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REVIEW

# Long COVID: lights and shadows on the clinical characterization of this emerging pathology

Viola Cogliandro[1], Paolo Bonfanti[1,2]

[1]*Infectious Disease Unit, Fondazione IRCCS San Gerardo dei Tintori, Monza, Italy;*
[2]*School of Medicine and Surgery, University of Milano Bicocca, Milan, Italy*

SUMMARY

More than 800 million individuals have contracted SARSCOV2 infection worldwide. It was estimated that almost 10-20% of these might suffer from Long COVID. It is a multisystemic syndrome, which negatively affects the quality of life with a significant burden of health loss compared to COVID negative individuals. Moreover, the risk of sequelae still remains high at 2 years in both non-hospitalized and hospitalized individuals. This review summarizes studies regarding long COVID and clarifies the definitions, the risk factors and the management of this syndrome. Finally, it delves into the most frequent long-term outcomes, especially postural orthostatic tachycardia syndrome" (POTS), myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), brain fog, and their therapeutical possibilities.

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## INTRODUCTION

Since the first cases in Wuhan in December 2019, Coronavirus virus – 2019 (COVID-19) has spread across the world. As of November 2023, there have been 772 million confirmed cases of COVID-19, including almost 7 million deaths, according to the World Health Organization (WHO) (World Health Organization. Weekly operational update on COVID-19 – 7 March 2023.). After the acute phase of COVID-19 infection, the duration of recovery is extremely variable. This may occur after days, weeks, or some symptoms may persist even for years after COVID-19 infection. This condition of persistent and new symptoms after COVID-19 was described for the first time by Paul Garner, an infectious disease professor, who reported his experience of seven weeks on a "roller-coaster of ill health" following COVID-19 on 5 May 2020 (Garner, 2020). Subsequently, the COVID-19 patient community also started talking about this condition and the term "long covid" was used for the first time on Twitter (#LongCovid) (Callard and Perego, 2021). In a short time, numerous scientific articles were published using different terms, such as "long covid", "long haul COVID", "post COVID syndrome", "post COVID sequalae" and "post COVID condition". Several definitions of Long COVID were initially proposed. In particular, the main differences concerned the timeframe to define long covid.

The publication of the National Institute for Health and Care Excellence (NICE) (National Institute for Health and Care Excellence (NICE), 2020), WHO (Soriano *et al.*, 2022) and Centers for Disease Control and Prevention (CDC) (Centers for Disease Control and Prevention) guidelines about the management of post COVID, have clarified the definition of long covid, although with some differences.

The NICE guidelines distinguish between *ongoing symptomatic COVID-19* and *Post COVID-19 syndrome*. The first definition includes "signs and symptoms of COVID-19 still present from 4 weeks up to 12 weeks;" whereas *post COVID-19 syndrome* is defined 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." Finally, the expert panel defines the common term *long COVID* as "signs and symptoms that continue or develop after acute COVID-19. It includes both ongoing symptomatic COVID-19 (from 4 to 12 weeks) and post-COVID-19 syndrome (12 weeks or more)"(National Institute for Health and Care Excellence (NICE), 2020).

The WHO guidelines use the term *post-COVID-19 condition* and define it as a condition that occurs "usually 3 months from the onset of COVID-19 with symptoms that last for at least 2 months and cannot be explained by an alternative diagnosis"(Soriano *et al.*, 2022). In contrast to the NICE guidelines, the ex-

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*Corresponding author:*
Viola Cogliandro
E-mail: viola.cogliandro@irccs-sangerardo.it

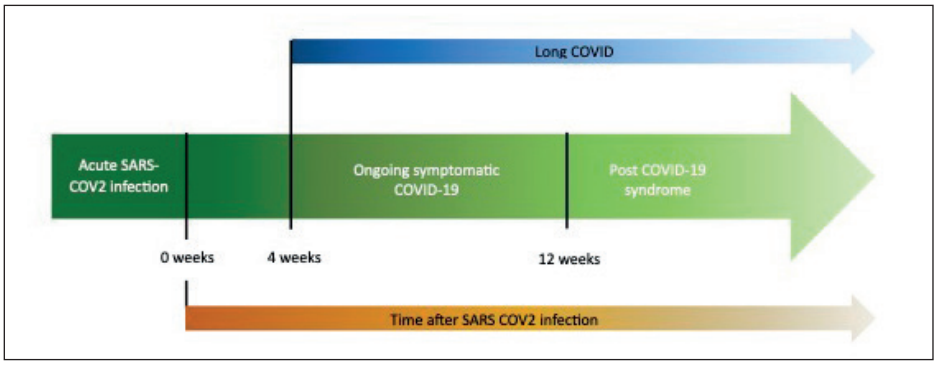


**Figure 1** - *Graphical representation of long COVID definitions according international guidelines (NICE, WHO and CDC).*

pert panel also comments on the minimum durations of symptoms to define post COVID condition.
Finally, even CDC uses the term *post COVID condition* but defines it as a condition that occurs after 4 weeks from acute COVID-19 infection, like the NICE guidelines (Center for Disease Control and Prevention).
In conclusion, after a post-acute phase which lasts the first 2-3 weeks after COVID 19 infection and is characterized mainly by respiratory symptoms, other symptoms and signs may persist or appear after 1 month. Although most patients resolve them within 12 weeks, a small proportion continues to be symptomatic for a long period. Given the broad spectrum of symptoms of long COVID, patients should be screened as early as 4 weeks after acute infection in order to identify patients who may suffer from persistent symptoms at an early stage (*Figure1*).

## EPIDEMIOLOGY

Long COVID is a multisystemic illness with multiple variable symptoms, which persist or fluctuate. According to WHO, in Europe, at least 17 million people experienced long COVID in the first two years of the pandemic (Wise, 2022). In the US, according to CDC data from the "Household Pulse Survey", 1 in 13 adults (7.5%) have long COVID symptoms three or more months after COVID-19 infection (CDC - Household Pulse Survey, no date). In UK, data from a "National Household Survey" estimate a long COVID prevalence after four weeks from acute infection of 3% (about 2 million people) as of February 2023 (UK - Office for National Statistics). A recent meta-analysis estimated that 45% of patients with COVID had at least one symptom at four months' follow-up, regardless of hospitalization status (O'Mahoney *et al.*, 2023).
It is difficult to estimate the real prevalence of this condition, because it varies according to geographical areas, length of follow up, severity of disease, and pandemic waves. Also, the heterogeneity of studies makes it difficult to compare the results. Most of them include only hospitalized patients, have no control group and include self-reported and not standardized symptoms. *Table 1* summarized studies that exploring the frequency and features of Long COVID. Indeed, whereas in hospital setting, most studies estimated that 50-80% of patients experienced persisting symptoms in the first seven months after acute infection (Carfì, Bernabei and Landi, 2020; Garrigues *et al.*, 2020; Arnold *et al.*, 2021; Huang *et al.*, 2021; Mandal *et al.*, 2021; Romero-Duarte *et al.*, 2021; Sigfrid *et al.*, 2021; Tleyjeh *et al.*, 2021; Venturelli *et al.*, 2021; Xiong *et al.*, 2021; Squillace *et al.*, 2023), the data were more discordant in the not-hospitalized population. In some cohort studies, which included mildly infected patients with a low proportion of the hospitalized population and excluded patients in intensive care units, the prevalence of persisting symptoms in the first five months was between 40% and 90% (Carfì, Bernabei and Landi, 2020; Carvalho-Schneider *et al.*, 2021; Davis *et al.*, 2021; Dennis *et al.*, 2021; Ballering *et al.*, 2022; Perlis *et al.*, 2022). In a cohort of home-isolated patients, 52% of young adults, aged 16-30 years, had persisting symptoms after 6 months from COVID19 infection (Blomberg *et al.*, 2021). In contrast, in a recent case-control study, conducted from January 2020 to April 2021, the prevalence of persisting symptoms related to COVID 19 was estimated at 5% (Subramanian *et al.*, 2022).
For longer follow-up in hospitalized population, the prevalence of long COVID seems to be between 40-50% (Bellan *et al.*, 2021; Fang *et al.*, 2022; Frontera *et al.*, 2022) at one year. In contrast, Lombardo et al. described a prevalence of 80% among hospitalized and not hospitalized individuals during the first wave (Lombardo *et al.*, 2021).
Two years after acute COVID-19 infection, in a Chinese cohort of hospitalized patients with a mainly mild infection, 19.8% still had symptoms (Yang *et al.*, 2022). Similar percentages were described in a Swedish cohort, in which almost 30% of survivors at four months after hospitalization were still symptomatic; and almost 80% of patients with long COVID at four months still reported symptoms affecting daily life at 24 months. Finally, no differences were found between ICU and non-ICU subjects, with regard to the persistence of symptoms (Wahlgren *et al.*, 2023).
A very important prevalence figure is the one derived

**Table 1** - *Summary of studies that exploring the frequency and features of Long COVID.*

| *Study reference* | *Number of subjects in study* | *Country of study* | *Study design* | *Hospitalized/not hospitalized* | *Time of infection* | *Time of follow up (average)* | *Symptomatic patients at follow up* |
|---|---|---|---|---|---|---|---|
| Carvalho-Schneider C, *et al.* 2020 | 150 | France | Cohort study | Hospitalized: n=53; Not hospitalized: n=97 | 17 March to June 2020 | 30 days after symptom onset | 103/150 (68%) |
| Halpin SJ, *et al.* 2020 | 100 | UK | Cross sectional | Hospitalized (ICU: n=32) | | 48 days after infection | 64/100 (64%) |
| Carfì A, *et al.* 2020 | 143 | Italy | Case series | Hospitalized (ICU: n=7) | 21 April - 29 May 2020 | 60 days after symptom onset | 125/143 (87%) |
| Garrigues E, 2020 | 120 | France | Cross sectional | Hospitalized (ICU: n=48) | 15 March - 14 April 2020 | 3 months after infection | 66/120 (55%) |
| Squillace N, *et at*. 2023 | 361 | Italy | Cohort study | Hospitalized (ICU: n=128) | 1 March - 31 May 2020 | 128 days after infection | 243/361 (67%) |
| Sigfrid L, *et al.* 2021 | 327 | UK | Prospective cohort study | Hospitalized (ICU: n=92) | 17 January - 5 October 2020 | 3 months after infection | 305/327 (93%) |
| Goertz YMJ, *et al.* 2020 | 2113 | Belgium, Netherlands | Cross sectional survey | Hospitalized: n=112 Not hospitalized: n=2001 | | 79 days after symptom onset | 2098/2113 (99%) |
| Mandal S, *et al.* 2020 | 384 | UK | Cross sectional | Hospitalized (ICU: n=54) | | 54 days after infection | 276/384 (72%) |
| Perlis RH, 2022 | 16.091 | USA | Cross sectional survey | | 5 February 2021 - 6 July 2022 | 2 months after infection | 2359/16091 (15%) |
| Venturelli S, *et al.* 2020 | 767 | Italy | Cross sectional | Hospitalized (ICU: n=66) | 1 March - 31 July 2020 | 81 days after infection | (51%) |
| Arnold TD, *et al.* 2020 | 110 | UK | Prospective cohort study | Hospitalized | 30 March - 3 June 2020 | 83 days after symptom onset | 81/110 (74%) |
| Xiong Q, *et al.* 2020 | 538 184 Covid negative | China | Cohort study | Hospitalized | 1 March - 30 April 2020 | 97 days after infection | 267/538 (50%) |
| Tleyjeh IM, *et al.* 2021 | 222 | Saudi Arabia | Prospective cohort study | Hospitalized (ICU: n=67) | May - July 2020 | 122 days after infection | 125/222 (56%) |
| Romero-Duarte A, *et al.* 2021 | 797 | Spain | Retrospective cohort study | Hospitalized (ICU: n=81) | 1 March - 15 April 2020 | 6 months after infection | 509/797 (64%) |
| Blomberg B, *et al.* 2021 | 312 | Norway | Prospective cohort study | Hospitalized: n=65 Not hospitalized: n=247 | 28 February - 4 April 2020 | 6 months after infection | 189/312 (61%) |
| Huang C, *et al.* 2021 | 1733 | China | Cohort study | Hospitalized (ICU: n=76) | 7 January - 29 May 2020 | 186 days after symptom onset | 1265/1733 (76%) |
| Davis HE, *et al.* 2021 | 3762 | USA | Cross sectional online survey | Hospitalized: n=317 Not hospitalized: n=2133 | December 2019 - May 2020 | 7.6 months after symptom onset | 3505/3762 (93%) |
| Dennis A, *et al.* 2021 | 201 | UK | Cross sectional | Hospitalized: n=37 Not hospitalized: n=164 (mild infections) | 1 April - 14 September 2020 | 141 days after symptom onset | 237/242 (98%) |
| Fang X, *et al.* 2022 | 1233 | China | Cohort study of older adults (> 60y) | Hospitalized (ICU: n=40) | 12 February - 10 April 2020 | 12 months after hospitalization | 630/1233 (50%) |

*Continue >>>*

*Continue >>>*

| *Study reference* | *Number of subjects in study* | *Country of study* | *Study design* | *Hospitalized/not hospitalized* | *Time of infection* | *Time of follow up (average)* | *Symptomatic patients at follow up* |
|---|---|---|---|---|---|---|---|
| Frontera JA, *et al.* 2021 | 242 | USA | Cohort study | Hospitalized (ICU: n=81) | 10 March - 20 May 2020 | 12 months after hospitalization | 122/242 (50%) |
| Lombardo MDM, *et al.* 2021 | 303 | Italy | Cohort study | Hospitalized: n=114 Not Hospitalized: n=189 | February - March 2020 | 12 months after infection | (81%) |
| Bellan M, *et al.* 2021 | 238 | Italy | Prospective cohort study | Hospitalized (ICU: n=23) | 1 March - 29 June 2020 | 12 months after infection | 79/242 (40%) |
| Ballering AV, *et al.* 2022 | 4231 8462 Covid negative | Netherlands | Prospective matched cohort study | Hospitalized: n=142 Not hospitalized: n=4089 | 31 March - 2 August 2020 | 90-150 days after infection | 13% |
| Durstenfeld MS, *et al.* 2022 | 1480 | USA | Cohort study | Hospitalized: n=7 Not hospitaziled: n=1473 | 26 March 2020 - 4 April 2022 | 12 months after symptom onset | 476/1480 (32%) |
| Subramanian A, *et al.* 2022 | 486.149 1.944.580 Covid negative | UK | Retrospective matched cohort study | Not hospitalized | 31 January - 15 April 2020 | 12 months after symptom onset | 20.864/384.137 (5%) |
| Ballouz T, *et al.* 2023 | 1106 608 Covid negative | Switzerland | Cohort study | Hospitalized: n=48 Not hospitalized: n=1058 | 6 August 2020 - 19 January 2021 | 6-12-18-24 months after infection | 23% at 6 months; 19% at 12 months; 19% at 18 months; 17% at 24 months |
| Yang X, *et al.* 2022 | 1864 | China | Cohort study | Hospitalized (ICU n=36) | 12 February - 10 April 2020 | 1-2 years after infection | 370/1864 (20%) |
| Wahlgren C, *et al.* 2023 | 165 | Sweden | Cohort study | Hospitalized (ICU n=47) | 1 March - 31 May 2020 | 4 months - 2 years after infection | 139/165 (84%) at 2 years |

from the paper by Gentilotti *et al.*, as in this study, data were collected prospectively, following patients from discharge for one year. At one year, 57% of patients had at least one symptom, and in particular: 42% of patients had a chronic fatigue-like syndrome, 23% a respiratory syndrome, 22% a chronic pain syndrome, and 11% a neurosensorial syndrome (Gentilotti *et al.*, 2023).

Concerning non-hospitalized population, a Swiss study conducted on a longitudinal population of 1069 adults with previous COVID-19 infection of whom only 5% were hospitalized. They evaluated the symptom trajectories with questionnaires at 6,12, 18 and 24 months after acute infection. They reported that the proportion of still symptomatic individuals was 22.9% at 6 months and decreased to 17.2% at 24 months (Ballouz, Menges, Anagnostopoulos, *et al.*, 2023).

Regarding the association among variants, vaccination status and long COVID, Ballouz *et al.* reported that the prevalence of long COVID at six months was similar among Wildtype, Delta and Omicron infection in non-vaccinated individuals (Ballouz, Menges, Kaufmann, *et al.*, 2023). However, there was strong evidence for a reduction in the odds among vaccinated individuals infected by Omicron variant (odds ratio 0.42, 95% CI 0.24-0.68, p= 0.0008) compared to unvaccinated individuals infected with Wildtype. No difference in the prevalence of Long COVID correlated with the number of vaccine doses (Ballouz, Menges, Kaufmann, *et al.*, 2023). Antonelli *et al.* also confirmed a reduction in odds of long COVID with the Omicron variant compared to the Delta variant of 0.24-0.50 depending on age and time since vaccination (Antonelli *et al.*, 2022); similar results were described by Hernandez-Aceituno *et al.* (Hernández-Aceituno, García-Hernández and Larumbe-Zabala, 2023). Fernandez-de-las-Peñas *et al.* reported that unvaccinated patients previously infected with Wildtype variant exhibited a greater number of long COVID symptoms than those non-vaccinated infected with Alpha or Delta variants, after 6 months of acute infection (Fernández-de-las-Peñas, Kin Israel Notarte, Peligro, Velasco, *et al.*, 2022). Azzolini *et al.* observed a prevalence of long COVID of 48.1% (95% CI 39.9-56.2%) with Wildtype variant, 35.9% (95% CI 30.5-41.6%) with Alpha variant, and 16.5% (95% CI 12.4-21.4%) with a mix of Delta and Omicron variant(Azzolini *et al.*, 2022).

## RISK FACTORS

Due to the lack of homogeneity in Long COVID studies, it is difficult to accurately predict its risk factors. Among the risk factors, the female sex is the most reported. In a cross-sectional study conducted through internet surveys, exploring the presence of Long COVID defined as the persistence of symptoms beyond 1 months after acute COVID-19 infection, the female sex was found to have a higher risk (aOR 1.84-1.91) of symptom persistence (Perlis *et al.*, 2022; Robertson *et al.*, 2023). Similar results were observed by Subramanian *et al.* in their case-control study of non-hospitalized patients three months after acute infection (Subramanian *et al.*, 2022). Even in hospitalized setting and for longer follow up, females may have a higher risk of Long COVID; in particular, Fernandez-de-las-Peñas *et al.* observed that females had a higher risk than males of complaining of more symptoms (aOR 2.54, 95% CI 1.671-3.865) (Fernández-de-las-Peñas, Martín-Guerrero, Pellicer-Valero, *et al.*, 2022).

With regard to age, however, there are conflicting data. Perlis *et al.* reported a higher risk of Long COVID in patients older than 40 years (aOR 1.15; 95% CI 1.12-1.19) (Perlis *et al.*, 2022). Lombardo *et al.* also described an increase in Long COVID with advancing age, although females between 40 and 75 years of age seemed to be at higher risk (Lombardo *et al.*, 2021). Sigfrid *et al.*, in a cohort of hospitalized individuals with a high percentage of patients with severe acute disease, reported that females under 50 years old were significantly more symptomatic than men of the same age; in particular, they had greater disability (aOR 4.22, 95% CI 1.12 to 15.94), reported worse fatigue (aOR 2.06, 95% CI 0.81 to 3.31) and became more breathless (aOR 7.15, 95% CI 2.24 to 22.83) (Sigfrid *et al.*, 2021). Instead, Subramanian *et al.* reported that the risk of symptom persistence in non-hospitalized individuals decreased with advancing age (Subramanian *et al.*, 2022).

Other risk factors described in hospitalized population are the severity of acute COVID-19 disease interpreted as admission in intensive care unit (Huang *et al.*, 2021; Sigfrid *et al.*, 2021; Vos *et al.*, 2022; Yang *et al.*, 2022; Squillace *et al.*, 2023) , length of hospitalization (Tleyjeh *et al.*, 2021), and persistent lung impairment (Bellan *et al.*, 2021).

Finally, in large population studies, ethnic minorities and lower socioeconomic status have been observed to be associated with long COVID. Pre-existing depression may also be associated with the persistence of symptoms after COVID (Subramanian *et al.*, 2022; Perlis *et al.*, 2022; Durstenfeld *et al.*, 2023; Bellan *et al.*, 2021).

## SYMPTOMS

According to WHO data, more than 200 symptoms are described in Long Covid syndrome. Different symptoms are present together and involve multiple organ systems. They can fluctuate over time and, in some cases, can relapse or resolve during follow up. Ziauddeen *et al.* observed that participants reported a mean of 12 initial symptoms and 10 ongoing symptoms. Furthermore, the majority of patients reported a course of illness that was fluctuating, with intensity of symptoms that change but symptoms that never completely disappear (57.7%) or symptoms 'coming and going'/relapsing (17.6%) (Ziauddeen *et al.*, 2022). Squillace *et al.* reported that 87.8% of patients complained of at least one symptoms cluster concerning the same system at discharge, 67.3% had persistence of them at follow-up, and 44% of the population experienced a new symptom cluster during follow-up. Moreover, in 27% of subjects with new symptoms, more than two organs or systems were involved (Squillace *et al.*, 2023). Ballouz *et al.* showed the symptoms trajectories at 12, 18 and 24 months after acute SARS COV2 infection: 68.4% of participants reported a continuous recovery over time, 5.2% reported worsening, 4.4% reported stable symptoms and 8.5% experienced alternating courses of recovery and healthy impairment (Ballouz, Menges, Anagnostopoulos, *et al.*, 2023). Similar data have been reported by Yang *et al.* (Yang *et al.*, 2022). Davis *et al.* observed that the relapses occurred in response to specific triggers such as physical activity (71%), stress (59%), exercise (54%), mental activity (46%), and fertile females experienced relapses mainly during (34%) or before (35%) menstruation (Davis *et al.*, 2021).

The multiorgan involvement in Long COVID was observed, also, by multiorgan MRI in a prospective study with population a low risk of COVID-19 mortality and follow-up at 5 months after acute infection. The most relevant impairment was observed in the pancreas (40%, of which 15% inflammation, 38% ectopic fat), liver (28%; of which 12% inflammation, 21% ectopic fat, 10% hepatomegaly), heart (26%, of which 19% myocarditis, 9% systolic dysfunction), lung (11%; reduced vital capacity), kidney (4%; inflammation), and spleen (4%; splenomegaly). 70% of the individuals had impairment in at least one organ and 29% had multiorgan impairment, with overlap across multiple organs (Dennis *et al.*, 2021).

In addition, the symptoms have a negative impact on health status and quality of life; a significant percentage of patients reports being unable to return to work due to their pre-acute health conditions (Goërtz *et al.*, 2020; Davis *et al.*, 2021; Dennis *et al.*, 2021; Huang *et al.*, 2022; Ballouz, Menges, Anagnostopoulos, *et al.*, 2023).

With regard to symptoms, the most common in the first phase after acute infection (the first two weeks) were: upper respiratory tract symptoms (sneezing, runny nose, sore throat), fever, chill, gastrointestinal ones (vomiting and diarrhea), which resolved within the first few months (Davis *et al.*, 2021; Subramanian

*et al.*, 2022; Ziauddeen *et al.*, 2022); instead, respiratory (shortness of breath, cough, chest pain), systemic (fatigue, pain, myalgia, post exertional malaise) and neuropsychiatric (sleep disorders, anxiety, palpitation, headache) persist over time (Garrigues *et al.*, 2020; Davis *et al.*, 2021; Huang *et al.*, 2021; Romero-Duarte *et al.*, 2021; Tleyjeh *et al.*, 2021; Xiong *et al.*, 2021; Ballering *et al.*, 2022; Subramanian *et al.*, 2022). In addition, cognitive dysfunctions, such as concentration and memory difficulties, brain fog, appear months after acute infection and persist over time (Davis *et al.*, 2021; Ziauddeen *et al.*, 2022) (*Figure 2*).
In studies with follow-up to two years, the number of symptoms and degree of severity reduced over time (Fernández-de-las-Peñas, Martín-Guerrero, Cancela-Cilleruelo, *et al.*, 2022; Huang *et al.*, 2022; Yang *et al.*, 2022; Ballouz, Menges, Anagnostopoulos, *et al.*, 2023; Wahlgren *et al.*, 2023) both in hospitalized and non-hospitalized population (Fernández-de-Las-Peñas *et al.*, 2022), although the female sex showed a longer persistence and a greater severity of COVID symptoms than the male sex (Ballering *et al.*, 2022).
The most persistent symptoms at two years are fatigue, myalgia, post-exertional malaise, sleep disorders, whereas sensorimotor deficits, affective symptoms and cognitive disfunctions are the disorders with better improvement (Wahlgren *et al.*, 2023). Dyspnea gradually improves at 24 months, especially if it was mild or moderate, but it still remains one of the most persistent symptoms (Yang *et al.*, 2022; Wahlgren *et al.*, 2023). Alteration of taste and smell, sexual dysfunctions, hair loss, and headache resulted among the main COVID related symptoms in studies with COVID negative control group both at 3-5 months of follow-up (Ballering *et al.*, 2022; Subramanian *et al.*, 2022) and at 12-24 months (Ballouz, Menges, Anagnostopoulos, *et al.*, 2023).
Concerning SARS-CoV-2 variants, Fernandez-de-las-Peñas *et al.* observed that subjects infected with Wuhan variant experienced a higher number of Long COVID symptoms; dyspnea was more frequent in patients with Wuhan variant, whereas hair loss was more common in patients with Delta variant. No difference between the variants was observed regarding fatigue (Fernández-de-las-Peñas, Ignacio Cancela-Cilleruelo, Rodríguez-Jiménez, Gómez-Mayordomo, *et al.*, 2022).
Although there is an improvement of symptoms impacting daily life and of self-rated general health with a reduction of the perception of anxiety and depression (Huang *et al.*, 2022; Wahlgren *et al.*, 2023), Huang *et al.* described that, compared to COVID negative, COVID patients continued to complain of more symptoms (65% vs 32%) and have more problems with usual activity (27% vs <1%), pain or discomfort (23% vs 5%), and anxiety or depression (12% vs 5%) (Huang *et al.*, 2022). Furthermore, in a recent study of 138,818 individuals with previous SARS-COV2 infection, the risk of death (RR 1.29, CI 95%) and of hospitalization (RR 2.57, CI 95%) remained significantly elevated through the 2 years after infection among the hospitalized population, compared to negative controls. Cumulatively at 2 years, Long COVID contributed 80.4 (95% CI: 71.6-89.6) and 642.8 (95% CI: 596.9-689.3) disability-adjusted life years (DALYs) per 1,000 persons among non-hospitalized and hospitalized individuals; 25.3% (18.9-31.0%) and 21.3% (18.2-24.5%) of the cumulative 2-year DALYs in non-hospitalized and hospitalized were from the second year (Bowe, Xie and Al-Aly, 2023). These findings highlight the substantial cumulative burden of health loss due to PASC and call for attention to the care needs of people with long-term health effects due to SARS-CoV-2.

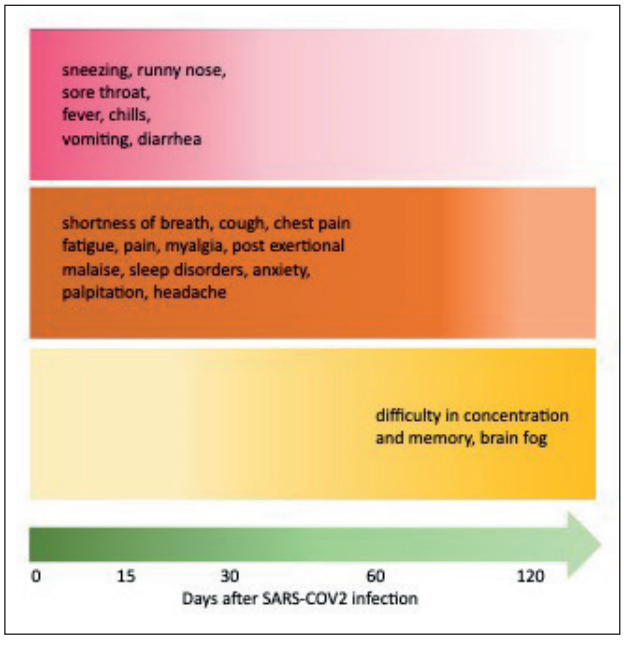


**Figure 2** - *Distribution of Long COVID symptoms over time after acute infection.*

## LONG TERM OUTCOMES AND THERAPEUTICAL STRATEGIES

The risk of sequelae remained elevated at 2 years in both non-hospitalized and hospitalized individuals. The most represented sequelae were musculoskeletal, pulmonary, neurologic, and cardiac (Bowe, Xie and Al-Aly, 2023).
From a pathogenetic point of view, a status of immune dysregulation with T cell alterations, including exhausted T cells, reduced numbers of $CD4^+$ and $CD8^+$ effector memory cells and elevated PD1 expression on central memory cells, persists for at least 13 months after COVID 19 infection (Klein *et al.*, 2023). Studies have also reported highly activated innate immune cells, a lack of naive T and B cells and elevated

expression of type I and type III interferons, persisting for at least 8 months (Phetsouphanh *et al.*, 2022). Other hypothesized mechanisms are: activation of autoimmune response including autoantibodies against ACE2; viral reactivation (EBV, HHV6), gut dysbiosis. These mechanisms appear to be differently involved depending on the type of sequelae.

### *Cardiac outcomes*

A study conducted on the US Veterans cohort of 153,760 COVID positive individuals matched with COVID negative individuals reported that people with COVID-19 exhibited increased risks and 12-month burdens of incident cardiovascular diseases (myocardial infarction, stroke, venous and arterial thromboembolism) independent of age, race, sex, other cardiovascular risk factors and severity of SARS-CoV-2 infection (Xie *et al.*, 2022). However, Ortega-Paz *et al.* did not observe an increase in mortality for cardiovascular events in COVID population at one-year follow-up (Ortega-Paz *et al.*, 2022).

Persistent cardiac damage after acute disease appears to be sustained by a chronic inflammatory response evoked by persistent viral reservoirs in the heart following acute infection, which might aggravate endothelial dysfunction. These processes might cause tissue damage, followed by chronic myocardial fibrosis leading to impaired ventricular compliance, altered myocardial perfusion, increased myocardial stiffness, reduced contractility and potential arrhythmias (Raman *et al.*, 2022). A second mechanism observed in cardiac damage was an autoimmune response to cardiac antigens through molecular mimicry (Raman *et al.*, 2022). Third, a status of hypercoagulability and endothelial dysfunction persists beyond the acute infection; in particular, an increased expression of prothrombotic factors (factor VIII, prothrombin, plasminogen activator inhibitor-1) and circulating microclots might contribute to the onset of thrombosis (Pretorius *et al.*, 2021).

Cardiac MRI is the best available modality to assess and quantify myocardial inflammation. The first studies reported high rates (up to 60%) of persistent myocardial inflammation in the first months after acute infection (Puntmann *et al.*, 2020). However, further studies with longer follow-up reported lower incidence of myocardial impairment. Dennis *et al.* reported, in a low-risk cohort at 5 months of follow up, that 19% of cardiac impairment observed through RMN was suggestive of myocarditis (Dennis *et al.*, 2021). A study of healthcare workers with mild infection and seronegative controls, reported a low prevalence of MRI abnormalities, with no significant difference in 6-month MRI tissue abnormalities between seropositive and seronegative healthcare workers (Joy *et al.*, 2021). The risk of developing myocarditis was found significant, especially in highly trained athletes. Most events were observed within 1–2 months of infection, even if the prevalence of myocarditis on objective examination was found to be generally low (0–3%) and studies assessing the burden of persistent symptom among athletes beyond 8 weeks of infection are lacking (Raman *et al.*, 2022). With regard to systolic dysfunction, follow-up studies with echocardiogram and MRI have confirmed that, even among subjects with severe acute infection, systolic impairment is rare, affecting up to 9-11% of patients (Dennis *et al.*, 2021; Raman *et al.*, 2022).

Tachycardia is another frequent symptom in patients with Long COVID, which reflects a cardiac deconditioning. It is an expression of dysautonomia disfunction and the normalization of heart rate is considered a prognostic indicator of favorable evolution of Long COVID. A particular dysautonomia condition is the "postural orthostatic tachycardia syndrome" (POTS). It is defined as chronic orthostatic intolerance with an increase of heart rate (HR) of ≥30 beats/min within 10 minutes of standing up and without significant hypotension. In addition to the rapid increase in HR, patients can experience palpitations, dyspnea, syncope, brain fog, lightheadedness, blurred/tunnel vision, tremulousness, fatigue, weakness, chronic pain, gastrointestinal issues, and sleep disorders (Sheldon *et al.*, 2015). The symptoms of POTS can overlap with those of myalgic encephalomyelitis and are associated with a worsening of life quality. Dysautonomia symptoms have previously been described after other viral illness, for example after SARS and MERS infection (Agarwal *et al.*, 2007; Ormiston, Świątkiewicz and Taub, 2022) . It has been estimated that 2-14% of COVID-19 survivors develop POTS within 6-8 months of infection (Blitshteyn and Whitelaw, 2021; Johansson *et al.*, 2021). Several mechanisms are involved in this syndrome. Firstly, hypovolemia, fever, anorexia, excessive sweating and the consequent physical deconditioning after acute infection cause low stroke volume. Secondly, SARS-CoV-2 might infect and destroy extracardiac postganglionic neurons of the sympathetic noradrenergic system (SNS), causing splanchnic venous pooling or a failure of reflexive mesenteric vasoconstriction during orthostatism. Third, SARS-CoV-2 could also invade the brainstem and alter functions of medullary centers that are responsible for SNS control and heart rhythm; this could explain the association with symptoms like brain fog (Marques, Quaresma and Falcão, 2023). The treatment of POTs primarily involves non-pharmacotherapy options, in entails increasing intake of fluid (2-3l/d) and salt (10-12 gr/d), avoiding excessive standing and dehydration, wearing compression socks, sleeping in the head-up position. Increasing physical exercise, which might be useful in some symptoms of Long COVID, in patients suffering with POTs and post-exertional malaise or chronic fatigue might exacerbate symptoms. Therefore, a gradual physical reconditioning with regular exercise is the

cornerstone of treatment for POTS. Physical countermeasures (e.g., squeezing a rubber ball, leg crossing and muscle tensing, muscle pumping, cough cardiopulmonary resuscitation) are suggested to manage acute clinical symptoms and prevent orthostatic intolerance. Moreover, occupational therapy, relaxation training, stress management and wellness instruction (e.g., sleep hygiene, healthy diet) could help manage symptoms (Fu and Levine, 2018). Pharmacological intervention (e.g., beta blockers, clonidine, ivabradine) might be useful if lifestyle modifications do not resolve POTS symptoms (Sheldon *et al.*, 2015; Ormiston, Świątkiewicz and Taub, 2022).

***Neurological outcomes***

In a US Veteran cohort of 154,068 individuals with COVID-19**,** matched with a negative control group, an increased risk (HR 1.42 with 95% CI 1.38-1.47) of neurologic sequelae including ischemic and hemorrhagic stroke, cognition and memory disorders, peripheral nervous system disorders, extrapyramidal and movement disorders, mental health disorders, musculoskeletal disorders, sensory disorders, Guillain–Barré syndrome were observed at one year of follow-up. The increased risk was independent of hospitalization during acute COVID-19 (Xu, Xie and Al-Aly, 2022). Even in an analysis of 2-year retrospective cohort studies of 1,487,712 patients with control group, the risk of neurological outcomes remained higher in the COVID group than in the control group. However, it was observed that the risk trajectory of psychiatric disorders (mood and anxiety disorders) returned to baseline after 1-2 months (mood disorders at 43 days, anxiety disorders at 58 days) and subsequently reached an overall incidence to the matched comparison group. In contrast, the risks of cognitive impairment, dementia, psychotic disorders, and epilepsy or seizures were still increased at the end of the 2-year follow-up period (HRs significantly greater than 1). These results show that the risk of developing psychiatric disorders seems to disappear after the first few months following acute infection, whereas a risk horizon is not reached for cognitive, psychotic disorders and epilepsy (Taquet *et al.*, 2022).

From a pathogenetic point of view, several hypotheses have been proposed. In patients with neurological symptoms, areas of hypometabolism were observed in the cortex, brainstem, cerebellum (Guedj *et al.*, 2021; Hugon *et al.*, 2022). Therefore, it was hypothesized that these regions experience high levels of microglial activation, cytotoxic T lymphocyte infiltration, oxidative stress, neurodegeneration and demyelination secondary to neuroinvasion. Microglial activation, together with systemic inflammation, appears to be a crucial point in the pathogenesis and persistence of neurocognitive disorders. This microglial reactivity seems to reduce hippocampal neurogenesis, loss of myelinating oligodendrocytes and oligodendrocyte precursors and subcortical areas (Leng *et al.*, 2023). Moreover, there was demonstrated extensive damage to the olfactory epithelium in individuals with persisting anosmia, which manifests as thinning of olfactory filia and reduction in olfactory bulb volumes. In addition, persisting local inflammation sustained the olfactory damage (Kandemirli *et al.*, 2021; Leng *et al.*, 2023).

As treatment of persistent anosmia, the use of intranasal insulin was proposed for its ability to regenerate olfactory mucosa. This therapy is used in non-COVID patients; actually, some clinical experiences have showed an improvement of anosmia, and a clinical trial is ongoing (NCT05104424) (Mohamad, Badawi and Mansour, 2021; Cherobin *et al.*, 2023; Daniel *et al.*, 2023). To contrast local inflammation on the olfactory epithelium, corticosteroids have been proposed to accelerate the recovery of smell. In 2021, an RCT of mometasone nasal spray, which included one hundred COVID-19 patients with post-infection anosmia, assigned patients to two treatment branches: mometasone furoate nasal spray with olfactory training for three weeks (N=50) or the control group with only olfactory training (N=50). There was no significant difference in duration of smell loss between groups ($p$=0.31). However, there was a significant improvement in score at week 3 in both groups (Abdelalim *et al.*, 2021). Nevertheless, Singh *et al.* were able to demonstrate significant improvements in smell (on day five) compared with baseline (day one) using fluticasone nasal spray compared to no intervention during acute COVID infection (Singh, Jain and Parveen, 2021). The results of these two studies suggest that corticosteroids could be used in the acute phase of infection to accelerate the recovery of smell.

Promising results emerged from the use of palmitoylethanolamide and luteolin (PEA-LUT) due to their neuroprotective and anti-inflammatory properties. Di Stadio *et al.* conducted an RCT in COVID cohort with persisting anosmia for > 6 months, dividing patients into two treatment branches: PEA-LUT plus olfactory training, and placebo plus olfactory training. The intervention group showed significantly greater improvement in olfactory threshold, discrimination, and identification scores compared to controls (92% vs 42%, $p$=0.0001) (Di Stadio *et al.*, 2022).

Concerning the treatment of brain fog and fatigue, RCTs evaluating the efficacy of conventional anti-inflammatory agents, such as steroids or IV immunoglobulin, are ongoing, whereas low-dose naltrexone, in a long COVID cohort, has shown good results in improving brain fog and fatigue (O'Kelly *et al.*, 2022); a forthcoming placebo-controlled RCT (NCT05430152) will provide greater clarity on its efficacy. In addition to drug therapy, neurorehabilitation seems to be effective in the management of neurocognitive and psychological disorders. Cognitive therapies and mindfulness techniques have been used with

improvement of symptoms; several RCTs are ongoing (Mathern *et al.*, 2022).

### *Musculoskeletal outcomes*

Chronic fatigue and post exertional malaise (PEM) are common symptoms in Long COVID syndrome. PEM is a worsening of symptoms (e.g., fatigue, weakness, orthostatic intolerance) and signs (e.g., heart rate variation, temperature dysregulation) after minimal physical or cognitive exertion, which can occur up to 72 hours after exertion and persist 24 hours or more. It can be triggered by daily activities such as sitting at the dining table, standing to make a salad, taking a shower, driving a car, grocery shopping, listening to a lecture, socializing, having a conversation, reading, or cleaning the house. In observational studies, PEM is present in almost half of Long COVID patients (Twomey *et al.*, 2022; Vernon *et al.*, 2023).

Moreover, PEM is also pathognomonic of myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and a diagnosis of them is necessary. ME/CFS is a complex chronic multi-system illness associated with a variety of constitutional and neurocognitive symptoms. It has a prevalence of 0.17-0.89% in the general population and occurs more frequently in women. The Institute of Medicine's most recent diagnostic criteria, published in 2015, characterize ME/CFS as a spectrum of five core symptoms: fatigue, post-exertional malaise, cognitive changes (impaired memory, concentration, information processing), sleep disturbance (unrefreshing sleep, circadian rhythm reversal), and orthostatic intolerance. Symptoms must not be relieved by rest and must persist for more than 6 months in the absence of any significant clinical or laboratory findings. Up to 75% of people with ME/CFS cannot work full-time and 25% are bedridden, have extreme sensitivity to sensory input and are dependent on others for care (Bateman *et al.*, 2021).

ME/CFS might be triggered by infection. Indeed, many cases are reported after infections such as EBV, West Nile virus, SARS and MERS, Q fever. Given the high prevalence of symptoms such as fatigue, PEM, sleep disorders in Long COVID syndrome, it was hypothesized that SARS COV2 could also be an infectious trigger of ME/CFS. However, there are still no observational studies examining the post-acute symptoms of COVID-19 as defined by the criteria of ME/CFS (Poenaru *et al.*, 2021).

The pathogenesis is not well understood; mechanisms linking acute infection and chronic dysregulation of the immune system, characterized by decreased natural killer cell function, T-cell depletion, mitochondrial dysfunction, and vascular and endothelial dysfunction have been proposed. In particular, a dysfunction of mitochondrial enzymes involved in inflammatory and anti-oxidant pathways could be the driver of orthostatic intolerance and post-exertional malaise, due to their involvement in peripheral vasodilation and autonomic regulation of the cardiovascular system.

As therapeutical approaches, The National Institute for Health and Care Excellence (NICE) guidelines on ME/CFS currently recommends graded exercise therapy (GET) and cognitive behavioral therapy. However, more recent evidence suggests that graded exercise therapy may accentuate post-exertional malaise, especially in patients with post COVID-19 symptoms. For this reason, in July 2020 NICE released a statement urging caution when implementing GET for people recovering from COVID 19 (Torjesen, 2020).

Another management strategy for fatigue and PEM is pacing; it is a coping strategy whereby patients manage tasks and activities to avoid over-exertion and exacerbating fatigue (Goudsmit *et al.*, 2012). In a prospective study of 31 patients who complained of persisting PEM after 17 months from acute COVID infection, the use of a pacing protocol statistically and clinically reduced the average number of PEM episodes (from 3.4 episodes in Week 1 to 1.1 in Week 6) and improved quality of life and tolerability to physical activity (Parker *et al.*, 2023). RCTs regarding therapeutical strategy for fatigue and PEM are ongoing.

## PNEUMOLOGICAL OUTCOMES

Shortness of breath is another persistent symptom after acute COVID infection. Several studies have included serial lung function testing up to 1 year after COVID-19 (Blanco *et al.*, 2021; Faverio *et al.*, 2022, 2023; Eizaguirre *et al.*, 2023). On average, an abnormal ratio of $FEV_1$ to forced vital capacity has been reported, with a normal forced expiratory flow rate between 25% and 75% of maximum. Moreover, most of these studies reported abnormal lung diffusion in approximately a third of patients. Similarly, imaging studies have identified chest CT abnormalities in up to 25% of cases after 1-year follow-up. These abnormalities range from limited ground-glass opacity and subpleural reticulation to more extensive ground-glass opacity, traction bronchiectasis, and honeycombing (Luger *et al.*, 2022; Stewart *et al.*, 2023). This last pattern is more prevalent in patients who were hospitalized with severe COVID 19 and is an expression of fibrotic evolution. The occurrence of fibrotic lesions is mainly associated with older age, ARDS, longer hospital stays, tachycardia, mechanical ventilation, and higher initial chest CT score. A multicenter cohort study is ongoing to determine the prevalence of post-COVID-19 fibrosis and to identify risk factors in patients admitted to hospital and in those managed at home (Stewart *et al.*, 2023).

SARS-CoV-2 virus causes an alveolar injury that can continue even after viral clearance. The binding between the virus and the ACE receptor leads to the internalization of the ACE2 receptor and, consequently, to an increase profibrotic angiotensin 1 and 2 signal-

ing and alveolar TGF-β signaling, which are known to stimulate fibrotic pathways such as fibronectin production, collagen synthesis, and fibroblast proliferation. Moreover, a pro-inflammatory immune set-up with high concentrations of eosinophils, mast cells, and lymphocytes, particularly $CD8^+$ cells, was correlated with worse lung function and increased radiological abnormalities after COVID-19. Innate immunity, in particular the excessive macrophages and neutrophil activation, might be also involved in fibrosis (Giacomelli *et al.*, 2021; Singh *et al.*, 2023).

The introduction of steroids as therapy for acute COVID pneumoniae significantly improved prognosis and lung outcome, and might protect by fibrotic evolution. Steroids, due to their anti-inflammatory action, have been proposed in the treatment of pulmonal sequelae. However, there are no placebo-controlled trials of steroids in post-COVID-19 interstitial lung disease (ILD), although an open-label study of high-dose (40 mg reducing to 10 mg) versus low-dose (10 mg stable dose) steroids showed that only 16 of 65 (24.6%) patients responded to the high-dose regimen, which was not significantly different from the response rate for the low- dose regimen (12 of 65 patients; 18.5%) (Dhooria *et al.*, 2022).

Another possible pulmonary sequela is thromboembolic disease. It is known that vascular endothelial dysfunction, a hyperinflammatory immune response, and a hypercoagulant state in acute phase of infection, predispose to developing venous thromboses.

Fortunately, despite the high numbers of patients with COVID-19-related pulmonary emboli globally, an increase in chronic thromboembolic pulmonary hypertension (CTEPH), which is a potential complication of pulmonary embolism, has not been reported (Newman *et al.*, 2021). The apparent lack of COVID-19-related CTEPH might be due to the clot burden affecting more distal segmental anatomical locations within the pulmonary vasculature. This subsegmental disease could represent an in-situ thrombosis (immunothrombosis) due to local inflammation. However, the risk of future re-thrombosis has not been defined (Singh *et al.*, 2023).

Finally, dyspnea is not always related to a lung damage. In fact, this debilitating symptom can be influenced by pre-existing respiratory conditions, psychological conditions (anxiety, previous experiences of breathlessness) and the level of function (including deconditioning). The relative contribution of all these factors can vary from one individual to another.

The main treatment of breathlessness is represented by rehabilitation programs, which include aerobic exercise, resistance exercise, and education on symptom management such as breathing exercises that aim to control breath and to strengthen breathing. Complementary behavioral modification and psychological support may also be useful (Reinert *et al.*, 2022; Zheng *et al.*, 2022). However, the risks of physical rehabilitation must be considered. Indeed, it may not be suitable for survivors of critical COVID-19 with severe pulmonary or cardiac damage and in patients with ME/CFS or POTS(Torjesen, 2020).

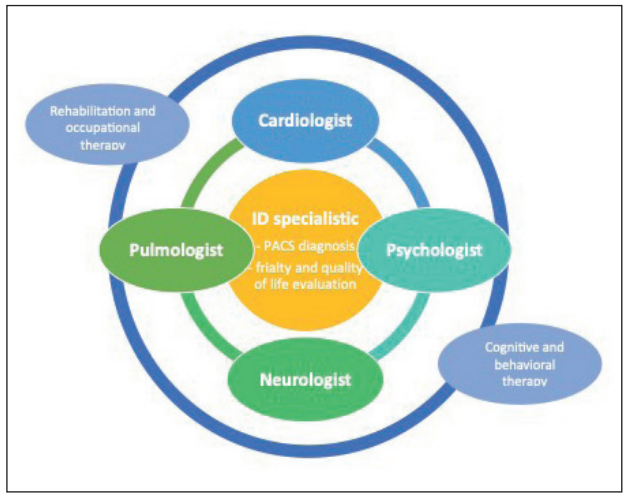


**Figure 3** - *Multidisciplinary approach to Long COVID syndrome. PACS: post acute COVID sequelae.*

## CONCLUSION

Long COVID is a complex syndrome, with fluctuant and varied symptoms. Thaweethai *et al.* tried to identify the most specific Long COVID symptoms and proposed a score to diagnose the syndrome. This score might be useful to screen populations and to refer them to specialized centers (Thaweethai *et al.*, 2023). Indeed, even international guidelines (Centers for Disease Control and Prevention, no date; National Institute for Health and Care Excellence (NICE), 2020; Soriano *et al.*, 2022) suggest that patients with more than one symptom related to Long Covid should be treated by multidisciplinary centers. Considering the most frequent long-term outcomes, the professional figures involved in these clinics should be: the infectious disease specialist as coordinator, cardiologist, pulmonologist, phycologist, neurologist as co-participant to guide the sequelae diagnosis, and neuropsychologist, physiotherapist to set up a rehabilitation treatment. According to our experience (Squillace *et al.*, 2023), this interplay with different specialists is useful because it allows a more accurate and quicker diagnose, and investigation of therapeutical possibilities (*Figure* 3).

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