidence has been presented in favour of this hypothesis (Blagova et al, 2021)

Early data from a patient-led observational study has hinted at the possibility of long COVID symptoms being alleviated through vaccination. (Strain et al., 2021) Of 900 people with long COVID, 56.7% of those vaccinated saw an overall improvement, 18.7% a deterioration, and 24.6% were unchanged post-vaccination. In another survey study (COVID symptom app study) (Antonelli et al., 2021; Antonelli et al., 2022) the odds of experiencing symptoms more than 28 days post-vaccination were halved by two vaccinations (n = 906). Some experts posit that an accelerated viral clearance and a muted chronic inflammatory response could explain symptom reduction following vaccination (Levine et al., 2021)

Myocarditis, potentially due to an autoimmune response (triggered by molecular mimicry between spike protein and self-antigen; see above), tends to be more common after the second mRNA vaccine dose but is comparatively less life-threatening as most cases spontaneously resolves. (Larson et al., 2021; Kim et al, 2021).

2c) Neuromuscular system

Muscle weakness, fatigue and exercise intolerance are among the most frequent symptoms of *PASC*. Myalgia is also observed in several patients and the symptoms may persist for several weeks or months (Soares et al, 2022). This condition, which is more frequent in patients who were hospitalized for COVID-19, but is also seen in non-hospitalized patients, is similar to the *chronic fatigue syndrome (CFS)*, also called *Myalgic Encephalomyelitis (ME)* or *ME/CFS* that may occur following different viral infections, thus also referred to as *Post-viral Fatigue Syndrome (PVFS)*.

The pathogenesis of all these conditions is unclear. To dissect the causes of muscle fatigue in PASC, it is useful to consider the pathogenesis of neuromuscular symptoms also during the acute phase of severe COVID-19.

Muscle wasting in COVID-19 patients admitted to ICU. Intensive-care patients with severe COVID- 19 show dramatic muscle wasting and weakness, a condition related to the *Critical Illness Myopathy* due

to immobilization and mechanical ventilation seen in many patients admitted to ICU, independently of the cause of the disease (Brown et al, 2019). This condition is followed, for those who survive, by sustained physical disability and requires a long rehabilitation process. Both myogenic mechanisms, with loss of myosin from the muscle fibers, and neurogenic factors, with slowing of nerve conduction velocities and axonal degeneration, may contribute to the *Critical Illness Myopathy* seen in patients with severe COVID19 (Soares et al, 2022). In addition, other factors, including systemic inflammation with increased cytokine levels (cytokine storm), hypoxaemia, which is present in all patients with severe disease, malnutrition due to loss of appetite, loss of smell and alteration in taste, likely contribute to promote muscle wasting.

Viral infection of skeletal muscles. It is not clear whether viral infection of muscles is involved in muscle changes during and after COVID-19. Evidence for myositis has been reported in deceased patients with COVID-19. However, detection of viral load was low or negative in most skeletal muscles, and probably attributable to circulating viral RNA rather than direct infection of muscle cells (Aschman et al, 2021).

Peripheral neuropathy during or after SARS-CoV-2 infection. Several COVID-19 patients show symptoms of peripheral neuropathy, such as painful paresthesia (numbness and tingling) either during or after SARS-CoV-2 infection (Rory et al, 2022). In some of these patients a diagnosis of small fiber neuropathy was supported by skin biopsy, and autonomic dysfunction was demonstrated by autonomic function testing. Combined involvement of motor and sensory nerves was seen only in occasional patients, for example patients showing bifacial weakness and paraesthesia (Stuby J et al, 2021). These cases are consistent with conditions related to various forms of *Guillain-Barré Syndrome (GBS*), probably caused by autoimmunity, thus different to other sensory disfunctions seen in COVID-19, such as anosmia and dysgeusia, which seem to reflect a direct viral infiltration of the nervous system.

Neurological complications following anti-COVID-19 vaccination. Different types of neurological complications, including muscle weakness and fatigue, are also seen after anti-Covid vaccinations but are up to 617-fold more frequent following acute SARS-CoV-2 infection than after COVID vaccination (Frontera et al, 2022).

PASC-anticipating risk factors. A multi-omic, longitudinal investigation of 309 COVID-19 patients from initial diagnosis to convalescence (2–3 months later), integrated with clinical data and patient-

reported symptoms, was recently used to identify major risk factors of PASC (Su et al, 2022). The patients were studied at three stages: i) clinical diagnosis, ii) acute disease stage and iii) 2-3 months after the onset of initial symptoms. Four major risk factors were identified: type 2 diabetes, SARS-CoV-2 RNAemia, Epstein-Barr virus viremia, and specific autoantibodies. Immunological analyses supported the hypothesis that unresolved/persistent immune activation and PASC are associated. This study is relevant not only for understanding PASC but also to develop treatments.

2d) Neurodeficits in PASC

It is now clear that many brain functions are affected for a long time after COVID-19 infection, in patients both with severe and mild symptomatology (Blomberg, et al., 2021; Liu, et al., 2022). The PASC syndrome includes cognitive, neurological and psychiatric diseases and distressing symptoms such as memory loss, fatigue, anosmia and dysgeusia.

The peculiar sensory deficits, anosmia/dysgeusia, that characterized the early symptoms of COVID-19 were manifested in more than 40% of COVID-19 patients infected with Delta or previous variants. It affected patients of all ages and the impairment lasted on average for 2-3 months after the end of the infection. However, even in young adults with no severe COVID, loss of taste and/or smell (about 28% of prevalence) were present at 6 months post infection. These sensory deficits are amongst the brain function deficits with a faster recovery in PASC (Su, et al., 2022) (Davis, et al., 2021).

Cognitive dysfunction in PASC is very broad, affecting attention, executive function, problem solving, and decision making. The most prevalent dysfunction concerns memory, affecting up to 73% (in an interview study on 2739 patients), inducing both short-term and long-term memory loss (Davis, et al., 2021). The time course of the loss and of the possible recovery of the many affected brain functions are variable: cognitive dysfunction increased over the first three months post infection, then decreased slightly in the following 7 months. The probability of experiencing memory symptoms increased over the first few months, with 56% reporting memory symptoms at month 4 and 50% at month 7. While age is an important factor in cognitive and memory disfunction, it is worrying that non-hospitalized, young people (16–30 years old) suffer potentially severe symptoms, such as concentration and memory problems, half a year after infection (Blomberg, et al., 2021; Davis, et al., 2021).

The study of the anatomical or functional imaging of brain alterations in PASC shows consistent changes in many brain areas, including the somatosensory cortex, rectal/orbital gyrus (including the olfactory system), temporal lobe (including the amygdala, piriform cortex and the hippocampus), hypothalamus/ thalamus, brainstem, and cerebellum (Najt et al., 2021).

18F-FDG brain PET studies in COVID-19 patients have revealed prominent hypometabolism in many of the above areas. However, during the PASC phase a reversibility of the decreased neocortical glucose metabolism is evident, which importantly is associated with an improvement in cognitive function. Interestingly, the spatial covariance pattern of the hypometabolism correlates with the cognitive impairment (MoCA) (Blazhenets, et al., 2021).

The preliminary evidence of brain alterations has been corroborated by a larger study that could compare in the single patient (55-75 years of age) the brain anatomy before and about 5 months after the covid-19 infection (Douaud, et al., 2022). Again, this study recruited patients in 2020 and early 2021, and hence does not include infections with Omicron Variants.

Both grey and white matter of many brain areas change. The changes are subtle but are consistent across individuals and highly significant. Grey matter, evaluated by cortical thickness, is reduced in many regions of the orbito/frontal cortex and limbic system that include olfactory cortex, piriform cortex, amygdala, parahippocampal and hippocampal cortex and insula. The changes are consistent with the white matter alterations, measured with mean diffusivity, in regions functionally connected with the piriform cortex, olfactory tubercle and anterior olfactory nucleus. These altered structures participate in the perception of taste, smell, emotion, memory and spatial navigation, functions that are strongly compromised during PASC.

The correspondence between the major cognitive and neurological dysfunction in PASC and the neuronal substrate that mediates these functions suggest that the observed symptoms result from insults, although small, to the brain in consequence of the infection. The mechanism that generates the insults is still to be defined (see 3a section).

A pronounced loss of grey matter was also observed in crus II, part of the cognitive, and olfactory-related lobule VII of the cerebellum. Interestingly, the amount of grey matter loss correlated well with the patient individual performance in a spatial attention task widely used as a neuropsychological test. Again this demonstrate a causal link between brain alterations and behavioural deficits.

Despite these highly localized deficits there is also an increase in Cerebro Spinal Fluid (CSF) volume

and decrease of whole brain volume respect to the controls, suggesting an additional diffuse loss of grey matter.

The anatomical deficits increase with age between 60 and 75 and are likely to be modest in the age group of 55. This reinforces neuropsychological data that showed COVID-19 as a risk factor to develop dementia, neurodegenerative diseases and mild cognitive impairments even in 50-year-old adults (Taquet, Geddes, Husain, Luciano, & Harrison, 2021).

At present there are no anatomical brain data comparing vaccinated with non-vaccinated patients in PASC.

2e) Metabolic disorders and diabetes

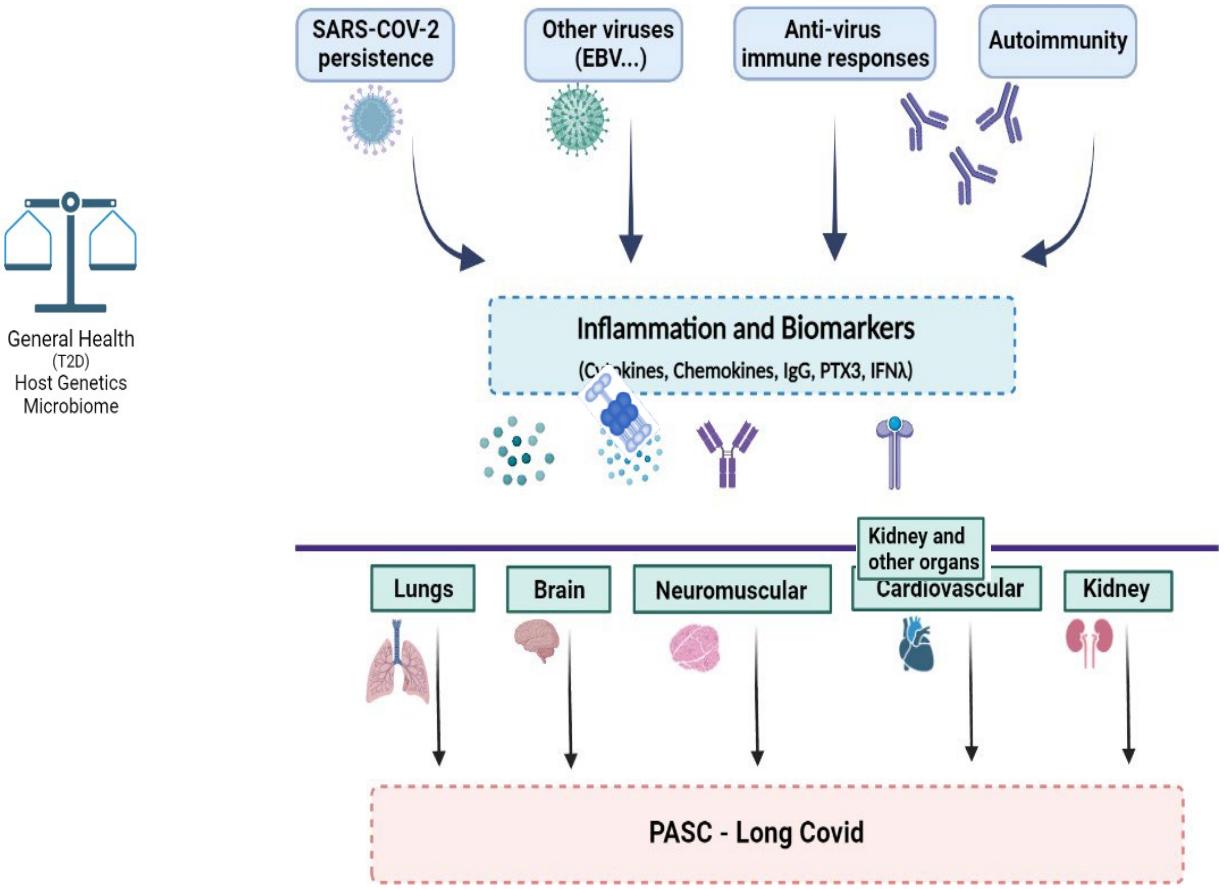
Metabolic dysfunctions, such as obesity and insulin resistance, and metabolic diseases, such as diabetes, were recognized as predisposing risk factors for severe acute COVID-19 since the early stages of the pandemic. Now emerging evidence supports the notion that these conditions also predispose to long COVID (PASC). For example, lipid metabolism disorders and obesity were found to be age-independent risk factors for the development of PASC, as shown in a retrospective study involving more than 50.000 patients with a confirmed diagnosis of COVID-19 treated by general practitioners in Germany (Loosen et al, 2022).

Type 2 diabetes is a well-established PASC-anticipating risk factor (Su et al, 2022) and several reports now support the notion that the incidence of diabetes is increased after COVID-19 (see review by Scherer et al, 2022). Abnormalities in glycometabolic control, insulin resistance and beta cell function were detected in patients with COVID-19 without any pre-existing history or diagnosis of diabetes and persist even after recovery (Montefusco et al, 2021).

The study by Xie and Al-Aly (2022) stands out for its large sample size. A cohort of more than 180.000 participants who had a positive COVID-19 test were followed up for about one year and found to have an increased risk of incident diabetes compared with a contemporary control group (> 4 M individuals) who had not contracted SARS-CoV-2 and an historical control group (> 4 M individuals) from the prepandemic era (Xie and Al-Aly, 2022). The risk was found to increase according to the severity of disease during the acute phase of the infection, as determined by

comparing three groups of patients (non-hospitalised, hospitalised, and admitted to intensive care), but was highly significant also among the non-hospitalised group. The excess burden of diabetes among non-hospitalised individuals (8.3 per 1000 people at 12 months) points to the magnitude of the problems that health systems might face, considering the hundreds of millions of people infected globally. Given the importance of this major risk of long COVID, it will be important to support the conclusion of these reports with prospective epidemiological studies (Paneni and Patrono, 2022). An immediate implication of the studies is the necessity of screening for hyperglycemia not only during the acute phase of COVID-19 but also during the follow-up.

Which are the factors responsible for the emergence of diabetes as a component of the post- COVID syndrome? As discussed also in greater detail below (sections 3a and 3b), the following factors might play a role: SARS-COV-2 RNAemia and persistence of SARS-COV-2 in tissues, including endothelial cells and adipose cells (Martínez-Colón et al, 2021), reactivation of latent viruses as revealed by Epstein-Barr virus (EBV) viremia (Su et al, 2022), systemic inflammation, with higher cytokine levels detected in infected patients long after recovery from COVID-19 (Su et al, 2022).



3a) Pathogenesis and Biomarkers

Understanding fundamental mechanisms underlying the pathogenesis of long COVID (LC) is in its infancy and represents a major challenge. Candidate mechanisms of pathogenesis can be classified along four major lines: persistence of SARS-CoV-2; reactivation of other viruses, in particular Epstein-Barr virus (EBV); autoimmunity triggered by the virus; persistent tissue damage and immunity-triggered inflammation (Nalbandian et al., 2021).

Persistence of the virus and viral fragments has been proposed to represent a driver sustaining long term sequelae of PASC (see below, 3b) (Ramakrishnan et al., 2021). Gastrointestinal (GI) viral shedding has been associated in some patients with persistent disease following the acute phase of the disease (Parasa et al., 2020). Consistent with the view of an important role of virus persistence are preliminary observations of vaccination of long-COVID patients being associated with resolution (Arnold et al., 2020, Knight et al, 2021).

Consistent with an important role of the virus itself, SARS-CoV-2 RNAmia was recently identified as a risk factor for PASC at the time of initial diagnosis (Su et al., 2022). In this longitudinal cohort of 209 patients investigated using a multi-omic approach, additional risk factors included diabetes, circulating EBV, and auto-antibodies. The EBV data suggest that viral reactivation may contribute to the pathogenesis.

Immune activation and autoimmunity have long been associated with PASC (Galeotti et al., 2020). Indeed, autoantibodies have been shown to contribute to severe COVID-19 disease (Casanova et al., 2021). Activation of autoreactive T cells has been observed in infection settings including COVID- 19 (Woodruff et al, 2020; Getts et al., 2014). In a recent study GI-PASC was found to correlate with newly expanded cytotoxic CD8+ and CD4+ T-cell populations. These new populations include SARS-CoV-2 reactive clones and their activation occurred during convalescence from acute disease. Concomitantly, non-specific activation of CMV-specific T cells was observed in subjects with GI-PASC (Su et al., 2022).

A limited number of prospective studies with validation cohorts have been conducted to investigate pathogenesis and prediction of evolution to PASC. In a study involving 215 subjects and a 395 individuals validation cohort (Cervia et al, 2022), an antibody signature (IgM and IgG3) together with a set of clinical variables was able to predict PASC. A study conducted on 147 patients in addition

to normal subjects, included controls who had been infected with prevalent coronaviruses other than SARS-CoV-2 (Phetsouphanh et al., 2022). Eight months following mild-to-moderate SARS-CoV-2 infection profound perturbations were found in COVID-19 patients. Myeloid cells showed an activated phenotype and alterations of naive T cells were observed. A combination of analyses was associated with PASC with a 78-81% accuracy. This set of biomarkers included cytokines (IFN-⍺, IFN-⍺, IFN-⍺ and IL-6) and the fluid phase pattern recognition molecule PTX3 (Brunetta et al., 2021).

Thus, the pathogenesis of PASC is complex, at the interception between virus persistence, activation of, and response to, endogenous viruses (EBV and possibly others), activation of antiviral and autoimmune responses, sustained inflammation. Given the diversity and pleiomorphic nature of PASC manifestations, it is tempting to speculate that the relative importance of different pathogenic components may vary depending on the spectrum of organs involved.

3b) Persistence/Virology

A growing number of studies provide evidence that in some PASC patients SARS-CoV-2 can persist in several tissue reservoirs after acute infection. In addition to the respiratory tract, SARS-CoV-2 viral proteins and/or RNA have in fact been detected throughout the cardiac and renal systems, GI tract, muscles as well as in the brain and lymph nodes, months after the infection (reviewed in Mehandru & Merad, 2022).

Recently, in one of the most comprehensive analyses to date of SARS-CoV-2 persistence across the body and brain in a diverse autopsy cohort collected in the United States, the authors report that, whereas the most common location in which SARS-CoV-2 RNA tends to linger is the respiratory tract, in more than 50% of the cases the virus was detected also in extrapulmonary tissues, including the myocardium, lymph nodes and all sampled areas of the brain, except the dura mater (Chertow et al., 2021). The data also indicate that SARS-CoV-2 can replicate within different tissues for over 3 months after infection. In some individuals, viral RNA could be detected in multiple compartments for up to 230 days after the primary infection (Chertow et al., 2021). The authors suggest that the persistence of viral genomic and subgenomic RNA may represent infection with defective virus, which has been described in persistent infection with other viruses, including the measles virus.

In addition to autopsy findings, persistence of SARS-CoV-2 RNA was detected in intestinal enterocytes of 5 out of 14 intestinal biopsies obtained from asymptomatic individuals at 4 months after the onset of COVID-19 (Gaebler et al., 2021). Interestingly, a recent study also revealed the

presence of virus transcripts and of SARS-CoV-2–infected cells in the olfactory mucosa of patients with long-term persistence of COVID-19–associated anosmia who were negative to nasopharyngeal swab SARS-CoV-2 RNA tests (de Melo et al., 2021).

Persistence of SARS-CoV-2 in some COVID-19 patients is not unexpected. Several studies have shown that coronaviruses are capable of establishing persistent infections *in vitro* as well as *in vivo*. Starting from the initial studies on the beta-coronavirus MHV (murine hepatitis virus) that was extensively investigated for its ability to cause persistent infection in the central nervous system also in primates, in some cases associated with demyelination (Bergmann et al., 2006; Pan et al, 2020), several studies have shown that persistent infection of FCoV (feline coronavirus) can often occur in cats (Kipar et al., 2010). Regarding human coronaviruses (HCoV), the ability of establishing persistent infection in cell cultures has been demonstrated for the seasonal coronaviruses HCoV-OC43 and HCoV-229E (Arbour et al, 1999; Arbour et al., 1999 b), as well as for the SARS-CoV-2 phylogenetically related SARS-CoV and MERS-CoV (Chan et al., 2004; Banerjee et al., 2020). In the case of these two highly pathogenic coronaviruses, it should be noted that a subset of individuals who survived SARS or MERS were reported to experience, in addition to persistent impairment of pulmonary function, protracted neuropsychiatric symptoms, sleep abnormalities, fatigue, myalgias and functional disabilities reminiscent of long COVID (reviewed in Mehandru & Merad, 2022).
In the case of SARS-CoV-2, it has been recently shown that the virus can establish a long-term, non-productive persistent infection in different types of cells (Sunhee et al., 2020; Gamage et al., 2022). The molecular mechanisms governing the establishment of RNA virus persistent infections have attracted considerable attention but remain elusive. Concurrence of molecular and immunological events is usually required to allow the virus to direct a transcriptional program enabling a long-lasting virus-host interaction, by regulating its replication without killing the host cells and by evading the immune response. In the case of SARS-CoV-2, establishment of persistent infection has been associated with immunosuppression (Moran et al., 2021; Yang et al., 2021), reduced expression of ribosomal proteins (Yang et al., 2021) and possible integration of selected SARS-CoV-2 sequences into the genome of infected cells (Zhang et al, 2021).
Contribution of SARS-CoV-2 persistence to PASC pathogenesis is not currently understood, but it could be hypothesized that viral RNA and/or selected viral proteins might act as constant stimuli that maintain an inflammatory condition contributing to pathogenesis until viral clearance is achieved.

This possibility is supported by reports of improved clinical symptoms after administration of anti-SARS-CoV-2 vaccines in PASC patients (Arnold et al, 2021).

4) Protection by vaccination

Early data from a patient-led observational study has hinted at the possibility of long COVID symptoms being alleviated through vaccination83. Of 900 people with long COVID, 56.7% of those vaccinated saw an overall improvement, 18.7% a deterioration, and 24.6% were unchanged post-vaccination. In another survey study (COVID symptom app study) (Antonelli et al, 2022) the odds of experiencing symptoms more than 28 days post- vaccination were halved by two vaccinations (n = 906). It has been suggested that an accelerated viral clearance and a muted chronic inflammatory response could explain symptom reduction following vaccination (Levine-Tiefenbrun et al, 2021). Early evidence was obtained in Israel that childhood vaccination against COVID-19 protects against both, the direct acute and the long-term effects of COVID-19 disease (Stein et al, 2022).

Recent studies have investigated the impact of vaccination on PASC following breakthrough infection (BTI). In a large study condicted on the US Department of Veterans Affairs database it was observed that vaccination with a single dose of the Ad26.CoV2.S or two doses of a mRNA vaccine confered only limited, but significant, protection against Long COVID after BTI (Al-Aly et al., 2022). Limitations of this study include the time window of observation (January through October 2021), the low number of females (<10%), the suboptimal vaccine schedule. Protection by vaccination against PASC after BTI was also observed in a survey on Long Covid (Kuodi et al, 2022) conducted in Israel.

Assessment of protection against PASC after BTI poses methodological challenges with limitations which are inherent to longitudinal versus case-control studies, usage of different vaccines or number of jabs, representation of different prevailing virus variants. However, in spite of these limitations, available information obtained using different approaches strongly suggest that full vaccination with mRNA vaccines confers protection against the development of PASC after BTI. The duration of protection and its significance to future variants remains to be defined.

5) Concluding Remarks and Perspective

Progress has been made in defining key cardinal aspects of PASC (neurocognitive; cardiorespiratory; fatigue, etc) and its prevalence, but important aspects remain undefined. These include the actual boundaries of the PASC symptom constellation, its similarity and peculiarities in relation to other

viral diseases, its actual frequency and relevance in the pediatric population.

Some of the symptoms and imbalances characteristic of PASC tend to last up to months, but are ultimately going to disappear, although in a minority of patients, anosmia, brain "fog", DPCO and dyspnea can persist after one year even among young and middle-aged adults after mild acute SARS-CoV-2 infection and impact on general health and working capacity (Seeßle et al., 2021; Fortini et al., 2022; Fortunato et al., 2022; Peter et al 2022). Females showed significantly more neurocognitive symptoms than males. It has been observed that among patients symptomatic after 2 months, 85% still reported symptoms one year after their symptom onset, while evolution of symptoms showed a decreasing prevalence over time for 27/53 symptoms (e.g., loss of taste/smell); a stable prevalence over time for 18/53 symptoms (e.g., dyspnoea), and an increasing prevalence over time for 8/53 symptoms (e.g., paraesthesia) (Tran et al, 2022) Of major concern are the reported increase in incidence, following COVID-19 infection, of Diabetes and cerebrovascular events, notably acute ischemic strokes. In addition, covid-19 is a risk factor for deep vein thrombosis, pulmonary embolism, and bleeding (Katsoularis et al., 2022) and coagulopathies (dysfunctions of the blood coagulation system), possibly related to fibrin amyloid microclots (Kell et al., 2022), that persist long after the initial infection. Alterations (reduction in thickness) of the brain cortex as a sequel of COVID-19 infection was observed in specific areas, mainly related to olfact sensibility, but it is not known if such derangements are going to persist in time. Recent data (May 2022) from Wuhan indicate that COVID-19 survivors still had more prevalent symptoms and more problems in pain or discomfort, as well as anxiety or depression, at 2 years than did controls (Huang et al., 2022).

Current understanding of pathogenesis is in its infancy. Evidence suggests that persistence of COVID-19, reactivation of other viruses, autoimmunity and uncontrolled inflammation are major determinants of PASC. Given the diversity of organ involvement and manifestations, it is tempting to speculate that the relative importance of pathogenic mechanisms may vary in different tissue and organ contexts. Several neurocognitive symptoms were associated with anti-nuclear antibodies (ANA) titre elevations. This may indicate autoimmunity as cofactor in aetiology of long COVID (Seeßle et al. 2021).

Most of the reported observations on the sequels to COVID-19 infections are related to early variants of the virus: we do not know and only time will tell if the now prevailing Omicron Variants induce similar effects (Huang 2022; Peter et al, 2022). It is tempting to speculate that the lower intrinsic pathogenicity of omicron and the dramatic impact on disease severity of vaccination will translate in

lower risk of PASC at the individual level. However, given the increase in transmission of omicron variants, including children, the potential PASC disease burden at the population/society level should not be underestimated and deserves careful assessment. It is reasonable to assume that prevention via vaccination and early treatment of the acute phase of COVID-19 represent invaluable assets to address the challenge of PASC at the level of individuals and society. Early evidence has been obtained in Israel that childhood vaccination against COVID-19 protects against both, the direct acute and the long-term effects of COVID-19 disease (Stein et al., 2022).

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