Universitat Autònoma de Barcelona

Headache attributed to COVID-19: an opportunity to advance in understanding the pathophysiology of headache disorders

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Doctoral Thesis

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At the time of the writing of this thesis, in January 2022, two years have gone since the first Chinese reports of COVID-19, the disease caused by the infection of the novel SARS-CoV-2.

Soon after its onset, COVID-19 became a pandemic and turned into one of the main global health issues of our times.

Globally **298.915.721 COVID-19 confirmed cases** and **5.469.303 deaths** have been reported to the World Health Organization since the beginning of the pandemic; and in Spain, specifically, 9.660.208 confirmed cases and 92.767 deaths.

We have all faced and have been impacted physically, emotionally and psychologically by COVID-19.

Healthcare professionals have put unprecedented efforts and courage, not without experiencing fear and grief, in helping those in need. Scientists have devoted time and perseverance to finding vaccines and treatments for the disease. Indeed, this has been an unprecedented collaborative effort to fight this virus together.

So, with this doctoral thesis I have tried to make my contribution to understanding COVID-19, and its neurological impact, with the goal of helping to transform this incredible challenge into an opportunity.

This thesis wants to honor and is dedicated to all the people that suffered or are still suffering from COVID-19 or its consequences, to their families and, to all the healthcare professionals and scientists that have fought and fight COVID-19.

To my Parents,

For their love and for always putting my dreams before theirs

To my Best Friends,

For always being there for me, no matter the distance

To my Mentors,

For showing me the right path in life

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The word *thesis* defines a main idea that someone has to prove.

When I think about it, our life is itself a *thesis.*

We have an idea of ourselves that we want to prove through our actions, accomplishments and achievements. This doctoral thesis is a fundamental step in the idea, or better, *thesis* of myself of becoming a researcher.

Yet, as any great accomplishment in my life, this doctoral thesis would not have been possible without the efforts, the learnings and the support of the people I would like to acknowledge.

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Finally, the last paragraph of my acknowledgements is the dedicated to a person that has marked these last years since I become a PhD candidate: my thesis Director Dr Patricia Pozo-Rosich. To her, my gratitude for giving me the opportunity to start my research career in her group and for her constant teachings, professionally and in daily life, suggestions, support and encouragements all along this path. She represents a model of researcher and mentor I believe in and I aspire to be.

Abbreviations

ACE2 Angiotensin-converting enzyme 2

ARDS Acute Respiratory Distress Syndrome

BBB blood–brain barrier

CGRP Calcitonin gene-related peptide

CNS central nervous system

CoV Coronavirus

COVID-19 Coronavirus disease 2019

CSF cerebrospinal fluid

DMV Double-Membrane Vesicle

E envelope

EMA European Medicines Agency

ER emergency room

GBS Guillain–Barre´ Syndrome

HIV Human Immunodeficiency Virus

ICHD-3 International Classification of Headache Disorders-3rd edition

ICU intensive care unit

IFN interferon

IL Interleukin

IRF3/7 Interferon Regulatory Factor 3/7

M membrane

mAbs monoclonal antibodies

MERS-CoV Middle East respiratory syndrome coronavirus

MHC Major Histocompatibility Complex

N nucleocapsid

NETs Neutrophil Extracellular Traps

NK-kB nuclear factor-κB

NLRP3 NOD-like Receptor Protein 3

Nsps non-structural proteins

ORF Open Reading Frame

PNS peripheral nervous system

PRR Pattern Recognition Receptors

RBD Receptor-Binding Domain

RTC Replication and Transcription Complex
RT-PCR Reverse Transcriptase-Polymerase Chain Reaction
S spike
SARS-CoV Severe Acute Respiratory Syndrome Coronavirus
SARS-CoV-2 Severe Acute Respiratory Syndrome Coronavirus-2
sg RNAs subgenomic mRNAs
ssRNA single-stranded, positive-sense RNA genome
TLRs Toll-Like Receptors
TMPRSS2 Transmembrane Protease Serine 2
TNF Tumor Necrosis Factors
TRPV1 Transient Receptor Potential Vanilloid 1 channel
TRS Transcription Regulatory Sequence
VWF von Willebrand factor
WHO World Health Organization

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Summary

At the end of 2019, a new coronavirus, the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), emerged in China. The severe respiratory infectious disease, for which SARS-CoV-2 was responsible, was named coronavirus disease 2019 (COVID-19). SARS-CoV-2 rapidly spread world-wide and COVID-19 became a pandemic. Spain was hit in March 2020. At that time, the number of infected patients was so high that, no matter the specialization, any healthcare professional had to attend COVID-19 patients. In this setting, headache was a commonly reported symptom of SARS-CoV-2 infection, often severe and difficult-to-treat, though some patients were not experiencing it. As neurologists, we wanted to better understand the headache attributed to this infection and why some patients had it while others did not. To do so, we could not count on any literature specifically published on this matter.

This motivated to start a research project and plan a doctoral thesis whose first objective was to describe the characteristics and evolution of headache attributed to COVID-19. We conducted a prospective study in a cohort of COVID-19 patients attended at the ER, where we observed that around 75% of them had headache. Of them, around 25% had a disabling, severe headache with migraine-like features. This phenotype was present even in those patients without personal or family migraine history and clearly pointed to the activation of the trigeminovascular system, from a pathophysiological standpoint. Following up patients at 6 weeks, we soon realized that headache could persist in around 30% of patients. This was in line with the emergence of the “post-COVID” syndrome, that defined a spectrum of symptoms persisting in COVID-19 patients after the resolution of the infection. Headache was one of them and, as part of the evolution of headache attributed to this infection, we decided to analyze it at even longer term, by conducting a new study. We collected data at 9 months from our cohort of COVID-19 patients attended at the ER and gathered them with the cohorts from other Spanish centers, observing that around 16% of patients were still experiencing headache at this timepoint, mainly with migraine-like features. In parallel, the increasing number of referrals for persistent headache after COVID-19 to our headache clinic urged us to start-up a specific clinic which allowed us to gather a cohort of outpatients with persistent headache. This led us to observe three main patient phenotypes: 1) patients with personal migraine history and

sudden headache worsening in the context of the infection; 2) patients without personal migraine history and a *de novo* headache since the acute phase of the infection; and, 3) patients without personal migraine history and a *de novo* headache starting after the resolution of the infection.

Further, the prospective study on COVID-19 patients attended at the ER was also designed to investigate the relationship between inflammation and COVID-19 headache, as a way to better understand its pathophysiology. The study of inflammatory biomarkers was specifically motivated by the evidence that the SARS-CoV-2 was able to trigger an hyperinflammatory response, known as *cytokine storm*, that specifically involved IL-6. Although IL-6 is known to be able to sensitize the trigeminovascular system in a rodent preclinical model of migraine and could be therefore involved in the pathophysiology of headache, we observed that COVID-19 patients with headache surprisingly had lower and more stable levels of IL-6 during the acute phase of COVID-19, This finding probably suggests, from one side that IL-6 may play a less relevant role in COVID-19 headache, from the other, that patients with headache have better control of inflammatory responses, considering that higher IL-6 levels are associated with more severe COVID-19 disease.

Calcitonin gene-related peptide (CGRP), was one of the other molecules that we focused on due to its well-known mechanism in the pathophysiology of migraine. CGRP, however, also has several other roles in the human body, including the regulation of inflammatory responses in the lungs after infections and therefore could be important in COVID-19 pathogenesis. The difficulties in obtaining blood samples from hospitalized patients during the COVID-19 pandemic, prevented us from directly analyzing the relationship between circulating levels of CGRP and the presence of headache. However, considering that monoclonal antibodies against CGRP are an approved and available treatment for migraine prevention, we focused instead our attention on determining whether the antagonism of CGRP could have a potential beneficial or detrimental effect in terms of COVID-19 prognosis in currently treated migraine patients. In order to increase the number of patients in treatment with anti-CGRP monoclonal antibodies for migraine, we developed a multicentric Spanish web-

based survey, which did not support neither a major risk of infection nor worsening outcomes in migraine patients with COVID-19 that were exposed to anti-CGRP treatment compared to those that were not using this treatment.

The final objective of this thesis was to analyze the relationship between the presence of headache in the acute phase of the infection and COVID-19 prognosis. The reason was mainly motivated by the fact that headache is usually considered an unspecific and irrelevant symptom during an infection, but our clinical observations with COVID-19, from the very beginning, were in contrast to this idea: headache had specific characteristics and was not always present. This fact could reflect that some patients activate certain pathophysiological mechanisms as a consequence of the infection, leading to headache, while others do not. The prospective design of our study on patients attended at the ER allowed us to observe that patients with headache had a better prognosis in terms of one-week shorter COVID-19. This unprecedented finding urged us to meta-analyze studies on COVID-19 mortality that reported headache as a symptom of the infection. We found that headache as a symptom among COVID-19 patients presenting to hospitals was correlated with enhanced COVID-19 survival. This led us to the conclusion that the pathophysiological mechanisms underlying headache in the acute phase of a viral infection could be associated with enhanced host defenses against the pathogen.

This thesis represents a milestone in the headache field, providing with its findings the first longitudinal and well-characterized description of headache attributed to COVID-19 as an acute and post-acute (post-covid) symptom. The migraine-like features of headache attributed to COVID-19, that represents a secondary headache, raises new questions on the pathophysiological similarities with primary headache disorders, such as migraine, and on whether certain infections in predisposed individuals can trigger an underlying migraine biology. Further, this thesis clearly demonstrates that headache is not an unspecific symptom but rather a marker of enhanced survival during a viral infection. As a consequence of our findings, we are the first ones in hypothesizing the revolutionary concept of headache as a defensive mechanism in humans against infections. COVID-19 therefore has represented an

opportunity to better understand headache disorders. Yet, future studies are warranted to prove these new theories and advance in the knowledge of the relationship between viruses and headache and its function in human biology.

Resumen

A finales de 2019, surgió en China un nuevo coronavirus, el síndrome respiratorio agudo severo coronavirus-2 (SARS-CoV-2 por su acrónimo en inglés). La enfermedad infecciosa respiratoria grave, de la que fue responsable el SARS-CoV-2, se denominó enfermedad por coronavirus 2019 (COVID-19 por su acrónimo en inglés). La rápida difusión del SARS-CoV-2 por todo el mundo convirtió la COVID-19 en una pandemia. España vio un rápido aumento de los casos a partir de marzo de 2020. En ese momento, el número de pacientes infectados era tan alto que, independientemente de la especialidad, cualquier profesional sanitario tuvo que atender a los pacientes con COVID-19. En este contexto, la cefalea era un síntoma de la infección por SARS-CoV-2 comúnmente reportado, a menudo intenso y difícil de tratar, aunque llamaba la atención que algunos pacientes no lo experimentaban. Como neurólogos, queríamos comprender mejor la cefalea atribuida a esta infección y por qué algunos pacientes la tenían y otros no. Sin embargo, no podíamos contar con ningún tipo de literatura publicada específicamente sobre este tema.

Esto nos motivó a iniciar un proyecto de investigación y planificar una tesis doctoral cuyo primer objetivo fue describir las características y evolución de la cefalea atribuida al COVID-19. Realizamos un estudio prospectivo en una cohorte de pacientes con COVID-19 atendidos en urgencias, donde observamos que alrededor del 75% presentaba cefalea. De ellos, alrededor del 25% tenía una cefalea severa e incapacitante con características similares a las de la migraña. Este fenotipo estaba presente incluso en aquellos pacientes sin antecedentes personales o familiares de migraña y apuntaba claramente a la activación del sistema trigeminovascular, desde el punto de vista fisiopatológico. Al hacer un seguimiento de los pacientes a las 6 semanas, pronto nos dimos cuenta que la cefalea podía persistir en alrededor del 30% de los pacientes. Esto reflejaba la aparición del síndrome “post-COVID”, que se iba definiendo como un espectro de síntomas persistentes en pacientes con COVID-19 después de la resolución de la infección. La cefalea era uno de estos síntomas y, como parte de la evolución de la cefalea atribuida a COVID-19, decidimos analizarla a más largo plazo, realizando un nuevo estudio. Recogimos datos a los 9 meses de nuestra cohorte de pacientes con COVID-19 atendidos en urgencias y los juntamos con las cohortes de otros centros españoles, observando que alrededor del 16% de

los pacientes seguía con cefalea, principalmente con características similares a las de la migraña. Paralelamente, el creciente número de derivaciones a nuestra consulta de cefalea por cefalea persistente después de COVID-19 nos impulsó a crear una consulta especializada en esta patología, y poder recoger una cohorte de pacientes ambulatorios con cefalea persistente observando que existían tres fenotipos principales de pacientes: 1) pacientes con antecedentes personales de migraña y cefalea con rápido empeoramiento en el contexto de la infección; 2) pacientes sin antecedentes personales de migraña y con aparición de una cefalea *de novo* desde la fase aguda de la infección; y, 3) pacientes sin antecedentes personales de migraña y con aparición de una cefalea *de novo* después la resolución de la infección.

Además, en nuestra hipótesis inicial estaba analizar el papel de la inflamación en este contexto. En la cohorte de pacientes recogidos en urgencias se estudiaron biomarcadores inflamatorios. Parecía evidente que el SARS-CoV-2 podía desencadenar una respuesta inflamatoria exagerada, conocida como *tormenta de citoquinas*, que involucraba en concreto a la IL-6. Aunque se sabe que la IL-6 puede sensibilizar el sistema trigeminovascular en modelos animales de migraña y, por lo tanto, podría estar involucrada en la fisiopatología de la cefalea, observamos que los pacientes con COVID-19 con cefalea sorprendentemente tenían niveles más bajos y más estables de IL- 6 durante la fase aguda del COVID-19. Este hallazgo probablemente sugiere, por un lado, que la IL-6 puede desempeñar un papel menos relevante en la cefalea por COVID-19, y por otro, que los pacientes con cefalea tienen un mejor control de la respuesta inflamatoria, considerando que los niveles más altos de IL-6 están asociados con una enfermedad COVID-19 más grave.

Entre otras moléculas que nos interesaba estudiar, también estaba el péptido relacionado con el gen de la calcitonina (CGRP por su acrónimo en inglés). El CGRP tiene un mecanismo bien conocido en la fisiopatología de la migraña, pero también tiene otras funciones en el cuerpo humano, incluida la regulación de respuestas inflamatorias en los pulmones después de las infecciones y, por lo tanto, podría ser importante en la patogénesis del COVID-19. Las dificultades para obtener muestras de sangre de pacientes hospitalizados durante la pandemia de COVID-19 nos impidió

analizar directamente la relación entre los niveles circulantes de CGRP y la presencia de dolor de cabeza. Sin embargo, considerando que los anticuerpos monoclonales contra el CGRP son un tratamiento aprobado y disponible para la prevención de la migraña, centramos nuestra atención en determinar si el antagonismo de CGRP podría tener un efecto potencialmente beneficioso o perjudicial en términos del pronóstico del COVID-19 en pacientes con migraña actualmente tratados. Para poder contestar a esta pregunta, diseñamos un estudio multicéntrico español utilizando una encuesta online. Este estudio no evidenció ni un mayor riesgo de infección ni peor pronostico en pacientes con migraña con COVID-19 que estaban tratados con anticuerpos contra el CGRP en comparación con aquellos que no usaban este tratamiento.

Por último, el objetivo final de esta tesis fue un analizar la relación entre la presencia de cefalea en la fase aguda de la infección y el pronóstico del COVID-19. La razón fue motivada principalmente por el hecho de que la cefalea generalmente se considera un síntoma inespecífico e irrelevante durante una infección, pero nuestras observaciones clínicas desde el principio de la pandemia contrastaban con esta idea: la cefalea tenía características específicas y no siempre estaba presente. Este hecho podría reflejar que algunos pacientes activan determinados mecanismos fisiopatológicos como consecuencia de la infección, dando lugar a la presencia de cefalea, mientras que otros no. Nuestro estudio prospectivo en pacientes con COVID-19 atendidos en urgencias nos permitió observar que los pacientes con cefalea tenían mejor pronóstico con una semana menos de duración de COVID-19. Este hallazgo sin precedentes nos llevó a realizar un meta-análisis de los estudios sobre mortalidad por COVID-19 que reportaban la cefalea como síntoma de la infección. Descubrimos que la cefalea como síntoma entre los pacientes con COVID-19 que acudieron a los hospitales se correlacionaba con una mayor supervivencia de COVID-19. Esto nos llevó a concluir que los mecanismos fisiopatológicos subyacentes a la cefalea en la fase aguda de una infección viral podrían estar asociados con una mejor capacidad del huésped de defenderse contra el patógeno.

Esta tesis representa un hito en el campo de las cefaleas, proporcionando con sus hallazgos la primera descripción longitudinal y bien caracterizada de la cefalea atribuida al COVID-19 tanto como síntoma agudo y como post-agudo (post-covid). Las características similares a la migraña de la cefalea atribuida a COVID-19, que representa una cefalea secundaria, plantean nuevas preguntas sobre las similitudes fisiopatológicas con las cefaleas primarias, como la migraña. Sobre todo, nos hacen reflexionar sobre si ciertas infecciones en individuos genéticamente predispuestos pueden en realidad activar directamente el substrato biológico de la migraña. Además, esta tesis demuestra claramente que la cefalea no es un síntoma inespecífico, sino un marcador de supervivencia durante una infección viral. A raíz de nuestros hallazgos, formulamos la novedosa hipótesis de la cefalea como mecanismo adaptativo y protector en la especie humana frente a infecciones. Por lo tanto, el COVID-19 ha representado una oportunidad para entender mejor las cefaleas y su relevancia. Sin embargo, son necesarios nuevos estudios para investigar estas nuevas teorías y avanzar en el conocimiento de la relación entre los virus y la cefalea en la biología humana.

1. Introduction

1.1. Headache disorders

1.1.1. Primary and Secondary Headache Disorders

Headache disorders are among the most common disorders of the nervous system and according to the World Health Organization, it has been estimated that almost half of the adult population have had a headache at least once within the last year (1). According to the International Classification of Headache Disorders, 3rd edition (ICHD-3) (2), **headache disorders could be classified into primary or secondary**. The adjective *primary* refers to those headaches, not caused by or attributed to another disorder. Migraine, tension-type headache and trigeminal autonomic cephalalgias are the three most common ones. On the contrary, the term *secondary headache disorder* is used when another underlying disorder is able to cause headache. According to the ICHD-3, the causative disorder must be specified by using the word *attributed to*. The different secondary headache disorders are listed in Table 1.

Table 1. Secondary Headache Disorders according to the ICHD-3

	Part II: The secondary headaches
5	Headache attributed to trauma or injury to the head and/or neck
6	Headache attributed to cranial or cervical vascular disorder
7	Headache attributed to non-vascular intracranial disorder
8	Headache attributed to a substance or its withdrawal
9	Headache attributed to infection
10	Headache attributed to disorder of homoeostasis
11	Headache or facial pain attributed to disorder of the cranium, neck, eyes, ears, nose, sinuses, teeth, mouth or other facial or cervical structure
12	Headache attributed to psychiatric disorder

Although several headache disorders exist, at present, **the pathophysiological mechanisms underlaying headache as a symptom are best understood in relation to migraine**.

Migraine is a chronic neurological disease that occurs in the form of recurrent attacks of headache accompanied by other neurological symptoms. Migraine is considered to have a strong inherited genetic component (3), although this does not apply to all individuals, considering that some do not have any family history of migraine. This fact may implicate the existence of other factors able to produce epigenetic changes and trigger migraine.

Migraine attacks usually last 4 to 72 hours. **Migraine characteristics** include: unilateral headache, throbbing quality, moderate or severe intensity and worsening with movement or exercise. Accompanying symptoms are the following: sensitivity to light or sound (photophobia or phonophobia), nausea and/or vomiting. Some patients present, in addition to headache, transient neurological symptoms (visual, sensory, etc) in some or all of the attacks, the so-called aura. Interestingly, migraine-like features are also observed in headache attributed to trauma or injury of the head, a secondary headache disorders, which may imply that headache mechanisms in migraine could be partially shared by other headache disorders (4).

For this reason, the knowledge acquired so far in migraine pathophysiology is useful to better characterize some of the underlying mechanisms and clinical aspects related to headache as a symptom, in general.

At present, it is known that at beginning of a migraine attack, a transient depolarization wave, called the cortical spreading depression, appears and extends through the cerebral cortex (5). This phenomenon, that is clinically correlated with aura, causes release of molecules such as substance P, calcitonin gene-related peptide (CGRP) and neurokinin A, which produce hyperemia and vasodilatation of the meningeal vessels (sterile neurogenic inflammation) (6). **The meningeal vessels are in intimate contact with the trigeminal nerve terminals and together constitute the trigeminovascular system**. The vasodilation is able to activate the nociceptors of the trigeminal nerve terminals at the perivascular level and this **is responsible for the onset of pain**. **The trigeminovascular system therefore plays a key role in**

the pathophysiology of headache (7). The information is transmitted to the trigeminal ganglion through the branches of the trigeminal nerve and then to the nucleus of the trigeminal nerve in the brainstem (7). From here, it reaches the thalamus and hypothalamus, and subsequently to the somatosensory cortex, but also projects to other cortical areas such as insula, amygdala, cingulate cortex, etc, that are related to the emotional experience and interpretation of pain (8). The pain process and modulation are therefore very complex and involve several areas of the nervous system that are responsible not only for headache but also for the different accompanying symptoms.

1.1.2. Headache attributed to systemic viral infection

Among secondary headache disorders, Section 9 of the ICHD-3 is dedicated to *Headache attributed to infection* (2). The subsection 9.1 defines *Headache attributed to intracranial infection* whereas 9.2 *Headache attributed to systemic infection*. Among the different systemic infections, the ICHD-3 distinguishes between *9.2.1 Headache attributed to systemic bacterial infection, 9.2.2 Headache attributed to systemic viral infection* and *9.2.3 Headache attributed to other systemic infection.*

This definition of ***9.2.2 Headache attributed to systemic viral infection*** **requires that headache is caused by and occurring in association with other symptoms and/or clinical signs of a systemic viral infection, in the absence of meningitis or encephalitis**. Evidence of causation should be demonstrated by at least two of the following: (1) headache has developed in temporal relation to onset of the systemic viral infection, (2) headache has significantly worsened in parallel with worsening of the systemic viral infection, (3) headache has significantly improved or resolved in parallel with improvement in or resolution of the systemic viral infection, and (4) headache has either or both of the following characteristics: (a) diffuse pain and (b) moderate or severe intensity (*see Table 2*). Moreover, according to ICHD-3, it could be defined as **acute** if a headache has been present for < 3 months or **chronic** if present for > 3 months; the systemic viral infection still being active or having resolved within the last 3 months.

Table 2. Diagnostic Criteria for 9.2.2 Headache attributed to systemic viral infection according to the ICHD-3

A	**Headache of any duration fulfilling criterion C**
B	**Both of the following:**
	1. systemic viral infection has been diagnosed
	2. no evidence of meningitic or encephalitic involvement
C	**Evidence of causation demonstrated by at least two of the following:**
	1. headache has developed in temporal relation to onset of the systemic viral infection
	2. headache has significantly worsened in parallel with worsening of the systemic viral infection
	3. headache has significantly improved or resolved in parallel with improvement in or resolution of the systemic viral infection
	4. headache has either or both of the following characteristics:
	a) diffuse pain
	b) moderate or severe intensity
D	**Not better accounted for by another ICHD-3 diagnosis.**

Although clinically it is frequently reported by patients in the setting of an acute viral infection, such as flu or colds (9), the real epidemiology of headache attributed to a systemic viral infection is unknown. Moreover, multiple viruses with different characteristics including morphology, transmission, tropism for specific organs and pathogenicity exist and this could substantially influence the possibility of presenting headache in the setting of the infection. Unfortunately, **very few data are available in the literature on the characteristics and variability, in relation to the pathogen involved, of headache attributed to a systemic viral infection**.

So, although potentially headache may be a symptom associated with a variety of viral infections, in literature headache features were best and almost uniquely described in the context of the acute phase of severe viral infections such as Dengue (10) and Zika (11) and during the acute or chronic infection of Human Immunodeficiency Virus (HIV) (12). In the case of Dengue, one study specifically observed that almost all patients had severe, bilateral, throbbing, frontal and/or retro-orbital pain, associated as well with nausea and/or vomiting and photophobia and/or phonophobia (13). These **migraine-like features** were also observed in HIV patients during the chronic phase of the infection (14). However, in the case of HIV, another

study found extremely difficult to distinguish headache attributed purely to HIV infection from the primary headaches (i.e. migraine) reported by most HIV patients, that often had begun subsequent to the HIV diagnosis (15).

Chronic headaches attributed to systemic viral infection, often presenting as new daily persistent headaches, were described after acute viral infections such as Dengue (16), Epstein-Barr virus (17) and as a consequence of the 1890 Russian/Asiatic flu (18).

The underlying pathophysiological mechanisms of headache attributed to a systemic viral infection are currently unknown. Nevertheless, probably indirect mechanisms involving fever may coexist with direct effects of the microorganisms themselves on the nervous system (19). In fact, headache is commonly associated with fever and may be dependent on it, but headache can also occur in its absence (19). Fever can be mediated by exogenous (e.g. toxins) or endogenous pyrogens (e.g. cytokines such as tumor necrosis factor [TNF], interferons [IFN], interleukin [IL]-1, etc) (20,21). Exogenous pyrogens are able to induce the release of endogenous pyrogens by host immune cells (macrophages). Endogenous pyrogens induce the synthesis of prostaglandins such as PGE2, that act on the hypothalamic thermoregulatory center, raising the thermostatic set point to initiate the febrile response (22). Yet, PGE2 has also vasoactive properties and could be indirectly implicated in any vascular component of headache (23). TNFs and IFNs were implicated in headache during flu or colds (24), based on the evidence that their administration provoked headache in human beings (25) and that headache was a common side-effect of IFN beta-1a, as observed for the treatment of multiple sclerosis (26). Thus, in the past, a cytokine theory, involving these molecules, was postulated to provide a unified mechanism to explain headache not only in the context of an infection but also triggered by food or secondary to trauma (25). However, this hypothesis is too simplistic to explain the complexity of headache disorders and, when considering viral infections, we have to keep in mind that there are multiple viruses with different characteristics and probably different propensity in causing headache. **Multiple pathogen-specific pathophysiological mechanisms can be therefore implicated a part from the indirect ones, such as fever or unspecific inflammation**. For example, some

viruses may trigger a specific inflammatory response. In the case of Zika, IL-5 levels correlated with headache as a symptom of the infection (27). In addition, some viruses are neurotropic and may exert their effects directly on the central nervous system (CNS). For example, during primary HIV infection neurological symptoms could correlate with cerebrospinal fluid (CSF) viral load (28,29). Some studies on HIV specifically focused on headache and suggested that one of its possible mechanisms was a dysregulation in the cortical excitability, considering that viral proteins such as *Tat* and gp120 could produce neurotoxicity by increasing glutamate excitotoxicity (30). In more advanced cases of HIV, it was proposed the hypothesis that HIV headache, in the absence of a secondary cause, could mimic an aseptic meningitis (31), through its ability to Infect macrophages and glial cells, resulting in brain toxicity. The specific characteristics of HIV infection led this headache disorder to being coded separately in the Appendix of the ICHD-3 (2) *as A9.3 Headache attributed to human immunodeficiency virus (HIV) infection,* considering that (1) HIV infection is always both systemic and within the CNS, (2) the CNS infection may progress independently of the systemic infection and (3) HIV infection is still not curable.

So, our current knowledge of headache attributed to systemic viral infections is scarce. The lack of data on the differential ability of the multiple existing viruses in causing headache is reflected by the ICHD-3·that, at present, only provides a general description of this headache disorder.

1.2. Viral infections

1.2.1. General characteristics

Viruses are small obligate intracellular parasites, which by definition contain a nucleic acid (either a RNA or DNA genome) surrounded by a protective, virus-coded protein coat, called capsid(32). However, the extreme structural simplicity does not match with the complexity from the biological and functional point of view. They depend on the metabolism of the host cell (parasites), and are forced to complete their lifecycle within it (obligate intracellular). The viral genome, transferred from cell to cell, must contain sufficient information to ensure the virus survival and propagation. Viruses have therefore evolved to contain the molecular machinery necessary for efficient and specific transfer of their genome to a new host cell. They are inert only as long as they are outside the host cell; once inside they are able to alter the metabolism of the cell and use it to their own advantage. The different ways viruses have to do so determine the multiple types of viral structure found in nature today.

The spectrum of virus-infected hosts is very broad: cellular microorganisms such as mycoplasma, algae and bacteria, plants and animals. However, **viral infections are not always associated with disease**, on the contrary, it is a common opinion that most of the viruses affecting the human species today are associated with subclinical or paucisymptomatic infections. This consideration arises from the hypothesis that there is a sort of balance, established along the joint evolution of viruses and the human species, between the host and the virus. The host tries to counteract the harmful effects of the viral infection through its defensive mechanisms, and **the virus tends to become "attenuated" in order not to kill the host to ensure its permanence in the environment**. However, this equilibrium can be broken following the decrease in the efficiency of the host defenses, as in the case of immunocompromised subjects. Furthermore, the evolutionary process towards the attenuation of viruses can take a very long time, so **when the human species is infected by a new virus, for example from animals or that emerged after**

recombination events, epidemics or pandemics of considerable gravity may happen.

So, viral infections are the result of a conflict between the invasive potential and characteristics of the pathogen and those of the host**. The stages common to almost all viral infections are the following: (1) virus entry, (2) replication at the site of first implantation, (3) dissemination to target other organs and (4) transmission of the virus from the infected host to the environment or to an uninfected individual**. Viruses can be transmitted vertically (from mother to child) or horizontally (from an infected subject to an uninfected one), which can be direct or indirect (through objects/vehicles/fomites). In the case of direct horizontal transmission, the entry of the virus can occur through various routes, the most common being the respiratory and digestive ones. Given that most viruses enter through mucosae, the chances of a successful infection are higher if the virus is able to carry out its replication cycle in epithelial cells. At this point the infection can remain localized to the tissue where the virus gained entry and firstly replicated, or it can spread to the rest of the organism. This characterizes all systemic infections and presupposes the dissemination of the virus through the bloodstream (viremia). However, for some viruses, such as herpesviruses (33), the dissemination can take place along the peripheral nerves: the virus reaches a nerve terminal and through trans-synaptic transfer enters the brain. Of note, **viral infections can be acute** (the virus rapidly reaches a maximum replication which subsequently decreases and is stopped when effective specific immune responses appear) **or persistent** (after the primary infection the virus persists in some organs). Both of these forms of viral infection can be clinically **symptomatic or asymptomatic**.

The symptomatic infection is the expression of a disease, where a structural and/or functional damage is caused directly or indirectly by the virus to the infected tissues. Target organs are, to a certain extent, characteristic of each virus and this defines the viral tropism. The onset of the disease is related to the cytopathic effect and consequent lysis of the infected cell, however, in addition to this direct damage, indirect mechanisms, such as the same host defenses can have an immunopathogenic role. Mechanisms representing cell-mediated and humoral

responses potentially contribute to tissue damage through inflammation, immune complexes formation, by triggering of autoimmune processes, etc. **Although the infection may be acute and resolves, the tissue damage caused could be permanent.**

Considering that this thesis is focused on analyzing headache specifically in the setting of the SARS-CoV-2 infection, we will now review the biology and pathogenicity of this virus.

1.2.2. SARS-CoV-2

1.2.2.1. Coronaviruses and their origins

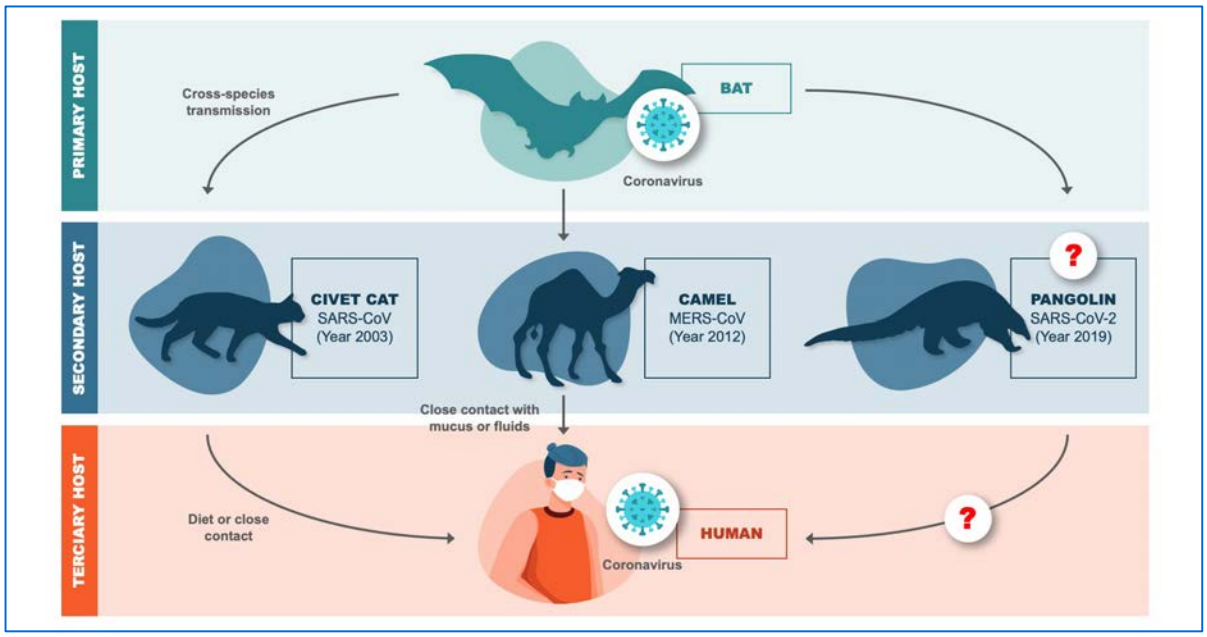
Taxonomy distributes the known 39 species of coronaviruses (CoVs) in 27 subgenera, 5 genera, and 2 subfamilies that are categorized under family *Coronaviridae*, suborder *Cornidovirineae*, order *Nidovirales* (34,35). CoVs infect humans, other mammals and avian species. **Human CoVs are responsible for multiple human respiratory diseases with different severity, ranging from common cold to bronchiolitis or even pneumonia** (36). Human CoVs can cause outbreaks of human fatal pneumonia, as demonstrated in 2002 by the severe acute respiratory syndrome coronavirus (SARS-CoV) and in 2012 by the Middle East respiratory syndrome coronavirus (MERS-CoV) (37).

At the end of 2019, the novel SARS-CoV-2 emerged in the city of Wuhan, China (38), representing the third major human CoV outbreak.

CoVs have a **zoonotic origin** but it is not infrequent the interchange of species and crossing of species barrier as well as genomic recombination in these viruses, especially in the setting of expedited urbanization and poultry farming (39). Therefore, **HCoV rise from the infection of a primary host before infecting humans** *(see Figure 1).* Bats harbor a great diversity of CoVs (40) and recently the sequence analysis of SARS-CoV-2 established a shared 96% genome with two SARS-like CoVs, viz. bat-SL- CoVZX45 and bat-SL-CoVZX2, from bats (41,42). Moreover, factors related to densely packed colonies in bats, their longevity, close social interaction,

and ability to fly make them a plausible primary host for viruses and specifically CoVs (43,44). Nevertheless, the direct transmission from bats to human has not been established and transmission by intermediary hosts has been suggested, making humans tertiary hosts. Palm civets have been considered as intermediate hosts in SARS-CoV (45), as well as, camels in MERS-CoV (46). Recently, CoVs in pangolins have demonstrated to share 99% of genetic homology with SARS-CoV-2 (47), pointing these animals as possible intermediate hosts, but concerns on the genetic techniques used in this study have been raised (48). Thus, data at the moment are not conclusive and it still needs to be clarified how SARS-CoV-2 emerged and transmitted to humans (*see Figure 1*)

Figure 1. Human Coronaviruses and their origins



***Modified from** (49)*

1.2.2.2. Biology and Lifecycle of SARS-CoV-2

Human CoVs are enveloped virus which contain non-segmented, single-stranded, positive-sense RNA genome (ssRNA) (50). The CoV virion consists of structural proteins: spike (S), envelope (E), membrane (M), nucleocapsid (N). The S protein is divided into two functionally distinct parts: the surface-exposed S1 that exhibits the receptor-binding domain (RBD) and the transmembrane S2 that is

involved in the fusion of viral and cellular membranes (50) (*see Figure 2*). The ssRNA is large and encapsidated by the nucleocapsid. The ssRNA has 5′ and 3′ untranslated regions that contain secondary RNA structures involved in its synthesis. At the 5′ end, there are two open reading frames (ORFs; ORF1a and ORF1b) that encode **non-structural proteins (Nsps), necessary to assemble the replication and transcription complex (RTC)** *(see Figure 2)* (50). The ORFs encoding the structural proteins (S, E, M and N) are located in the 3' one-third of coronavirus genome. Interspersed between these ORFs there are other ORFs encoding for so-called accessory proteins. These accessory proteins are variable among CoVs and, although they are not involved in virus replication directly, they are thought to have a relevant role in host responses and therefore in CoV pathogenicity (50,51).

In order to gain cell entry, the S protein binds with its specific receptor *(see Figure 2)*. Studies on the 2002 SARS-CoV identified **the angiotensin-converting enzyme 2 (ACE2)** as the virus functional receptor (52). This receptor was also confirmed to enable cell entry for SARS-CoV-2, consistent with the finding of a high structural homology between the S proteins of SARS-CoV and SARS-CoV-2 (53–55). Nevertheless, it is important to remark that the S gene shows recombination breakpoints, representing genome sites susceptible of frequent exchange of genetic material between related viruses during co-infection of the same host cell, favoring the emergence of new CoVs (53,56).

After binding to the receptor, the S protein of SARS-CoV-2, similarly to SARS-CoV, needs a proteolytic cleavage by host cell-derived proteases in order to achieve fusion (57). The transmembrane protease serine 2 (TMPRSS2) is responsible for this action and its inhibition has proven to block SARS-CoV-2 cell entry (58) *(see Figure 2)*. However, cathepsin L can also cleavage SARS-CoV-2 S protein in endosomes and can compensate for entry into cells that lack TMPRSS2 (58). Moreover, SARS-CoV-2 S protein shows a unique characteristic: the presence of a furin cleavage site at the S1/S2 junction, that allows a more efficient proteolytic cleavage process (59).

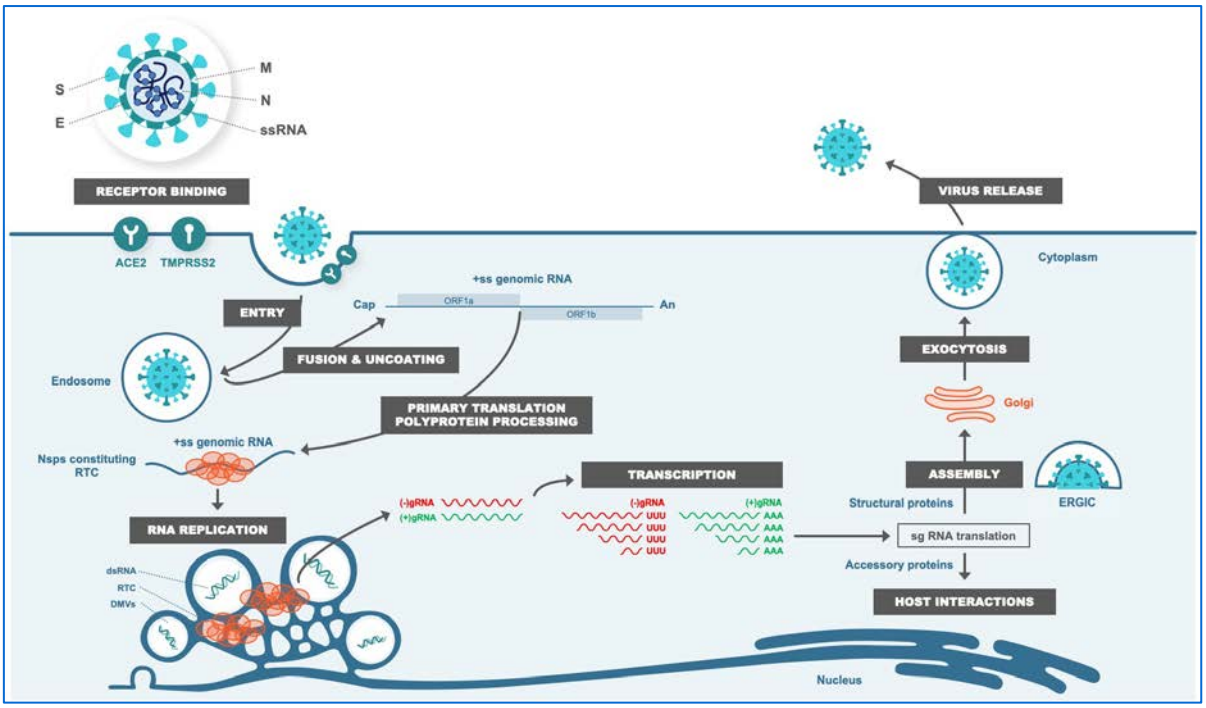
Following entry, SARS-CoV-2 genome is released into the host cell cytoplasm to start replication (60) *(see Figure 2)*. The genomic regions ORF1a and ORF1b are

immediately translated, leading to the production of two polyproteins, pp1a and pp1ab, from which Nsps are derived after proteolytic cleavage (60) *(see Figure 2)*. Specifically, Nsp2–16 compose the viral RTC, which is essential for viral replication and includes the RNA-dependent RNA polymerase. The synthesis starts with full-length negative-sense genomic copies, which function as templates for the generation of new positive ssRNA (60). However, during the negative-strand RNA synthesis, the RTC interrupts the transcription when it encounters specific regions called transcription regulatory sequences (TRS) (60,61) *(see Figure 2)*. This is typical for CoVs and the synthesis of the negative-strand RNA is re-initiated at another TRS (61). This discontinuous synthesis results in the production of negative-strand subgenomic mRNAs (sgRNAs) from which a nested set of positive-sense sgRNAs are derived and then translated into structural and accessory proteins (60,61).

In CoVs, replication organelles are characteristically involved in the process. They consist of characteristic perinuclear double-membrane vesicles (DMVs), convoluted membranes and small open double-membrane spherules that create a protective microenvironment for viral genomic RNA replication, avoiding exposure to host cytosolic innate immune mechanisms (62) *(see Figure 2)*.

Finally, translated structural proteins translocate into endoplasmic reticulum (ER) membranes and transit through the ER-to-Golgi intermediate compartment (60). The interaction between these proteins and new N-encapsidated genomic ssRNA results in budding into the lumen of secretory vesicular compartments (60). Then, virions are released by the infected cell by exocytosis (60) (*see Figure 2*).

Figure 2. Lifecyle of SARS-CoV-2



Modified from (60)

1.2.2.3. Transmission and Tropism of SARS-CoV-2

Human CoVs transmission occurs mainly through **respiratory droplets** (>5 μm) (*see Figure 3*). The fact that SARS-CoV-2 replication is observed in both the upper respiratory tract and the lower one makes the droplets transmission as one of the most consistent routes also in SARS-CoV-2 (63,64). However, human-to-human transmission could be also mediated by direct contact from one infected individual to a second, especially between people with close interactions (65), whereas the contagiousness of SARS-CoV-2 after disposition on fomites (e.g., door handles) still needs further investigation (65). Airborne transmission (66) and other forms of transmission such as the fecal-oral route are considered possible, but still have to be clearly demonstrated. In support of this latter route, SARS-CoV-2 RNA has been observed in feces, even after resolution of respiratory symptoms (67), and ACE2 is expressed by the human intestinal enterocytes (68).

Once SARS-CoV-2 enters the airways, it primarily target alveolar epithelial cells, vascular endothelial cells and alveolar macrophages to gain cell entry (68,69) (*see Figure 3*). This fact depends on the expression of the virus entry receptor ACE2 (70), that determine its tropism and pathogenicity. However, in these subsets of cells. ACE2 expression is rather low compared with other extrapulmonary tissues (71), so the tissue distribution of the receptor cannot entirely explain the viral tropism and other factors can make these cells more permissive to SARS-CoV-2, contributing to the infection.

Examples are the presence of TMPRSS2 and the active furin, that are highly expressed in the human respiratory tract, or the fact that ACE2 gene expression can be upregulated by type I and II IFNs in human airway epithelial cells during viral infection (72).

However, SARS-CoV-2 has higher binding affinity to the ACE2, thanks to mutations in the receptor-binding domain of the S protein (73,74). This higher affinity may explain why, compared to SARS-CoV, SARS-CoV-2 more efficiently infect the upper respiratory tract, eventually increasing its transmissibility and infectivity (55,75). Nevertheless, the increased viral load in the upper respiratory tract of SARS-CoV-2 infected patients does not correlate with disease severity, suggesting that infectivity does not fully reflect pathogenicity (76,77).

1.2.2.4. Pathogenesis of SARS-CoV-2 Infection

Through inhalation, SARS-CoV-2 reaches the respiratory tract deep into the lower lung, where, as mentioned, **it infects alveolar epithelial cells, vascular endothelial cells, and alveolar macrophages** (68,78) (*see Figure 3*). Once entered in the cells, the **viral nucleic acid is detected by pattern recognition receptors** (PRRs) consisting of host cytosolic innate immune sensors as well as endosomal toll-like receptors (TLRs) (78) (*see Figure 3*). This recognition activates a downstream signal that is able to recruit adaptor proteins on the mitochondrial or endoplasmic reticulum membrane surface (78). The interaction between PRR and adaptor proteins allows further recruitment of kinases, **leading to phosphorylation of interferon regulatory factor 3/7 (IRF3/7) and nuclear factor-κB (NF-κB)** (78) (*see Figure 3*). All these

processes culminate in the transcription and production of type-I/III IFNs, that are fundamental during early stages of viral infections as well as other pro-inflammatory cytokines (79) (*see Figure 3*).

CoVs have a characteristic sensitivity to IFNs, reason why they have adapted to antagonize the induction of type-I IFN from infected cells (80). Specifically, CoVs can (1) avoid immune sensing by the formation of DMVs that sequester viral nucleic acid from being recognized by PRRs; and (2) can target several components of IFN-I signaling, through specific viral proteins like the Nsp1 (80,81) (*see Figure 3*). SARS-CoV-2 suppresses type I and type III IFN expression especially in bronchial epithelial cells (82). This suppressed IFN expression is fundamental in the immunopathology associated with SARS-CoV-2 for three main reasons: (1) it allows higher viral replication, (2) it promotes induction of more tissue damage and (3) triggers more immune response as the immune system struggles to limit viral replication. In support of this hypothesis, patients with severe COVID-19 show imbalanced immune response with high concentrations of inflammatory cytokines/chemokines, but little IFN-β or IFN-λ, resulting in persistent viremia (83).

In SARS-CoV-2 **another pathological mechanism** has been recently demonstrated. A specific TLR, the TLR3, activates the transcription of the NOD-like receptor protein 3 (NLRP3) gene, contributing to the formation of an immune cytosolic multiprotein complexes, namely NLRP3 inflammasome (84) (*see Figure 3*). **The NLRP3, once assembled, causes the maturation and secretion from the cell of two key proinflammatory cytokines (interleukin (IL) -1β and IL-18)** and triggers cell death through gasdermin D (*see Figure 3*). IL-1β then activates monocytes, which secrete IL-6, tumor necrosis factor and IL-8 (*see Figure 3*). It has been observed that NLRP3 activation correlates with COVID-19 disease severity (85) and that this pathway can also trigger the coagulation cascade, for example via the extracellular release of gasdermin D (86), which lead to the formation of **neutrophil extracellular traps (NETs), which can recruit platelets and promote hypercoagulability and thrombotic events** (87) (*see Figure 3*).

The dysregulation of proinflammatory cytokines represent a relevant pathophysiological mechanism during SARS-CoV-2 infection (*see Figure 3*). Once they are produced and released through the activation of immune sensing mechanisms (NF-κB, NLPR3 etc), the higher their concentrations the greater the tissue is damaged. IL-1β and IL-6, for example, can downregulate adherens junctions in endothelial cells, increasing their permeability and creating endothelial dysfunction, that further allows recruitment of neutrophils and macrophages, contributing to tissue damage (87) (*see Figure 3*). In fact, these innate immune cells promote further local inflammation, by releasing more even cytokines (IL-1, IL-6 and TNF) (88,89) and creating a positive feedback loop of cytokine production, that in SARS-CoV-2 critically ill patients could be so exaggerated to be defined as a ***cytokine storm*** *(see Figure 3)*. The cytokine storm **contributes to the occurrence of acute respiratory distress syndrome (ARDS)** (90,91), where as a result of these inflammatory mechanisms, lung edema occurs, limiting gas exchange and leading to irreversible lung damage and respiratory failure (92–94). This was confirmed by studies that compared non-ICU patients with ICU patients, having the latter higher levels of plasma cytokines, which suggests an immunopathological process caused by the cytokine storm (95,96).

Considering that an excessive inflammatory response is therefore detrimental, it is critical for the host to try to control it. Recently, there is growing evidence of the existence of **neuroimmune unit in the lungs able to regulate pulmonary inflammatory responses, through the vagus nerve** and the activation of the transient receptor potential vanilloid 1 (TRPV1) channel that involve the calcitonin gene-related peptide (CGRP) release (97) (*see Figure 3*). According to this mechanism, following viral infection, inflammatory cytokines are further produced locally by immune cells, but their levels are potentially reduced by vagus nerve activity, leading to resolution of inflammation (97).

However, it has been demonstrated that not all patients display the same profile of cytokines, as **immunity in SARS-CoV-2 may be differentially activated and expressed**. In more detail, a recent study has investigated immune responses in 113 SARs-CoV-2 infected patients with moderate or severe disease and identified four

immune signatures, each one reflecting the activation of specific immunity type and related cytokines expression (98). Signature A was enriched with growth factors (EGF, PDGF, VEGF), signature B with cytokines belonging to type 2 (IL-4, IL-5 and IL-13) and type 3 (IL-1α, IL-1β, IL-17A, IL-17E and IL-22) immunity. Signature C was a mix of type-1 (IFNγ, IL-12 p70, IL-15, IL-2 and TNF), type 2 and type 3 cytokines and Signature D was rich in chemokines involved in leukocyte trafficking (like CCL1, CCL2 etc). These signatures correlated with three distinct disease trajectories, defined as clusters. Cluster 1 primarily included patients with moderate disease, enriched in tissue reparative growth factor belonging to signature A, whereas the profiles of those who developed severe disease had elevated levels of all four signatures, especially Cluster 3. These findings are important to underline individual differences in COVID-19 outcomes from the immunological perspective and further support the detrimental role of cytokines in COVID-19 pathogenesis and their relation with disease severity (99).

Adaptive immunity is also important during viral infections. CD8+ T cells directly kills virus-infected cells while CD4+ T cells promote the secretion of pathogen-specific antibodies by inducing T- dependent B cells (100). Antigen presentation is also able to stimulate adaptive immunity and major histocompatibility complex (MHC) class II molecules are responsible for presenting pathogen-derived peptide to T cells (101). However, a recent study on SARS-CoV-2 infected patients showed reduced expression of MHC genes in severe disease (93), correlating with greater infiltration of neutrophils, rather than T cells. However, T cells are still relevant during SARS-CoV-2 infection as they produce proinflammatory cytokines, such as IL-2, granulocyte-macrophage colony-stimulating factor (GM-CSF) and IFN-γ (88,89), as a consequence of the proinflammatory milieu previously mentioned and amplifying it even more. Recently, the study of the immunological profile of SARS-CoV-2 infection has led to the observation of 3 different “immunotypes” in hospitalized patients (102). Immunotype 1 was associated with disease severity and showed robust activation CD4 T cells, hyperactivated or exhausted CD8 T cells, and plasmablasts. Immunotype 2 was characterized by less CD4 T cell activation and Immunotype 3, lacked activated T and B cell response. However, the interesting finding is that mortality occurred for

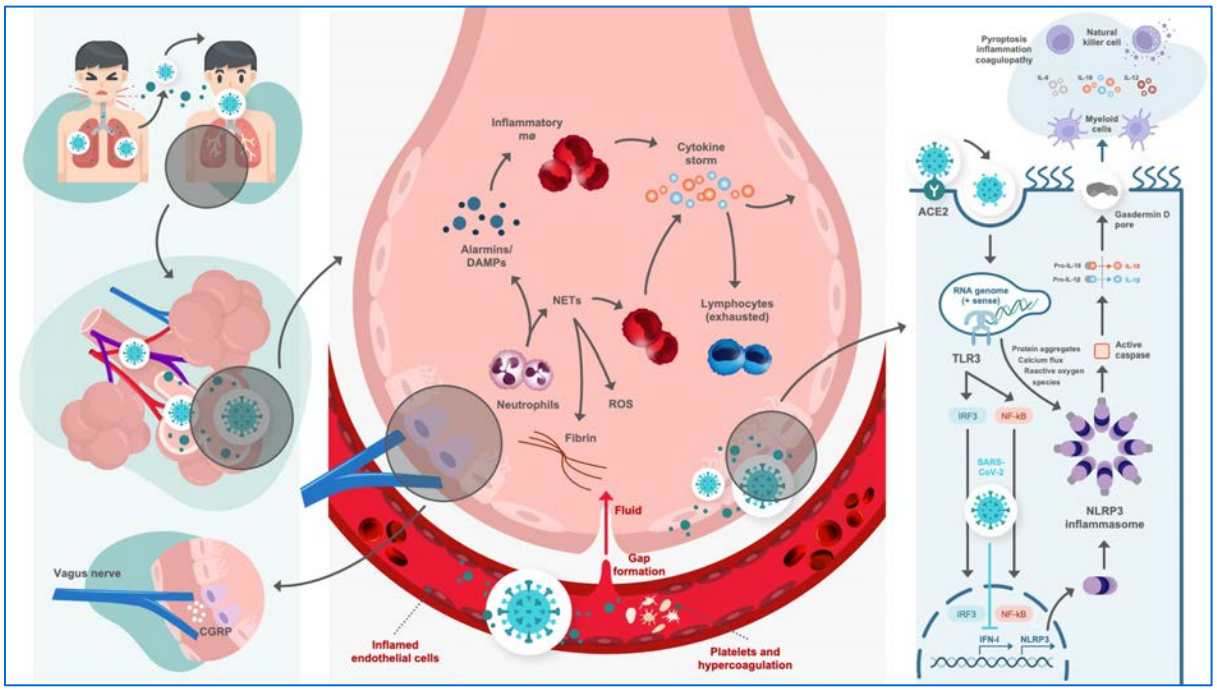
patients with all three immunotypes and from this perspective, it is possible that the **pathogenesis of SARS-CoV-2 infection is complex and can reflect immune responses that are either too weak (no T and B cell activation), resulting in virus-induced pathology, or too strong (exhausted CD8 T cells), leading to immunopathology**. Factors such as host genetics and underlying co-morbidities may also influence the immunotypes (102).

Finally, humoral immunity is required for controlling infections, and in the case of SARS-CoV-2, >90% of infected individuals present seroconversion a few weeks after initial infection (103,104). A recent study, investigating humoral immunity in a cohort of 87 individuals assessed at 1.3 and 6.2 months after infection with SARS-CoV-2, found that although titres of IgM and IgG antibodies against the RBD of the S protein of SARS-CoV-2 decreased significantly over this time period, the number of RBD-specific memory B cells remained unchanged at 6.2 months after infection. This fact suggests that individuals who are infected with SARS-CoV-2 could rapidly and efficiently respond to the virus upon re-exposure (105). It has been postulated also that pre-existing immunity, linked to prior exposure to common-cold coronaviruses, can modulate COVID-19 disease severity (106). For example, IgG specific to SARS-CoV-2 S protein has been detected in unexposed individuals, showing neutralizing activity against SARS-CoV-2 (107) (68). This fact could point to a potentially protective effect against severe forms of the infection, however the previous findings were not confirmed by another study (108).

However, **humoral responses could also represent a pathogenetic mechanism in COVID-19**. Antibody-dependent enhancement (ADE), a phenomenon described in MERS (109) has been involved in severe COVID-19. ADE occurs when antibodies target a virus without neutralizing it, leading instead to a facilitation of the endocytosis of the virus and therefore enhanced viral replication.

It is clear that the pathophysiology of SARS-CoV-2 infection has still to be fully clarified, however more and more studies, specifically investigating the pathogenicity of the virus and the immunological host responses, are providing new data to better understand this complex disease.

Figure 3. Transmission of SARS-CoV-2 and Pathogenesis of SARS-CoV-2 infection



Modified from: (78,87,97,110)

1.3. COVID-19

1.3.1. Overview of the Pandemic

The first recognized patients, infected by SARS-CoV-2 developed symptoms on December 2019 after which rapid human-to-human transmission and intercontinental spread occurred. The disease, for which SARS-coV-2 was responsible, was named COVID-19 by the World Health Organization (WHO) in February 2020 (111). On March 11th, 2020, the WHO officially characterized the global COVID-19 outbreak as a **pandemic** (111).

Since the end of 2019, the world has been struggling with COVID-19, facing different waves of the pandemic due to the emergence of SARS-CoV-2 variants.

A **variant** refers to a viral genome that may contain one or more mutations. In some cases, a group of variants with similar genetic changes, such as a lineage or group of lineages, can be considered by public health organizations as a Variant of Concern or a Variant of Interest. A **Variant of Concern** is specifically designated when there is evidence of increased transmissibility, more severe disease (for example, increased hospitalizations or deaths), significant reduction in neutralization by antibodies generated during previous infection or vaccination, reduced effectiveness of treatments or vaccines, or diagnostic detection failures (112) (*see Table 3*).

Table 3. Current Variants Of Concern in Europe (European Centre for Disease Prevention and Control

WHO label	Lineage + additional mutations	Country first detected (communitv)	Spike mutations of interest	Year and month first detected	Impact on transmissibil ity	Impact on immunity	Impact on severity	Transmissio n in EU/EEA
Beta	**B.1.351**	South Africa	K417N, E484K N501Y, D614G, A701V	September 2020	Increased (v) (1)	Increased (v) (2, 3)	Increased (v) (4, 5)	Community
Gamma	**P.1**	Brazil	K417T, E484K, N501Y, D614G, H655Y	December 2020	Increased (v) (6)	Increased (v) (7)	Increased (v) (5)	Community
Delta	**B.1.617.2**	India	1452R, T478K, D614G, P681R	December 2020	Increased (v) (8)	Increased (v) (9-11)	Increased (v) (10, 12)	Community
Omicron	**B.1.1.529**	South Africa and Botswana	(x)	November 2021	Unclear (v) (13-15) a	Increased (v) (16)	Unclear (v) (17, 18) b	Community

Modified from (113)

However, since its very beginning, a lot of progress has been made in fighting against COVID-19. Most of the advances come from studies done during the first wave of the pandemic that, through a better knowledge of the SARS-CoV-2 biology and the pathophysiology of COVID-19, led to (1) better techniques for virus detection (2) effective prevention strategies against SARS-CoV-2 infection and (3) new treatments for the acute phase of the infection.

Specifically, better preventive strategies have been achieved through greater accessibility to SARS-CoV-2 detection tests, better health and social measures at the community level, but above all through immunization. The incredibly rapid development of new **vaccines against SARS-CoV-2** available for global administration represents an unprecedented result for the mankind and a success for science. As of 5 January 2022, a total of 9.118.223.397 vaccine doses have been administered (111) (*see Table 4*).

Table 4. Current vaccines against SARS-CoV-2, approved by the EMA or under study

PHARMACEUTICAL COMPANY	TYPE OF VACCINE	IMMUNISATION SCHEDULE	PROCESS
Pfizer/BioNTech COMIRNATY	mRNA encoding S protein encapsulated in lipid nanoparticles.	2 doses 0-21 days	Authorised
Moderna	mRNA encoding S- protein encapsulated in lipid particles.	2 doses 0-28 days	Authorised
Oxford/Astrazeneca	Non-replicative chimpanzee adenovirus carrying the S-protein	2 doses 0-28 davs	Authorised
J&J/Janssen	Human adenovirus carrying the S-protein.	1or 2 doses	Authorised
Sanofi/CSK	S protein purified with the AS03 adjuvant	2 doses 0-28 days	Phase III
Novavax	S-protein nanoparticle with saponin- based MI- matrix adjuvant	2 doses 0-21 days	Authorised
Curevac	mRNA encoding a stabilised form of s- protein encapsulated in lipid nanoparticles	2 doses 0-28 days	Phase III

Modified from: https://www.ema.europa.eu/en/human-regulatory/overview/public-health-threats/coronavirus-disease-covid-19/treatments-vaccines/covid-19-vaccines

Also, **new drugs** or the repurposing of already existing ones have been investigated and, in some cases, approved to treat the active SARS-CoV-2 infection (*see Table 5*).

However, we have to keep in mind that the virus has mutated in the meantime and the efficacy of these treatments and vaccines on the new current variants or future ones will have to be assessed.

Table 5. Current drugs authorized by the European Medicines Agency (EMA) to treat COVID-19

Currently under rolling review	Marketing authorisation application submitted	Authorised for use in the European Union
• **Evusheld** (tixagevimab/ cilgavimab)	• **Lagevrio** (molnupiravir)	• **Kineret** (anakinra)*
• **Paxlovid** (PF-07321332/ ritonavir)	• **Olumiant** (baricitinib)*	• **Regkirona** (regdanvimab)
		• **RoActemra** (tocilizumab)*
		• **Ronapreve** (casirivimab/imdevimab)
		• **Veklury** (remdesivir)
		• **Xevudy** (sotrovimab)

Modified from: https://www.ema.europa.eu/en/human-regulatory/overview/public-health-threats/coronavirus-disease-covid-19/treatments-vaccines/covid-19-treatments

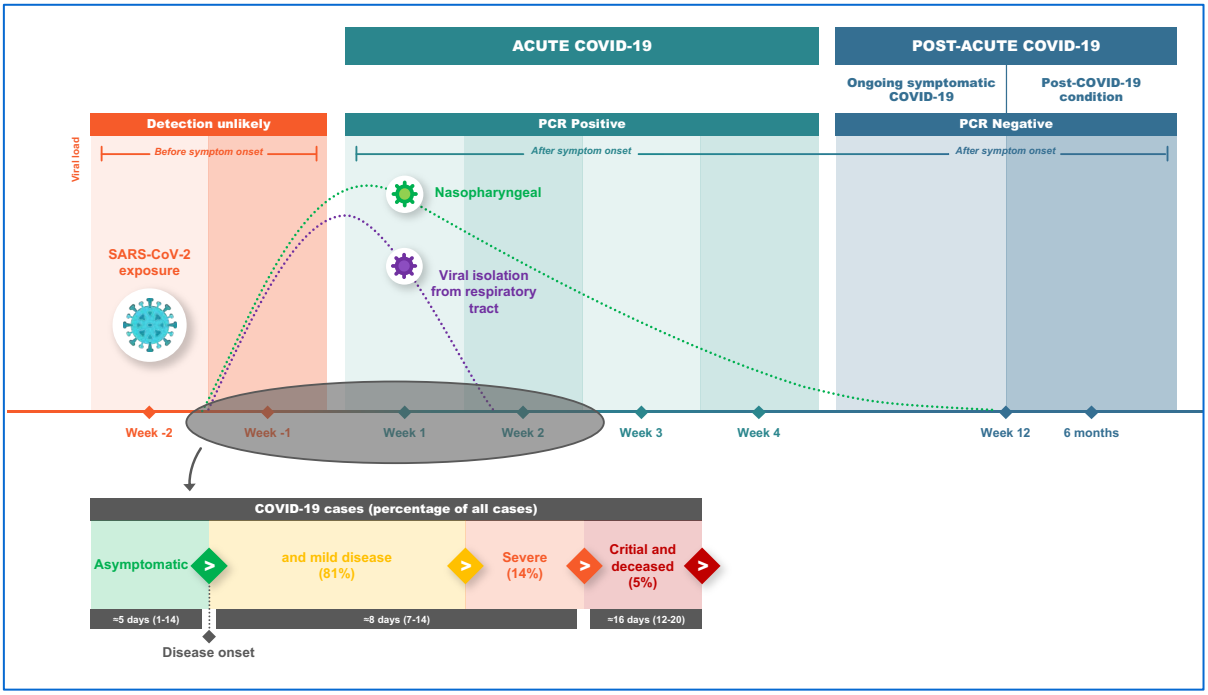
1.3.2. Clinical Presentation and Evolution

Human CoVs are primarily recognized for producing respiratory symptoms and, occasionally, gastrointestinal involvement. In the case of SARS-CoV-2, being highly pathogenic, infected people may experience a **variety of symptoms** that include respiratory, gastrointestinal, renal, hematological, neurological etc, that could range from mild to severe (38). All ages of the population are susceptible to SARS-CoV-2 infection; however, the median age of infection is around 50 years (63,114,115).

The **incubation period** in COVID-19 is around 5 days (114), phase in which the individuals are asymptomatic (*see Figure 4*). In the case of young people and children, SARS-CoV-2 infection can stay asymptomatic or they may develop only a mild form of disease (116,117). According to a study that included 72,314 COVID-19 cases in China, **81% of the cases were mild, 14% were severe requiring ventilation in an intensive care unit (ICU) and a 5% were critical** (patients with respiratory failure, septic shock and/or multiple organ dysfunction or failure) (114) (*see Figure 4*). The overall case fatality rate was 2.3 percent; no deaths were reported among noncritical cases (114).

Mild disease exhibits among the most common symptoms fever, cough and fatigue, although individuals may also present ground-glass opacities and mild pneumonia (63,115,116,118). Less common symptoms are considered diarrhea, anorexia, sore throat, chest pain, chills, headache and nausea/vomiting (63,115,116,118) (*see Figure 5*). Severe disease usually develops ~8 days after symptom onset, with more marked dyspnea and pneumonia, requiring ventilatory support and ICU (114). Eventually, some patients may develop ARDS that can be accompanied by acute cardiac injury and multi-organ failure, defining a critical state that can lead to death (95). The **critical disease and death occur at ~16 days from disease onset** (95,114) (*see Figure 4*).

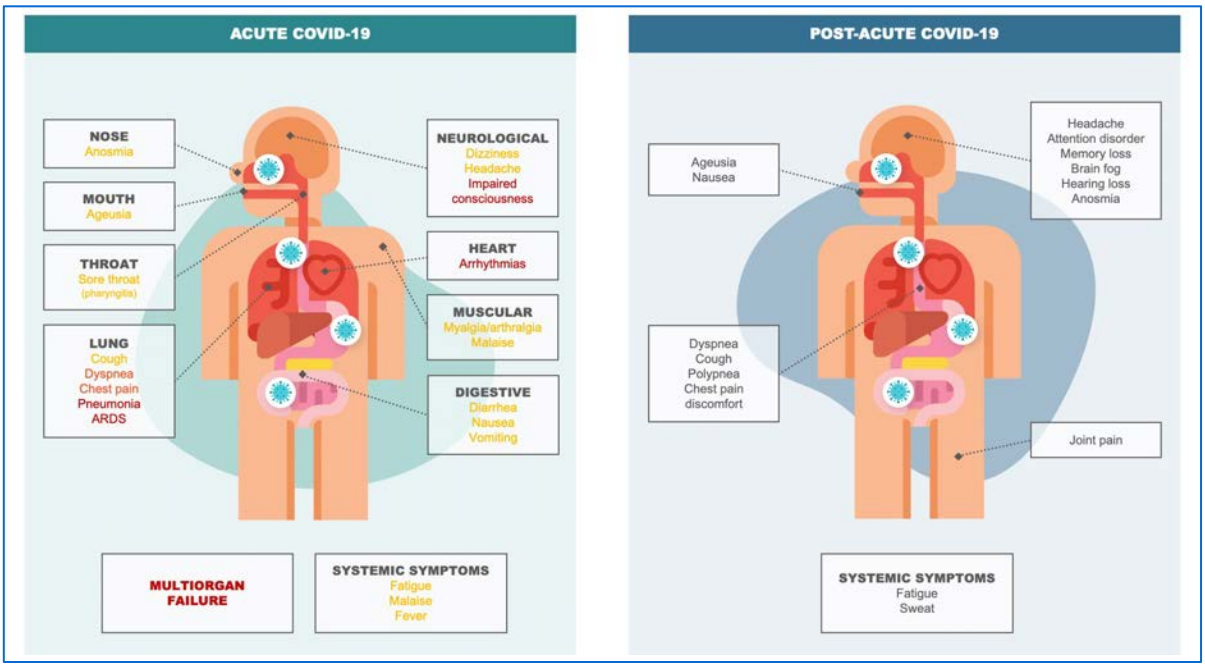
Figure 4. Evolution of SARS-CoV-2 Infection



Modified from: (38,119)

Particular laboratory features have been associated with worse outcomes, including lymphopenia, thrombocytopenia, elevated liver enzymes, lactate dehydrogenase, inflammatory markers (e.g. C-reactive protein [CRP], ferritin), inflammatory cytokines (e.g. IL-6, TNF-alpha), D-dimer, prothrombin time (PT) etc (120–122).

Figure 5. COVID-19 symptoms in the acute and post-acute phase of the infection



Modified from: (78,123)

Concerning recovery, individuals with mild infection usually recover within two weeks (124), although this may depend on other factors such as age and pre-existing comorbidities. Individuals with severe disease have usually a longer time to recovery (125). However, according to the Chinese study on 72,314 COVID-19 patients, most of them recovered enough to be released from hospital in 2 weeks (114).

As time is passing, it is becoming more and more evident that **certain patients present persisting symptoms**, pointing to the existence of subacute and long-term effects of COVID-19, affecting multiple organ systems (126).

Different terms have been used to define the long-term sequelae of COVID-19 such as “post-acute COVID-19”, “post-COVID syndrome”, “Long COVID” etc. However, it is more widely accepted that ***post-acute COVID-19* or *Long COVID*** refer to all persistent symptoms and/or delayed or long-term complications of SARS-CoV-2 infection beyond 4 weeks from the onset of symptoms (127,128) 1 (*see Figure 4*).

Then, based on recent literature, it is possible to further divide this phase into (1) *ongoing symptomatic COVID-19*, which includes symptoms and abnormalities present from 4–12 weeks beyond acute COVID-19; and (2) *post COVID-19 condition*, which includes symptoms and abnormalities persisting or present beyond 12 weeks of the onset of acute COVID-19 and not attributable to alternative diagnoses(128,129). The choice of using 4 weeks since symptoms onset as a cut-off to distinguish the acute and post-acute phase relies on the evidence that replication-competent SARS-CoV-2 has not been isolated after this timepoint (130).

More consistent data on post-acute COVID-19 are only currently being published. One study on 1,250 patients discharged alive, for example, shows 32.6% of them reporting persistent symptoms at 60 days (131). Studies have shown that most common persistent symptoms include fatigue, dyspnea, chest pain, cognitive disturbances, arthralgia, which affect quality of life (132–134) (*see Figure 5).*

Of note, although persistent symptoms are common in hospitalized patients (135), recent data suggest that **even patients with less severe disease** who were never hospitalized, including those with self-reported COVID-19, experience prolonged and persistent symptoms (133,136–138).

1.3.3. Risk factors for severe COVID-19

Several risk factors are considered to determine COVID-19 severity and are therefore responsible for a different course of the disease.

Age is considered a major risk factor, being associated with greater COVID-19 morbidity, admittance to the ICU, progression to ARDS and greater mortality rates (96,139,140). As a marker of more severe disease, older patients (≥65-years old) reports higher proportions of lymphocytopenia, neutrophilia and elevated inflammatory and coagulation-related biomarkers (63,121,141). Moreover, markers predictive of severity in COVID-19 such as IL-6, IL-12 and IL-1β are considered to be involved in inflammation-related aging (142) . However, age as a risk factor in COVID-19 could also be related, pathophysiologically, to weaker type I IFN responses upon viral infection (143) as well as decreased vagal immunomodulatory function in the

lungs via CGRP mechanisms (97) and impairment in T-cell activation (144). Thus, in older individuals impaired immune cell response to pathogens, combined with increased levels of pro-inflammatory cytokines, could contribute to the induction of a cytokine storm (97).

Studies on COVID-19 showed that **males** have a higher rate of respiratory intubation and longer length of hospital stay compared to females (145). Higher death rates in males are significant even when compared across age groups, ethnicity and comorbidity (146). This may involve the fact that women elicit stronger type I IFN responses upon stimulation with toll-like receptor 7 (TLR7) ligands (147). The deleterious effect may affect predominantly males as TLR7 is on the X chromosome and its mutation may result in immune cells that fail to produce normal amounts of IFN, correlating with more severe COVID-19 (148).

Concerning ethnicity, Black, Hispanic, and Southern Asian individuals have been strongly affected by COVID-19 (149) although, part of this finding could be related to underlying disparities in the social determinants of health (150,151).

A study has also shown genetic factors associated with severe COVID-19. Two genomic regions were identified: one on chromosome 3, which contains a cluster of six genes, and one region on chromosome 9 that determines ABO blood groups (152,153) . However, a dataset was released by the COVID-19 Host Genetics Initiative in which **the region on chromosome 3 is the only one that is significantly associated with severe COVID-19 at the genome-wide level** (154). The risk variant in this region confers an odds ratio for requiring hospitalization of 1.6 (95% confidence interval, 1.42–1.79). Thus, these genes may have functions that are potentially relevant to COVID-19, but causative genes cannot be reliably implicated.

Finally, it is worth mentioning that several **comorbidities**, for example chronic obstructive pulmonary disease, cerebrovascular disease and coronary heart disease were associated with more severity and worse prognosis of COVID-19 (114,120,155). Also, deficiencies in micronutrients, especially vitamin D have been associated with

more severe disease, but multiple confounders likely impact the observed associations in these studies (156,157).

A special comment should be done for **migraine**. At the time that this thesis started, no study aimed to investigate migraine as a risk factor for COVID-19. However, the accumulating evidence on CGRP regulatory mechanisms in the neuroimmune unit of the lungs, as previously mentioned, warranted the need to study the migraine population during the pandemic. This is because anti-CGRP monoclonal antibodies (mAbs) are available treatments for migraine prevention (158,159), but there were no reports on whether they could be beneficial or detrimental in the context of the SARS-CoV-2 infection when the pandemic started.

1.4. COVID-19 and The Nervous System

1.4.1. Neurological Manifestations of COVID-19

Among COVID-19 symptoms, **the neurological ones have emerged as relevant clinical manifestations of the SARS-CoV-2 infection**, involving either the CNS or the peripheral nervous system (PNS) (160).

In the acute phase of the infection, reports of neurological manifestations of COVID-19 include case series (161,162) and single and/or regional cohorts(163–166) with varying data definitions and methodologies, limiting the ability to accurately estimate the incidence of COVID-19 neurological manifestations. For example, a recent study conducted on hospitalized patients showed that the prevalence of neurological disorders was globally 13.5% (164), whereas another study has observed that over a third (36.4%) of patients had some degree of neurological involvement (163). However, studies agree in that neurological manifestations seem to be associated with COVID-19 worse prognosis and mortality (167,168).

In Spain, the NeuroCOVID registry, a multicentre study of patients with neurological manifestations of COVID-19 during the first wave of the pandemic, showed that the most frequently reported ones were: stroke (27%), neuromuscular symptoms (23.6%), altered mental status (23.6%), anosmia (17.6%), headache (12.9%), and seizures (11.6%) (169). However, **symptoms such as anosmia and headache may be less likely to be reported compared to other neurological diseases that are considered more severe** (cerebrovascular, autoimmune diseases etc). Anosmia, for example, according to other studies is the most common sudden neurologic symptoms of COVID-19 and seems to develop in the early stages of the disease, to the point of being considered as a useful diagnostic marker (170). As it will be discussed later, this symptom has specifically raised general interest for being considered linked to SARS-CoV-2 neurotropism, this is the ability of the virus to directly affect the nervous system.

Several meta-analyses have tried to summarize and estimate the prevalence of COVID-19 neurological symptoms(171–173) (*see Table 6*).

Table 6. Pooled prevalence of the most frequently reported neurological symptoms (171)

	Number of studies (N)	Summary estimate (%)	95% CI	2
Smell disturbances	17	35.8	(21.4, 50.2)	99.87
Taste disturbance	14	38.5	(24.0, 53.0)	99.65
Headache	54	14.7	(10.4, 18.9)	99.09
Myalgia	38	19.3	(15.1, 23.6)	9898
Disturbances in consciousness/altered mental status	9	9.6	(4.9, 14.3)	98.26
Dizziness	12	6.1	(3.1, 9.2)	93.44
Acute cerebrovascular disease	8	2.3	(1.0,3.6)	96.61
Ischaemic stroke	7	2.1	(0.9,3.3)	96.67
Hemorrhagic stroke	7	0.4	(0.2, 0.6)	62.36
Cerebral venous thrombosis	2	0.3	(0.1.0.6)	0.00
Syncope	3	1.8	(0.9, 4.6)	98.48
Ataxia	2	0.3	(0.1.0.7)	0.00
Seizure	5	0.9	(0.5, 1.3)	9.03

Another recent meta-analysis, on the contrary, has focused on neurological symptoms, signs or diagnoses identified in case reports of patients with COVID-19(174). It was observed that acute ischemic stroke was the most commonly reported disease (16.5%), followed by Guillain–Barre´ syndrome (GBS) (15.5%), cranial neuropathies (7.7%), encephalitis/meningitis (7.0%), cerebral venous thrombosis (3.9%), intracerebral hemorrhage (3.7%), myelitis/myelopathy (3.2%), para-infectious (autoimmune) encephalopathies (3.0%), other peripheral neuropathies (2.8%), posterior reversible encephalopathy syndrome (2.5%), acute necrotizing encephalopathy, and seizures/epilepsy (2.3%). Thus, it seems that, in the context of COVID-19, **cerebrovascular disorders, followed by immune-mediated peripheral neuropathies are the most documented neurological complications** (174). However, the results of case reports are implicitly limited because the role of chance cannot be excluded, yet, they postulate possible associations that could require further investigations from the pathophysiological standpoint. Table 7 shows all the neurological manifestations that have been associated with COVID-19.

Table 7. Summary of all neurological manifestations that have been associated with COVID-19 (173)

Neurological symptoms	Neurological manifestations and complications
Gustatory dysfunctions	Stroke
Olfactorydysfunctions(hyposmia/anosmia)	Epilepsy and seizures
Myalgia	Cerebral venous (sinus) thrombosis
Headache	Meningitis, encephalitis, meningoencephalitis
Altered mental status	Guillan-Barrésyndrome
Dizziness	Miller Fisher syndrome/Bickerstaff's encephalitis
Nausea and vomiting	Acute myelitis
Neuralgia	Posterior reversible encephalopathy syndrome (PRES)
Ataxia	Acute hemorrhagic necrotizing encephalopathy
Myoclonus	Acute demyelinating encephalomyelitis (ADEM)-like pathology
Diplopia	Posthypoxic necrotizing leukoencephalopathy
Vision loss	CNS vasculitis
Stupor	Acute cerebellitis
Meningism	Movement disorders
Dysexecutive syndrome	Intensive-care-unit acquired neuropathy
Bilateral leg stiffening	Rhabdomyolysis
Sustained upward gaze	Critical illness myopathy Necrotizing autoimmune myositis (NAM) Acute mesenteric ischemia

Finally, recent studies are showing that **neurological symptoms can be persisting** and are therefore common also in the post-acute phase of COVID-19, including ‘brain fog’, headache, anosmia, dysgeusia and myalgia (119,123,134). These symptoms have been observed also in patients that during the acute phase of COVID-19 did not require hospitalization (175)

So, a variety of neurological manifestations has been reported in COVID-19. However, at present no straightforward data on their prevalence is available. The variability in estimates mainly depends on the (1) the origins of the investigated individuals and cohorts; (2) the limited subspecialty of clinicians reporting cases; (3) the variability of infection control measures within specific geographic areas; (4) the use of diagnostic tests with suboptimal sensitivity and specificity; (5) demographic, cultural, ethnic, health, and nutritional differences of the populations studied (176); and (6) the lack of

adequate control groups within the study design (177,178). Therefore, more standardized studies are needed to elucidate the spectrum of the neurological involvement of COVID-19.

1.4.2. SARS-CoV-2 Pathogenesis in The Nervous System

The spectrum of neurological manifestations of COVID-19 suggests different underlying pathophysiological mechanisms.

As previously mentioned, cerebrovascular diseases has been reported as one of the most common neurological complications in COVID-19 (179). The mechanism underlying this group of neurological manifestations is considered to be based on the **hypercoagulability induced by systemic and local inflammation**, which can lead to both arterial and venous thromboembolic events (180) (*see Figure 6*). In addition, also microvascular thrombosis is frequently observed and has been demonstrated in post-mortem samples (181). In support to the involvement of inflammation, several studies have shown higher levels of IL-6, IL-8 and TNF-a in COVID-19 patients with stroke (182,183). IL-8 and TNF-a promote the release of von Willebrand factor (VWF), a marker of endothelial damage, whose cleavage is inhibited by IL-6, leading to accumulation of multimers that facilitate platelet aggregation (184). These findings suggest prothrombotic manifestations as being, in reality, the result of endothelial damage with augmented VWF release, platelet activation, and finally hypercoagulability (*see Figure 6)*. The post-mortem brain samples in COVID-19 patients show signs of endothelial injury and thrombotic microangiopathy rather than prototypic vasculitis, supporting the **inflammation and the subsequent damage of the endothelium of cerebral vessels, namely endothelitis, as one of the main determinants in SARS-CoV-2 neuropathology** and specifically in cerebrovascular disease (181).

However, not only systemic inflammation is probably responsible for this endothelial damage. Mechanisms that could involve **direct viral infection of endothelial cells or endothelial damage as a result of immune cells infiltration and activation** have been postulated in COVID-19 (99,185,186). Of note, endothelial damage itself produces local inflammation that further upregulates this mechanism (*see Figure 6)*.

In this context, the **neurotropism of SARS-CoV-2**, that would enable the direct infection not only of endothelial cells but potentially also of neurons and glia within the nervous system resulting in acute cell death, is still under investigation (*see Figure 6)*. The possibility of direct CNS invasion for SARS-CoV-2 has been suggested initially by the neurotropism showed by SARS-CoV and MERS-CoV (187). This mechanism for example is reported also for other viruses such as herpes simplex virus (HSV-1) and is considered the cause of the encephalitis (33). Encephalitis is also reported for SARS-CoV-2 (188) but encephalitis-like presentations could be in reality associated with inflammation (189,190) rather than direct viral infection of the CNS. The fact that detection of SARS-CoV-2 in CSF or brain autopsies is rare can imply that inflammation and immune-mediated damage is more important than viral replication in neurons (191,192). However, it has been observed that SARS-CoV-2 is able to infect neurons in vitro (191) and, more recently, also in vivo in mice models (193), causing neuronal death. As for the COVID-19 encephalitis, other proposed mechanisms consider viral antigens into the CNS as possible triggers of inflammation. In support of this hypothesis, SARS-CoV-2 S protein subunit S1 seems able to cross the blood–brain barrier (BBB) via absorptive transcytosis when administered intravenously and intra-nasally in a murine model (194).

The **integrity of the BBB** could play a key role in the neuropathogenesis of COVID-19, since a more permissive BBB can represent a portal for virus entry. Permeability can be **altered either as a consequence of endothelial damage and inflammation, as previously mentioned, or by the action of the virus itself** found in the bloodstream. In this latter case a recent study has demonstrated that the S1 subunit of the S protein promotes loss of BBB integrity (195). However also patients' comorbidities can influence the BBB properties (196) . Lastly, through bloodstream dissemination, the virus can reach sites that physiologically lack of BBB, such as the pituitary and median eminence of the hypothalamus. Interestingly these areas are rich in ACE2 and TMPRSS2, making the virus entry theoretically possible (197).

To gain access to a cell type, SARS-CoV-2 needs to bind to ACE2 receptor which is expressed by neurons (198) and astrocytes (199) as well as in pericytes and smooth

muscle cells of cerebral blood vessels (200). However, SARS-CoV-2 may use alternative docking receptors including neuropilin-1 (201), that are found at higher levels in the CNS, and furin proteases to counterbalance low levels of TMPRSS2 expression (202).

In order to exert a direct effect on the CNS, other anatomic routes of neuroinvasion have been proposed for SARS-CoV-2. **The fact that anosmia is a very common symptom of SARS-CoV-2** infection has raised the possibility that the virus is able to invade and damage olfactory nerve projections in the nasal cavity and **enter the central nervous system through trans-synaptic pathways** (203). Moreover, this mechanism has been observed in other CoVs (204). However, the ACE2 is only expressed in the nasal mucosa on epithelial cells rather than olfactory neurons, which makes this hypothesis controversial. Therefore, other mechanisms such as paracellular migration of cytokines or virions, transported across the cribriform plate from infected olfactory epithelial tissue to the olfactory bulbs, has been implicated in anosmia in COVID-19 (205). Nevertheless, recently viral RNA has been found in the olfactory bulb, as well as in other neuroanatomical areas, in post-mortem brain samples of COVID-19 patients, supporting CNS entry and olfactory transmucosal invasion by SARS-CoV-2. However, the association between these findings and neurological injury is not established (206).

So, it is clear that other studies are needed to fully understand the potential ability of SARS-CoV-2 to invade the CNS and infect neurons. Nevertheless, a new study has observed another potential mechanism through which SARS-CoV-2 can mediate neuronal damage (207). In fact, the study, conducted on post-mortem brain samples, could not detect virus RNA or protein, but observed that peripheral SARS-CoV-2 infection was able to inflame brain-barrier cells in the choroid plexus. Authors also demonstrated that this inflammation is then relayed into the brain parenchyma, since they found an increase in inflammatory pathways from the choroid plexus epithelium to brain astrocytes, oligodendrocytes, microglia. Among neuronal subtypes that were most affected in COVID-19, the study showed downregulation of layer 2/3 excitatory neurons and concomitant upregulation in proximal VIP inhibitory neurons, findings

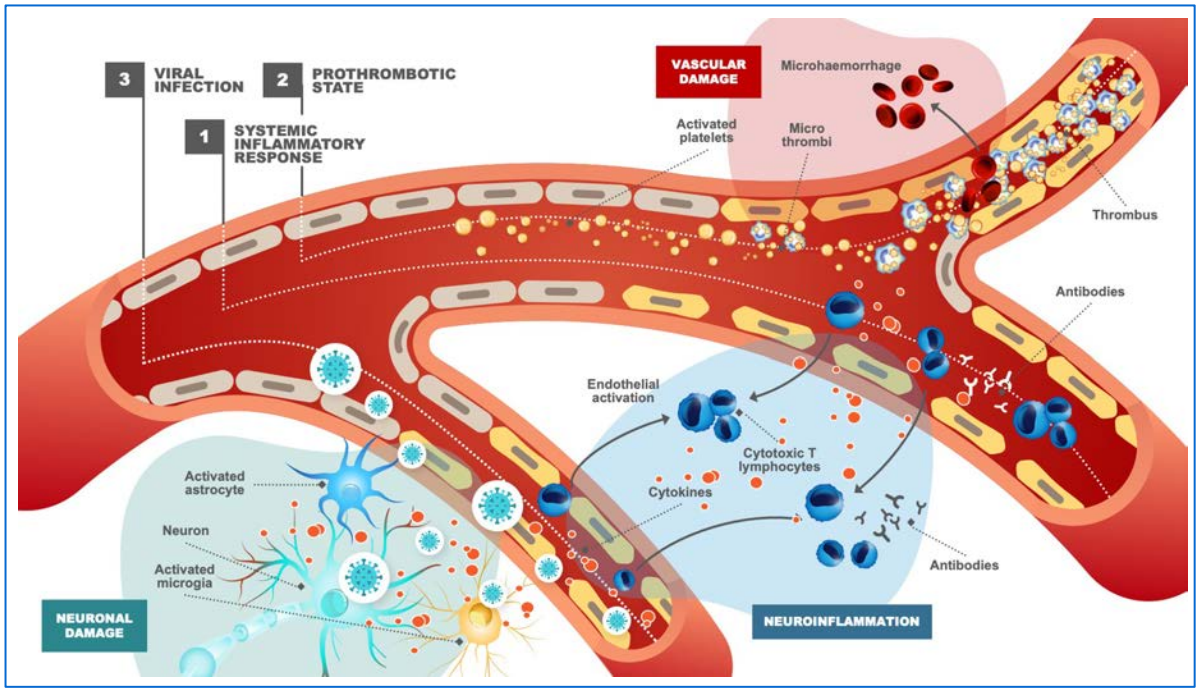
that suggest dysfunction in upper-layer cortical circuitry and could explain neuropsychiatric symptoms.

In COVID-19 neuropathogenesis it is, therefore, important to take into consideration the possibility of widespread **activation of astrocytes in the brain and activation of microglia** more specifically in brainstem and cerebellum, as observed in another study of post-mortem samples (208). The autopsies conducted also demonstrated cytotoxic T-cells mainly in the brainstem and in the meninges in several patients (208). Moreover, the involvement of host neuroimmune responses is suggested by CSF studies, showing activation of innate immune responses by the presence of elevated levels of biomarkers such as b2-microglobulin and neopterin and the presence of dedifferentiated monocytes (209,210). All these cell types, but specifically microglia, once activated, are responsible for the **continuous release of cytokines which perpetuate inflammatory mechanisms, leading to a local persistent neuroinflammation, that further contributes to more endothelial and neuronal damage** (211) (*see Figure 6*).

Finally, considering neuroimmune responses, reported cases of PNS pathology such as GBS or CNS diseases such as acute disseminated encephalomyelitis, point also to the existence of **autoimmune damage of neural tissues**. In this context, the production of **autoantibodies** (e.g. aGQ1b, a-NMDA-R, a-CASPR2 and aLGI2) that target a range of neural antigen has been observed, potentially caused by molecular mimicry mediated by SARS-CoV-2 (212). However, it has been proposed that T-cell exhaustion may also contribute to autoimmune neuropathogenesis in COVID-19 (209).

Thus, according to the findings presented, the COVID-19 neuropathology is still unclear and, partially, controversial probably due to the difficulties in reproducing the results form one study to another. Especially the mechanisms of direct viral damage on the CNS are still matter of debate. Nevertheless, accumulating evidence and new research will help clarify these aspects of COVID-19 pathology in the future.

Figure 6. Pathogenesis of SARS-CoV-2 in the nervous system



Modified from (211)

1.5. Headache attributed to COVID-19

At the beginning of March 2020, in Spain, COVID-19 cases started to be reported. As other viral infections we expected that headache could be associated with SARS-CoV-2. In the following days, due to the rapid increase in the number of COVID-19 patients, we started working as general clinicians in the emergency room (ER) attending people infected by SARS-CoV-2. From this perspective we noticed something that we could not have imagined at the very beginning: **headache was one of the major complains of COVID-19 patients**. Interestingly, although common, not all patients were experiencing it. We were then interested in better understanding this headache, so we searched for reports on the clinical characteristics of COVID-19 and found that, **according to the WHO, headache was listed in the category of less common symptoms of COVID-19** (213). We also looked into the first Chinese studies that were available at that moment. Headache was reported among COVID-19 symptoms but its prevalence was only around 10% (163). Nevertheless, there were **no data at all describing** neither **the characteristics of headache attributed to COVID-19**, nor its association with COVID-19 evolution or outcomes. Its pathophysiology was also unknown. So, the lack of these scientific data led to the hypothesis of this thesis.

2. Hypothesis

Considering that SARS-CoV-2 is a new pathogen, and that headache is among its symptoms; we wondered why headache was so associated with COVID-19. We wanted to better understand the mechanism behind it and decided to describe its specific characteristics, evolution and whether its presence and features could depend on factors reflecting some of the pathophysiological mechanisms activated during this infection.

We were particularly interested in investigating inflammatory mechanisms, since (1) data on other viral infections seem to suggest a key role for cytokines and pyrogens in headache, although mainly attributed to fever, and (2) SARS-CoV-2 triggers an exaggerated inflammatory response, known as *cytokine storm*. We hypothesized that the study of inflammation could not only give insights into the pathophysiology of COVID-19 headache, but also generate new hypothesis on headache attributed to systemic viral infections in general, that is scarcely known at present.

Moreover, we sought to elucidate the relationship between migraine and headache in the setting of COVID-19, as a way to better understand both primary and secondary headache disorders. We hypothesized the existence of shared mechanisms between them, based on (1) previous reports on phenotypical similarities between migraine and headache attributed to other viral infections (Dengue, HIV) and (2) on the emergence of more data on COVID-19. For example, COVID-19 severity was hypothesized to depend also on dysregulation of certain peptides such as CGRP, that is a well-known mechanism in the pathophysiology of migraine. The role of CGRP also led us to other reflections. Considering that monoclonal antibodies against CGRP are an approved and available treatment for migraine prevention, we wondered whether they could have a potential beneficial or detrimental effect in currently treated patients during the SARS-CoV-2 pandemic.

Finally, following the hypothesis of the existence of pathophysiological mechanisms in COVID-19 leading to headache, that are not expressed in patients without it, we also wondered whether they could be associated with a specific disease prognosis. If this was true, headache, often regarded as an unspecific and irrelevant symptom, would clearly play a role in defining COVID-19 evolution and outcomes.

3. Objectives

Main objective:

To describe headache characteristics and evolution in patients with COVID-19

Secondary objectives:

1. To analyze the relationship between headache and inflammation in COVID-19 during the acute phase of the infection

2. To assess the role of CGRP in COVID-19, by correlating the use of anti-CGRP monoclonal antibodies in a migraine cohort with COVID-19 outcomes

3. To analyze the relationship between the presence of headache and COVID-19 prognosis

4. Methods

This thesis focuses on understanding headache in the context of COVID-19.

To answer all of the formulated hypothesis, we planned and carried out different studies with strict work methodologies, that reinforce the value of our findings. Both prospective and retrospective studies have been conducted and are based on self-administered questionnaires with closed options or structured neurologist interviews, special attention was given to the data collected and recognition of possible confounding factors. The studies have been approved by the *Clinical Research Ethics Committee* of Vall d'Hebron University Hospital and participants gave their consent to participate. Data collection was performed in coded databases in accordance with the *Personal Data Protection Law* and statistical analyses were carefully done under the counselling of an expert statistician.

However, the development of this thesis has encountered some challenges as well. First of all, when we planned the first study to prospectively describe headache during the first wave of the pandemic, the pressure on healthcare professional due to the huge number of patients coming to the ER was high. This, added to the fact that other symptoms, such as the respiratory ones, needed more attention in that setting; and, that headache, generally, does not raise interest in general clinicians, made it difficult to find collaborations to our project. However, young neurologists and neurologists-in-training took the study as a learning opportunity, making a substantial contribution.

The pressure on the healthcare system also made difficult to conduct studies requiring blood analyses not included into the ones routinely assessed in clinical practice. For example, we would have liked to analyze CGRP in COVID-19 inpatients but both the logistic difficulties related to extraction and the fact that there are not standardized measurement of serum CGRP, made this option impossible.

Another aspect to consider was the evolution of COVID-19 pandemic since its beginning. Presenting with different waves, patients' recruitment has not always been easy in periods with low rate of virus spread in the population and especially when

the study population needed to have specific characteristics (e.g. migraine patients with treatment with anti-CGRP monoclonal antibodies). Therefore, in order to increase the validity of the results by including a large sample of patients in each study, we collaborated with different other headache clinics in Spain and designed multicenter studies.

It is also important to consider that some studies were planned when this thesis had already started. For example, the relevant number of referrals for persisting headache after COVID-19 resolution was something we could not foresee and led to the design of new studies later on. Also, the encouraging results of our prospective study made us conduct a meta-analysis on the basis of new hypotheses we wanted to test.

Some results have been already published in scientific journals indexed and specifically belonging to the headache field, which guarantees the scientific quality of the work done in this doctoral thesis. Others have been presented as oral communications or posters in scientific congresses and are under peer-review in indexed journals at the time of the writing of this thesis.

4.1. COVID-19 Headache: Characteristics and Evolution

As no data on COVID-19 headache existed at that time, **we first decided to describe headache characteristics and evolution in patients with COVID-19**. This is the first objective of this thesis.

To do so, we designed a **prospective study**, involving all the neurologists who had started working 24/7 at the ER as general clinicians during the COVID-19 pandemic. This study has already been published (*Appendix 1*). We recruited, during a 3-week period (28 March to 22 April 2020), all the consecutive patients with COVID-19 symptoms attended by us (ER patients were assigned randomly by triage to neurologists to be visited), but including only those who could give consent and undergo a full interview. COVID-19 symptoms were pre-specified for all neurologists and were based on the list of symptoms reported by the World Health Organization

(213). We collected demographic data, COVID-19 symptoms, family and personal history of any headache disorder (ICHD-3) (2), categorizing patients as episodic or chronic. If patients had experienced headache at any time during COVID-19, we collected the date of onset and cessation in relation to other COVID-19 symptoms as well as headache characteristics. Headache pain severity was defined as mild if patients considered that, in the absence of other COVID-19 symptoms, headache alone would allow them to carry on with their daily activities as usual; moderate if they had to reduce their daily activities, and severe if they had to stop doing any kind of task. Then, we analyzed all data, described the cohort of patients with headache associated with COVID-19, and compared them with those not having headache. **After 6 weeks from admission, we followed up patients** by phone call to evaluate persistence of headache and its characteristics as well as other COVID-19 symptoms through a structured survey. Then, we compared baseline and post-6-week data.

The statistical analysis for this study was done using the SPSS, version 21.0 for Windows. We reported nominal (categorical) variables as frequencies (percentages), and continuous variables as mean standard deviation or median and interquartile range (IQR), depending on the normality of the distribution. We checked the normality assumption of quantitative variables through visual methods (Q-Q plots) and normality tests (Kolmogorov-Smirnov test). We assessed statistical significance for intergroup variables by Pearson's chi- square when comparing categorical variables. In the case of having an expected count of less than 5 in more than 20% of cells in the contingency table, we used Fisher's exact test. We used linear trend chi-square for ordinal variables, independent t-test for continuous variables that followed a normal distribution and the Mann-Whitney U test for the rest of the continuous variables. We did not conduct a statistical power calculation prior to the study because the sample size was based on the available data. Missing values were imputed using the MICE (Multivariate Imputation via Chained Equations) package from R (v3.8.0)(214). Concerning missing values, there was <5% of missingness in nominal variables (headache localization, quality of pain, and pain severity). Hence, we used a Bayesian polytomous regression as a method of imputation for headache localization and quality of pain and a proportional odds model for headache pain severity. p-values

presented are for a two-tailed test and we considered p-values < 0.05 statistically significant.

Then, **to describe the evolution of headache after COVID-19**, we did not exclusively evaluate our prospective cohort of patients attended at the ER at 6 weeks, but **we designed a new study**. The need to better investigate headache after the resolution of the acute phase of the SARS-CoV-2 infection emerged also due to the growing number of referrals of people reporting persistent headache to our outpatient headache clinic, in the months following the first wave of the pandemic.

This new **study is multicenter and prospective**. The primary aim was specifically to describe the duration of headache over time, and the proportion of patients in which headache persisted. The study population was composed by patients that had been included in studies, conducted in Spain, that specifically analyzed headache in COVID-19 and whose methods and results had already been published (215–220). Therefore, our cohort (219) of patients attended at the ER was one of those included in the study (*Appendix 1*). All the cohorts had in common that had systematically screened the presence of new-onset headache during the course of COVID-19 and whenever present, a neurologist had administered in-person or telephonic questionnaires to describe headache. Moreover, all the studies were conducted between March 1 and April 27, 2020 and had been approved by local Ethic Committees. For the present study, all the participant sites completed **at least 9 months follow-up** in those patients in which headache persisted at the time of the original study completion. The study was done according to the Strengthening the Reporting of Observational Studies in Epidemiology (221). We harmonized the databases and combined a series of demographic variables, including age at the moment of COVID-19 infection, sex, and prior history of any headache disorder. As clinical variables, we assessed the time elapsed between the first COVID-19 symptom and the headache onset. We assessed whether the patient was managed in an outpatient setting or was hospitalized. The severity of COVID-19 was categorized into mild-disease, pneumonia, severe pneumonia, ARDS and death. We also evaluated the same headache characteristics assessed during the acute phase, including the

localization, quality, intensity of headache and the presence of associated symptoms, such as photophobia/ phonophobia, nausea or worsening by physical activity. Then, we explored which variables were associated with a more prolonged duration of headache. One of the centers, the *Hospital Clinico Universitario de Valladolid* was in charge of the statistical analysis, which was conducted similarly to the other above-mentioned studies, using SPSS (version 26.0) for Mac (IBM Corp. Armonk, NY). We used Kaplan-Meier survival curves to represent the duration of headache over time. For the exploratory analysis of which variables were associated with a more prolonged headache duration, Cox-regression was done, and those variables with a p value <0.2 in the univariate analysis were included in a multivariate analysis. We then present Hazard ratio (HR) and 95% confidence intervals (CI). Missing data were managed with complete case analysis.

Finally, t**o better describe the characteristics of headache in the post-acute COVID-19**, this is after the resolution of the infection, we designed another prospective study that **included all the consecutive outpatients referred to our headache clinic** from October 2020 **for persistent headache** after COVID-19. Demographic data, previous headache history, headache characteristics, and other COVID-19 symptoms were collected in regards to both the acute and post-acute COVID-19 phases. We described this outpatient cohort and compared patients on the basis of the presence of personal history of migraine, disease severity (hospitalization yes/no) and headache onset (prior/concomitant to other COVID-19 symptoms or later than other COVID-19 symptoms). The statistical analysis was similar to the one used to analyze the prospective cohort of patients attended at the ER, as mentioned above.

4.2. COVID-19 Headache: Possible pathophysiological mechanisms

4.2.1. Systemic Inflammation

The first data on the presence of a cytokine storm in COVID-19 from the Chinese studies, made us wonder whether there could be a correlation between inflammation and the presence of headache during the acute phase of COVID-19. Moreover, considering that headache during a viral infection could be attributed to the presence of cytokines in the context of fever, we also wanted to investigate this symptom.

So, **we conducted the prospective study on patients admitted at the ER not only with the objective of describing headache but also of evaluating its relationship with inflammatory biomarkers** (*Appendix 1*). In our hospital, according to the COVID-19 ER protocol at the moment, we recorded vital signs and performed a physical examination and a chest X-ray to rule out pneumonia. At the ER, patients with COVID-19 symptoms but with normal vital signs, negative X-ray and normal physical examination could be immediately discharged without undergoing nasopharyngeal swabs to confirm SARS-CoV-2 infection (this was mainly due in the shortage of supplies at the time the study was conducted). In all other cases, patients were admitted for further testing and/or treatment, including a real-time reverse transcriptase polymerase -chain reaction (RT-PCR) assay by nasopharyngeal swabs to confirm SARS-Cov-2 and blood testing with inflammatory markers (C-reactive protein– CRP: 0.03–0.50 mg/dL; IL-6: 0–4.3 pg/ mL; ferritin: 25–250ng/mL; Lactate Dehydrogenase–LDH: 0–248UI/L; D-dimer: 0–243ng/mL). We included for the analysis of inflammatory biomarkers and fever (>37.5C) only a subgroup of patients with ongoing headache at the ER and compared them with an equally age/gender-matched group without headache in order to avoid biased results. Then, **we also conducted a longitudinal data analysis in order to model the inflammatory biomarker changes over the course of COVID-19 between patients with and without headache**. We only selected hospitalized patients with at least three available blood tests that had been done at the same timepoints in the course of COVID-19 disease, starting from the onset of their COVID-19 symptoms. This longitudinal analysis was performed using linear mixed-effects models fitted by maximum likelihood and adjusted by age. Models were computed using the nlme (v3.1-144) package from R. The missingness in continuous variables (temperature, CRP, IL-6, D-dimer, ferritin, LDH) was rated between 2% (temperature) to 18% (LDH). In that case, we used random forest imputations in order to estimate these values according to their other variables.

4.2.2. CGRP

One of the other secondary objectives of this thesis was **to assess the role of CGRP in COVID-19**. This idea was based on the evidence of a neuroimmune regulation of pulmonary inflammatory responses during an infection, mediated by the vagus nerve and the release of neuropeptides such as CGRP. However, directly evaluating CGRP levels in COVID-19 patients had to face the limitations previously mentioned. Yet, as neurologists interested in headache disorders, we were familiar with CGRP considering that it represents a well-known mechanism in migraine pathophysiology, whose **antagonism (anti-CGRP mAbs) is an available treatment for migraine prevention**. So, we wondered **what was the impact of these treatments in patients with migraine during the COVID-19 pandemic and if this could correlate with different outcomes of COVID-19**.

We designed a **multicenter cross-sectional study** in which different headache clinics in Spain participated. The study has been already published (*Appendix 2*). Outpatients with migraine, who were under treatment with anti-CGRP mAbs, were invited to fill in an online survey available on the website of the Spanish Neurological Society. The same questionnaire was filled in, at the same time, by age-and sex-matched random outpatients with migraine but without anti-CGRP treatment. Demographic data, presence of symptoms suggestive of COVID-19 and headache, including its characteristics, acute medication intake, type of anti-CGRP mAb and other preventive treatment were collected through the survey. COVID-19 symptoms were selected according to the list of symptoms reported by the WHO(213). We also collected data about healthcare resource utilization (outpatient visits, ER admission, hospitalization) in relation to COVID-19 as indicators of disease severity. Then, we compared participants with and without anti-CGRP mAbs and conducted a subanalysis in those patients that had a confirmed diagnosis of COVID-19 or represented suspected cases of COVID-19. We defined confirmed cases as those participants who reported a SARS-CoV-2 positive RT-PCR assay by nasopharyngeal swabs. We defined suspected cases as those with three or more of the COVID-19 symptoms reported by WHO either with negative RT-PCR assay or if no confirmatory test had been performed, following the definition used in a national epidemiological

study(222). The statistical analysis was conducted in our center similarly to the one previously mentioned.

4.3. Headache and COVID-19 Prognosis

At the time **we designed the first study to describe COVID-19 headache in patients admitted at the ER,** we also wanted **to investigate the relationship between the presence of headache and COVID-19 prognosis** (*Appendix 1*).

In our cohort, to define prognosis, we used these variables: COVID-19 disease duration, defined as the number of days between the onset of the first and the resolution of the last COVID-19 symptom, hospital length of stay, and all-cause in-hospital mortality. Data on hospital and ICU length of stay and mortality were obtained by periodically revising electronic medical charts. Then, we compared these variables between patients reporting headache as a COVID-19 symptom and those without it. From the statistical point of view, in order to evaluate the COVID-19 prognosis (COVID-19 disease duration, hospital length of stay and mortality) at the follow-up, we used one-way analysis of covariance (ANCOVA), adjusted for the effect of age and gender, for the group with and without headache. The false discovery rate with Benjamini-Hochberg procedure was used to correct p-values for multiple comparisons.

The results observed by this analysis (*see Results, 6.4 Headache and COVID-19 Prognosis*), led us to **meta-analyze available studies reporting headache as a COVID-19 symptom to determine whether headache is associated with relative risk of COVID survival**.

For the meta-analysis search strategy, we conducted a systematic literature search of PubMed (April 1, 2020 to December 22, 2020) to identify all COVID-19 clinical inpatient series in accordance with the PRISMA guideline(223). We also included 6 studies published between December 2019 and March 2020 from a previous meta-analysis(224). There was no restriction on study design, language, nor laboratory confirmation of COVID-19 diagnosis. Studies were included if they clearly presented in their results or in the supplementary material: (1) study design; (2) COVID-19

confirmation method; (3) patients' demographics; (4) ratio of COVID-19 survivors and non-survivors and (5) the presence of headache as a symptom of the infection in both cohorts (*see Appendix 3, Table 1*). We excluded review articles, opinion articles, case-reports, preprint server articles, and studies performed either on populations <18 years old or animal models. The full references for the 48 studies included in the meta-analysis are listed in the *Appendix 3* (*Meta-Analysis References*). **The protocol was registered in PROSPERO** (225) on 17 June 2021, prior to the final analysis being undertaken (registration number CRD42021260151). For all eligible studies, we extracted information on study country, study size, COVID-19 confirmation, patients' characteristics, including demographics, presence of other COVID-19 accompanying symptoms and comorbidities (cardiovascular diseases, chronic kidney diseases, chronic liver diseases, chronic respiratory and diabetes). The presence of headache represented our COVID-19 confirmation outcome of interest. The risk of bias was assessed using the Newcastle–Ottawa Scale (NOS) to evaluate the quality of studies included in the meta-analysis(226). Concerning the data analysis, random-effects pooling models were computed in order to estimate the effect size of the following binary outcome data: presence of headache in survived vs. non-survived COVID-19 cohorts(227). Pooled headache prevalence and 95% CIs were presented from selected publications. Risk ratio (RR) with 95% CIs were used to estimate the risk of experiencing headache in both COVID-19 cohorts: survivors and non-survivors(228). RR was computed using the Mantel-Haenszel method(229). Headache prevalence and RR from each publication were reported using forest plots. Headache RR was also analyzed in different COVID-19 subgroups using moderator analysis in order to study if some covariables (gender and age) had a significant effect on the observed effect size(230) and adjusted RR was computed through meta-regression random-effects models. Between-study heterogeneity was assessed using the I^2 statistic and the Cochran's Q-test for statistical significance(231). Outlier publications were discarded in the sensitivity analysis in order to check the robustness of our results. We repeated the same analysis for the other COVID-19 symptoms and patients' comorbidities collected, although not all publications recorded the same COVID-19 symptomatology or patients' comorbidities. Hence, we analyzed their pooled

prevalence and risk ratio in publications where headache was reported. In case of higher heterogeneity (I^2 > 75%) in the RR analysis, if the publication's CI did not overlap with the CI of the pooled effect, we considered these studies as outliers. Influence analyses of effect size between publications were also computed in order to assess whether the influence of a particular publication distorted the overall pooled effect. Other strategies considered in the sensitivity analysis were excluding small studies (n < 250); excluding studies lacking validated COVID-19 confirmation methods, and considering only prospective studies. Finally, publication bias was assessed through visual inspection (funnel plot) and significance test (Egger's test). All of the statistical analysis and plots were generated using metaprop (version 2.4-0), meta (version 4.15-1) and dmetar (version 0.0.9) packages of R (version 4.0.3) software.

5. Results

We now report the main results for each of the objective of this thesis.

5.1. COVID-19 Headache: Characteristics and Evolution

We first described headache characteristics in patients admitted at the ER and reporting headache (*see Appendix 1*).

We included 130 adult **patients at the ER** (*see Table 8*). From them, **74.6% (97/130) had experienced headache** as a COVID-19 symptom, while the other 33 did not.

Table 8. Patients' characteristics and COVID-19 symptoms reported at ER

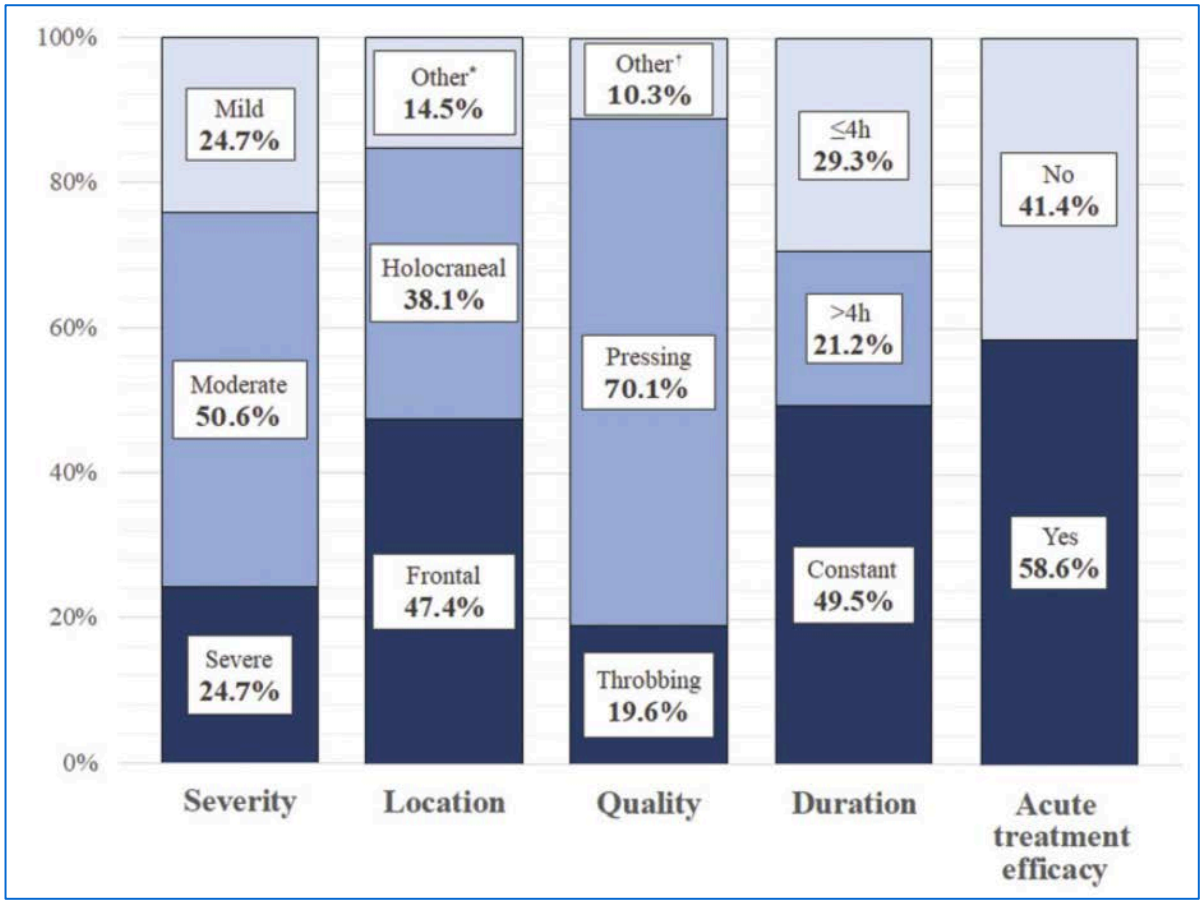
Demographic characteristics (n = 130)		
Sex, n (%)	Male	(64) 49.2%
	Female	(66) 50.8%
Age, n (%)	Mean (SD)	53.9 (16.4)
	<34 y	16 (12.3%)
	35-44 y	23 (17.7%)
	45-54 y	30 (23.1%)
	55-64 y	25 (19.2%)
	≥65 y	36 (27.7%)
COVID-19 characteristics		
Reported symptoms at ER, n (%)	Headache	97 (74.6%)
	Fever	115 (88.5%)
	Malaise	60 (46.2%)
	Myalgia	39 (30.0%)
	Dizziness	19 (14.6%)
	Cough	105 (80.2%)
	Dyspnea	81 (62.3%)
	Chest pain	4 (3.0%)
	Expectoration	19 (14.6%)
	Odynophagia	12 (9.2%)
	Loss of smell/taste	59 (45.4%)
	Diarrhea	36 (27.7%)
Radiological findings at ER, n (%)	Pneumonia	103 (79.2%)
	Bilateral pneumonia	77 (59.2%)
COVID-19 Confirmation (positive RT-PCR), n (%)		89 (68.5%)
Hospitalization, n (%)		104 (80.0%)
Vital signs and inflammatory markers at the ER		
O2 requirements, n (%)		31 (23.8%)
Fever, n (%)		45 (34.6%)
Lymphopenia, n (%)		70 (53.8%)
CRP, Mean ± SD, mg/ml		9.3 ± 8.4
IL-6, Median (IQR), pg/ml		34.0 (62.1)
D-dimer, Median (IQR), ng/ml		231.0 (243)
Ferritin, Median (IQR), ng/ml		354.0 (406)
LDH, Mean ± SD, UI/L		369.4 ± 221.4

ER = Emergency room; SD = standard deviation; IQR = interquartile range; ICU = Intensive Care Unit; RT-PCR = real-time reverse transcriptase polymerase -chain reaction; O2 = Oxygen; Lymphopenia (< 1.0x109/L); CRP = C-reactive protein; IL-6 = interleukin-6; LDH= Lactate Dehydrogenase

In our cohort of headache patients, 57.7% (56/97) were female, the mean age was 50.6±15.3 years old and 19.6% (19/97) had a personal history of episodic migraine. No patients had a history of chronic migraine. Headache-associated symptoms reported by patients were nausea and vomiting (25/97), worsening with movement

(12/97) photo/phonophobia (10/97), vertigo (4/97) and subjective neck stiffness (3/97) (*see Figure 7*).

Figure 7. Headache characteristics in COVID-19 patients in the acute phase



At the ER, the neurological examination together with symptoms evaluation, performed by neurologists, ruled out meningitis in all recruited patients with headache. Based on the striking clinical observation of some patients with severe headache at ER, we compared **patients with severe headache (24/97)** with the ones with mild-moderate pain (73/97), specifically analyzing migraine-like features (*see Table 9*). In the first group, there were more females (83.3% vs. 49.3%; p=0.004) but **no differences in personal migraine history** (25.0% vs.17.8%; p=0.175). Moreover, the severe group had more proportions of headache starting before the first COVID-19 symptom (25.0% vs. 4.1%; p=0.007) and of certain **migraine-like features**: throbbing quality (37.5% vs. 13.7%; p=0.017), nausea and vomiting (45.8% vs. 19.2%;

p=0.015), a trend suggesting more worsening with movements (25.0% vs. 8.2%; p=0.66), but not more photo/phonophobia (20.8% vs. 6.8%; p=0.114). Severe headache had **less response to acute treatment** (37.5% vs. 65.8%; p=0.018).

Table 9. Comparison between patients with severe and mild-moderate headache at ER

		Severe Headache (n=24)	Mild-Moderate Headache (n=73)	Adj. p value[a]
Demographic characteristics				
Age, years old, mean ± SD		44.8 ± 14.9	52.5 ± 15.1	0.049*
Sex (female), n (%)		20 (83.3%)	36 (49.3%)	0.004**
History of any type of headache, n (%)		10 (41.7%)	21 (28.8%)	0.313
History of migraine, n (%)		6 (25.0%)	13 (17.8%)	0.554
Headache characteristics				
Onset before another COVID-19 symptom, n (%)		6 (25.0%)	3 (4.1%)	0.007**
Time since headache onset to ER presentation, days, median (IQR)		10.5 (10.0)	6.0 (6.0)	0.002**
Holocraneal pain, n (%)		9 (37.5%)	28 (38.4%)	1.000
Pain quality, n (%)	Pressing	11 (45.8%)	57 (78.1%)	0.004**
	Throbbing	9 (37.5%)	10 (13.7%)	0.017*
Other migraine features, n (%)	Worsening with movement	6 (25.0%)	6 (8.2%)	0.066
	Nausea and vomiting	11 (45.8%)	14 (19.2%)	0.015*
	Photo/Phonophobia	5 (20.8%)	5 (6.8%)	0.114
Daily constant pain, n (%)		18 (75.0%)	30 (41.1%)	0.005**
Response to acute treatment, n (%)		9 (37.5%)	48 (65.8%)	0.018*

[a] Adjusted P value with Benjamini-Hochberg procedure

*p value ≤ 0.05

**p value ≤ 0.01

ER = Emergency room; SD = standard deviation; IQR = Interquartile Range

Then, comparing patients with or without headache, we observed that the first group were younger (50.6 ±15.3 vs. 63.6 ± 15.7; p < 0.0001), there were more females (57.7% vs. 30.3%; p=0.009) and reported higher headache history of any type (32.0% vs. 12.1%; p=0.039). In regards to COVID-19 symptoms, the most relevant result was that in the **headache group more patients had anosmia/ageusia** (54.6% vs. 18.2%; p < 0.0001) (*see Table 10*).

Table 10. Comparison between COVID-19 patients with and without headache at the ER

		No headache (n=33)	Headache (n=97)	Adj. p value[a]
Demographic characteristics				
Age, years old, mean ± SD		63.6±15.7	50.6±15.3	<0.0001**
Sex (female), n (%)		10 (30.3%)	56 (57.7%)	0.009**
History of any type of headache, n (%)		4 (12.1%)	31 (32.0%)	0.039*
History of migraine, n (%)		2 (6.1%)	19 (19.6%)	0.099
COVID-19 characteristics				
Reported Symptoms at ER, n (%)	Fever	28 (84.8%)	87 (89.7%)	0.529
	Malaise	8 (24.2%)	52 (53.6%)	0.004**
	Myalgia	7 (21.2%)	32 (33.0%)	0.272
	Dizziness	1 (3.0%)	18 (18.6%)	0.042*
	Cough	24 (72.7%)	81 (83.5%)	0.204
	Dyspnea	21 (63.6%)	60 (61.9%)	1.000
	Chest pain	2 (6.1%)	1 (1.0%)	0.158
	Expectoration	1 (3.0%)	18 (18.6%)	0.042*
	Odynophagia	1 (3.0%)	11 (11.3%)	0.294
	Loss of smell/taste	6 (18.2%)	53 (54.6%)	<0.0001**
	Diarrhea	8 (24.2%)	28 (28.9%)	0.660
	Pneumonia	25 (75.8%)	78 (80.4%)	0.622
	Bilateral pneumonia	21 (63.6%)	56 (57.7%)	0.682
COVID-19 confirmation (RT-PCR), n (%)		23 (69.7%)	66 (68.0%)	1.000
Hospitalization, n (%)		27 (81.8%)	77 (79.4%)	1.000

[a] Adjusted p value with Benjamini-Hochberg procedure

*p value ≤ 0.05

**p value ≤ 0.01

ER = Emergency room; RT-PCR = real-time reverse transcriptase polymerase-chain reaction

Then, to study headache evolution, we conducted different analyses and designed as well new studies, as mentioned in the *Methods.*

First, **after 6 weeks**, we followed up with patients that we recruited at the ER (*see Appendix 1*). We could get in touch with 74 of the 97 headache patients. Of these, **37.8% (28/ 74) still had headache**. Those patients whose headache had stopped had a mean duration of this symptom of 15.4 ± 11.1 days. Then, we analyzed patients with ongoing headache after 6 weeks, observing that **50% of them (14/28) had never suffered from recurrent headache before**. A total of 60.7% of patients (17/28) had daily constant headache. **Response to acute treatment was insufficient both at baseline and follow-up,** without statistically significant differences at the two timepoints (32.1% vs. 28.6%; p=0.701). Then, we compared patients with ongoing headache after 6 weeks with those who were headache free. Significant variables

associated with persisting headache were female sex (81.2% vs. 47.8%; p=0.004), history of headache disorder (50% vs. 26.1%; p=0.047) and onset of headache before the other COVID-19 symptoms (21.4% vs. 4.4%; p=0.010) (*see Table 11*).

Table 11. Comparison between patients with ongoing headache and with headache resolution during follow-up

		Ongoing Headache (n=28)	Headache Resolution (n=46)	Adj. p value[a]
Demographic characteristics				
Age, years old, mean ± SD		49.2 ±15.7	52.5 ±15.7	0.386
Sex (female), n (%)		23 (82.1%)	22 (47.8%)	0.004**
History of any type of headache, n (%)		14 (50.0%)	12 (26.1%)	0.047*
History of migraine, n (%)		8 (28.6%)	8 (17.4%)	0.383
COVID-19 characteristics				
Reported Symptoms at ER, n (%)	Fever	25 (89.3%)	41 (89.1%)	1.000
	Malaise	15 (53.6%)	26 (56.5%)	0.815
	Myalgia	12 (42.9%)	14 (30.4%)	0.321
	Dizziness	9 (19.6%)	5 (17.9%)	1.000
	Cough	22 (78.6%)	40 (87.0%)	0.352
	Dyspnea	22 (78.6%)	26 (56.5%)	0.079
	Chest pain	1 (3.6%)	0 (0.0%)	0.717
	Expectoration	3 (10.7%)	7 (15.2%)	0.733
	Odynophagia	4 (14.3%)	3 (6.5%)	0.415
	Loss of smell/taste	16 (57.1%)	29 (63.0%)	0.632
	Diarrhea	5 (17.9%)	17 (37.0%)	0.116
	Pneumonia	39 (84.8%)	21 (75.0%)	0.364
	Bilateral pneumonia	28 (60.9%)	15 (53.6%)	0.629
Persistent symptom at follow-up, n (%)		19 (67.9%)	9 (10.6%)	< 0.001**
COVID-19 disease duration, days, Median (IQR)		26.5 (21.5)	23.0 (12.5)	0.126
Hospitalization, n (%)		21 (75.0%)	39 (89.4%)	0.364
Days of hospitalization, median (IQR)		6.0 (13.5)	5.5 (7.5)	0.971
ICU, n (%)		4 (14.3%)	4 (8.7%)	0.467
Headache characteristics				
Onset before another COVID-19 symptom, n (%)		6 (21.4%)	2 (4.4%)	0.010**
Holocraneal pain, n (%)		10 (35.7%)	18 (39.1%)	0.809
Pain quality, n (%)	Pressing	19 (67.9%)	33 (71.7%)	0.796
	Throbbing	5 (17.9%)	9 (19.6%)	1.000
Moderate-severe pain, n (%)		25 (89.3%)	32 (69.6%)	0.085
Daily constant pain, n (%)		17 (60.7%)	22 (47.8%)	0.341
Headache Associated Symptoms at ER, n (%)	Nausea and vomiting	9 (32.1%)	12 (26.1%)	0.604
	Photo/Phonophobia	1 (3.6%)	6 (8.1%)	0.242
	Vertigo	1 (3.6%)	2 (4.4%)	1.000
	Neck stiffness	1 (3.6%)	1 (2.2%)	1.000
	Worsening with movement	6 (21.4%)	6 (13.0%)	0.352
Response to acute treatment, n (%)		9 (32.1%)	34 (73.9%)	0.001**

[a] Adjusted P value with Benjamini-Hochberg procedure

*p value ≤ 0.05

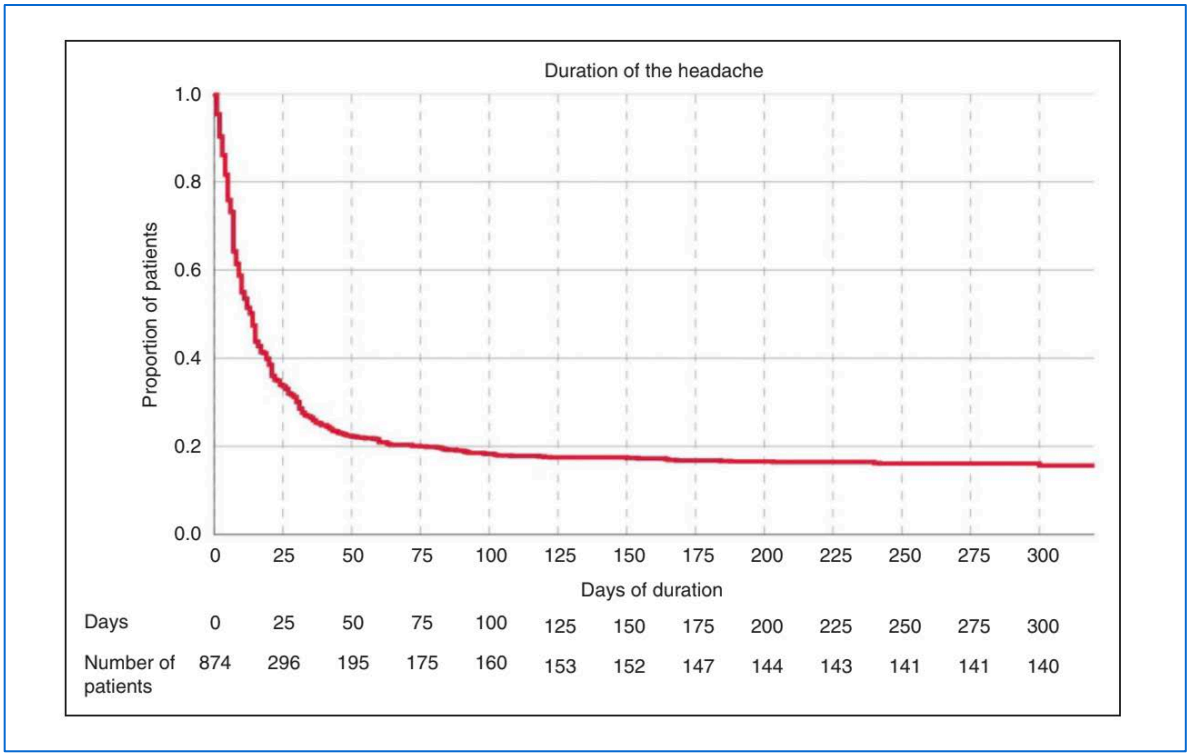
**p value ≤ 0.01

ER = Emergency room; SD = standard deviation; IQR = interquartile range; ICU = Intensive Care Unit

We also conducted a **multicenter study**, in which data from our cohort of ER patients were gathered with the ones of other 4 Spanish centers, that systematically evaluated the presence of headache. Headache was described in 821 out of 3698 patients (22.2%; 95% CI: 20.9-23.6%). We also added data about 112 headache patients from an additional sixth study which did not report the total number of screened patients, reaching a total of 933 COVID-19 patients with headache. Long-term follow-up data was available in 905/933 (97.0%) headache patients. Patients had been hospitalized

in 457/905 (50.5%) cases and managed in outpatient setting in 448/905 (49.5%) cases. The precise **duration of the headache** was available in 874/905 (96.6%) cases and **length 14 [6-39] days in median**, similar to what we found in our cohort of patients attended at the ER. The **proportion of patients in which the headache persisted** after the first month was 272/874 (31.1%; 95% CI: 28.1-34.3%) patients, 188/874 (21.5%; 95% CI: 18.9-24.4%) after the second month, 166/874 (19.0%; 95% CI: 16.5-21.8%) after the third month, **147/874 (16.8%; 95% CI: 14.4-19.5%) after six months and 140/874 (16.0%; 95% CI: 13.7-18.7%) after nine months** (*see Figure 8*).

Figure 8. Survival curve of the headache duration in the entire study sample.



Patients with persistent headache after 9 months were older, more frequently female, had less frequency of pneumonia, milder intensity of the headache, and had **higher frequency of throbbing quality of pain, photophobia or phonophobia and worsening by physical activity,** but lower frequency of pressing headache (*see Table 12*).

Table 12. Demographic and Clinical differences between patients with and without persistent headache after 9 months in the multicenter study

Variable	Entire study sample (n=905)	Non-persistent headache <9 months (n=735)	Persistent headache ≥9 months (n=140)	P value
Age (years) (Median, IQR)	51 [41.5-61]	47 [37-57]	52 [42-61]	0.001
Female sex (n, %)	592/875 (67.7%)	486 (66.1%)	106 (75.7%)	0.030
Prior headache history	348/846 (41.1%)	297/706 (42.1%)	51/140 (36.4%)	0.223
Difference in start of COVID symptoms and headache (days) (Median, IQR)	1 [0-3]	1 [0-3]	1 [0-3]	0.435
Diagnosis of pneumonia	403/875 (46.1%)	352 (47.9%)	51 (36.4%)	0.013
COVID-19 severity (median severity, IQR)	Mild [mild-severe pneumonia]	Mild [mild-pneumonia]	Mild [mild-severe pneumonia]	0.003
Headache phenotype				
Intensity of headache (median, IQR)	2.4 [2-3]	3 [3-3]	3 [2-3]	<0.001
Holocranial headache	614/831 (73.9%)	513/692 (74.1%)	101/139 (72.7%)	0.751
Throbbing quality	182/868 (21.0%)	126/730 (17.3%)	56/138 (40.6%)	<0.001
Pressing quality	519/868 (59.8%)	463/730 (63.4%)	56/138 (40.6%)	<0.001
Photophobia / phonophobia	314/875 (35.9%)	250/735 (34.0%)	64/140 (45.7%)	0.009
Nausea	200/875 (22.9%)	171/735 (23.3%)	29/140 (20.7%)	0.583
Worsening by physical activity	314/875 (35.9%)	250/735 (34.0%)	64/140 (45.7%)	0.009

The differing denominators used indicate missing data.

Abbreviations: IQR: Inter-quartile range.

These statistically significant variables in the univariate analysis were introduced in the multivariate one, where only headache intensity during the acute phase of the headache remained statistically significant (HR 0.655; 95%CI: 0.582-0.737, p<0.001).

Finally, we then conducted a study to better describe the characteristics of persistent headache after COVID-19. We recruited 32 patients, after the first wave of the pandemic, that were referred to our headache clinic for this reason. The mean age was 47.3 ±12.6; 87.5% (28/32) were women and 65.6% (21/32) had a personal history of migraine. Moreover, 65.5% (21/32) had suffered a mild COVID-19 infection (i.e. no pneumonia). At the first visit, headache was moderate-severe in all the cases, 65.5% (21/32) had a throbbing quality and in 81.2% (26/32) of cases was daily. The most frequent accompanying symptoms were insomnia 84.4% (27/32), fatigue 56.2% (18/32), anxiety/depression 46.9% (15/32) (*see Table 13*).

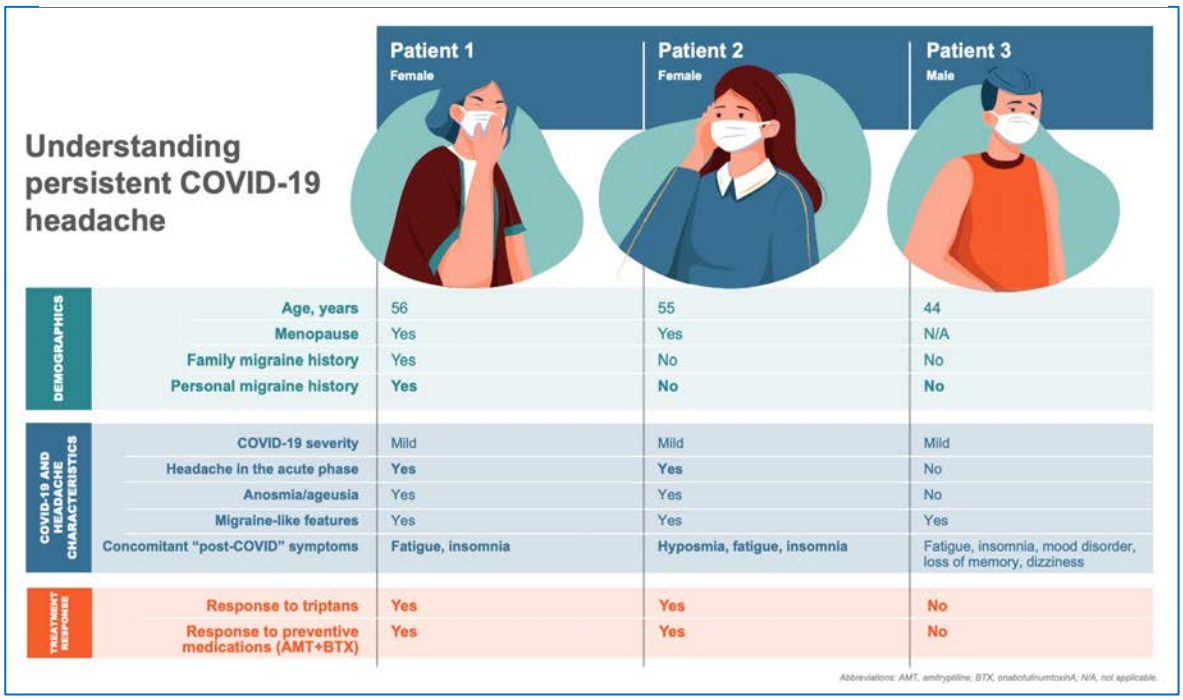
Table 13. Demographics and headache characteristics in the cohort of outpatients referred for persistent headache after COVID-19

	n = 32
Age, mean (SD),	47.3 (12.6)
Female, n (%)	28 (87.5%)
Ethnicity (Caucasian), n (%)	23 (71.9%)
History of migraine, n (%)	21 (65.6%)
Previous preventive treatment, n (%)	4 (12.5%)
COVID-19 duration*, d, Me [IQR]	32.5 [56.5]
Inpatients, n (%)	11 (34.4%)
Anosmia, n (%)	20 (62.5%)
Headache onset**, n (%) Before/Concomitant After	 20 (62.5%) 12 (37.5%)
Localization (unilateral), n (%)	8 (25.0%)
Quality, n (%) Pressing Throbbing	 11 (34.4%) 21 (65.6%)
Intensity, n (%) Moderate Severe	 18 (56.2%) 14 (43.8%)
Constant pain, n (%)	26 (81.2%)
Nausea/Vomiting, n (%)	16 (50.0%)
Photo-phonophobia, n (%)	23 (71.9%)
Worsening with movements, n (%)	16 (50.0%)
Dizziness, n (%)	6 (18.8%)
Insomnia, n (%)	27 (84.4%)
Dysautonomia (e.g. palpitations), n (%)	6 (18.8%)
Brain Fog, n (%)	9 (28.1%)
Fatigue, n (%)	18 (56.2%)
Anxiety/Depression, n (%)	15 (46.9%)

We compared patients according to presence of migraine history, which resulted associated with unilateral location (38.1% vs. 0.0%, p=0.029) of post-acute COVID-19 headache and less fatigue (38.1% vs. 90.9%, p=0.008). There were no differences comparing patients according to the severity of the SARS-CoV-2 infection or the time when headache started. The description of this cohort, led to the identification of **3 different patient prototypes**, these case-series has already been published (*see Appendix 4*). Although all these 3 cases had **persistent headache with migraine-like features and had suffered a mild COVID-19**, they were substantially different in other clinical aspects, such as previous personal migraine history, time of headache onset and associated symptoms (*see Figure 9*).

Figure 9. Three cases of persistent headache with migraine-like features after mild COVID-19

Figure 9. Three cases of persistent headache with migraine-like features after mild COVID-19



5.2. COVID-19 Headache: Possible pathophysiological mechanisms

5.2.1. Systemic Inflammation

To study the relationship between inflammatory biomarkers in COVID-19 and headache, we selected from our cohort of COVID-19 patients that had been admitted at the ER, those with ongoing headache at admission, when vital signs and blood samples were collected, and an equally age/gender-matched group without headache (*see Appendix 1*). We included 60 patients, 36 with headache and 24 without. We observed statistically significantly l**ower levels of IL-6 and LDH in those patients reporting headache at the ER** (*see Table 14*). We found no differences in presence of fever at the ER between the two groups.

Table 14. Comparison in inflammatory biomarkers between age/gender-matched COVID-19 patients with and without headache

	Headache (n=36)	No headache (n=24)	Adj. p value[a]
Demographic characteristics			
Age, years old, mean ± SD	59.1 ±14.2	61.1 ±14.9	0.594
Sex (female), n (%)	21 (58.3%)	15 (41.7%)	0.280
COVID-19 characteristics			
COVID-19 confirmation (RT-PCR), n (%)	25 (69.4%)	15 (62.5%)	0.708
COVID-19 disease duration at ER, days, Median (IQR)	8.5 (7.5)	9.0 (9.8)	0.623
Vital Signs and inflammatory biomarkers			
Fever, n (%)	11 (30.6%)	4 (16.7%)	0.482
Lymphopenia, n (%)	20 (55.6%)	16 (66.7%)	0.432
CRP, Mean ± SD, mg/ml	8.9 ±7.9	11.7 ±9.8	0.381
IL-6, Median (IQR), pg/ml	22.9 (57.5)	57.0 (78.6)	0.036*
D-dimer, Median (IQR), ng/ml	300.0 (3300)	250.0 (1593.0)	0.481
Ferritin, Median (IQR), ng/ml	488.0 (466.0)	287.0 (110.0)	0.052
LDH, Mean ± SD, UI/L	302.8 ±107.7	457.1 ± 207.6	0.016*

[a] Benjamini-Hochberg Adjusted p value with a False Discovery Rate greater than 0.05

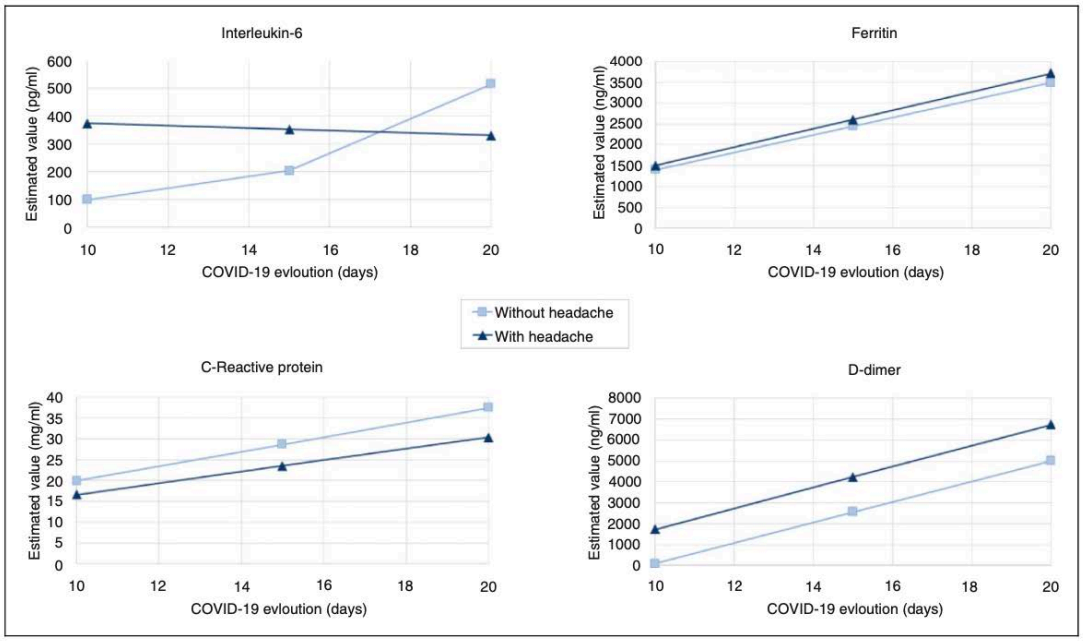
*p value ≤ 0.05

**p value ≤ 0.01

SD = standard deviation; RT-PCR = real-time reverse transcriptase polymerase -chain reaction; ER = Emergency room; IQR = interquartile range; CRP = C-reactive protein; IL-6 = interleukin-6; LDH= Lactate Dehydrogenase

Then, to specifically analyze the evolution of inflammatory biomarkers over time, we included a subset of 24 patients who had been hospitalized and for whom at least three available blood tests had been done at the same timepoints (*see Methods*): 18 had headache, while six did not. There were no statistically significant differences either in patients' age (headache: 56.6±9.8 vs. no-headache: 63.3 ±6.7 years; p=0.130) or in sex (female – headache: 55.6% vs. no-headache: 66.7%; p=1.000). Only IL-6 significantly changed **over time** between the two groups (p=0.003), observing **more stable levels of IL-6 during COVID-19 in patients with headache** (*see Figure 10)*.

Figure 10. Evolution of inflammatory biomarkers (IL-6, CRP, ferritin and D-dimer) during the progression of COVID-19 disease



5.2.2. CGRP

One of our objectives was to assess the role of CGRP in COVID-19, by **analyzing the relationship between the use of anti-CGRP mAbs in a cohort of migraine patients with COVID-19 outcomes,** as these treatments are available for migraine prevention.

We conducted a web-based multicenter study in which 300 patients with migraine participated. Of them, 51.7% (155/300) were treated with anti-CGRP mAbs. The mean age was 47.1 ± 11.6 years old and 87.3% were women (*see Table 15)*.

Table 15. Characteristics of the cohort of migraine patients with and without anti-CGRP mAbs treatment

		Total (n=300)	Without MAbs (n=145)	With MAbs (n=155)	Adj. p-value[a]
Demographic characteristics					
Sex, n (%)	Female	262 (87.3%)	132 (91.0%)	130 (83.9%)	0.219
Age, n (%)	Mean ± SD, y	47.1 ± 11.6	45.7 ± 12.5	48.3 ± 10.7	0.208
	<30 y	28 (9.3%)	18 (12.4%)	10 (6.5%)	0.240
	30-39 y	38 (12.7%)	16 (11.0%)	22 (14.2%)	
	40-49 y	106 (35.3%)	55 (37.9%)	51 (32.9%)	
	50-59 y	91 (30.3%)	41 (28.3%)	50 (32.3%)	
	≥60 y	37 (12.3%)	15 (10.3%)	22 (14.2%)	
Migraine treatment					
Concomitant preventive treatment, n (%)		254 (84.7%)	126 (86.9%)	128 (82.6%)	0.386
Adherence to preventive treatment†, n (%)		231/254 (90.9%)	106/126 (84.1%)	125/128 (97.6%)	**0.008**
Adherence to MAbs†, n (%)		N/A	N/A	147 (94.8%)	N/A
Response to acute medication, n (%)		207 (69.0%)	95 (65.5%)	112 (72.3%)	0.320
COVID-19					
COVID-19 cases‡, n (%)		41 (13.7%)	16 (11.0%)	25 (16.1%)	0.320
Previous history of pneumonia, n (%)		63 (21.0%)	29 (20.0%)	34 (21.9%)	0.777

In **bold** are marked statistically significant variables (P value ≤ 0.05)

[a] Adjusted P value with Benjamini-Hochberg procedure

†Period starting from Feb 1st, 2020 until participants' submission of the survey

‡Participants with confirmed SARS-CoV-2 real-time reverse transcriptase polymerase-chain reaction (RT-PCR) assay by nasopharyngeal swabs or with >2 of the COVID-19 symptoms either in the absence of a confirmatory test or with negative RT-PCR assay

MAbs = anti-CGRP monoclonal antibodies; SD = standard deviation; N/A = not applicable

In this cohort, 13.7% (41/300) met the criteria for either confirmed or suspected case of COVID-19, 5 of them required hospital admission (*see Table 16*). Headache was the most frequent symptom in 82.9% of patients (34/41) (*see Table 16*)

Comparing migraine patients with and without anti-CGRP mAbs, no differences were found in terms of baseline characteristics or proportion of COVID-19 cases.

In the subgroup of COVID-19 cases, **there were no differences in COVID-19 symptoms except for diarrhea** (without anti-CGRP mAbs: 68.8% vs. with mAbs: 28.0%; p=0.022). **Healthcare resource utilization related to COVID-19** and adherence to other preventive medications **was similar** between the two groups (*see Table 16*). Two patients in this group discontinued anti-CGRP mAbs, reporting that it

was for fear of possible interactions with the concomitant COVID- 19 infection. We finally performed a sensitivity analysis of confirmed COVID-19 patients (12/41) and we also found no statistically significant differences between patients with anti-CGRP mAbs vs. without mAbs.

Table 16. Characteristics of the COVID-19 subgroup in the cohort of migraine patients with and without anti-CGRP mAbs

		Total† (n=41)	Without MAbs (n=16)	With MAbs (n=25)	Adj. p-value[a]
Demographic characteristics					
Sex, n (%)	Female	37 (90.2%)	15 (93.8%)	22 (88.8%)	1.000
Age, n (%)	Mean ± SD, y	42.6 ± 13.1	41.3 ± 12.5	45.4 ± 11.6	0.282
	<30 y	9 (22.0%)	5 (31.3%)	4 (16.0%)	0.620
	30-39 y	5 (12.2%)	1 (6.3%)	4 (16.0%)	
	40-49 y	11 (26.8%)	3 (18.8%)	8 (32.0%)	
	50-59 y	13 (31.7%)	7 (43.8%)	6 (24.0%)	
	≥60 y	3 (7.3%)	0 (7.3%)	3 (12.0%)	
Migraine treatment					
Concomitant preventive treatment, n (%)		29 (70.7%)	11 (68.8%)	18 (72.0%)	1.000
Adherence to preventive treatment‡, n (%)		27/29 (93.1%)	11/11 (100.0%)	16/18 (88.9%)	0.512
Adherence to MAbs‡, n (%)		N/A	N/A	23/25 (92.0%)	N/A
Response to acute medication, n (%)		21 (51.2%)	6 (37.5%)	15 (60.0%)	0.208
COVID-19					
COVID-19 confirmed (RT-PCR), n (%)		12 (29.3%)	3 (18.8%)	9 (36.0%)	0.305
Previous history of pneumonia, n (%)		11 (26.8%)	3 (18.8%)	8 (32.0%)	0.478
Reported symptoms, n (%)	Headache	34 (82.9%)	13 (81.3%)	21 (84.0%)	1.000
	Cough	32 (78.0%)	13 (81.2%)	19 (76.0%)	1.000
	Fever	25 (61.0%)	9 (56.3%)	16 (64.0%)	0.746
	Myalgia	31 (75.6%)	14 (87.5%)	17 (68.0%)	0.265
	Dyspnea	21 (51.2%)	9 (56.3%)	12 (48.0%)	0.757
	Anosmia	25 (61.0%)	9 (56.3%)	16 (64.0%)	0.746
	Diarrhea	18 (43.9%)	11 (68.8%)	7 (28.0%)	0.022
	Odynophagia	17 (41.5%)	7 (43.8%)	10 (40.0%)	1.000
	Expectoration	17 (41.5%)	6 (37.5%)	11 (44.0%)	0.753
Healthcare Resource Utilization in relation to COVID-19					
Outpatient visits, n (%)		37 (90.2%)	15 (93.8%)	22 (88.0%)	1.000
Telephonic visits, n (%)		25 (61.0%)	12 (75.0%)	13 (52.0%)	0.195
Emergency room, n (%)		20 (48.8%)	5 (31.3%)	15 (60.0%)	0.111
Hospitalization, n (%)		5 (12.2%)	1 (6.3%)	4 (16.0%)	0.632

In **bold** are marked statistically significant variables (P value ≤ 0.05)

[a] Adjusted P value with Benjamini-Hochberg procedure

†Participants with confirmed SARS-CoV-2 real-time reverse transcriptase polymerase-chain reaction (RT-PCR) assay by nasopharyngeal swabs or with >2 of the COVID-19 symptoms either in the absence of a confirmatory test or with negative RT-PCR assay

‡Period starting from Feb 1st, 2020 until participants' submission of the survey

MAbs = anti-CGRP monoclonal antibodies; SD = standard deviation; RT-PCR = reverse transcriptase polymerase-chain reaction; N/A = not applicable

5.3. Headache and COVID-19 Prognosis

To study the association between headache as a symptom of the acute infection and COVID-19 prognosis, we followed up the COVID-19 patients recruited at the ER. Of 130 patients, 80.0% (104/130) were hospitalized after ER evaluation. In all hospitalized patients, we could follow their clinical course by electronic chart and

observed that 8.5% (11/130) required ICU. Mortality was 3.1% (4/130, one patient belonging to the headache group and three to the group without headache).

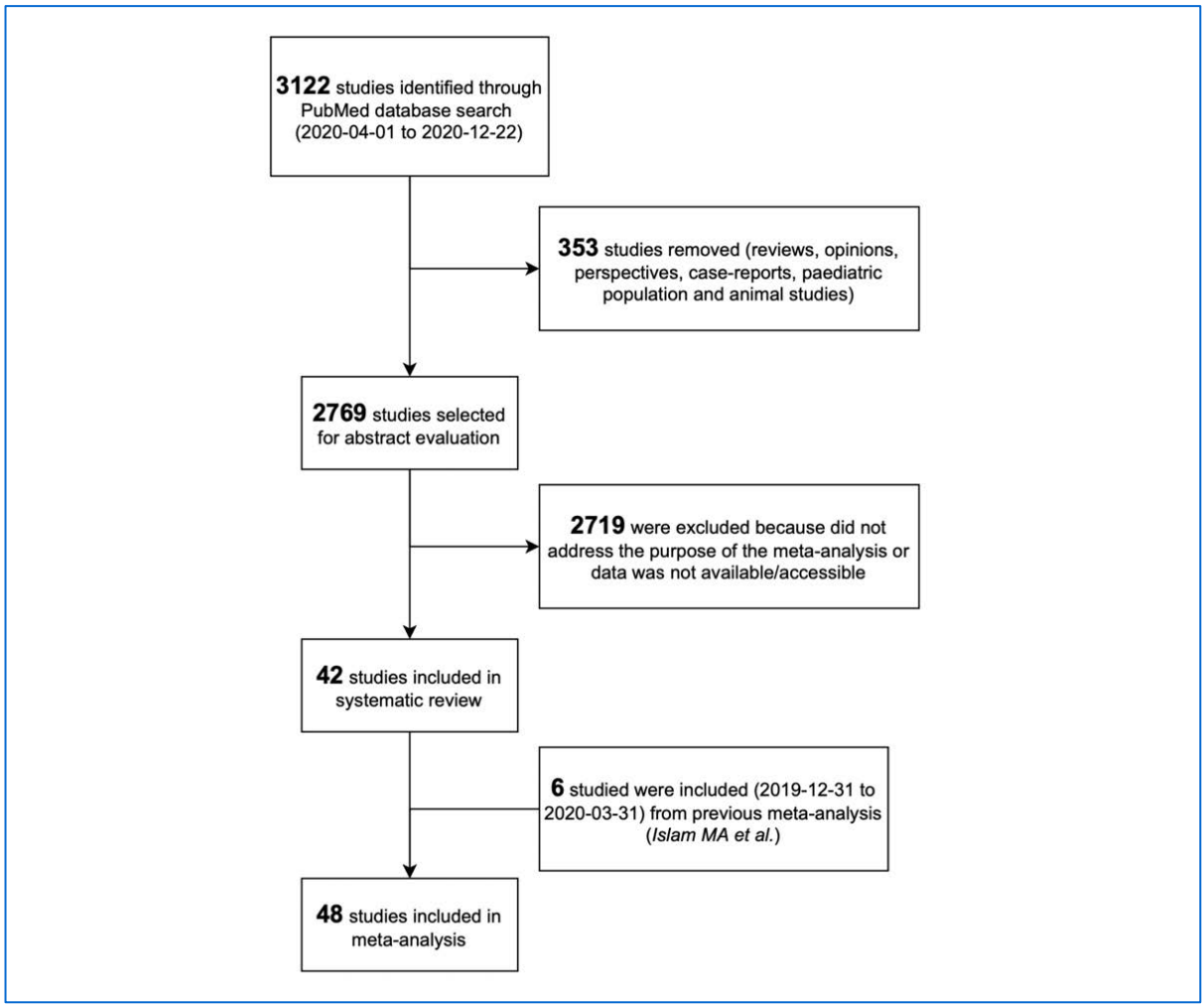
As previously mentioned, we re-assessed patients by phone calls after 6 weeks. Globally, we could get in touch with 100 patients of our cohort (74 with headache and 26 without headache) and interviewed them about disease evolution. There were no statistically significant differences with regard to the demographic variables between patients that were and were not followed up. From the follow-up group, 27.0% (27/100) was still experiencing at least one symptom of COVID-19 other than headache. Those without any more symptoms of COVID-19 had a mean duration of disease of 25.8 ± 11.9 days.

Interestingly, comparing patients with and without headache, for whom data were available at follow-up, and adjusting for age and gender, **we observed shorter COVID-19 disease duration in the headache group (23.9 ± 11.6 vs. 31.2 ± 12.0 days; p=0.028)**. We did not observe any difference in mortality (no mortality in this subgroup) or hospital length of stay (9.1±9.0 vs. 10.9 ± 9.0 days; p=0.854).

The finding of a COVID-19 disease duration one-week shorter in patients reporting headache, made us conduct a meta-analysis to determine whether headache was associated with relative risk of COVID survival.

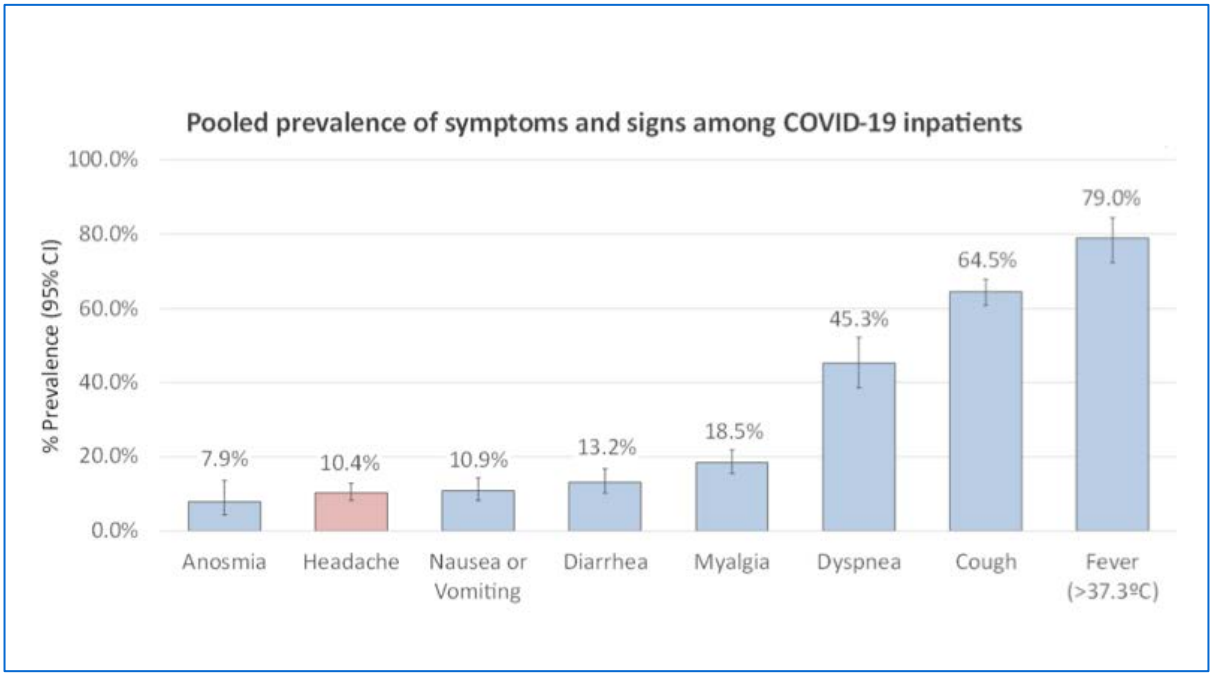
The meta-analysis included a total of 48 full-text peer-reviewed publications of COVID-19 inpatient mortality studies that also reported headache as a COVID-19 symptom (*see Figure 11 and Appendix 3, Table 2*).

Figure 11. The PRISMA flow diagram of the meta-analysis



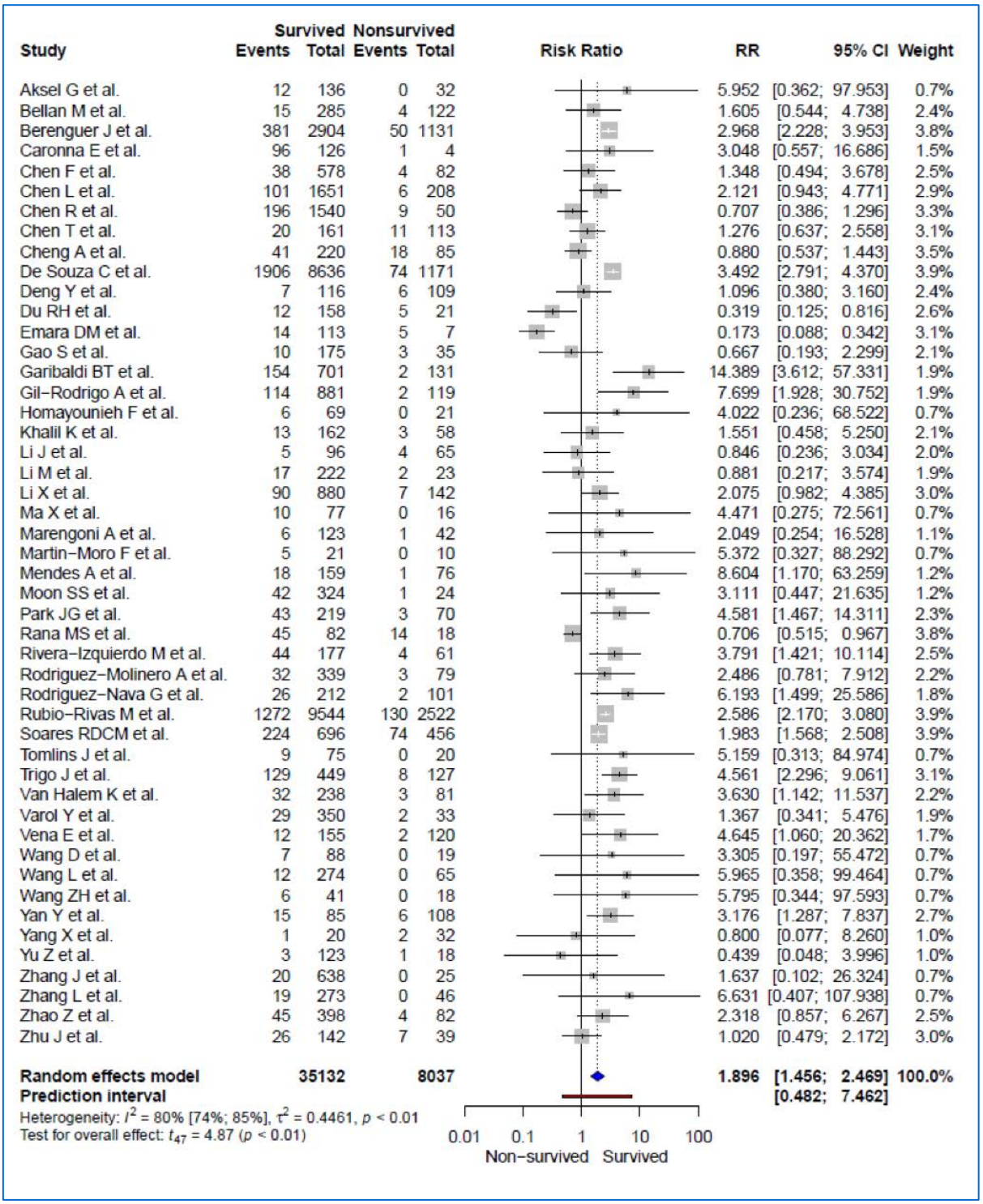
Although there was statistically significant heterogeneity between studies, the overall pooled prevalence of headache as a symptom among COVID-19 inpatients was 10.4% [8.3% - 12.9%] (*see Figure 12A*). Removing outlier studies for a sensitivity analysis, the estimated pooled prevalence of headache was 9.7% [7.8%-12-0%] (*see Appendix 3, Table 3*).

Figure 12. Pooled prevalence of symptoms and signs among COVID-19 inpatients



Regarding the risk of headache relative to mortality, **we observed a higher risk ratio of headache among COVID-19 inpatients who survived, compared to those who did not (RR: 1.90 [1.46-2.47], p<0.0001)** (*see Figure 13*). Further, we performed sensitivity analyses of headache RR, and consistently observed higher RR of headache among COVID-19 inpatients who survived. Excluding studies with lower quality (NOS score < 7), headache RR increased without a statistically significant heterogeneity between studies (RR: 2.60 [2.03-3.32], p<0.0001; I2=23.6%, p=0.180) (*see Appendix 3, Table 4*). Moreover, risk of headache did not exhibit statistically significant publication bias following visual inspection and Egger's test (*see Appendix 3, Figure 1*).

Figure 13. Risks of headache among survived (recovered or discharged) vs non-survived COVID-19 inpatients



6. Discussion

This thesis supports that headache in the context of SARS-CoV-2 infection is not just an unspecific symptom, but rather the result of mechanisms (1) specifically targeting the trigeminovascular system and is (2) associated with a better COVID-19 prognosis. According to these findings, this thesis generates new hypothesis on (1) the similarities existing between primary and secondary headache disorders and (2) whether headache could be an adaptive mechanism to protect from external attacks such as infections.

6.1. COVID-19 Headache: Characteristics and evolution

In order to answer the first objective of this thesis, we sought to describe headache characteristics and evolution in the setting of COVID-19, considering the complete lack of data existing on this matter at the time this thesis started. Our results were published in 2020, providing the first prospective description of COVID-19 Headache ever done.

6.1.1. Headache in the acute phase of the infection

In our prospective cohort of patients with COVID-19 attended at the ER, we observed that around **70% had headache** in the acute phase. This was clearly **in contrast** with the first Chinese studies published in early 2020 (163) and even **with the WHO definition that included headache as a less common COVID-19 symptom** (213). However, as more literature started to emerge other studies with a similar design supported a prevalence of headache in COVID-19 higher than 50% (218,232–234), whereas others, mainly retrospective, around 10–20% (163,217,220,224). The differences in prevalence may be certainly accounted on the design (retrospective vs prospective) but also on other factors such as objective of the study (assessing specifically headache vs. COVID-19 symptoms in general or the researchers involved in patient evaluation (neurologists vs. non-neurologists). Of note, prevalence **data on headache associated with COVID-19 mainly come from studies involving the inpatient population** with the limitation of often excluding severe patients, due to the

difficulty in their recruitment, as well as of not being representative of the outpatient population with milder disease.

So, although its real prevalence is still a matter of debate, we observed in our study that headache in COVID-19 patients was common in both genders and middle-aged people, while patients without headache were older and male. Another study later supported these findings (217) and according to a web-based study, having male gender together with bilateral localization, duration over 72h, and analgesic resistance were important variables to differentiate between COVID-19 positive patients from negative ones (235). We also observed that headache was also more common in patients with primary headache disorders but not exclusive of this population.

Concerning headache characteristics in COVID-19, in our cohort, we observed that, in the majority of patients, **headache was mild/moderate similar to a tension-type headache, while one fourth, especially women and younger subjects, had a severe "migraine-like" headache**. It is true that migraine itself is more prevalent in young women and one study showed that individuals with history of migraine or other primary headaches presented headache in the setting of COVID-19 more similar to their headache history, for example, showing more throbbing pain in the group with migraine history (216). However, we observed that migraine-like features were expressed as well in patients without personal migraine history. In fact, these migraine-like characteristics are not unique for headache in the context of SARS-CoV-2 infection, but have been described in other viral infections such as HIV (14) and Dengue (13).

We also observed that, although headache in the setting of COVID-19 usually started with the other COVID-19 symptoms, patients with severe pain more often had it as a prodromal symptom. Headache as the first COVID-19 symptom has been later reported by another study (218).

A very interesting finding from our cohort is that the presence of headache in COVID-19 was significantly associated with the other major neurological symptoms at a

cranial level: anosmia and ageusia. Our observations were later confirmed by other studies (217,233).

6.1.2. Headache evolution

After describing headache characteristics in the acute phase, we analyzed its evolution. Surprisingly, we observed that one third of followed-up patients had persistent disabling daily headache after 6 weeks, with poor response to acute treatment and, in more than 30%, representing the only symptom left of COVID- 19. Interestingly, 50% of these patients had no personal history of recurrent headache at all. Moreover, in a relevant number of these cases, headache was an initial prodromal symptom of COVID-19. In our study, headache was more likely to persist in females and patients with a headache history, although no patients had a chronic primary headache before COVID-19.

As the first wave of the pandemic was coming to an end, the scientific community started to investigate more carefully the sequelae of the SARS-CoV-2 infection and tried to give a better definition of them. As persisting symptoms after the resolution of the infection were more and more reported, terms such as post-COVID, long-COVID, post-acute COVID started to be used. However, an agreement among experts was reached in considering the acute phase until week 4 since the onset of the symptoms, then an ongoing symptomatic phase from week 4 to 12, and post-COVID condition after 12 weeks. So, considering that the follow-up of our study included patients in the ongoing symptomatic phase, we further followed up this cohort of patients in our outpatient headache clinic in order to describe the evolution of headache at a longer term. However, to do so, it was necessary to increase the size of the sample, reason that led to a multicenter study in Spain that described the evolution of COVID-19 patients at 9 months. Of the 905 patients with headache in the acute phase of SARS-CoV-2 infection that were finally included, we observed a median duration of headache of two weeks, but approximately 16% of patients both at 6 and at 9 months had a persistent headache with a chronic pattern. This percentage has been confirmed recently by a meta-analysis (236). This result also points to the fact that

after 6-months patients that are still reporting headache are unlikely to see a remission after 9-12 months which is the time of reported follow-up.

6.1.3. Headache in the post-acute phase of the infection

As mentioned above, the months following the first wave of the pandemic saw a raising in cases of patients with persisting COVID-19 symptoms. Headache became a common reason to seek specialized medical care in outpatient headache clinics. We therefore gathered data of patients with persisting headache after 6 weeks from our prospective cohort with data from new patients that were referred to our headache clinic. We sought to better describe headache in the post-acute phase of the infection and observed that patients with persistent headache had frequently migraine-like features and had suffered from mild COVID-19. These findings were also supported by our collaborative multicenter study with 9-month follow-up. Moreover, personal migraine history was really common (65.6%) in our outpatient cohort of persistent COVID-19 headache and the most frequent associated symptoms were fatigue, insomnia and mood disorder. Yet, the most interesting finding in our cohort was that patients, although exhibiting a phenotypically similar headache (i.e. headache with migraine-like features in patients who had a mild COVID-19 infection), still had considerably different characteristics. We therefore described three **patient prototypes**: (1) **patients with a personal migraine history** that experience a sudden worsening of headache following acute SARS-CoV-2 infection; (2) patients without personal or family history of migraine that experience ***de novo* headaches since the acute phase** of the infection and (3) patients without personal or family history of migraine that experience ***de novo* headaches only as a delayed symptom** in the post-acute phase of COVID-19. Frequently these cases had daily headache. New daily persistent headache is defined by the ICHD-3 as a persistent headache with a distinct and clearly-remembered onset, with pain becoming continuous and unremitting within 24 hours and present for more than 3 months (2). Viral infections have been previously postulated as possible pathophysiological mechanisms of new daily persistent headache and cases have been reported for Dengue (16), EBV (17) and the 1890 Russian/Asiatic flu (18).

We also observed that the spectrum of associated symptoms (insomnia, memory loss, dizziness, fatigue, etc.) that identify the post-COVID-19 condition is extremely variable among patients. A recent study, for example, seems to indicate that headache in the acute phase is associated specifically with headache and fatigue at long term (237). However, literature on persistent headache after COVID-19 is still too limited to further discuss our findings. Yet, our clinical observations may suggest that the spectrum of persistent headache after the resolution of the infection harbors different subtypes with distinct pathophysiological mechanisms.

6.2. COVID-19 Headache: Possible pathophysiological mechanisms

While describing the characteristics of COVID-19 headache, we wanted to investigate the possible pathophysiological mechanisms underlying this condition. The observation that, in our cohort, headache in the acute phase of COVID-19 had **migraine-like features, even in patients without previous migraine history,** clearly pointed to the possible **activation of the trigeminovascular system** as a consequence of the infection.

We therefore postulated the existence of specific pathophysiological mechanisms activated by the SARS-CoV-2 itself, potentially through (1) a direct viral invasion of the nervous system or (2) systemic factors with indirect brain effects. At that time, systemic inflammatory mechanisms were at the center of scientific interest, considering the emerging evidence of their implication in the cytokine storm that had been observed in severe COVID-19 patients.

However, it is important to mention that an **unspecific mechanism such as fever** also deserved attention, considering that usually patients and clinicians blame it for headache during a viral infection, but its real implication is not known. Nevertheless, the scarce literature available on the pathophysiology of headache attributed to systemic viral infection was mainly supporting the idea of fever and its related increase of proinflammatory cytokines (238). In our cohort, we observed that headache was not associated with presence of fever at the ER nor as a reported symptom,

supporting the need to further evaluate whether fever, in reality, has a minor role in directly causing headache. Later another study showed that headache in COVID-19 was present independently from fever (216), whereas another work observed higher odds of having headache in COVID-19 patients with fever (217). So, the relationship between fever and headache is still a matter of debate. However, it is more likely that fever either could contribute to headache in the early stages of the infection, rather than representing a sustained mechanism for headache all over the infection period, or could represent a factor able to increase frequency and severity of headaches (232).

6.2.1. Systemic Inflammation

Then, we focused on analyzing inflammatory biomarkers in our prospective cohort of patients attended at the ER, collecting data from blood tests that were routinely done, once hospitalized. We were the first one, world-wide, in conducting a study on inflammatory biomarker and correlate them with presence of headache.

At the time of our study, scientific evidence on the hyperinflammatory state of COVID-19 considered IL-6 as one of the main determinants in the cytokine storm (239,240), whose levels seem to correlate with dysregulation of other coagulation and inflammatory biomarkers (241) as well as disease severity (242). For this reason, it was assessed at the ER and during hospitalization. **The IL-6** was extremely interesting from the headache perspective, considering that its role has been also demonstrated in neuroinflammation (243) and specifically in migraine, observing that its meningeal application is able to sensitize dural afferents, leading to migraine-related behavior in mice (244,245). Therefore, it was logical to wonder whether the suggested inflammatory state in COVID-19 was also responsible for neuroinflammation, leading to headache. Specifically, **systemic inflammatory molecules, by reaching the meningeal vessels, could cause endothelial dysfunction and lead to an increased local inflammatory state, able to sensitize the trigeminovascular system** (*See Figure 14*). Surprisingly, we observed that, although elevated in both groups, IL-6 were lower at the ER in COVID-19 patients with headache compared to those without it. This finding could not be explained by different stages or severity of the disease between groups neither by age or sex, as

the groups were matched. Moreover, we observed that during hospitalization, levels of IL-6 seemed to be more stable in patients with headache compared to the ones without it.

A more recent study has also investigated IL-6, observing, on the contrary, higher levels in COVID-19 patients with headache compared to patients without it (246). However, we have to consider that the patient selection was different from our study (for example only patients with severe headache with a visual analog scale (VAS) > 7 were included) and that the headache group showed a statistically significant higher proportion of pneumonia, factor that could affect the results. When authors compared IL-6 levels between headache patients with and without pneumonia, they expectedly resulted significantly higher in patients with pneumonia.

So, although it seems that at present, published findings are still conflictive on the relationship between IL-6 and COVID-19 headache, our results of IL-6 lower levels in COVID-19 patients with headache, compared to those without it, probably point to a less prominent role of IL-6 as a cause of headache in COVID-19 patients.

For this reason, recently, counting on the advances made in the understanding of COVID-19 pathophysiology, new inflammatory molecules are being studied. The NLRP3 inflammasome, as mentioned in the introduction, is activated by SARS-CoV-2 directly or by host-intrinsic mechanisms (87). However, it is also crucial for the regulation of neuroinflammation by microglia. Recently, a study conducted using an experimental mouse model of chronic migraine showed that repeated nitroglycerin administration induced acute and chronic mechanical hyperalgesia and increased expression of NLRP3 and IL-1β in the trigeminal *nucleus caudalis* (247). So, in the context of COVID-19, viral or host-intrinsic mechanisms can activate inflammasomes producing neuroinflammation, leading to activation of the trigeminal system and headache (248). Recently, the same study that investigated IL-6, also studied NLRP3 levels in serum and observed that they were higher in COVID-19 patients with headache, however, we should consider these results carefully, taking into account the limitations previously explained (246).

6.2.2. CGRP

As the pandemic advanced, a specific molecule emerged in COVID-19 pathophysiology and drove the attention from the headache perspective: **CGRP**. This is a 37-amino acid peptide with strong vasodilating properties, which has a **fundamental role in migraine pathophysiology** and, for many, its antagonism is clinically effective in treating migraine (159). However, CGRP has several other functions in the human body (249) and, depending on the situation, for example, may promote inflammation or protect from it (250). Specifically, CGRP seems to be **involved in the neuroimmune regulation of pulmonary inflammatory responses** during an infection (97). Therefore, CGRP could be involved in the systemic inflammation produced by SARS-CoV-2 and acts, as well, in the brain. CGRP is elevated during migraine attacks (251) and headache during COVID-19 might represent an increase in CGRP levels as a host response. Considering the risk of SARS-COV-2 transmission, that limited the possibility of conducting studies requiring blood samples from hospitalized patients during the first wave of the pandemic, and that techniques for measuring CGRP in serum are still not well-standardized, we were not able to assess CGRP levels in our cohort of patients admitted at the ER and therefore determine its association with COVID-19 headache. We decided, instead, to evaluate the role of CGRP with another purpose and methodology, specifically in regards to COVID-19 outcomes, as an indirect way to assess its potential role in the pathophysiology of COVID-19 (*See Prognosis*). However, concerning headache, recently one study has measured serum CGRP in COVID-19 patients with or without headache, observing no differences in its levels between these two groups (246). In addition, another study has observed that CGRP levels in COVID-19 patients are generally reduced compared to controls (252), though it is still unclear whether this is pathological or compensatory. So, at the moment, there is no clear data supporting that systemic circulating CGRP may play a major role in COVID-19 headache. Nevertheless, we still have to keep in mind the technical limitations in CGRP serum measurement and the fact that these findings on circulating CGRP do not exclude the presence of CGRP-dependent inflammatory mechanisms within the nervous system

that are activated in response to neural activity during the infection, for example at the trigeminal level.

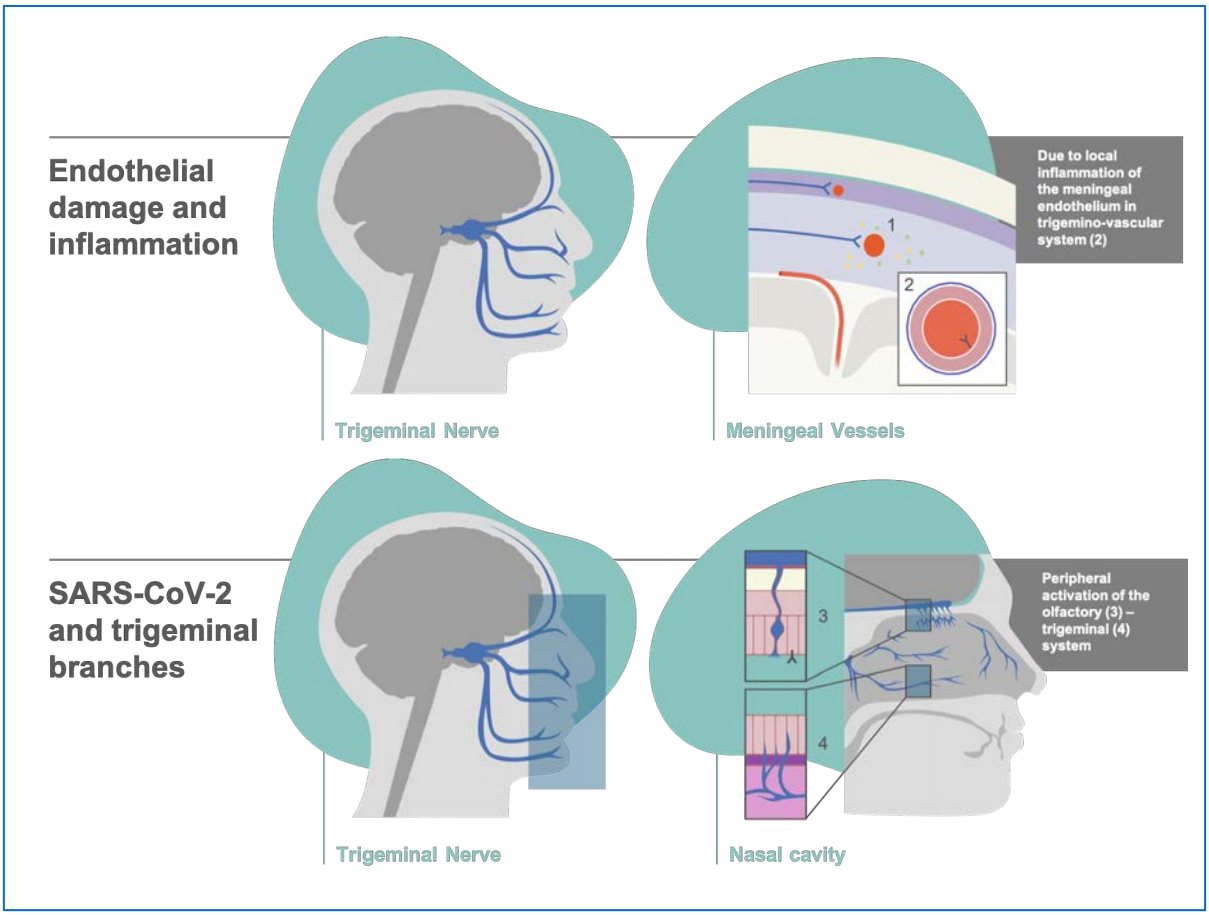
6.2.3. Direct viral damage

It is particularly intriguing the finding, in our cohort of patients attended at the ER, that headache was associated with anosmia, another cranial neurological symptom. In this context, we can hypothesize the involvement not only of systemic inflammatory mechanisms, but also of neurotropism leading to a direct viral effect on the nervous system. A direct viral damage may sensitize the trigeminal nerve and therefore the trigeminovascular system, being responsible for COVID-19 headache pathophysiology. Although, as mentioned in the introduction, the evidence of direct invasion of the nervous system is still conflicting, a recent study has showed that this phenomenon (206) seems to be possible on the basis that SARS-CoV-2 RNA has been detected not only in olfactory mucosa but also in the olfactory bulb and different branches of the trigeminal nerve (including conjunctiva, cornea, mucosa covering the uvula and the respective trigeminal ganglion (206). Although it is true that the ACE2 receptor is present in the nasal mucosa on epithelial cells and not on olfactory neurons (253), these data support a peripheral neurotropism that takes place inside the nasal cavity. Here the **activation of the trigeminovascular system may be mediated by the pathogen itself on trigeminal branches present at this level or through olfactory-trigeminal interactions, potentially explaining the association between headache and anosmia** (*See Figure 14*).

However, the same study showed immunoreactivity to SARS-CoV-2 protein in cerebral and leptomeningeal endothelial cells (206), finding that seems to confirm the possibility of another pathophysiological mechanism, consisting on the virus reaching the meninges through bloodstream dissemination and damaging endothelial cells. Endothelial damage produces local inflammation and leads to BBB instability, further promoting inflammation. The final result of this mechanism is the activation of the trigeminovascular system, causing headache (244,254) (*see Figure 14*). However, SARS-CoV-2 may also enter the brain in regions with a leaky BBB due to fenestrated capillaries, such as the median eminence of the hypothalamus and other

circumventricular organs (197,255). A dysfunction in these areas cannot be ruled out, with potential consequences on headache, as the hypothalamus is a well-known region in migraine pathophysiology (256).

Figure 14. Possible pathophysiological mechanisms of headache in the acute phase of COVID-19, involving the trigeminovascular system



6.2.4. Pathophysiological mechanisms in persistent COVID-19 headache

From a pathophysiological standpoint, other aspects to be analyzed are the mechanisms underlying persistent headache in the context of COVID-19, which could be different from the headache experienced in the acute phase.

We observed that **persistent headache may also have migraine-like features, probably reflecting the sensitization of the trigeminovascular system that persists once the viral infection has resolved.** This sensitization may be due to persistent local inflammation as a consequence of maintained activation of microglia

and release of inflammatory mediators, including interleukins, TNF-α.7 and complement proteins (257,258). Increased quinolinic acid can also lead to higher glutamate and upregulation of NMDA receptors, causing altered neurotransmission and neuronal damage. Glutamate is reported to be involved in migraine pathophysiology and at present treatments to block the NMDA receptor, such as memantine, are used in migraine prevention (259).

However, many other different mechanisms could be involved in the persistence of headache after COVID-19 and this concept is supported by the spectrum of headache subtypes that we described from our outpatient cohort. For example, headache as a long-lasting symptom since its onset in the acute phase of COVID-19 may be pathophysiologically different from headache appearing exclusively as a delayed symptom when the infection is about to resolve. Also, the expression of different comorbid conditions could point to the involvement of some pathophysiological pathways rather than others (e.g., brainstem in insomnia (260), cortical areas in neuropsychiatric symptoms (257)), although eventually they could all play a role in headache.

Recently, some authors have postulated that the persistence of symptoms after COVID-19 may be related to a constant immune activation (261). It cannot be ruled out that this phenomenon could be favored by the presence of SARs-CoV-2 antigens that persist in some tissues despite the fact that the virus is no more isolated by PCR at the nasopharyngeal level (105). Other authors support the existence of autoimmune mechanisms generated against host epitopes in the acute phase of infection (262) as responsible for post-COVID condition. However, the relationship between immunity and persistent symptoms is not clear at present and further studies are needed in this field to deeply understand the complexity of the post-COVID condition and, in this setting, headache.

6.3. Headache and COVID-19 Prognosis

According to our initial hypothesis, we wanted to investigate whether patients experiencing headache in the acute phase of COVID-19 had a different prognosis compared to those without it. In our prospective cohort we found that **inpatients reporting headache on admission had COVID-19 symptoms for one less week than inpatients not reporting headache**.

This finding could be supported by the fact that, in our cohort, IL-6 levels were lower and more stable during hospitalization in the headache group, probably indicating a better regulation of inflammatory mechanisms that may be kept on a more localized level.

More recent studies have gone on the same direction. The presence of headache was inversely associated with worse outcomes in one study involving 1000 patients attended at the ER (263). In regards to inflammatory biomarkers, lower C-reactive protein levels (218,220) have been observed in COVID-19 patients with headache compared to those without it.

At the same time, as previously mentioned, CGRP was gaining interest on the basis of its involvement in the regulation of inflammatory mechanisms in the lungs, although it may either promote or downregulate inflammation depending on the situation (250). In terms of COVID-19 prognosis, we were particularly concerned about people with migraine who have either higher levels of CGRP (264) compared to people without it or may receive treatment consisting of monoclonal antibodies antagonizing the CGRP, that are available medications for migraine prevention. This led us to design a multicenter study to assess COVID-19 outcomes in people with migraine, comparing patients with and without anti-CGRP mAbs. **We found that prevalence of COVID-19 in people with migraine was similar to the one of the general population** at the time the study was conducted, suggesting that they have not an increased risk on the basis of their migraine history. Moreover, we observed that the prevalence of COVID-19 was similar in migraine patients with and without anti-CGRP mAbs, suggesting that **antagonism of CGRP does not predispose to COVID-19**. Finally, patients with confirmed or suspected COVID-19 under anti-CGRP mAbs treatment did not seem to

have a worse course of the disease compared with migraine patients taking other preventive treatments. All these findings, although preliminary, suggest that drugs **antagonizing the CGRP pathway do not influence COVID-19 outcomes**, at least in patients with migraine. Yet, it is still an open question whether these drugs could be, on the contrary, beneficial in specific situations. Recently a clinical trial has started to investigate intranasal vazegepant, a new anti-CGRP molecule for migraine therapy, to treat COVID-19 (265), but results have not still been published.

In light of our prior observations that headache as a symptom was associated with reduced length of COVID-19 disease, we hypothesized that headache, as a COVID-19 symptom, is a putative marker of favorable COVID-19 clinical outcomes, specifically mortality. For this reason, we performed a meta-analysis of 48 published COVID-19 inpatient mortality studies which captured headache as a symptom.

This analysis indicates an unprecedented finding: **inpatients that experience headache in the setting of the SARS-CoV-2 infection are approximately twice as likely to survive, compared to those without headache**. Thus, headache in the setting of COVID-19 may be a marker of host defense responses to enhance survival.

It is notable that headache was reported as a symptom in only 10.4% of COVID-19 inpatients included in the meta-analysis. If headache is indeed a marker for reduced relative risk of mortality for COVID-19 inpatients, then it appears that this may affect a small minority of COVID-19 patients. However, this may be a misleading conclusion; COVID-19 outpatients with headache may be less likely to visit the ER or become hospitalized. Moreover, it is important to underline that, to avoid introduction of potentially confounding categorical variables, we excluded studies published following either the introduction of COVID-19 vaccines, or the appearance of the more virulent SARS-CoV-2 variant strains (e.g. Delta); and that data included in studies was collected retrospectively; patients were not all tested for COVID-19; as this was not widely available or reliable, during the first year of the pandemic. We are also aware that in specifically analyzing studies of COVID-19 patients who reported either the presence or the absence of headache symptoms, we may have introduced bias against inclusion of patients who would be unable to report these symptoms (e.g.

patients who were intubated at the time of presentation to hospital). In addition, data about previous headache history in these patients is scarce and may lead to bias. However, we have to consider that in our meta-analysis the prevalence of headache in these patients was similar to the presence of anosmia, a widely recognized COVID-19 symptom.

6.4. Insights into Primary and Secondary Headache Disorders

This thesis provides new insights into the understanding of both primary and secondary headache disorders.

6.4.1 Similarities among Secondary Headache Disorders

Our finding of migraine-like features in relation to COVID-19 headache supports previous similar observations made in the context of other viral infections (13). All together these results suggest **the activation of the trigeminovascular system in some patients during viral infections**, although the underlying mechanisms are not completely understood and could be different according to the pathogen involved. Even though there could be SARS-CoV-2-specifc mechanisms, the substantial lack of studies on headache associated with other viral infections, makes it difficult to fully compare COVID-19 headache and, consequently, separate it from the general definition of *9.2.2 headache attributed to systemic viral infection* (2).

However, it is important to emphasize the **limitation of the current classification for this definition**. The ICHD guides clinicians in the diagnosis of headache but, also, offers researchers a more systematic approach to investigate different headache types, promoting reproducibility. In this regard, a relevant conceptual change would be the inclusion of the two main headache phenotypes (i.e. migraine-like or tension-type-like) that (1) have emerged not only in SARS-CoV-2 but also in other viral infections and (2) probably reflects different pathophysiological mechanisms which might also be correlated with different treatment responses. *9.2.2 Headache attributed to systemic viral infection* in the ICHD, for example, could include these different phenotypes as an alternative for criterion C.4 and when coding this diagnosis specify if its migraine-like or tension-type-like.

Another relevant consideration, in light of our results, must be done. **Migraine-like headaches are also observed in other secondary headache disorders**, such as post-traumatic headache. So, not only different pathogens, but probably also completely **different *noxae*** (infection, trauma, vascular disorders etc) **can elicit a cascade of pathophysiological mechanisms that may end up in common a pathway**. This could apply also for persistent headaches, considering for example similarities in the manifestations between the post-COVID condition and the post-concussion syndrome. Thus, studies are needed to fully understand whether common mechanisms could be activated after different types of injury occur to the brain, fact that will help discover new treatments, targeting at the same time a broader number of secondary headache disorders, with currently scarcely available therapeutic options.

6.4.2. Similarities between Primary and Secondary Headache Disorders

The migraine-like phenotype, observed in COVID-19 headache but found in other secondary headaches, definitely suggests the **possibility of shared pathophysiological mechanisms with primary headache disorders such as migraine**.

In the case of COVID-19, considering its pathophysiology, circulating molecules such as IL-6 and CGRP have been postulated in COVID-19 headache, also on the basis on their well-known role in migraine. Yet, we demonstrated that IL-6, for example, was unexpectedly lower in patients with COVID-19 headache and for CGRP no clear clues on its implication in COVID-19 headache are available. Thus, these molecules may only have a marginal role in COVID-19 headache. Probably more relevant mechanisms still need to be discovered and some may significantly represent the common pathways between COVID-19 headache and migraine.

Some of the answers may in future also come from genetics. Recently, the COVID-19 Host Genetics Initiative has found multiple **genomic loci that are associated with SARS-CoV-2** infection or COVID-19 outcomes (266) and at least four of these GWAS loci **span genes that might influence headache or migraine susceptibility**: IFNAR2(267), LINC02210-CRHR1(268), TAC4(269), and ICAM1(270). However, it is

unknown whether these or other genetic variants link COVID-19 to specific headache pathophysiological mechanisms.

In future, investigating the mechanisms of COVID-19 may not only help understanding headache in this setting, but could also give new perspective on the pathophysiology and similarities between primary and secondary headaches disorders.

6.4.3. Viral Infections in the Pathophysiology of Headache Disorders

Our work has also demonstrated that COVID-19 patients with a migraine-like phenotype of headache may or may not have a personal migraine history. This applies for headache both in the acute and the post-acute phase of the infection, where persistent, disabling and difficult-to-treat headaches are frequently observed. These observations remark two fundamental concepts on the role of viral infections in the pathophysiology of headache disorders:

First, **in patients with migraine history, SARs-CoV-2**, but maybe other types of viruses as well, may act as **factors of sudden chronification**, rather than causing a new headache type. This is supported by the great proportion of patients with personal migraine history in our cohort of outpatients with persistent headaches after COVID-19. In this context, we can hypothesize that the infection can elicit pathophysiological mechanisms, such as inflammation, that target the trigeminovascular system and cause its continuous activation, eventually leading to central sensitization (271). This is relevant as, at present, several factors are usually considered to potentially cause the progression from episodic to chronic migraine (272), but viral infections are not included usually among them and therefore have never been fully investigated from this perspective. We therefore consider that from now on it is important to pay attention to the onset of headache attributed to a systemic viral infection in migraine patients, as this could engender a rapid migraine chronification, that, as clinicians, we should try to avoid.

Second, in patients without personal history of migraine, **SARS-CoV-2 is responsible for a *de novo* headache similar to migraine**. This raises two main questions in the headache field: (1) whether SARS-CoV-2 and potentially other viruses are able to

directly cause migraine or (2) whether they are able to trigger migraine in people with a latent migraine biology. Studies are warranted to investigate these hypotheses. The first case is related to the concept of migraine as either an acquired (e.g. due to infections) or a congenital condition (migraine as a primary headache disorder). This could be supported by the fact that migraine heritability is estimated only around 30-60%(273). The second case is related to the idea of migraine biology as a frequent trait in humans, that certain injuries to the nervous system may bring to light. If the latter was the case, studies should then clarify why such trait is so prevalent and which are its implications for human evolution.

6.4.4. Headache Disorders as Protective Mechanisms against Infections

With this thesis, we sought to investigate headache not only to better define it during COVID-19, but also to understand its role during an infection. For example, fever is a protective mechanism to reduce pathogens survival and enhance cytoprotection through changes in body temperature (274,275), while cough enables pathogens clearance (276). If headache could also have a protective role during an infection is still unknown.

The findings from our prospective study and meta-analysis have led to the conclusion that **headache in the acute phase is a marker of better prognosis** and specifically of enhanced survival. So, these surprising results **may indicate that, underlying headache, protective mechanisms may be present**. This is a revolutionary conceptual change, since headache so far has been mainly considered an unspecific symptom and nothing more than a pathological manifestation of the ongoing infection. However, our findings should be further replicated and this intriguing hypothesis specifically tested by other studies, aiming to assess the link between headache and viral infections.

As a final remark, it is therefore logical to wonder whether **not only headache as a symptom but also primary headache disorders may be protective in viral infections**. In that case, questions may raise on whether primary headache disorders, such as migraine, have **emerged as adaptive responses** in human evolution **to enhance survival and consequently genetically selected in the population in**

response to certain stimuli such as viruses (277). Although our preliminary study on a migraine cohort during the pandemic showed a similar prevalence of COVID-19 compared to the general population, the study of the link between migraine and COVID-19 represents an opportunity to advance in the knowledge of this extraordinarily common genetic disorder.

7. Conclusions

1. Headache in the acute phase of SARS-CoV-2 infection is common, disabling and difficult-to-treat, exhibiting migraine-like features, that point to the activation of the trigeminovascular system. In a relevant number of patients, headache persists after the resolution of the infection and displays as well a migraine-like phenotype, forcing patients to seek specific medical care.

2. Headache in the acute phase of COVID-19 is associated with lower and more stable markers of inflammation, specifically IL-6, suggestive of a better control of inflammatory responses in patients reporting this symptom.

3. The antagonism of CGRP, a molecule involved in migraine pathophysiology and in the regulation of lung inflammatory responses during infection, does not seem to be correlated with worse COVID-19 outcomes, in a cohort of patients with migraine treated with anti-CGRP drugs.

4. Headache as a symptom among COVID-19 patients presenting to hospitals is correlated with enhanced COVID-19 survival.

8. Future Research

The results of this thesis have had a relevant impact in the scientific community, providing the first prospective and one of the most accurate descriptions of the characteristics and evolution of headache in the setting of SARS-CoV-2 infection. Our finding of a better course of COVID-19 in patients with headache as a symptom of the infection was further confirmed by the observation of a reduced mortality in these patients in the meta-analysis that we conducted. This represents a sensational result that may suggest that headache arising secondary to a respiratory infection is a marker of enhanced likelihood of survival.

Following this line of thought, we aim to keep investigating in this field by studying the relationship between COVID-19 and primary headache disorders. Our hypothesis is that not only headache as a symptom of the infection but also primary headache disorders such as migraine might encompass a phenotype of heightened sensitivity to detect the presence of viruses, which might lead to earlier symptoms of viral infections and more effective overall defenses against them. Common behaviors associated with migraine attacks, for example, include withdrawal from environmental stimuli and social interactions (social distancing) that could represent defensive mechanisms. In this context, we have to keep in mind that migraine is among the most prevalent and burdensome diseases and it is highly heritable. From an evolutionary perspective, it seems contradictory for such an impairing disease to be so common. However, this observation can be conciliated by the fact that adaptive pressures may have selected for migraine susceptibility alleles to improve fitness (278). Thus, we hypothesize that viral selection pressures may have increased the prevalence of headache disorders and, specifically, migraine as an adaptive mechanism to enhance surviva

9. Bibliographic References

1. World Health Organization. Headache Disorders. [Internet]. [cited 2021 Mar 7]. Available from: https://www.who.int/news-room/fact-sheets/detail/headache-disorders

2. Headache Classification Committee of the International Headache Society (IHS) The International Classification of Headache Disorders, 3rd edition. Cephalalgia. 2018; 38(1):1-211

3. Gormley P, Anttila V, Winsvold BS, Palta P, Esko T, Pers TH, et al. Meta-analysis of 375,000 individuals identifies 38 susceptibility loci for migraine. Nat Genet. 2016; 48(8):856-66

4. Lucas S, Ahn AH. Posttraumatic Headache: Classification by Symptom-Based Clinical Profiles. Headache. 2018;58(6):873-882

5. Hadjikhani N, Sanchez Del Rio M, Wu O, Schwartz D, Bakker D, Fischl B, et al. Mechanisms of migraine aura revealed by functional MRI in human visual cortex. Proc Natl Acad Sci U S A. 2001;98(8):4687–92.

6. Moskowitz MA. The neurobiology of vascular head pain. Ann Neurol. 1984;16(2):157–68.

7. Moskowitz MA, buzzi MG. Migraine: General aspects. Handb Clini Neurol. 2010;97:253–266.

8. Dodick DW. A Phase-by-Phase Review of Migraine Pathophysiology. Headache. 2018;58:4–16.

9. Eccles R, Loose I, Jawad M, Nyman L. Effects of acetylsalicylic acid on sore throat pain and other pain symptoms associated with acute upper respiratory tract infection. Pain Med. 2003;4(2):118–24.

10. Schatzmayr HG, Nogueira RM, Travassos da Rosa AP. An outbreak of dengue virus at Rio de Janeiro--1986. Mem Inst Oswaldo Cruz. 1986;81(2):245–6.

11. Guanche Garcell H, Gutiérrez García F, Ramirez Nodal M, Ruiz Lozano A, Pérez Díaz CR, González Valdés A, et al. Clinical relevance of Zika symptoms in the context of a Zika Dengue epidemic. J Infect Public Health. 2020;13(2):173–6.

12. Sampaio Rocha-Filho PA, Torres RCS, Ramos Montarroyos U. HIV and Headache: A Cross-Sectional Study. Headache. 2017; 57(10):1545-1550

13. Domingues RB, Kuster GW, Onuki De Castro FL, Souza VA, Levi JE, Pannuti CS. Headache features in patients with dengue virus infection. Cephalalgia. 2006;26(7):879–82.

14. Holloway RG, Kieburtz KD. Headache and the Human Immunodeficiency Virus Type I Infection. Headache J Head Face Pain. 1995;35(5):245–55.

15. Kirkland KE, Kirkland K, Many WJ, Smitherman TA. Headache among patients with HIV disease: Prevalence, characteristics, and associations. Headache. 2012;52(3):455–66.

16. de Abreu LV, Oliveira CB, Bordini CA, Valença MM. New Daily Persistent Headache Following Dengue Fever: Report of Three Cases and an Epidemiological Study. Headache. 2020;60(1):265–8.

17. Diaz-Mitoma F, Vanast WJ, Tyrrell DLJ. Increased Frequency of Epstein-Barr Virus Excretion in Patients With New Daily Persistent Headaches. Lancet. 1987;329(8530):411–5.

18. Rozen TD. Daily persistent headache after a viral illness during a worldwide pandemic may not be a new occurrence: Lessons from the 1890 Russian/Asiatic flu. Cephalalgia. 2020; 40(13):1406-1409

19. Marinis M de, Welch K. Headache associated with non-cephalic infections: Classification and mechanisms. Cephalalgia. 1992;12(4):197–201.

20. Morimoto A, Murakami N, Nakamori T, Watanabe T. Multiple control of fever production in the central nervous system of rabbits. J Physiol.

1988;397(1):269–80.

21. Morimoto A, Nakamori T, Watanabe T, Ono T, Murakami N. Pattern differences in experimental fevers induced by endotoxin, endogenous pyrogen, and prostaglandins. Am J Physiol - Regul Integr Comp Physiol. 1988;254(4).

22. Bleich HL, Boro ES, Dinarello CA, Wolff SM. Pathogenesis of Fever in Man. N Engl J Med. 1978;298(11):607–12.

23. Omori K, Kida T, Hori M, Ozaki H, Murata T. Multiple roles of the PGE2-EP receptor signal in vascular permeability. Br J Pharmacol. 2014;171(21):4879–89.

24. Eccles R. Understanding the symptoms of the common cold and influenza. Lancet Infect Dis. 2005;5(11):718–25.

25. Smith RS. The cytokine theory of headache. Med Hypotheses. 1992;39(2):168–74.

26. Gold R, Rieckmann P, Chang P, Abdalla J. The long-term safety and tolerability of high-dose interferon β-1a in relapsing-remitting multiple sclerosis: 4-Year data from the PRISMS study. Eur J Neurol. 2005;12(8):649–56.

27. Barros JB de S, da Silva PAN, Koga R de CR, Gonzalez-Dias P, Filho JRC, Nagib PRA, et al. Acute Zika virus infection in an endemic area shows modest proinflammatory systemic immunoactivation and cytokine-symptom associations. Front Immunol. 2018;9:821.

28. Tambussi G, Gori A, Capiluppi B, Balotta C, Papagno L, Morandini B, et al. Neurological symptoms during primary Human Immunodeficiency Virus (HIV) infection correlate with high levels of HIV RNA in cerebrospinal fluid. Clin Infect Dis. 2000;30(6):962–5.

29. Lavreys L, Baeten JM, Overbaugh J, Panteleeff DD, Chohan BH, Richardson

BA, et al. Virus load during primary human immunodeficiency virus (HIV) type 1 infection is related to the severity of acute HIV illness in Kenyan women. Clin Infect Dis. 2002;35(1):77–81.

30. Nath A, Haughey NJ, Jones M, Anderson C, Bell JE, Geiger JD. Synergistic neurotoxicity by human immunodeficiency virus proteins Tat and gp120: Protection by memantine. Ann Neurol. 2000;47(2):186–94.

31. Brew BJ, Miller J. Human immunodeficiency virus-related headache. Neurology. 1993;43(6):1098–100.

32. Gelderblom HR. Structure and Classification of Viruses [Internet]. Medical Microbiology. 1996. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21413309

33. Bradshaw MJ, Venkatesan A. Herpes Simplex Virus-1 Encephalitis in Adults: Pathophysiology, Diagnosis, and Management. Neurotherapeutics. 2016;13(3): 493–508.

34. Gorbalenya AE, Baker SC, Baric RS, de Groot RJ, Drosten C, Gulyaeva AA, et al. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol. 2020;5(4):536–44.

35. Siddell SG, Walker PJ, Lefkowitz EJ, Mushegian AR, Adams MJ, Dutilh BE, et al. Additional changes to taxonomy ratified in a special vote by the International Committee on Taxonomy of Viruses (October 2018). Arch Virol. 2019;164(3):943–6.

36. Pene F, Merlat A, Vabret A, Rozenberg F, Buzyn A, Dreyfus F, et al. Coronavirus 229E-Related Pneumonia in Immunocompromised Patients. Clin Infect Dis. 2003;37(7):929–32.

37. Banerjee A, Kulcsar K, Misra V, Frieman M, Mossman K. Bats and coronaviruses. Viruses. 2019;11(1):41

38. Hu B, Guo H, Zhou P, Shi ZL. Characteristics of SARS-CoV-2 and COVID-19. Nature Reviews Microbiology. 2021;19(3):141-154

39. Jones BA, Grace D, Kock R, Alonso S, Rushton J, Said MY, et al. Zoonosis emergence linked to agricultural intensification and environmental change. Proc Natl Acad Sci U S A. 2013;110(21):8399–404.

40. Olival KJ, Weekley CC, Daszak P. Are Bats Really “Special” as Viral Reservoirs? What We Know and Need to Know. In: Bats and Viruses: A New Frontier of Emerging Infectious Diseases. 2015; 281–94.

41. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med. 2020;8(4):420-422

42. Zhou P, Yang X Lou, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270–3.

43. Calisher CH, Childs JE, Field HE, Holmes K V., Schountz T. Bats: Important reservoir hosts of emerging viruses. Clin Microbiol Rev. 2006;19:531–45.

44. Luis AD, Hayman DTS, O’Shea TJ, Cryan PM, Gilbert AT, Pulliam JRC, et al. A comparison of bats and rodents as reservoirs of zoonotic viruses: Are bats special? Proc R Soc B Biol Sci. 2013;280(1756).

45. Wang LF, Shi Z, Zhang S, Field H, Daszak P, Eaton BT. Review of bats and SARS. Vol. 12, Emerg Infect Dis. 2006;12:1834–40.

46. Hemida MG, Chu DKW, Poon LLM, Perera RAPM, Alhammadi MA, Ng HY, et al. Mers coronavirus in dromedary camel herd, Saudi Arabia. Emerg Infect Dis. 2014;20(7):1231–4.

47. Yi Y, Lagniton PNP, Ye S, Li E, Xu RH. COVID-19: What has been learned and to be learned about the novel coronavirus disease. Int J Biol Sci. 2020;16(10):1753–66.

48. Callaway E, Cyranoski D. Why snakes probably aren't spreading the new China virus. Nature. 2020; [online ahead of print]

49. Kirtipal N, Bharadwaj S, Kang SG. From SARS to SARS-CoV-2, insights on structure, pathogenicity and immunity aspects of pandemic human coronaviruses. Infect Genet Evol. 2020;85:104502

50. Masters PS. The Molecular Biology of Coronaviruses. Adv Virus Res. 2006;65:193–292.

51. Liu DX, Fung TS, Chong KKL, Shukla A, Hilgenfeld R. Accessory proteins of SARS-CoV and other coronaviruses. Antiviral Res. 2014;109: 97–109.

52. Li W, Moore MJ, Vasllieva N, Sui J, Wong SK, Berne MA, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003;426(6965):450–4.

53. Lan J, Ge J, Yu J, Shan S, Zhou H, Fan S, et al. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature. 2020;581(7807):215–20.

54. Shang J, Ye G, Shi K, Wan Y, Luo C, Aihara H, et al. Structural basis of receptor recognition by SARS-CoV-2. Nature. 2020;581(7807):221–4.

55. Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science (80-). 2020;367(6485):1444–8.

56. Letko M, Marzi A, Munster V. Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. Nat Microbiol. 2020;5(4):562–9.

57. Matsuyama S, Nagata N, Shirato K, Kawase M, Takeda M, Taguchi F. Efficient Activation of the Severe Acute Respiratory Syndrome Coronavirus Spike Protein by the Transmembrane Protease TMPRSS2. J Virol. 2010;84(24):12658–64.

58. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell. 2020;181:271-280.e8

59. Hoffmann M, Kleine-Weber H, Pöhlmann S. A Multibasic Cleavage Site in the Spike Protein of SARS-CoV-2 Is Essential for Infection of Human Lung Cells. Mol Cell. 2020;78(4):779-784.e5.

60. V'kovski P, Kratzel A, Steiner S, Stalder H, Thiel V. Coronavirus biology and replication: implications for SARS-CoV-2. Nat Rev Microbiol. 2021;19:155–70.

61. Sawicki SG, Sawicki DL. Coronaviruses use discontinuous extension for synthesis of subgenome-length negative strands. Adv Exp Med Biol. 1995; 380:499–506.

62. Överby AK, Popov VL, Niedrig M, Weber F. Tick-Borne Encephalitis Virus Delays Interferon Induction and Hides Its Double-Stranded RNA in Intracellular Membrane Vesicles. J Virol. 2010;84(17):8470–83.

63. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical Characteristics of 138 Hospitalized Patients with 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA - J Am Med Assoc. 2020;323(11):1061–9.

64. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, et al. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus–Infected Pneumonia. N Engl J Med. 2020;382(13):1199–207.

65. Meyerowitz EA, Richterman A, Gandhi RT, Sax PE. Transmission of sars-cov-2: A review of viral, host, and environmental factors. Ann Intern Med. 2021;174:69–79.

66. Stadnytskyi V, Bax CE, Bax A, Anfinrud P. The airborne lifetime of small speech droplets and their potential importance in SARS-CoV-2 transmission. Proc Natl Acad Sci U S A. 2020;117(22).

67. Xiao F, Tang M, Zheng X, Liu Y, Li X, Shan H. Evidence for Gastrointestinal

Infection of SARS-CoV-2. Gastroenterology. 2020;158(6):1831-1833.e3.

68. Hamming I, Timens W, Bulthuis MLC, Lely AT, Navis GJ, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol. 2004; 203:631-7

69. Jia HP, Look DC, Shi L, Hickey M, Pewe L, Netland J, et al. ACE2 Receptor Expression and Severe Acute Respiratory Syndrome Coronavirus Infection Depend on Differentiation of Human Airway Epithelia. J Virol. 2005;79(23):14614–21.

70. Ziegler CGK, Allon SJ, Nyquist SK, Mbano IM, Miao VN, Tzouanas CN, et al. SARS-CoV-2 Receptor ACE2 Is an Interferon-Stimulated Gene in Human Airway Epithelial Cells and Is Detected in Specific Cell Subsets across Tissues. Cell. 2020;181(5):1016-1035.e19.

71. Sun K, Gu L, Ma L, Duan Y. Atlas of ACE2 gene expression in mammals reveals novel insights in transmisson of SARS-Cov-2. bioRxiv. 2020;

72. Smith JC, Sausville EL, Girish V, Yuan M Lou, Vasudevan A, John KM, et al. Cigarette Smoke Exposure and Inflammatory Signaling Increase the Expression of the SARS-CoV-2 Receptor ACE2 in the Respiratory Tract. Dev Cell. 2020;53(5):514-529.e3.

73. Hui KPY, Cheung MC, Perera RAPM, Ng KC, Bui CHT, Ho JCW, et al. Tropism, replication competence, and innate immune responses of the coronavirus SARS-CoV-2 in human respiratory tract and conjunctiva: an analysis in ex-vivo and in-vitro cultures. Lancet Respir Med. 2020;8(7):687–95.

74. Ou J, Zhou Z, Dai R, Zhang J, Zhao S, Wu X, et al. V367F Mutation in SARS-CoV-2 Spike RBD Emerging during the Early Transmission Phase Enhances Viral Infectivity through Increased Human ACE2 Receptor Binding Affinity. J Virol. 2021;95(16).

75. Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, et al. Virological assessment of hospitalized patients with COVID-2019. Nature. 2020;581(7809):465–9.

76. Abdulrahman A, Mallah SI, Alqahtani M. COVID-19 viral load not associated with disease severity: findings from a retrospective cohort study. BMC Infect Dis. 2021;21(1).

77. Korber B, Fischer WM, Gnanakaran S, Yoon H, Theiler J, Abfalterer W, et al. Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases Infectivity of the COVID-19 Virus. Cell. 2020;182(4):812-827.e19.

78. Harrison AG, Lin T, Wang P. Mechanisms of SARS-CoV-2 Transmission and Pathogenesis. Trends Immunol. 2020;41:1100–15.

79. Totura AL, Baric RS. SARS coronavirus pathogenesis: Host innate immune responses and viral antagonism of interferon. Curr Opin in Virol. 2012;2:264–75.

80. Snijder EJ, van der Meer Y, Zevenhoven-Dobbe J, Onderwater JJM, van der Meulen J, Koerten HK, et al. Ultrastructure and Origin of Membrane Vesicles Associated with the Severe Acute Respiratory Syndrome Coronavirus Replication Complex. J Virol. 2006;80(12):5927–40.

81. Kindler E, Thiel V, Weber F. Interaction of SARS and MERS Coronaviruses with the Antiviral Interferon Response. Adv Virus Res. 2016;96: 219–43.

82. Blanco-Melo D, Nilsson-Payant BE, Liu WC, Uhl S, Hoagland D, Møller R, et al. Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. Cell. 2020;181(5):1036-1045.e9.

83. Hadjadj J, Yatim N, Barnabei L, Corneau A, Boussier J, Smith N, et al. Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. Science. 2020;369(6504):718–24.

84. Nieto-Torres JL, Verdiá-Báguena C, Jimenez-Guardeño JM, Regla-Nava JA,

Castaño-Rodriguez C, Fernandez-Delgado R, et al. Severe acute respiratory syndrome coronavirus E protein transports calcium ions and activates the NLRP3 inflammasome. Virology. 2015;485:330–9.

85. Rodrigues TS, de Sá KSG, Ishimoto AY, Becerra A, Oliveira S, Almeida L, et al. Inflammasomes are activated in response to SARS-cov-2 infection and are associated with COVID-19 severity in patients. J Exp Med. 2020;218(3).

86. Wu C, Lu W, Zhang Y, Zhang G, Shi X, Hisada Y, et al. Inflammasome Activation Triggers Blood Clotting and Host Death through Pyroptosis. Immunity. 2019;50(6):1401-1411.e4.

87. Vora SM, Lieberman J, Wu H. Inflammasome activation at the crux of severe COVID-19. Nat Rev Immunol. 2021;21:694–703.

88. Mangalmurti N, Hunter CA. Cytokine Storms: Understanding COVID-19. Immunity. 2020;53:19–25.

89. Nile SH, Nile A, Qiu J, Li L, Jia X, Kai G. COVID-19: Pathogenesis, cytokine storm and therapeutic potential of interferons. Cytokine Growth Factor Rev. 2020;53:66–70.

90. Channappanavar R, Fehr AR, Zheng J, Wohlford-Lenane C, Abrahante JE, Mack M, et al. IFN-I response timing relative to virus replication determines MERS coronavirus infection outcomes. J Clin Invest. 2019;129(9):3625–39.

91. Reghunathan R, Jayapal M, Hsu LY, Chng HH, Tai D, Leung BP, et al. Expression profile of immune response genes in patients with severe acute respiratory syndrome. BMC Immunol. 2005;6.

92. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, et al. Dysregulation of immune response in patients with coronavirus 2019 (COVID-19) in Wuhan, China. Clin Infect Dis. 2020;71(15):762–8.

93. Chen H, Liu W, Liu D, Zhao L, Yu J. SARS-CoV-2 activates lung epithelia cell proinflammatory signaling and leads to immune dysregulation in COVID-19

patients by single-cell sequencing. medRxiv. 2020;

94. Channappanavar R, Perlman S. Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology. Semin Immunopathol. 2017;39:529–39.

95. Chen T, Wu D, Chen H, Yan W, Yang D, Chen G, et al. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: Retrospective study. BMJ. 2020;368.

96. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med. 2020;8(5):475–81.

97. De Virgilis, Francesco; Di Giovanni S. Lung innervation in the eye of a cytokine storm: neuroimmune interactions and COVID-19. Nat Rev Neurol. 2020;16(11):645-652

98. Lucas C, Wong P, Klein J, Castro TBR, Silva J, Sundaram M, et al. Longitudinal analyses reveal immunological misfiring in severe COVID-19. Nature. 2020;584(7821):463–9.

99. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 2020;395:1033–4.

100. Hosseini A, Hashemi V, Shomali N, Asghari F, Gharibi T, Akbari M, et al. Innate and adaptive immune responses against coronavirus. Biomed Pharmacother. 2020;132:110859

101. Ting JPY, Trowsdale J. Genetic control of MHC class II expression. Cell. 2002;109 Suppl:S21-33

102. Mathew D, Giles JR, Baxter AE, Oldridge DA, Greenplate AR, Wu JE, et al. Deep immune profiling of COVID-19 patients reveals distinct immunotypes

with therapeutic implications. Science. 2020;369(6508).

103. Gudbjartsson DF, Norddahl GL, Melsted P, Gunnarsdottir K, Holm H, Eythorsson E, et al. Humoral Immune Response to SARS-CoV-2 in Iceland. N Engl J Med. 2020;383(18):1724–34.

104. Wajnberg A, Amanat F, Firpo A, Altman DR, Bailey MJ, Mansour M, et al. Robust neutralizing antibodies to SARS-CoV-2 infection persist for months. Science (80-). 2020;370(6521):1227–30.

105. Gaebler C, Wang Z, Lorenzi JCC, Muecksch F, Finkin S, Tokuyama M, et al. Evolution of antibody immunity to SARS-CoV-2. Nature. 2021;591(7851):639–44.

106. Lipsitch M, Grad YH, Sette A, Crotty S. Cross-reactive memory T cells and herd immunity to SARS-CoV-2. Nat Rev Immunol. 2020;20:709–13.

107. Ng KW, Faulkner N, Cornish GH, Rosa A, Harvey R, Hussain S, et al. Preexisting and de novo humoral immunity to SARS-CoV-2 in humans. Science. 2020;370(6522):1339–43.

108. Anderson EM, Goodwin EC, Verma A, Arevalo CP, Bolton MJ, Weirick ME, et al. Seasonal human coronavirus antibodies are boosted upon SARS-CoV-2 infection but not associated with protection. Cell. 2021;184(7):1858-1864.e10.

109. Wan Y, Shang J, Sun S, Tai W, Chen J, Geng Q, et al. Molecular Mechanism for Antibody-Dependent Enhancement of Coronavirus Entry. J Virol. 2020;94(5).

110. Paludan SR, Mogensen TH. Innate immunological pathways in COVID-19 pathogenesis. Sci Immunol. 2022;7(67).

111. WHO. World Health Organization. Coronavirus disease 2019 (COVID-19) [Internet]. Vol. 2019, Situation Report, 32. 2020. p. 1–16. Available from: https://covid19.who.int

112. CDC. SARS-CoV-2 Variant Classifications and Definitions [Internet]. Cdc. 2021. p. 1–12. Available from: https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-info.html#Interest

113. ECDC. SARS-CoV-2 variants of concern as of 5 January 2022 [Internet]. Https://Www.Ecdc.Europa.Eu/En/Covid-19/Variants-Concern. 2022. Available from: https://www.ecdc.europa.eu/en/covid-19/variants-concern

114. Wu Z, McGoogan JM. Characteristics of and Important Lessons from the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72314 Cases from the Chinese Center for Disease Control and Prevention. JAMA. 2020;323:1239–42.

115. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020; 395:497-506

116. Guan W, Ni Z, Hu Y, Liang W, Ou C, He J, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med. 2020;382(18):1708–20.

117. Lu X, Zhang L, Du H, Zhang J, Li YY, Qu J, et al. SARS-CoV-2 Infection in Children. N Engl J Med. 2020;382(17):1663–5.

118. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020; 395:507-513

119. Nalbandian A, Sehgal K, Gupta A, Madhavan M V., McGroder C, Stevens JS, et al. Post-acute COVID-19 syndrome. Nat Med. 2021;27:601–15.

120. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395(10229):1054–62.

121. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, et al. Risk Factors Associated with Acute Respiratory Distress Syndrome and Death in Patients with Coronavirus

Disease 2019 Pneumonia in Wuhan, China. JAMA Intern Med. 2020;180(7):934–43.

122. Liao D, Zhou F, Luo L, Xu M, Wang H, Xia J, et al. Haematological characteristics and risk factors in the classification and prognosis evaluation of COVID-19: a retrospective cohort study. Lancet Haematol. 2020;7(9):e671–8.

123. Lopez-Leon S, Wegman-Ostrosky T, Perelman C, Sepulveda R, Rebolledo PA, Cuapio A, et al. More than 50 long-term effects of COVID-19: a systematic review and meta-analysis. Sci Rep. 2021;11(1).

124. World Health Organization Director-General's opening remarks at the media briefing on COVID-19 - 24 February 2020. [Internet]. Available from: https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---24-february-2020

125. McCue C, Cowan R, Quasim T, Puxty K, McPeake J. Long term outcomes of critically ill COVID-19 pneumonia patients: early learning. Intensive Care Med. 2021;47:240–1.

126. Gupta A, Madhavan M V., Sehgal K, Nair N, Mahajan S, Sehrawat TS, et al. Extrapulmonary manifestations of COVID-19. Nat Med. 2020;26:1017–32.

127. Datta SD, Talwar A, Lee JT. A Proposed Framework and Timeline of the Spectrum of Disease Due to SARS-CoV-2 Infection: Illness beyond Acute Infection and Public Health Implications. JAMA. 2020;324: 2251–2.

128. Greenhalgh T, Knight M, A'Court C, Buxton M, Husain L. Management of post-acute covid-19 in primary care. BMJ. 2020;370.

129. Shah W, Hillman T, Playford ED, Hishmeh L. Managing the long term effects of covid-19: Summary of NICE, SIGN, and RCGP rapid guideline. BMJ. 2021;372.

130. van Kampen JJA, van de Vijver DAMC, Fraaij PLA, Haagmans BL, Lamers MM, Okba N, et al. Duration and key determinants of infectious virus shedding

in hospitalized patients with coronavirus disease-2019 (COVID-19). Nat Commun. 2021;12(1).

131. Chopra V, Flanders SA, O'Malley M, Malani AN, Prescott HC. Sixty-day outcomes among patients hospitalized with COVID-19. Ann Intern Med. 2021;174:576–8.

132. Docherty AB, Harrison EM, Green CA, Hardwick HE, Pius R, Norman L, et al. Features of 20 133 UK patients in hospital with covid-19 using the ISARIC WHO Clinical Characterisation Protocol: Prospective observational cohort study. BMJ. 2020;369.

133. Tenforde MW, Kim SS, Lindsell CJ, Billig Rose E, Shapiro NI, Files DC, et al. Symptom Duration and Risk Factors for Delayed Return to Usual Health Among Outpatients with COVID-19 in a Multistate Health Care Systems Network — United States, March–June 2020. MMWR Morb Mortal Wkly Rep. 2020;69(30):993–8.

134. Huang C, Huang L, Wang Y, Li X, Ren L, Gu X, et al. 6-month consequences of COVID-19 in patients discharged from hospital: a cohort study. Lancet. 2021; 397:220-232

135. Carfì A, Bernabei R, Landi F. Persistent symptoms in patients after acute COVID-19. JAMA. 2020;324:603–5.

136. Xiong Q, Xu M, Li J, Liu Y, Zhang J, Xu Y, et al. Clinical sequelae of COVID-19 survivors in Wuhan, China: a single-centre longitudinal study. Clin Microbiol Infect. 2021;27(1):89–95.

137. Goërtz YMJ, Van Herck M, Delbressine JM, Vaes AW, Meys R, Machado FVC, et al. Persistent symptoms 3 months after a SARS-CoV-2 infection: the post-COVID-19 syndrome? ERJ Open Res. 2020; 6(4):00542-2020

138. Nehme M, Braillard O, Chappuis F, Courvoisier DS, Guessous I. Prevalence of symptoms more than seven months after diagnosis of symptomatic covid-

19 in an outpatient setting. Ann Intern Med. 2021;174(9):1252–60.

139. Wu JT, Leung K, Bushman M, Kishore N, Niehus R, de Salazar PM, et al. Estimating clinical severity of COVID-19 from the transmission dynamics in Wuhan, China. Nat Med. 2020;26(4):506–10.

140. Chow RD, Majety M, Chen S. The aging transcriptome and cellular landscape of the human lung in relation to SARS-CoV-2. Nat Commun. 2021;12(1).

141. Liu K, Chen Y, Lin R, Han K. Clinical features of COVID-19 in elderly patients: A comparison with young and middle-aged patients. J Infect. 2020;80:e14–8.

142. McGonagle D, Sharif K, O'Regan A, Bridgewood C. The Role of Cytokines including Interleukin-6 in COVID-19 induced Pneumonia and Macrophage Activation Syndrome-Like Disease. Autoimmun Rev. 2020. 19(6):102537

143. Molony RD, Nguyen JT, Kong Y, Montgomery RR, Shaw AC, Iwasaki A. Aging impairs both primary and secondary RIG-I signaling for interferon induction in human monocytes. Sci Signal. 2017;10(509).

144. Zhou R, To KKW, Wong YC, Liu L, Zhou B, Li X, et al. Acute SARS-CoV-2 Infection Impairs Dendritic Cell and T Cell Responses. Immunity. 2020;53(4):864-877.e5.

145. Takahashi T, Ellingson MK, Wong P, Israelow B, Lucas C, Klein J, et al. Sex differences in immune responses that underlie COVID-19 disease outcomes. Nature. 2020;588(7837):315–20.

146. Nguyen NT, Chinn J, de Ferrante M, Kirby KA, Hohmann SF, Amin A. Male gender is a predictor of higher mortality in hospitalized adults with COVID-19. PLoS One. 2021;16:e0254066

147. Berghöfer B, Frommer T, Haley G, Fink L, Bein G, Hackstein H. TLR7 Ligands Induce Higher IFN-α Production in Females. J Immunol. 2006;177(4):2088–96.

148. Schoggins J. Defective viral RNA sensing linked to severe COVID-19. Science. 2021;374(6567):535.

149. Williamson EJ, Walker AJ, Bhaskaran K, Bacon S, Bates C, Morton CE, et al. Factors associated with COVID-19-related death using OpenSAFELY. Nature. 2020; 584:430-436

150. Moore JT, Ricaldi JN, Rose CE, Fuld J, Parise M, Kang GJ, et al. Disparities in Incidence of COVID-19 Among Underrepresented Racial/Ethnic Groups in Counties Identified as Hotspots During June 5–18, 2020 — 22 States, February–June 2020. MMWR Morb Mortal Wkly Rep. 2020;69(33):1122–6.

151. Price-Haywood EG, Burton J, Fort D, Seoane L. Hospitalization and Mortality among Black Patients and White Patients with Covid-19. N Engl J Med. 2020;382(26):2534–43.

152. Severe COVID GWAS Group. Genomewide Association Study of Severe Covid-19 with Respiratory Failure. N Engl J Med. 2020; 383:1522-1534

153. Pairo-Castineira E, Clohisey S, Klaric L, Bretherick A, Rawlik K, Parkinson N, et al. Genetic mechanisms of critical illness in Covid-19. Nature. 2020; 591:92-98

154. Zeberg H, Pääbo S. The major genetic risk factor for severe COVID-19 is inherited from Neanderthals. Nature. 2020; 587:610-612

155. Fang X, Li S, Yu H, Wang P, Zhang Y, Chen Z, et al. Epidemiological, comorbidity factors with severity and prognosis of COVID-19: A systematic review and meta-analysis. Aging (Albany NY). 2020;12(13):12493–503.

156. Maghbooli Z, Sahraian MA, Ebrahimi M, Pazoki M, Kafan S, Tabriz HM, et al. Vitamin D sufficiency, a serum 25-hydroxyvitamin D at least 30 ng/mL reduced risk for adverse clinical outcomes in patients with COVID-19 infection. PLoS One. 2020;15:e0239799

157. Munshi R, Hussein MH, Toraih EA, Elshazli RM, Jardak C, Sultana N, et al.

Vitamin D insufficiency as a potential culprit in critical COVID-19 patients. J Med Virol. 2021;93:733–40.

158. Caronna E, Starling AJ. Update on Calcitonin Gene-Related Peptide Antagonism in the Treatment of Migraine. Neurol Clin. 2021;39:1–19.

159. Charles A, Pozo-Rosich P. Targeting calcitonin gene-related peptide: a new era in migraine therapy. Lancet. 2019;394:1765–74.

160. Pezzini A, Padovani A. Lifting the mask on neurological manifestations of COVID-19. Nat Rev Neurol. 2020; 16:636-644

161. Toscano G, Palmerini F, Ravaglia S, Ruiz L, Invernizzi P, Cuzzoni MG, et al. Guillain–Barré Syndrome Associated with SARS-CoV-2. N Engl J Med. 2020;382(26):2574–6.

162. Xiong W, Mu J, Guo J, Lu L, Liu D, Luo J, et al. New onset neurologic events in people with COVID-19 in 3 regions in China. Neurology. 2020;95(11):e1479–87.

163. Mao L, Jin H, Wang M, Hu Y, Chen S, He Q, et al. Neurological Manifestations of Hospitalized Patients with COVID-19 in Wuhan, China. JAMA 2020; 77:683-690

164. Frontera JA, Sabadia S, Lalchan R, Fang T, Flusty B, Millar-Vernetti P, et al. A Prospective Study of Neurologic Disorders in Hospitalized Patients With COVID-19 in New York City. Neurology. 2021;96(4):e575–86.

165. Liotta EM, Batra A, Clark JR, Shlobin NA, Hoffman SC, Orban ZS, et al. Frequent neurologic manifestations and encephalopathy-associated morbidity in Covid-19 patients. Ann Clin Transl Neurol. 2020;7(11):2221–30.

166. Benussi A, Pilotto A, Premi E, Libri I, Giunta M, Agosti C, et al. Clinical characteristics and outcomes of inpatients with neurologic disease and COVID-19 in Brescia, Lombardy, Italy. Neurology. 2020;95(7):e910–20.

167. Kim SW, Kim SM, Kim YK, Kim JY, Lee YM, Kim BO, et al. Clinical Characteristics and Outcomes of COVID-19 Cohort Patients in Daegu Metropolitan City Outbreak in 2020. J Korean Med Sci. 2021;36(1).

168. Chou SHY, Beghi E, Helbok R, Moro E, Sampson J, Altamirano V, et al. Global Incidence of Neurological Manifestations among Patients Hospitalized with COVID-19 - A Report for the GCS-NeuroCOVID Consortium and the ENERGY Consortium. JAMA Netw Open. 2021; 4:e2112131

169. García-Azorín D, Abildúa MJA, Aguirre MEE, Fernández SF, Moncó JCG, Guijarro-Castro C, et al. Neurological presentations of COVID-19: Findings from the Spanish Society of Neurology neuroCOVID-19 registry. J Neurol Sci. 2021;423.

170. Cooper KW, Brann DH, Farruggia MC, Bhutani S, Pellegrino R, Tsukahara T, et al. COVID-19 and the Chemical Senses: Supporting Players Take Center Stage. Neuron. 2020;107:219–33.

171. Favas TT, Dev P, Chaurasia RN, Chakravarty K, Mishra R, Joshi D, et al. Neurological manifestations of COVID-19: a systematic review and meta-analysis of proportions. Neurol Sci. 2020;41(12):3437–70.

172. Whittaker A, Anson M, Harky A. Neurological Manifestations of COVID-19: A systematic review and current update. Acta Neurol Scand. 2020;142:14–22.

173. Harapan BN, Yoo HJ. Neurological symptoms, manifestations, and complications associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease 19 (COVID-19). J Neurol. 2021; 268:3059–71.

174. Beghi E, Giussani G, Westenberg E, Allegri R, Garcia-Azorin D, Guekht A, et al. Acute and post-acute neurological manifestations of COVID-19: present findings, critical appraisal, and future directions. J Neurol. 2021;1-10

175. Graham EL, Clark JR, Orban ZS, Lim PH, Szymanski AL, Taylor C, et al.

Persistent neurologic symptoms and cognitive dysfunction in non-hospitalized Covid-19 “long haulers.” Ann Clin Transl Neurol. 2021;8(5):1073–85.

176. Munõz-Price LS, Nattinger AB, Rivera F, Hanson R, Gmehlin CG, Perez A, et al. Racial Disparities in Incidence and Outcomes among Patients with COVID-19. JAMA Netw Open. 2020;3(9).

177. Collantes ME V., Espiritu AI, Sy MCC, Anlacan VMM, Jamora RDG. Neurological Manifestations in COVID-19 Infection: A Systematic Review and Meta-Analysis. Can J Neurol Sci. 2021;48:66–76.

178. Akhvlediani T, Jelcic I, Taba P, Pfausler B, Steiner I, Sellner J. What did we learn from the previous coronavirus epidemics and what can we do better: a neuroinfectiological point of view. Eur J Neurol. 2020;27: e69–72.

179. Hernandez-Fernandez F, Sandoval Valencia H, Barbella-Aponte RA, Collado-Jimenez R, Ayo-Martin O, Barrena C, et al. Cerebrovascular disease in patients with COVID-19: Neuroimaging, histological and clinical description. Brain. 2020;143(10):3089–103.

180. Malas MB, Naazie IN, Elsayed N, Mathlouthi A, Marmor R, Clary B. Thromboembolism risk of COVID-19 is high and associated with a higher risk of mortality: A systematic review and meta-analysis. EClinicalMedicine. 2020;29–30.

181. Kantonen J, Mahzabin S, Mäyränpää MI, Tynninen O, Paetau A, Andersson N, et al. Neuropathologic features of four autopsied COVID-19 patients. Brain Pathol. 2020;30:1012–6.

182. Yaghi S, Ishida K, Torres J, Mac Grory B, Raz E, Humbert K, et al. SARS-CoV-2 and Stroke in a New York Healthcare System. Stroke. 2020;2002–11.

183. Qin C, Zhou L, Hu Z, Yang S, Zhang S, Chen M, et al. Clinical Characteristics and Outcomes of COVID-19 Patients with a History of Stroke in Wuhan, China. Stroke. 2020;2219–23.

184. Goshua G, Pine AB, Meizlish ML, Chang CH, Zhang H, Bahel P, et al. Endotheliopathy in COVID-19-associated coagulopathy: evidence from a single-centre, cross-sectional study. Lancet Haematol. 2020;7(8):e575–82.

185. Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, et al. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. N Engl J Med. 2020;383(2):120–8.

186. Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, et al. Endothelial cell infection and endotheliitis in COVID-19. Lancet. 2020;395:1417–8.

187. Bergmann CC, Lane TE, Stohlman SA. Coronavirus infection of the central nervous system: Host-virus stand-off. Nat Rev Microbiol. 2006;4:121–32.

188. Pilotto A, Masciocchi S, Volonghi I, Crabbio M, Magni E, De Giuli V, et al. Clinical Presentation and Outcomes of Severe Acute Respiratory Syndrome Coronavirus 2-Related Encephalitis: The ENCOVID Multicenter Study. J Infect Dis. 2021;223(1):28–37.

189. Alexopoulos H, Magira E, Bitzogli K, Kafasi N, Vlachoyiannopoulos P, Tzioufas A, et al. Anti-SARS-CoV-2 antibodies in the CSF, blood-brain barrier dysfunction, and neurological outcome: Studies in 8 stuporous and comatose patients. Neurol Neuroimmunol neuroinflammation. 2020;7(6).

190. Pilotto A, Masciocchi S, Volonghi I, de Giuli V, Caprioli F, Mariotto S, et al. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Encephalitis Is a Cytokine Release Syndrome: Evidences from Cerebrospinal Fluid Analyses. Clin Infect Dis. 2021;73(9):E3019–26.

191. Yang L, Han Y, Nilsson-Payant BE, Gupta V, Wang P, Duan X, et al. A Human Pluripotent Stem Cell-based Platform to Study SARS-CoV-2 Tropism and Model Virus Infection in Human Cells and Organoids. Cell Stem Cell. 2020;27(1):125-136.e7.

192. Solomon IH, Normandin E, Bhattacharyya S, Mukerji SS, Keller K, Ali AS, et al. Neuropathological Features of Covid-19. N Engl J Med. 2020;383:989-992

193. Song E, Zhang C, Israelow B, Lu-Culligan A, Prado AV, Skriabine S, et al. Neuroinvasion of SARS-CoV-2 in human and mouse brain. J Exp Med. 2021;218(3).

194. Rhea EM, Logsdon AF, Hansen KM, Williams LM, Reed MJ, Baumann KK, et al. The S1 protein of SARS-CoV-2 crosses the blood–brain barrier in mice. Nat Neurosci. 2021;24(3):368–78.

195. Buzhdygan TP, DeOre BJ, Baldwin-Leclair A, Bullock TA, McGary HM, Khan JA, et al. The SARS-CoV-2 spike protein alters barrier function in 2D static and 3D microfluidic in-vitro models of the human blood–brain barrier. Neurobiol Dis. 2020;146.

196. Yang Y, Rosenberg GA. Blood-brain barrier breakdown in acute and chronic cerebrovascular disease. Stroke. 2011;42(11):3323–8.

197. Nampoothiri S, Sauve F, Ternier G, Fernandois D, Coelho C, Imbernon M, et al. The hypothalamus as a hub for putative SARS-CoV-2 brain infection. bioRxiv. 2020;

198. Xu J, Lazartigues E. Expression of ACE2 in Human Neurons Supports the Neuro-Invasive Potential of COVID-19 Virus. Cell Mol Neurobiol. 2022;42(1):305–9.

199. Chen R, Wang K, Yu J, Howard D, French L, Chen Z, et al. The Spatial and Cell-Type Distribution of SARS-CoV-2 Receptor ACE2 in the Human and Mouse Brains. Front Neurol. 2021;11.

200. Allen AM, O'Callaghan EL, Mendelsohn FAO, Chai SY. Neuronal Angiotensin. Encyclopedia of Neuroscience. 2009;697–702.

201. Cantuti-Castelvetri L, Ojha R, Pedro LD, Djannatian M, Franz J, Kuivanen S, et al. Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity. Science.

2020;370(6518).

202. Shang J, Wan Y, Luo C, Ye G, Geng Q, Auerbach A, et al. Cell entry mechanisms of SARS-CoV-2. Proc Natl Acad Sci U S A. 2020;117(21).

203. Zubair AS, McAlpine LS, Gardin T, Farhadian S, Kuruvilla DE, Spudich S. Neuropathogenesis and neurologic manifestations of the coronaviruses in the age of coronavirus disease 2019: A review. JAMA Neurol. 2020;77:1018–27.

204. Dubé M, Le Coupanec A, Wong AHM, Rini JM, Desforges M, Talbot PJ. Axonal Transport Enables Neuron-to-Neuron Propagation of Human Coronavirus OC43. J Virol. 2018;92:e00404

205. Xydakis MS, Albers MW, Holbrook EH, Lyon DM, Shih RY, Frasnelli JA, et al. Post-viral effects of COVID-19 in the olfactory system and their implications. Lancet Neurol. 2021;20:753–61.

206. Meinhardt J, Radke J, Dittmayer C, Franz J, Thomas C, Mothes R, et al. Olfactory transmucosal SARS-CoV-2 invasion as a port of central nervous system entry in individuals with COVID-19. Nat Neurosci. 2021;24:168-175

207. Yang AC, Kern F, Losada PM, Agam MR, Maat CA, Schmartz GP, et al. Dysregulation of brain and choroid plexus cell types in severe COVID-19. Nature. 2021;595(7868):565–71.

208. Matschke J, Lütgehetmann M, Hagel C, Sperhake JP, Schröder AS, Edler C, et al. Neuropathology of patients with COVID-19 in Germany: a post-mortem case series. Lancet Neurol. 2020;19(11):919–29.

209. Heming M, Li X, Räuber S, Mausberg AK, Börsch AL, Hartlehnert M, et al. Neurological Manifestations of COVID-19 Feature T Cell Exhaustion and Dedifferentiated Monocytes in Cerebrospinal Fluid. Immunity. 2021;54(1):164-175.e6.

210. Edén A, Kanberg N, Gostner J, Fuchs D, Hagberg L, Andersson LM, et al. CSF Biomarkers in Patients With COVID-19 and Neurologic Symptoms: A

Case Series. Neurology. 2021;96(2):e294–300.

211. Solomon T. Neurological infection with SARS-CoV-2 — the story so far. Nat Rev Neurol. 2021;17:65–6.

212. Balcom EF, Nath A, Power C. Acute and chronic neurological disorders in COVID-19: potential mechanisms of disease. Brain. 2021;144(12):3576–88.

213. Organization WH. World Health Organization Coronavirus Disease Dashboard [Internet]. 12/5/2020. Available from: https://covid19.who.int/?gclid=CjwKCAiA_Kz-BRAJEiwAhJNY74BJHWPUTVpLi2lBpngW2Cosw7Rpt8BXLW5gd3HYIDc_Eyg2hf_9tBoCDk4QAvD_BwE

214. van Buuren S, Oudshoorn CGM. MICE: multivariate imputation by chained equations. R Packag version. 2007;1:2007.

215. Romero-Sánchez CM, Díaz-Maroto I, Fernández-Díaz E, Sánchez-Larsen Á, Layos-Romero A, García-García J, et al. Neurologic manifestations in hospitalized patients with COVID-19: The ALBACOVID registry. Neurology. 2020;95(8):e1060–70.

216. Porta-Etessam J, Matías-Guiu JA, González-García N, Gómez Iglesias P, Santos-Bueso E, Arriola-Villalobos P, et al. Spectrum of Headaches Associated With SARS-CoV-2 Infection: Study of Healthcare Professionals. Headache. 2020;60(8):1697–704.

217. Trigo J, García-Azorín D, Planchuelo-Gómez Á, Martínez-Pías E, Talavera B, Hernández-Pérez I, et al. Factors associated with the presence of headache in hospitalized COVID-19 patients and impact on prognosis: A retrospective cohort study. J Headache Pain. 2020;21(1).

218. Membrilla JA, de Lorenzo Í, Sastre M, Díaz de Terán J. Headache as a Cardinal Symptom of Coronavirus Disease 2019: A Cross-Sectional Study. Headache. 2020; 60:2176-2191

219. Caronna E, Ballvé A, Llauradó A, Gallardo VJ, María Ariton D, Lallana S, et al. Headache: A striking prodromal and persistent symptom, predictive of COVID-19 clinical evolution. Cephalalgia. 2020;40:1410-1421

220. Gonzalez-Martinez A, Fanjul V, Ramos C, Serrano Ballesteros J, Bustamante M, Villa Martí A, et al. Headache during SARS-CoV-2 infection as an early symptom associated with a more benign course of disease: a case–control study. Eur J Neurol. 2021;28(10):3426–36.

221. Vandenbroucke JP, von Elm E, Altman DG, Gøtzsche PC, Mulrow CD, Pocock SJ, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): Explanation and elaboration. Int J Surg. 2014;12(12):1500–24.

222. Estudio ENE-COVID19: Primera Ronda Estudio Nacional de Sero-Epidemiología de la Infección por SARS-CoV-2 en España. Informe preliminar 13 de Mayo 2020.

223. Moher D, Liberati A, Tetzlaff J, Altman DG, Altman D, Antes G, et al. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. PLoS Medicine. 2009. 6:e1000097

224. Islam MA, Alam SS, Kundu S, Hossan T, Kamal MA, Cavestro C. Prevalence of Headache in Patients With Coronavirus Disease 2019 (COVID-19): A Systematic Review and Meta-Analysis of 14,275 Patients. Front Neurol. 2020;11(562634).

225. wwwmetaxiscom. PROSPERO International prospective register of systematic reviews [Internet]. Available from: http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42018088322

226. Wells G, Shea B, O'Connell D, Peterson J. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses [Internet]. Ottawa, ON: Ottawa Hospital Research Institute. 2000. Available

from: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp

227. Higgins JPT, Thompson SG, Spiegelhalter DJ. A re-evaluation of random-effects meta-analysis. J R Stat Soc Ser A Stat Soc. 2009;

228. Newcombe RG. Two-sided confidence intervals for the single proportion: Comparison of seven methods. Stat Med. 1998;17(8):857–72.

229. Robins J, Greenland S, Breslow NE. A general estimator for the variance of the mantel haenszel odds ratio. Am J Epidemiol. 1986. 124:719-23

230. Borenstein M, Higgins JPT. Meta-Analysis and Subgroups. Prev Sci. 2013;14(2):134–43.

231. Higgins JPT, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med. 2002; 21:1539-58

232. Magdy R, Hussein M, Ragaie C, Abdel-Hamid HM, Khallaf A, Rizk HI, et al. Characteristics of headache attributed to COVID-19 infection and predictors of its frequency and intensity: A cross sectional study. Cephalalgia. 2020;40(13):1422–31.

233. Rocha-Filho PAS, Magalhães JE. Headache associated with COVID-19: Frequency, characteristics and association with anosmia and ageusia. Cephalalgia. 2020; 40:1443-1451

234. Poncet-Megemont L, Paris P, Tronchere A, Salazard JP, Pereira B, Dallel R, et al. High Prevalence of Headaches During Covid-19 Infection: A Retrospective Cohort Study. Headache. 2020; 60:2578-2582

235. Uygun Ö, Ertaş M, Ekizoğlu E, Bolay H, Özge A, Kocasoy Orhan E, et al. Headache characteristics in COVID-19 pandemic-a survey study. J Headache Pain. 2020; 21(1):121

236. Fernández-de-las-Peñas C, Navarro-Santana M, Gómez-Mayordomo V, Cuadrado ML, García-Azorín D, Arendt-Nielsen L, et al. Headache as an

acute and post-COVID-19 symptom in COVID-19 survivors: A meta-analysis of the current literature. Eur J Neurol. 2021;28(11):3820–5.

237. Fernández-de-las-Peñas C, Gómez-Mayordomo V, Cuadrado ML, Palacios-Ceña D, Florencio LL, Guerrero AL, et al. The presence of headache at onset in SARS-CoV-2 infection is associated with long-term post-COVID headache and fatigue: A case-control study. Cephalalgia. 2021;41(13):1332–41.

238. Walter EJ, Hanna-Jumma S, Carraretto M, Forni L. The pathophysiological basis and consequences of fever. Crit Care. 2016;20(1).

239. Tanaka T, Narazaki M, Kishimoto T. Il-6 in inflammation, Immunity, And disease. Cold Spring Harb Perspect Biol. 2014; 6:a016295

240. Zhang C, Wu Z, Li J-W, Zhao H, Wang G-Q. The cytokine release syndrome (CRS) of severe COVID-19 and Interleukin-6 receptor (IL-6R) antagonist Tocilizumab may be the key to reduce the mortality. Int J Antimicrob Agents. 2020; 55:105954

241. D'Alessandro A, Thomas T, Dzieciatkowska M, Hill RC, Francis RO, Hudson KE, et al. Serum Proteomics in COVID-19 Patients: Altered Coagulation and Complement Status as a Function of IL-6 Level. J Proteome Res. 2020;19:4417-4427

242. Broman N, Rantasärkkä K, Feuth T, Valtonen M, Waris M, Hohenthal U, et al. IL-6 and other biomarkers as predictors of severity in COVID-19. Ann Med. 2021;53(1):410–2.

243. Zhou YQ, Liu Z, Liu ZH, Chen SP, Li M, Shahveranov A, et al. Interleukin-6: An emerging regulator of pathological pain. J Neuroinflammation. 2016;13:141

244. Sarchielli P, Alberti A, Baldi A, Coppola F, Rossi C, Pierguidi L, et al. Proinflammatory cytokines, adhesion molecules, and lymphocyte integrin expression in the internal jugular blood of migraine patients without aura assessed ictally. Headache. 2006;46(2):200–7.

245. Yan J, Melemedjian OK, Price TJ, Dussor G. Sensitization of dural afferents underlies migraine-related behavior following meningeal application of interleukin-6 (IL-6). Mol Pain. 2012; 8:6

246. Bolay H, Karadas Ö, Oztürk B, Sonkaya R, Tasdelen B, Bulut TDS, et al. HMGB1, NLRP3, IL-6 and ACE2 levels are elevated in COVID-19 with headache: a window to the infection-related headache mechanism. J Headache Pain. 2021;22(1).

247. He W, Long T, Pan Q, Zhang S, Zhang Y, Zhang D, et al. Microglial NLRP3 inflammasome activation mediates IL-1β release and contributes to central sensitization in a recurrent nitroglycerin-induced migraine model. J Neuroinflammation. 2019;16(1).

248. Kursun O, Yemisci M, van den Maagdenberg AMJM, Karatas H. Migraine and neuroinflammation: the inflammasome perspective. J Headache Pain. 2021;22(1):55

249. Russell FA, King R, Smillie SJ, Kodji X, Brain SD. Calcitonin gene-related peptide: physiology and pathophysiology. Physiol Rev. 2014;94:1099–142.

250. Holzmann B. Modulation of immune responses by the neuropeptide CGRP. Amino Acids. 2013;45:1-7

251. Goadsby PJ, Edvinsson L, Ekman R. Vasoactive peptide release in the extracerebral circulation of humans during migraine headache. Ann Neurol. 1990;28:183-7

252. Ochoa-Callejero L, García-Sanmartín J, Villoslada-Blanco P, Íñiguez M, Pérez-Matute P, Brody R, et al. Circulating levels of calcitonin gene-related peptide (CGRP) are lower in COVID-19 patients. medRxiv. 2020.

253. Brann DH, Tsukahara T, Weinreb C, Lipovsek M, Van Den Berge K, Gong B, et al. Non-neuronal expression of SARS-CoV-2 entry genes in the olfactory system suggests mechanisms underlying COVID-19-associated anosmia. Sci

Adv. 2020;6(31).

254. Conti P, D'Ovidio C, Conti C, Gallenga CE, Lauritano D, Caraffa A, et al. Progression in migraine: Role of mast cells and pro-inflammatory and anti-inflammatory cytokines. Eur J of Pharmacol. 2019;844:87–94.

255. Kaur C, Ling EA. The circumventricular organs. Histol Histopathol. 2017;32:879-892

256. Schulte LH, May A. The migraine generator revisited: Continuous scanning of the migraine cycle over 30 days and three spontaneous attacks. Brain. 2016;139(7):1987–93.

257. Boldrini M, Canoll PD, Klein RS. How COVID-19 Affects the Brain. JAMA Psychiatry. 2021;78:682–3.

258. Vasek MJ, Garber C, Dorsey D, Durrant DM, Bollman B, Soung A, et al. A complement-microglial axis drives synapse loss during virus-induced memory impairment. Nature. 2016;534:538-43

259. Noruzzadeh R, Modabbernia A, Aghamollaii V, Ghaffarpour M, Harirchian MH, Salahi S, et al. Memantine for Prophylactic Treatment of Migraine Without Aura: A Randomized Double-Blind Placebo-Controlled Study. Headache. 2016;56(1):95–103.

260. Vgontzas A, Pavlović JM. Sleep Disorders and Migraine: Review of Literature and Potential Pathophysiology Mechanisms. Headache. 2018;58:1030–9.

261. Files JK, Sarkar S, Fram TR, Boppana S, Sterrett S, Qin K, et al. Duration of post-COVID-19 symptoms is associated with sustained SARS-CoV-2-specific immune responses. JCI Insight. 2021;6(15).

262. Bhadelia N, Belkina AC, Olson A, Winters T, Urick P, Lin N, et al. Distinct Autoimmune Antibody Signatures Between Hospitalized Acute COVID-19 Patients, SARS-CoV-2 Convalescent Individuals, and Unexposed Pre-Pandemic Controls. medRxiv [Internet]. 2021;2021.01.21.21249176. Available

from: https://www.medrxiv.org/content/10.1101/2021.01.21.21249176v1%0Ahttps://www.medrxiv.org/content/10.1101/2021.01.21.21249176v1.abstract

263. Gil-Rodrigo A, Miró Ò, Piñera P, Burillo-Putze G, Jiménez S, Martín A, et al. Analysis of clinical characteristics and outcomes in patients with covid-19 based on a series of 1000 patients treated in spanish emergency departments. Emergencias. 2020;32(4):233–41.

264. Kamm K, Straube A, Ruscheweyh R. Calcitonin gene-related peptide levels in tear fluid are elevated in migraine patients compared to healthy controls. Cephalalgia. 2019;39(12):1535–43.

265. Safety and Efficacy Trial of Zavegepant* Intranasal for Hospitalized Patients With COVID-19 Requiring Supplemental Oxygen [Internet]. [cited 2020 Sep 16]. Available from: https://clinicaltrials.gov/ct2/show/NCT04346615

266. Mapping the human genetic architecture of COVID-19. Nature. 2021;600(7889):472–7.

267. Pathak GA, Singh K, Miller-Fleming TW, Wendt FR, Ehsan N, Hou K, et al. Integrative genomic analyses identify susceptibility genes underlying COVID-19 hospitalization. Nat Commun. 2021;12(1).

268. Meng W, Adams MJ, Hebert HL, Deary IJ, McIntosh AM, Smith BH. A Genome-Wide Association Study Finds Genetic Associations with Broadly-Defined Headache in UK Biobank (N = 223,773). EBioMedicine. 2018;28:180–6.

269. Aczél T, Kecskés A, Kun J, Szenthe K, Bánáti F, Szathmary S, et al. Hemokinin-1 gene expression is upregulated in trigeminal ganglia in an inflammatory orofacial pain model: Potential role in peripheral sensitization. Int J Mol Sci. 2020;21(8).

270. Martelletti P, Stirparo G, Morrone S, Rinaldi C, Giacovazzo M. Inhibition of

intercellular adhesion molecule-1 (ICAM-1), soluble ICAN- 1 and interleukin-4 by nitric oxide expression in migraine patients. J Mol Med. 1997;75(6):448–53.

271. Iyengar S, Johnson KW, Ossipov MH, Aurora SK. CGRP and the Trigeminal System in Migraine. Headache. 2019.59:659-681

272. Torres-Ferrús M, Ursitti F, Alpuente-Ruiz A, Brunello F, Chiappino D, De Vries T, et al. From transformation to chronification of migraine: Pathophysiological and clinical aspects. J Headache Pain. 2020;21(1):42

273. Sutherland HG, Albury CL, Griffiths LR. Advances in genetics of migraine. J Headache Pain. 2019;20(1):72

274. Hasday JD, Singh IS. Fever and the heat shock response: Distinct, partially overlapping processes. Cell Stress Chaperones. 2000;5(5):471–80.

275. Guihur A, Rebeaud ME, Fauvet B, Tiwari S, Weiss YG, Goloubinoff P. Moderate Fever Cycles as a Potential Mechanism to Protect the Respiratory System in COVID-19 Patients. Front Med. 2020;7:564170

276. Whitsett JA. Airway epithelial differentiation and mucociliary clearance. Ann Am Thorac Soc. 2018;15:S143–8.

277. Souilmi Y, Lauterbur ME, Tobler R, Huber CD, Johar AS, Moradi SV, et al. An ancient viral epidemic involving host coronavirus interacting genes more than 20,000 years ago in East Asia. Curr Biol. 2021;31(16):3504-3514.e9.

278. Loder E. What is the evolutionary advantage of migraine? Cephalalgia. 2002;22(8):624–32.

10. Appendix

Appendix 1: Headache: A striking prodromal and persistent symptom, predictive of COVID-19 clinical evolution

Caronna E, Ballvé A, Llauradó A, et al. Headache: A striking prodromal and persistent symptom, predictive of COVID-19 clinical evolution. Cephalalgia 2020;40(13):1410-1421.

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Headache: A striking prodromal and persistent symptom, predictive of COVID-19 clinical evolution

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Abstract

Objective: To define headache characteristics and evolution in relation to COVID-19 and its inflammatory response.
Methods: This is a prospective study, comparing clinical data and inflammatory biomarkers of COVID-19 patients with and without headache, recruited at the Emergency Room. We compared baseline with 6-week follow-up to evaluate disease evolution.
Results: Of 130 patients, 74.6% (97/130) had headache. In all, 24.7% (24/97) of patients had severe pain with migraine-like features. Patients with headache had more anosmia/ageusia (54.6% vs. 18.2%; $p < 0.0001$). Clinical duration of COVID-19 was shorter in the headache group (23.9 ± 11.6 vs. 31.2 ± 12.0 days; $p = 0.028$). In the headache group, IL-6 levels were lower at the ER (22.9 (57.5) vs. 57.0 (78.6) pg/mL; $p = 0.036$) and more stable during hospitalisation. After 6 weeks, of 74 followed-up patients with headache, 37.8% (28/74) had ongoing headache. Of these, 50% (14/28) had no previous headache history. Headache was the prodromal symptom of COVID-19 in 21.4% (6/28) of patients with persistent headache ($p = 0.010$).
Conclusions: Headache associated with COVID-19 is a frequent symptom, predictive of a shorter COVID-19 clinical course. Disabling headache can persist after COVID-19 resolution. Pathophysiologically, its migraine-like features may reflect an activation of the trigeminovascular system by inflammation or direct involvement of SARS-CoV-2, a hypothesis supported by concomitant anosmia.

Keywords
Headache, COVID-19, SARS-CoV-2, prognosis, loss of smell

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Introduction

The global pandemic caused by the Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) is putting our healthcare systems at stake. COVID-19 is the infectious disease caused by this viral pathogen, and which can lead, in some patients, to respiratory failure amongst other severe symptoms, requiring urgent medical aid. In Spain, COVID-19 had been diagnosed in as many

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as 200,210 people by 19 April 2020 (1), forcing hospitals to reorganise in a profound, quick way. Specifically, neurologists have been attending COVID-19 patients at the emergency room (ER), which has enabled them to observe the presence of neurological symptoms associated with COVID-19 otherwise not analysed in detail. At the ER, severe, difficult-to-treat headache, together with anosmia and ageusia, has been a major initial neurological complaint. Despite the fact that the Centres for Disease Control and Prevention (2) include headache as one of the main symptoms of COVID-19, a better definition of headache itself is lacking and no data on its evolution are available at present. Moreover, it is unknown whether headache may represent a relevant clinical symptom, predicting the course of COVID-19 itself, a fact that could guide clinicians in their evaluations of COVID-19 patients in future waves of the pandemic.

"Headache attributed to systemic viral infection" is included in the International Classification of Headache Disorders third edition (ICHD-3) (3) and, although commonly reported (4), specific data are lacking. COVID-19 gives the opportunity to define its headache characteristics, which until now have mainly been described in terms of prevalence, with discrepant results, the prevalence in studies based on clinical charts review in China (8 23%) (5 8) or Spain (14.1%) (9) being substantially different from interview-based cross-sectional study (70%) (10).

As studies related to headache associated with viral infections are lacking, and COVID-19 is clearly associated with headache, we decided to define and describe headache characteristics and evolution in relation to COVID-19, focusing on it as a possible prognostic factor. Moreover, we decided to correlate the presence of headache with systemic inflammatory responses and hypothesise about its pathophysiological mechanisms.

Methods

This is a prospective study conducted in a Spanish tertiary hospital in Barcelona (Vall d'Hebron Hospital).

During the COVID-19 pandemic, neurologists started working 24/7 at the ER as general clinicians. They recruited, during a 3-week period (28 March to 22 April 2020), all the consecutive patients with COVID-19 symptoms attended by them (ER patients were assigned randomly by triage to clinicians to be visited), including only those who could give consent and undergo a full interview. COVID-19 symptoms were pre-specified for all neurologists and were based on the list of symptoms reported by the World Health Organization (11). We collected demographic data, COVID-19 symptoms, family and personal history of any headache (ICHD-3) (3), categorising patients as episodic or chronic. If patients had experienced headache at any time during COVID-19, we collected the date of onset and cessation in relation to other COVID-19 symptoms as well as headache characteristics. Headache pain severity was defined as mild if patients considered that, in the absence of other COVID-19 symptoms, headache alone would allow them to carry on with their daily activities as usual; moderate if they had to reduce their daily activities, and severe if they had to stop doing any kind of task.

In our hospital, according to the COVID-19 ER protocol, we recorded vital signs and performed a physical examination and a chest X-ray to rule out pneumonia. At the ER, patients with COVID-19 symptoms but with normal vital signs, negative X-ray and normal physical examination could be immediately discharged without undergoing nasopharyngeal swabs to confirm SARS-CoV-2 infection. In all other cases, patients were admitted for further testing and/or treatment, including a real-time reverse transcriptase polymerase -chain reaction (RT-PCR) assay by nasopharyngeal swabs to confirm SARS-Cov-2 and blood testing with inflammatory markers (C-reactive protein CRP: 0.03 0.50 mg/dL; interleukin-6-IL-6: 0 4.3 pg/mL; ferritin: 25 250 ng/mL; Lactate Dehydrogenase LDH: 0 248 UI/L; D-dimer: 0 243 ng/mL). In hospitalised patients, we recorded treatment administered, presence of other medical complications, hospital and intensive care unit (ICU) length of stay and mortality, by periodically revising electronic medical charts. Then, we analysed all data, described the cohort of patients with headache associated with COVID-19, and compared them with those not having headache.

After 6 weeks from admission, we followed up patients by phone call to evaluate persistence of headache and its characteristics as well as other COVID-19 symptoms through a structured survey. Then, we compared baseline and post-6-week data.

To define prognosis in our cohort, we used these variables: COVID-19 disease duration, defined as the number of days between the onset of the first and the resolution of the last COVID-19 symptom, hospital length of stay, and all-cause in-hospital mortality.

Statistical analysis

We obtained descriptive and frequency statistics and made comparisons using the SPSS, version 21.0 for Windows. We reported nominal (categorical) variables as frequencies (percentages), and continuous variables as mean ± standard deviation (age, temperature, CRP and LDH) or median and interquartile range (IQR) (IL-6, D-dimer and ferritin). We checked the normality assumption of quantitative variables through visual methods (Q-Q plots) and normality tests (Kolmogorov-Smirnov test). We assessed statistical

significance for intergroup variables by Pearson's chi-square when comparing categorical variables. In the case of having an expected count of less than 5 in more than 20% of cells in the contingency table, we used Fisher's exact test. We used linear trend chi-square for ordinal variables, independent t-test for continuous variables that followed a normal distribution and the Mann-Whitney U test for the rest of the continuous variables. In order to evaluate the COVID-19 prognosis (COVID-19 disease duration, hospital length of stay and mortality) at the follow-up, we used one-way analysis of covariance (ANCOVA), adjusted for the effect of age and gender, for the group with and without headache. The false discovery rate (FDR) with Benjamini-Hochberg procedure was used to correct *p*-values for multiple comparisons.

Finally, we studied the role of inflammatory biomarkers and fever (≥37.5°C) in a subgroup of patients with ongoing headache at the ER and an equally age/gender matched group without headache in order to avoid biased results. We also conducted a longitudinal data analysis in order to model the inflammatory biomarker changes over the course of COVID-19 between patients with and without headache. We only selected hospitalised patients with at least three available blood tests that had been done at the same timepoints in the course of COVID-19 disease, starting from the onset of their COVID-19 symptoms. Longitudinal analysis was performed using linear mixed-effects models fitted by maximum likelihood and adjusted by age. Models were computed using the nlme (v3.1-144) package from R.

We did not conduct a statistical power calculation prior to the study because the sample size was based on the available data. Missing values were imputed using the MICE (Multivariate Imputation via Chained Equations) package from R (v3.8.0) (12). Concerning missing values, there was <5% of missingness in nominal variables (headache localisation, quality of pain, and pain severity). Hence, we used a Bayesian polytomous regression as a method of imputation for headache localisation and quality of pain and a proportional odds model for headache pain severity. The missingness in continuous variables (temperature, CRP, IL-6, D-dimer, ferritin, LDH) was rated between 2% (temperature) to 18% (LDH). In that case, we used random forest imputations in order to estimate these values according to their other variables.

p-values presented are for a two-tailed test and we considered p-values < 0.05 statistically significant.

Ethics approval and patients' consent

The study was approved by the Vall d'Hebron Ethics Committee (PR(AG)227/2020). All patients gave written informed consent for the analysis of patients' data which was collected according to Spanish regulation on clinical trials.

Results

We included 130 adult patients. Table 1 shows their characteristics and all COVID-19 symptoms reported at ER. From the 130 patients included, 74.6% (97/130) had experienced headache as a COVID-19 symptom, while the other 33 did not.

Table 1. Clinical characteristics of COVID-19 patients at ER.

Demographic characteristics (n = 130)	
Sex, n (%)	
Male	64 (49.2)
Female	66 (50.8)
Age (years), n (%)	
Mean (SD)	53.9 (16.4)
<34	16 (12.3)
35–44	23 (17.7)
45–54	30 (23.1)
55–64	25 (19.2)
≥65	36 (27.7)
COVID-19 characteristics	
Reported symptoms at ER, n (%)	
Headache	97 (74.6)
Fever	115 (88.5)
Malaise	60 (46.2)
Myalgia	39 (30.0)
Dizziness	19 (14.6)
Cough	105 (80.2)
Dyspnea	81 (62.3)
Chest pain	4 (3.0)
Expectoration	19 (14.6)
Odynophagia	12 (9.2)
Loss of smell/taste	59 (45.4)
Diarrhea	36 (27.7)
Radiological findings at ER, n (%)	
Pneumonia	103 (79.2)
Bilateral pneumonia	77 (59.2)
COVID-19 Confirmation (positive RT-PCR), n (%)	89 (68.5)
Hospitalisation, n (%)	104 (80.0)
Vital signs and inflammatory markers at the ER	
O_2 requirements, n (%)	31 (23.8)
Fever, n (%)	45 (34.6)
Lymphopenia, n (%)	70 (53.8)
CRP, mean ± SD, mg/ml	9.3 ± 8.4
IL-6, median (IQR), pg/ml	34.0 (62.1)
D-dimer, median (IQR), ng/ml	231.0 (243)
Ferritin, median (IQR), ng/ml	354.0 (406)
LDH, mean ± SD, UI/L	369.4 ± 221.4

ER: Emergency room; SD: standard deviation; IQR: interquartile range; ICU: intensive care unit; RT-PCR: real-time reverse transcriptase polymerase-chain reaction; O_2: oxygen; lymphopenia ($< 1.0 \times 10^9$/L); CRP: C-reactive protein; IL-6: interleukin-6; LDH: lactate dehydrogenase.

Headache and COVID-19 at ER

In our cohort of headache patients, 57.7% (56/97) were female, the mean age was 50.6 ± 15.3 years old and 19.6% (19/97) had a personal history of episodic migraine. No patients had a history of chronic migraine. Headache-associated symptoms reported by patients were nausea and vomiting (25/97), worsening with movement (12/97) photo/phonophobia (10/97), vertigo (4/97) and subjective neck stiffness (3/97). Specific headache features are reported in Figure 1.

At the ER, the neurological examination together with symptoms evaluation, performed by neurologists, ruled out meningitis in all recruited patients with headache. Based on the striking clinical observation of some patients with severe headache at ER, we compared patients with severe headache (24/97) with the ones with mild-moderate pain (73/97), specifically analysing migraine-like features (Table 2).

Then, comparing patients with or without headache, we observed that the first group were younger (50.6 ± 15.3 vs. 63.6 ± 15.7; $p < 0.0001$), there were more females (57.7% vs. 30.3%; $p = 0.009$) and reported higher headache history of any type (32.0% vs. 12.1%; $p = 0.039$). In regards to COVID-19 symptoms, the most relevant result was that in the headache group more patients had anosmia/ageusia (54.6% vs. 18.2%; $p < 0.0001$). All other variables are shown in Table 3.

COVID-19 evolution: Headache as a prognostic factor

Of 130 patients, 80.0% (104/130) were hospitalised after ER evaluation. In all hospitalised patients, we could follow their clinical course by electronic chart and observed that 8.5% (11/130) required ICU. Mortality was 3.1% (4/130, one patient belonging to the headache group and three to the group without headache). We did not detect new onset headaches through chart review during hospitalisation.

After 6 weeks, we could get in touch with 100 patients of our cohort (74 with headache and 26 without headache) and interviewed them about disease evolution. There were no statistically significant differences with regard to the demographic variables in patients that were not followed up.

From these, 27.0% (27/100) was still experiencing at least one symptom of COVID-19 other than headache. Those without any more symptoms of COVID-19 had a mean duration of disease of 25.8 ± 11.9 days.

Interestingly, comparing patients with and without headache, for whom data were available at follow-up, and adjusting for age and gender, we observed shorter COVID-19 disease duration in the headache group (23.9 ± 11.6 vs. 31.2 ± 12.0 days; $p = 0.028$). We did not observe any difference in mortality (no mortality

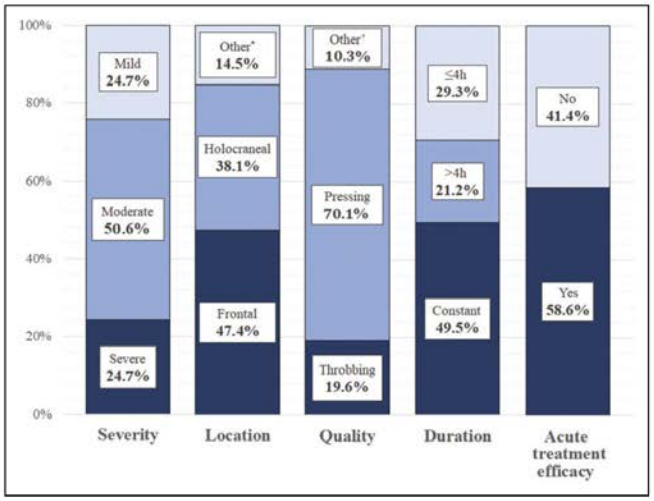

Figure 1. Headache associated with COVID-19 characteristics.
*Other headache locations reported were frontocervical (6.2%), hemicranial (5.2%) and cervical (4.1%).
†Other reported pain qualities were drilling (5.2%), shooting (4.1%) and burning (1.0%).

Table 2. Comparison between patients with severe and mild-moderate headache at ER (n = 97).

	Severe headache (n = 24)	Mild-moderate headache (n = 73)	Adjusted *p*-value[a]
Demographic characteristics			
Age (years), mean ± SD	44.8 ± 14.9	52.5 ± 15.1	0.049*
Sex (female), n (%)	20 (83.3)	36 (49.3)	0.004**
History of any type of headache, n (%)	10 (41.7)	21 (28.8)	0.313
History of migraine, n (%)	6 (25.0)	13 (17.8)	0.554
Headache characteristics			
Onset before another COVID-19 symptom, n (%)	6 (25.0)	3 (4.1)	0.007**
Time since headache onset to ER presentation (days), median (IQR)	10.5 (10.0)	6.0 (6.0)	0.002**
Holocraneal pain, n (%)	9 (37.5)	28 (38.4)	1.000
Pain quality, n (%)			
Pressing	11 (45.8)	57 (78.1)	0.004**
Throbbing	9 (37.5)	10 (13.7)	0.017*
Other migraine features, n (%)			
Worsening with movement	6 (25.0)	6 (8.2)	0.066
Nausea and vomiting	11 (45.8)	14 (19.2)	0.015*
Photo/phonophobia	5 (20.8)	5 (6.8)	0.114
Daily constant pain, n (%)	18 (75.0)	30 (41.1)	0.005**
Response to acute treatment, n (%)	9 (37.5)	48 (65.8)	0.018*

[a]Adjusted *p*-value with Benjamini-Hochberg procedure.
*p-value ≤ 0.05.
**p-value ≤ 0.01.
ER: Emergency room; SD: standard deviation; IQR: interquartile range.

Table 3. Comparison between COVID-19 patients with and without headache at the ER.

	No headache (n = 33)	Headache (n = 97)	Adjusted *p*-value[a]
Demographic characteristics			
Age (years), mean ± SD	63.6 ± 15.7	50.6 ± 15.3	<0.0001**
Sex (female), n (%)	10 (30.3)	56 (57.7)	0.009**
History of any type of headache, n (%)	4 (12.1)	31 (32.0)	0.039*
History of migraine, n (%)	2 (6.1)	19 (19.6)	0.099
COVID-19 characteristics			
Reported symptoms at ER, n (%)			
Fever	28 (84.8)	87 (89.7)	0.529
Malaise	8 (24.2)	52 (53.6)	0.004**
Myalgia	7 (21.2)	32 (33.0)	0.272
Dizziness	1 (3.0)	18 (18.6)	0.042*
Cough	24 (72.7)	81 (83.5)	0.204
Dyspnea	21 (63.6)	60 (61.9)	1.000
Chest pain	2 (6.1)	1 (1.0)	0.158
Expectoration	1 (3.0)	18 (18.6)	0.042*
Odynophagia	1 (3.0)	11 (11.3)	0.294
Loss of smell/taste	6 (18.2)	53 (54.6)	<0.0001**
Diarrhea	8 (24.2)	28 (28.9)	0.660
Pneumonia	25 (75.8)	78 (80.4)	0.622
Bilateral pneumonia	21 (63.6)	56 (57.7)	0.682
COVID-19 confirmation (RT-PCR), n (%)	23 (69.7)	66 (68.0)	1.000
Hospitalisation, n (%)	27 (81.8)	77 (79.4)	1.000

[a]Adjusted *p*-value with Benjamini-Hochberg procedure.
*p-value ≤ 0.05.
**p-value ≤ 0.01.
ER: Emergency room; RT-PCR: real-time reverse transcriptase polymerase-chain reaction.

in this subgroup) or hospital length of stay (9.1 ± 9.0 vs. 10.9 ± 9.0 days; $p = 0.854$).

Headache evolution

After 6 weeks, of the 74 headache patients, 37.8% (28/74) still had headache. Those patients whose headache had stopped had a mean duration of the symptom of 15.4 ± 11.1 days. Then, we analysed patients with ongoing headache after 6 weeks, observing that 50% of them (14/28) had never suffered from recurrent headache before. A total of 60.7% of patients (17/28) had daily constant headache. Response to acute treatment was insufficient both at baseline and follow-up, without statistically significant differences at the two timepoints (32.1% vs. 28.6%; $p = 0.701$).

Then, we compared patients with ongoing headache after 6 weeks with those who were headache free. Significant variables associated with persisting headache are shown in Table 4.

Headache related to inflammation and fever

We selected patients with ongoing headache at the ER, when vital signs and blood samples were collected, and an equally age/gender-matched group without headache. We included 60 patients, 36 with headache and 24 without. We observed statistically significantly lower levels of IL-6 and LDH. We found no differences in presence of fever at the ER between the two groups. Other variables are shown in Table 5.

To specifically analyse the evolution of inflammatory biomarkers over time, we included 24 patients in this sub-analysis: 18 had headache, while six did not. There were no statistically significant differences either in patients' age (headache: 56.6 ± 9.8 vs. no-headache: 63.3 ± 6.7 years; $p = 0.130$) or in gender (female headache: 55.6% vs. no-headache: 66.7%; $p = 1.000$). Only IL-6 significantly changed over time between the two groups ($p = 0.003$), observing more stable levels of IL-6 during COVID-19 in patients with headache (Figure 2).

Discussion

In March 2020, during the COVID-19 pandemic, we noticed that some patients were complaining of headache while others were not, which made us wonder why.

Our study aimed to first describe the characteristics and evolution of headache associated with COVID-19, to correlate it with the evolution of the disease, and generate a hypothesis on the underlying pathophysiological mechanisms, with the final goal of helping clinicians to better understand this symptom and its clinical relevance.

Clinical presentation of headache associated with COVID-19

Headache attributed to COVID-19 is common in both genders and middle-aged people, while patients without headache are older and male. History of headache of any type is more common in patients presenting headache as a COVID-19 symptom, but not exclusive. Interestingly, the presence of headache in COVID-19 is significantly associated with other cranial symptoms, anosmia/ageusia. Specifically, these symptoms have been frequently reported in case reports of patients with headache during COVID-19 (13 14).

Concerning headache characteristics in COVID-19, a recent study conducted in Spain (15) has detected different phenotypes on the basis of headache features and the presence of personal history of primary headaches. In our cohort, we observed that in the majority of patients headache is mild/moderate, while one fourth, especially women and younger subjects, have a severe "migraine-like" headache that is not associated with a more personal migraine history. Although headache attributed to COVID-19 usually starts with the other COVID-19 symptoms, patients with severe pain more often have it as a prodromal symptom. Headache as the first COVID-19 symptom has been described in a recent case report (16).

Concerning headache evolution, surprisingly we observed that one third of followed-up patients had persistent disabling daily headache after 6 weeks, with poor response to acute treatment and, in more than 30%, representing the only symptom left of COVID-19. Interestingly, up to 50% of these patients had no history of recurrent headache at all, a fact that could lead to the onset of a "new daily" headache. Moreover, in a relevant number of these cases, headache was an initial prodromal symptom of COVID-19. In our study, headache was more likely to persist in females and patients with a headache history, although no patients had a chronic primary headache before COVID-19.

Relevance of assessing headache in COVID-19 for prognosis

As of 13 May 2020, the seroprevalence of antibodies against COVID-19 in the Spanish population is only 5%, making SARS-CoV-2 an ongoing health threat in our country (17). In our study, we observed that, among patients' reported COVID-19 symptoms, only the presence of headache is predictive of better prognosis, in terms of an almost 1-week shorter COVID-19 disease. Although recent studies (18 19) have shown that anosmia could be useful in detecting COVID-19 cases, more specifically pointing to SARS-CoV-2

Table 4. Comparison between patients with ongoing headache and with headache resolution during follow-up (n = 76).

	Ongoing headache (n = 28)	Headache resolution (n = 46)	Adjusted p-value[a]
Demographic characteristics			
Age (years), mean ± SD	49.2 ± 15.7	52.5 ± 15.7	0.386
Sex (female), n (%)	23 (82.1)	22 (47.8)	0.004**
History of any type of headache, n (%)	14 (50.0)	12 (26.1)	0.047*
History of migraine, n (%)	8 (28.6)	8 (17.4)	0.383
COVID-19 characteristics			
Reported symptoms at ER, n (%)			
Fever	25 (89.3)	41 (89.1)	1.000
Malaise	15 (53.6)	26 (56.5)	0.815
Myalgia	12 (42.9)	14 (30.4)	0.321
Dizziness	9 (19.6)	5 (17.9)	1.000
Cough	22 (78.6)	40 (87.0)	0.352
Dyspnea	22 (78.6)	26 (56.5)	0.079
Chest pain	1 (3.6)	0 (0.0)	0.717
Expectoration	3 (10.7)	7 (15.2)	0.733
Odynophagia	4 (14.3)	3 (6.5)	0.415
Loss of smell/taste	16 (57.1)	29 (63.0)	0.632
Diarrhea	5 (17.9)	17 (37.0)	0.116
Pneumonia	39 (84.8)	21 (75.0)	0.364
Bilateral pneumonia	28 (60.9)	15 (53.6)	0.629
Persistent symptoms at follow-up, n (%)	19 (67.9)	9 (10.6)	<0.001**
COVID-19 disease duration (days), Median (IQR)	26.5 (21.5)	23.0 (12.5)	0.126
Hospitalisation, n (%)	21 (75.0)	39 (89.4)	0.364
Days of hospitalisation, median (IQR)	6.0 (13.5)	5.5 (7.5)	0.971
ICU, n (%)	4 (14.3)	4 (8.7)	0.467
Headache characteristics			
Onset before another COVID-19 symptom, n (%)	6 (21.4)	2 (4.4)	0.010**
Holocraneal pain, n (%)	10 (35.7)	18 (39.1)	0.809
Pain quality, n (%)			
Pressing	19 (67.9)	33 (71.7)	0.796
Throbbing	5 (17.9)	9 (19.6)	1.000
Moderate-severe pain, n (%)	25 (89.3)	32 (69.6)	0.085
Daily constant pain, n (%)	17 (60.7)	22 (47.8)	0.341
Headache associated symptoms at ER, n (%)			
Nausea and vomiting	9 (32.1)	12 (26.1)	0.604
Photo/phonophobia	1 (3.6)	6 (8.1)	0.242
Vertigo	1 (3.6)	2 (4.4)	1.000
Neck stiffness	1 (3.6)	1 (2.2)	1.000
Worsening with movement	6 (21.4)	6 (13.0)	0.352
Response to acute treatment, n (%)	9 (32.1)	34 (73.9)	0.001**

[a]Adjusted p-value with Benjamini-Hochberg procedure.
*p-value ≤0.05.
**p-value ≤0.01.
ER: Emergency room; SD: standard deviation; IQR: interquartile range; ICU: intensive care unit.

infection, we remark that headache represents a relevant symptom of COVID-19 in terms of prognosis, raising awareness of the importance of its assessment.

Hypothesis on underlying pathophysiological mechanisms

Usually patients and clinicians blame fever as the cause of headache associated with viral infections. However, its role in causing headache is still a matter of debate and possible mechanisms involve increase of proinflammatory cytokines (20). In a recent study on COVID-19, headache seemed to present independently from fever (15), in line with our findings observing that headache was not associated with presence of fever at the ER or as a reported symptom. Although our study was not specifically designed to address the relationship between fever and headache, our findings support the

Table 5. Comparison in inflammatory biomarkers between age/gender-matched COVID-19 patients with and without headache.

	Headache (n = 36)	No headache (n = 24)	Adjusted *p*-value[a]
Demographic characteristics			
Age (years), mean ± SD	59.1 ± 14.2	61.1 ± 14.9	0.594
Sex (female), n (%)	21 (58.3)	15 (41.7)	0.280
COVID-19 characteristics			
COVID-19 confirmation (RT-PCR), n (%)	25 (69.4)	15 (62.5)	0.708
COVID-19 disease duration at ER, days, median (IQR)	8.5 (7.5)	9.0 (9.8)	0.623
Vital signs and inflammatory biomarkers			
Fever, n (%)	11 (30.6)	4 (16.7)	0.482
Lymphopenia, n (%)	20 (55.6)	16 (66.7)	0.432
CRP, mean ± SD, mg/ml	8.9 ± 7.9	11.7 ± 9.8	0.381
IL-6, median (IQR), pg/ml	22.9 (57.5)	57.0 (78.6)	0.036*
D-dimer, median (IQR), ng/ml	300.0 (3300)	250.0 (1593.0)	0.481
Ferritin, median (IQR), ng/ml	488.0 (466.0)	287.0 (110.0)	0.052
LDH, mean ± SD, UI/L	302.8 ± 107.7	457.1 ± 207.6	0.016*

[a]Benjamini-Hochberg adjusted *p*-value with a false discovery rate greater than 0.05.
*p value ≤ 0.05.
**p value ≤ 0.01.
SD: standard deviation; RT-PCR: real-time reverse transcriptase polymerase-chain reaction; ER: emergency room; IQR: interquartile range; CRP: C-reactive protein; IL-6: interleukin-6; LDH: lactate dehydrogenase.

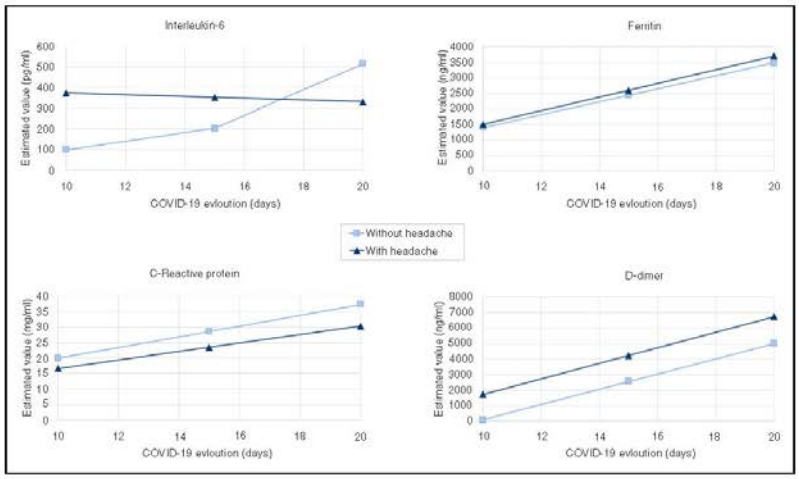

Figure 2. Evolution of inflammatory biomarkers (IL-6, CRP, ferritin and D-dimer) during the progression of COVID-19 disease. Once having analysed mean values of inflammatory biomarkers at the ER (baseline), we decided to exclusively evaluate the trends of their evolution over time. Of 24 hospitalised patients in whom IL-6, CRP, ferritin and D-dimer data were available at the same three points in time, 18 had headache while six did not. There were no differences in time passed between each blood test and COVID-19 onset. Only IL-6 changes over COVID-19 progression were significant when comparing patients with and without headache ($p = 0.003$) in the linear mixed-effects models adjusted by age.

need to further evaluate whether fever has a minor role in directly causing headache, being more directly related to the initial response to the pathogen.

Concerning specific pathophysiological mechanisms, it has been hypothesised that SARS-CoV-2 may trigger a hyperinflammatory state in some patients (cytokine storm) (21) especially through IL-6 (22 23), whose levels seem to correlate with dysregulation of other coagulation and inflammatory biomarkers in a recent proteomic study (24). The role of IL-6 has been also demonstrated in neuroinflammation (25) and specifically in migraine (26 27). Therefore, it is logical to wonder whether the suggested inflammatory state observed in COVID-19 is also responsible for neuroinflammation, leading to headache. We observed lower levels of IL-6 at the ER in the headache group, a finding that cannot be explained by different stages of the disease between groups. This fact is surprising, considering that COVID-19 patients with headache had more personal headache history. Moreover, we observed that during COVID-19 evolution, levels of IL-6 seemed to be more stable in patients with headache compared to the ones without it. Considering that headache patients also had shorter COVID-19 disease evolution, their lower and more stable IL-6 levels may indicate that inflammation may be kept at a more localised level.

In this context, considering that headache attributed to COVID-19 could mimic migraine even in individuals without a personal history of this condition, we hypothesise that there is a meningeal peripheral sensitisation and therefore an activation of the trigeminovascular system (28) underlying this headache type.

Amongst possible mechanisms that could lead to the activation of the trigeminovascular system, it is worth mentioning our finding on the association between headache and anosmia/ageusia as distinctive symptoms of COVID-19 (5,29). It has been hypothesised that SARS-CoV-2 has neurotropic characteristics, being able to invade peripheral nerve terminals and enter the central nervous system through trans-synaptic pathways (30 32). Inside the nasal cavity, concomitant anosmia may indicate a peripheral activation of the trigeminovascular system mediated by the pathogen itself, acting not only on the specialised olfactory epithelium, but also on trigeminal branches present at this level or through the olfactory-trigeminal interactions. As for other pathophysiological mechanisms, endothelitis may also have a relevant role in COVID-19 (33), since inflammatory reactions could be generated through the interaction between the virus and its receptor, angiotensin-converting enzyme 2 (ACE2), which is present in the endothelium of blood vessels. ACE2 is expressed in the endothelium of small vessels of cerebral circulation (34), that SARS-CoV-2 can reach through bloodstream dissemination (35). Thus, the presence of ACE2 in the meningeal endothelium, leading to trigeminovascular sensitisation, should be further investigated as a potential pathophysiological mechanism for COVID-19 headache.

All the previously mentioned pathophysiological mechanisms are shown in Figure 3.

It could be interesting for future studies to assess whether a sensitisation of the trigeminovascular system persists once these pathological *noxae* have resolved, leading to headache persistence and chronification. In this context, it is worth noticing that a relevant proportion of patients without a personal history of headache had persistent headache. This fact makes us wonder whether a post-infectious aetiology may represent, in our clinical practice, an underdiagnosed cause of new daily headache. However, we are aware that to fully assess headache persistence these patients should be followed up for at least 3 months.

This is, at present, the only prospective study conducted on headache and COVID-19. We report the highest proportion of headache observed in literature (5 9), which reflects the fact that, for the first time, neurologists, working as general clinicians at the ER, could properly directly assess headache in the acute phase of a viral disease. However, we cannot estimate in our study the real prevalence of headache attributed to COVID-19 since we only included those patients attended by the investigators, who were only a part of the huge number of patients visited at the ER daily during the study period. However, it is important to point out that our results are in line with that of the European COVID-19 cohort (10), where headache and other COVID-19 symptoms were also directly assessed by clinicians through a structured questionnaire. Our strength is that the neurologists' direct involvement made data collection on previous headache history and headache characteristics more reliable. As for the other limitations, we could not include severe COVID-19 patients due to the impossibility of conducting a full interview, or very mild patients who have gone undiagnosed and were unlikely to come to the ER, our cohort not being representative of the entire spectrum of COVID-19 patients. This fact makes it more difficult to properly assess possible associations between headache and the clinical evolution of COVID-19 in a population different from the hospitalised one. Nevertheless, a more homogeneous group makes our findings more generalisable in this specific group and useful to guide clinicians in case of future new disease peaks. Another limitation is represented by the fact that not all patients could have a confirmed diagnosis

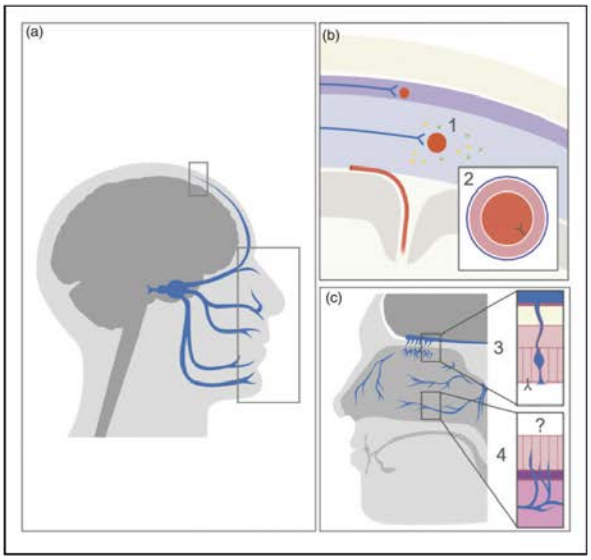

Figure 3. Possible pathophysiological mechanisms of headache associated with COVID-19. (a) Head section representing trigeminal innervation, including meninges and nasal cavities (selected areas that are enlarged in (b) and (c). (b) At meningeal level, the trigeminal afferents (blue arrows) innervate meningeal vessels (represented in red), creating the trigeminovascular system. Its activation may be due to i) systemic inflammation that may facilitate meningeal sensitisation leading to a local release of inflammatory peptides that stimulates trigeminal terminals; ii) direct binding of SARS-CoV-2 from the bloodstream on ACE2, which is expressed by the endothelium of meningeal vessels, causing endothelitis and therefore inflammation. (c) In the nasal cavities, both the specialised olfactory epithelium and the nasal epithelium are present, the latter being innervated by trigeminal nerve afferents. iii) The supporting cells of the olfactory epithelium, in which the olfactory neurons are embedded, express ACE2, where the SARSCoV- 2 may bind, causing anosmia, a symptom that is significantly associated with presence of headache. iv) At the level of the nasal epithelium, the trigeminal system may be peripherally activated by the direct action of SARS-CoV-2 on the nasal epithelium or the trigeminal branches, or by an indirect pathway involving the interactions between the olfactory and trigeminal innervation. These mechanisms should be further studied.

through an RT-PCR for SARS-CoV-2 due to hospital protocols, although all patients in our cohort were highly suspected cases. Moreover, we also have to consider that, as observed in a recent study, the false negative rate with RT-PCR can be high, reaching 29% (36). In addition, we did not investigate secondary causes of headache during disease evolution, making it difficult to exclusively attribute to SARS-CoV-2 possible changes in headache features during the course of COVID-19. Finally, we are also aware that the analysis of inflammatory biomarker evolution during hospitalisation counts on a small sample size. These findings should be further assessed in larger patient groups, although they represent a starting point in hypothesising and studying the link between headache, COVID-19 and IL-6.

Conclusions

Headache associated with COVID-19 is a frequent symptom, predictive of a shorter COVID-19 duration. A relevant number of patients without a headache history have headache persisting for more than 6 weeks even when other COVID-19 symptoms resolve. Persistent headache often represents a prodromal, difficult to treat and disabling symptom of COVID-19, for which patients may seek medical attention. Efforts in the future will have to focus on better understanding its pathophysiological mechanisms, possibly involving the peripheral activation of the trigeminovascular system by inflammation or a direct role of SARS-CoV-2, in order to provide better care and new therapeutic solutions to patients.

Clinical implications

- Headache is one of the most frequent and prominent symptoms of COVID-19, its presence being predictive of a shorter COVID-19 disease duration.
- Headache attributed to COVID-19 can have migraine-like features and in some cases can be persistent and disabling, even in patients without a previous personal headache history.
- Presence of headache, its different characteristics and evolution may reflect different pathophysiological mechanisms acting in COVID-19, making their study necessary to better understand both headache and COVID-19 itself and offer better therapeutic options.

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Declaration of conflicting interests

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Authors and contributions

PPR and EC made substantial contributions to conception and study design. EC, AB, AL, DA, SL, SL, MOG, LQ, JLR, MR, AV worked on acquisition of data. VJG, MH, and MM contributed to analysis and interpretation of data. EC and VJG wrote the first draft. PPR, JAS, RP, MTF and AA critically revised and finally approved the version to be published. All authors fully complied with and approved the version to be published.

Consent to publish

All patients consented to publication of anonymous individual data.

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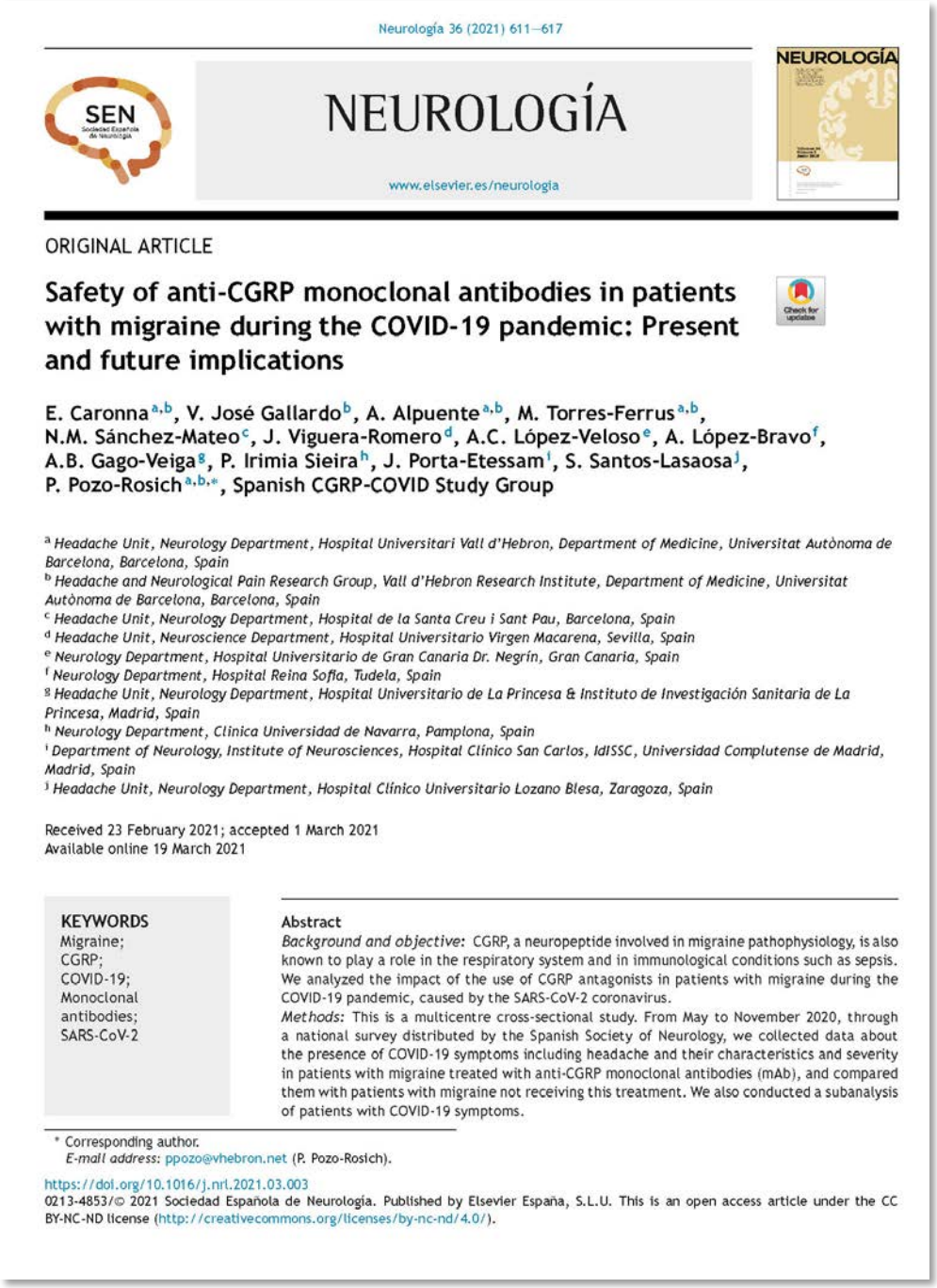
References

1. Actualización no 81. Enfermedad por el coronavirus (COVID-19). 20.04. 2020 (datos consolidados a las 21:00 horas del 19.04.2020). Centro de Coordinación de Alertas y Emergencias Sanitarias. Ministerio de Sanidad. Gobierno de España, https://www.mscbs.gob.es/profesionales/saludPublica/ccayes/alertasActual/nCov-China/documentos/Actualizacion_81_COVID-19.pdf (2020, accessed 21 April 2020).
2. Centers for Disease Control and Prevention, https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html (2020, accessed 17 June 2020).
3. Headache Classification Committee of the International Headache Society (IHS). The International Classification of Headache Disorders, 3rd edition. *Cephalalgia* 2018; 38: 1–211.
4. De Marinis M and Welch KMA. Headache associated with non-cephalic infections: Classification and mechanisms. *Cephalalgia* 1992; 12: 197–201.
5. Mao L, Jin H, Wang M, et al. Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in Wuhan, China. *JAMA Neurol* 2020; 77: 683–690.
6. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: A descriptive study. *Lancet* 2020; 395: 507–513.
7. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; 395: 497–506.
8. Chang D, Lin M, Wei L, et al. Epidemiologic and clinical characteristics of novel coronavirus infections involving 13 patients outside Wuhan, China. *JAMA* 2020; 323: 1092–1093.
9. Romero-Sánchez CM, Díaz-Maroto I, Fernández-Díaz E, et al. Neurologic manifestations in hospitalized patients with COVID-19: The ALBACOVID registry. *Neurology* 2020; 95: e1060–e1070.
10. Lechie JR, Chiesa-EstombaCM, Place S, et al. Clinical and epidemiological characteristics of 1,420 European patients with mild-to-moderate coronavirus disease 2019. *J Intern Med* 2020; 288: 335–344.
11. World Health Organization. https://www.who.int/health-topics/coronavirus#tab=tab_3 (2020, accessed 10 August 2020).
12. Buuren S and Groothuis-Oudshoorn K. Mice: Multivariate imputation by chained equations in R. *J Stat Softw* 2011; 45: 3–67.
13. Bolay H, Gül A and Baykan B. COVID-19 is a real headache! *Headache* 2020; 60: 1415–1421.

14. Belvis R. Headaches during COVID-19: My clinical case and review of the literature. *Headache* 2020; 60: 1422–1426.
15. Porta-Etessam J, Matías-Guiu JA, González-García N, et al. Spectrum of headaches associated with SARS-CoV-2 infection: Study of healthcare professionals. *Headache* 2020; 60: 1697–1704.
16. Singh J and Ali A. Headache as the presenting symptom in 2 patients with COVID-19 and a history of migraine: 2 case reports. *Headache* 2020; 60: 1773–1776.
17. Estudio ENE-COVID19: Primera Ronda Estudio Nacional de Sero-Epidemiología de la Infección por SARS-CoV-2 en España. Informe preliminar 13 de Mayo 2020, https://www.mscbs.gob.es/ciudadanos/ene-covid/docs/ESTUDIO_ENE-COVID19_PRIMERA_RONDA_INFORME_PRELIMINAR.pdf (2020, accessed 27 May 2020).
18. Kaye R, Chang CWD, Kazahaya K, et al. *COVID-19 Anosmia Reporting Tool: Initial findings. Otolaryngol Head Neck Surg* 2020; 163: 132–134.
19. Beltrán-Corbellini Á, Chico-García JL, Martínez-Poles J, et al. Acute-onset smell and taste disorders in the context of Covid-19: A pilot multicenter PCR-based case-control study. *Eur J Neurol* 2020; 27: 1738–1741.
20. Walter EJ, Hanna-Jumma S, Carraretto M, et al. The pathophysiological basis and consequences of fever. *Crit Care* 2016; 20: 200.
21. Mehta P, McAuley DF, Brown M, et al. COVID-19: Consider cytokine storm syndromes and immunosuppression. *Lancet* 2020; 395: 1033–1034.
22. Tanaka T, Narazaki M and Kishimoto T. Il-6 in inflammation, immunity, and disease. *Cold Spring Harb Perspect Biol* 2014; 6: a016295.
23. Zhang C, Wu Z, Li JW, et al. Cytokine release syndrome in severe COVID-19: Interleukin-6 receptor antagonist tocilizumab may be the key to reduce mortality. *Int J Antimicrob Agents* 2020; 55: 105954.
24. D'Alessandro A, Thomas T, Dzieciatkowska M, et al. Serum proteomics in COVID-19 patients: Altered coagulation and complement status as a function of IL-6 level. *J Proteome Res*. Epub ahead of print 29 July 2020. doi: 10.1021/acs.jproteome.0c00365.
25. Zhou YQ, Liu Z, Liu ZH, et al. Interleukin-6: An emerging regulator of pathological pain. *J Neuroinflammation* 2016; 13: 141.
26. Sarchielli P, Alberti A, Baldi A, et al. Proinflammatory cytokines, adhesion molecules, and lymphocyte integrin expression in the internal jugular blood of migraine patients without aura assessed ictally. *Headache* 2006; 46: 200–207.
27. Yan J, Melemedjian OK, Price TJ, et al. Sensitization of dural afferents underlies migraine-related behavior following meningeal application of interleukin-6 (IL-6). *Mol Pain* 2012; 8: 6.
28. Ramachandran R. Neurogenic inflammation and its role in migraine. *Semin Immunopathol* 2018; 40: 301–314.
29. Vaira LA, Salzano G, Deiana G, et al. Anosmia and ageusia: Common findings in COVID-19 patients. *Laryngoscope* 2020; 130: 1787.
30. Li YC, Bai WZ and Hashikawa T. The neuroinvasive potential of SARS-CoV2 may play a role in the respiratory failure of COVID-19 patients. *J Med Virol* 2020; 92: 552–555.
31. Zubair AS, McAlpine LS, Gardin T, et al. Neuropathogenesis and neurologic manifestations of the coronaviruses in the age of coronavirus disease 2019: A review. *JAMA Neurol* 2020; 77: 1018–1027.
32. Politi L, Salsano E and Grimaldi M. Magnetic resonance imaging alteration of the brain in a patient with coronavirus disease 2019 (COVID-19) and anosmia. *JAMA Neurol* 2020; 77: 1028–1029.
33. Varga Z, Flammer AJ, Steiger P, et al. Endothelial cell infection and endotheliitis in COVID-19. *Lancet* 2020; 395: 1417–1418.
34. Hamming I, Timens W, Bulthuis MLC, et al. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol* 2004; 203: 631–637.
35. Baig AM, Khaleeq A, Ali U, et al. Evidence of the COVID-19 virus targeting the CNS: Tissue distribution, host-virus interaction, and proposed neurotropic mechanisms. *ACS Chem Neurosci* 2020; 11: 995–998.
36. Woloshin S, Patel N and Kesselheim AS. False negative tests for SARS-CoV-2 infection – challenges and implications. *N Engl J Med* 2020; 383: e38.

Appendix 2: Safety of anti-CGRP monoclonal antibodies in patients with migraine during the COVID-19 pandemic: Present and future implications

Caronna E, Gallardo VJ, Alpuente A, et al. Safety of anti-CGRP monoclonal antibodies in patients with migraine during the COVID-19 pandemic: Present and future implications. Neurologia. 2021; 36(8):611-617.



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ORIGINAL ARTICLE

Safety of anti-CGRP monoclonal antibodies in patients with migraine during the COVID-19 pandemic: Present and future implications

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Migraine;
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Abstract
Background and objective: CGRP, a neuropeptide involved in migraine pathophysiology, is also known to play a role in the respiratory system and in immunological conditions such as sepsis. We analyzed the impact of the use of CGRP antagonists in patients with migraine during the COVID-19 pandemic, caused by the SARS-CoV-2 coronavirus.
Methods: This is a multicentre cross-sectional study. From May to November 2020, through a national survey distributed by the Spanish Society of Neurology, we collected data about the presence of COVID-19 symptoms including headache and their characteristics and severity in patients with migraine treated with anti-CGRP monoclonal antibodies (mAb), and compared them with patients with migraine not receiving this treatment. We also conducted a subanalysis of patients with COVID-19 symptoms.

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Results: We recruited 300 patients with migraine: 51.7% (155/300) were taking anti-CGRP mAbs; 87.3% were women (262/300). Mean age (standard deviation) was 47.1 years (11.6). Forty-one patients (13.7%) met diagnostic criteria for COVID-19, with no statistically significant difference between patients with and without anti-CGRP mAb treatment (16.1% vs 11.0%, respectively; $P = .320$). Of the patients with COVID-19, 48.8% (20/41) visited the emergency department and 12.2% (5/41) were hospitalised. Likewise, no clinical differences were found between the groups of patients with and without anti-CGRP mAb treatment.
Conclusion: Anti-CGRP mAbs may be safe in clinical practice, presenting no association with increased risk of COVID-19.

PALABRAS CLAVE
Migraña;
CGRP;
COVID-19;
Anticuerpos monoclonales;
SARS-CoV-2

Seguridad de los anticuerpos monoclonales anti-CGRP en pacientes con migraña durante la pandemia de COVID-19: implicaciones actuales y futuras

Resumen
Antecedentes y objetivo: El péptido relacionado con el gen de la calcitonina (CGRP, por sus siglas en inglés), es un neuropéptido involucrado en la fisiopatología de la migraña, que también es conocido por participar en la regulación del sistema respiratorio y en algunas enfermedades inmunológicas como la sepsis. Hemos analizado el impacto del uso de los antagonistas de CGRP en pacientes con migraña durante la pandemia de COVID-19, causada por el coronavirus SARS-CoV-2.
Métodos: Estudio transversal multicéntrico desarrollado entre mayo y noviembre de 2020, en el que la Sociedad Española de Neurología distribuyó a nivel nacional una encuesta de la que recogimos datos sobre la presencia, las características y la gravedad de síntomas de COVID-19, entre los que se encontraba la cefalea, en pacientes con migraña tratados con anticuerpos monoclonales (AcM) anti-CGRP, y los comparamos con los de pacientes con migraña que no recibían dicho tratamiento. También realizamos un subanálisis de los pacientes con síntomas de COVID-19.
Resultados: Identificamos 300 pacientes con migraña: 51,7% (155/300) recibían AcM anti-CGRP; el 87,3% eran mujeres (262/300) y la edad media (desviación estándar) de la muestra fue de 47,1 (11,6) años. Un total de 41 pacientes (13,7%) cumplían los criterios diagnósticos de COVID-19, sin diferencias estadísticamente significativas entre los pacientes que recibían tratamiento con AcM anti-CGRP y los que no (16,1% y 11,0%, respectivamente; p = 0,320). De los pacientes con COVID-19, el 48,8% (20/41) acudieron a urgencias y el 12,2% (5/41) fueron hospitalizados. Igualmente, no se detectaron diferencias clínicas entre los pacientes que recibían dicho tratamiento y los que no.
Conclusión: El tratamiento con AcM anti-CGRP parece un recurso seguro en la práctica clínica, y no se asocia a un mayor riesgo de COVID-19.

Introduction

Calcitonin Gene-Related Peptide (CGRP) is a 37-amino acid peptide with strong vasodilating properties, that has a fundamental role in migraine pathophysiology.[1] Its antagonism is effective in treating migraine and monoclonal antibodies targeting CGRP (MAbs) are currently one of the available anti-CGRP therapies,[2] but the only one available in Spain. However, CGRP has several other functions in the human body.[3] For example, by producing vasodilation and modifying vascular permeability, potentially allows the recruitment of inflammatory cells to the local area,[4] being involved in tissue inflammation and sepsis. However, CGRP, depending on the situation, may promote inflammation or protect from it, through its ability to increase cAMP.[5] Another function of CGRP is the regulation of the peripheral resistance of the cardiovascular and pulmonary systems during infection, and in this context CGRP antagonism may alter the ability to cope with acute lung injury.[6]

At present the world is facing the Coronavirus Disease 2019 (COVID-19) pandemic, caused by the Severe-Acute-Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) that is able to produce a respiratory infection associated with a severe inflammatory response.[7] Under the hypothesis that CGRP may play a role in the evolution of COVID-19, it was necessary to assess the impact of drugs promoting

its antagonism. Therefore, we decided to investigate the impact of the use of anti-CGRP MAbs in migraine patients during the COVID-19 pandemic, specifically analyzing their safety profile since new waves of the disease may be approaching.

Methods

This is a Spanish multicenter cross-sectional study, conducted between May and November 2020. The following represents the primary analysis of these data. Outpatients with migraine, who were under treatment with anti-CGRP MAbs, were invited to fill in an online survey available on the website of the Spanish Neurological Society. The same questionnaire was filled in, at the same time, to age- and sex-matched random outpatients with migraine but without anti-CGRP treatment. Demographic data, presence of symptoms suggestive of COVID-19 and headache, including its characteristics, acute medication intake, type of anti-CGRP MAb and other preventive treatment were collected through the survey. COVID-19 symptoms were selected according to the list of symptoms reported by the World Health Organization (WHO).[8] We also collected data about healthcare resource utilization (outpatient visits, ER admission, hospitalization) in relation to COVID-19 as indicators of disease severity. Then, we compared participants with and without anti-CGRP MAbs and conducted a subanalysis in those patients that had a confirmed diagnosis of COVID-19 or represented suspected cases of COVID-19. We defined confirmed cases as those participants who reported a SARS-CoV-2 positive real-time reverse transcriptase polymerase-chain reaction (RT-PCR) assay by nasopharyngeal swabs. We defined suspected cases as those with three or more of the COVID-19 symptoms reported by WHO either with negative RT-PCR assay or if no confirmatory test had been performed, following the definition used in a national epidemiological study.[9]

Statistical analysis

We obtained descriptive and frequency statistics and made comparisons using the SPSS, version 21.0 for Windows. We reported nominal (categorical) variables as frequencies (percentages) and continuous variables as mean ± standard deviation (age). We checked normality assumption of quantitative variables through visual methods (Q—Q plots) and normality tests (Kolmogorov—Smirnov test). For our preplanned analyses, we assessed statistical significance for intergroup variables by Pearson's chi-square when comparing categorical variables. In the case of having an expected count less than 5 in more than 20% of cells in the contingency table, we used Fisher's exact test. We used linear trend chi-square for ordinal variables and the independent *t*-test for the continuous variable that followed a normal distribution (age). The false discovery rate with Benjamini—Hochberg procedure was used to correct *P* values for multiple comparisons.

We did not conduct a statistical power calculation prior to the study because the sample size was based on the available data. All patients recruited completed the survey and there were no missing data.

P values presented are for a two-tailed test and we considered *P* values < 0.05 statistically significant.

Ethics approval and patients' consent

The study was approved by the Vall d'Hebron Ethics Committee (PR(AG)317/2020). All patients gave informed consent for the analysis of patients' data which was collected according to Spanish regulation on clinical trials. All patients consented to publication of anonymous individual data.

Results

Of the 300 participants with migraine who answered the survey, 51.7% (155/300) were treated with MAbs. 87.3% were women and mean age was 47.1 ± 11.6 years old (Table 1).

In our cohort, 13.7% (41/300) met the criteria for either confirmed or suspected case of COVID-19, 5 of them required hospital admission (Table 2). Headache was the most frequent symptom in 82.9% of patients (34/41) (Table 2). 47.1% (16/34) of patients with headache associated with COVID-19 reported that it had worsened compared to their usual migraine.

Comparing patients with and without MAbs, no differences were found in terms of baseline characteristics or proportion of COVID-19 cases, with the exception of adherence to migraine preventive treatment: patients with MAbs presented a statistically significant higher adherence than patients without MAbs (97.6% vs. 84.1%; *P* = 0.008) (Table 1). Discontinuation was due to lack of efficacy, impossibility in administering or collecting the medication.

In the subgroup of COVID-19 cases, there were no differences in COVID-19 symptoms except for diarrhea (without MAbs: 68.8% vs. with MAbs: 28.0%; *P* = 0.022). The proportions of patients with headache as a COVID-19 symptom as well as with worsening with respect to the usual migraine (47.1%, 16/34) were not different between the group with and without MAb. Healthcare resource utilization related to COVID-19 and adherence to other preventive medications was similar between the two groups (Table 2). Two patients in this group discontinued MAbs, reporting that it was for fear of possible interactions with the concomitant COVID-19 infection. We finally performed a sensitivity analysis of confirmed COVID-19 patients (12/41) and we also found no statistically significant differences between patients with MAbs vs. without MAbs.

Discussion

In our study we decided to evaluate de potential impact of CGRP antagonism through MAbs in patients with migraine during COVID-19 pandemic, considering that, at present, there are no clear data assessing the effects of this treatment during a viral infection.

Our findings are relevant for three main reasons:

First, we observed that prevalence of COVID-19 in people with migraine is similar to the general population, suggesting

Table 1 Characteristics of the study cohort.

	Total ($n = 300$)	Without MAbs ($n = 145$)	With MAbs ($n = 155$)	Adj. P-value[a]
Demographic characteristics				
Sex, n (%)				
Female	262 (87.3%)	132 (91.0%)	130 (83.9%)	0.219
Mean ± SD, y	47.1 ± 11.6	45.7 ± 12.5	48.3 ± 10.7	0.208
Age, n (%)				
<30 y	28 (9.3%)	18 (12.4%)	10 (6.5%)	0.240
30–39 y	38 (12.7%)	16 (11.0%)	22 (14.2%)	
40–49 y	106 (35.3%)	55 (37.9%)	51 (32.9%)	
50–59 y	91 (30.3%)	41 (28.3%)	50 (32.3%)	
≥60 y	37 (12.3%)	15 (10.3%)	22 (14.2%)	
Migraine treatment				
Concomitant preventive treatment, *n* (%)	254 (84.7%)	126 (86.9%)	128 (82.6%)	0.386
Adherence to preventive treatment[b], *n* (%)	231/254 (90.9%)	106/126 (84.1%)	125/128 (97.6%)	**0.008**
Adherence to MAbs[b], *n* (%)	N/A	N/A	147 (94.8%)	N/A
Response to acute medication, *n* (%)	207 (69.0%)	95 (65.5%)	112 (72.3%)	0.320
COVID-19				
COVID-19 cases[c], *n* (%)	41 (13.7%)	16 (11.0%)	25 (16.1%)	0.320
Previous history of pneumonia, *n* (%)	63 (21.0%)	29 (20.0%)	34 (21.9%)	0.777

In **bold** are marked statistically significant variables (P value ≤ 0.05).
[a] Adjusted P value with Benjamini–Hochberg procedure.
[b] Period starting from Feb 1st, 2020 until participants' submission of the survey.
[c] Participants with confirmed SARS-CoV-2 real-time reverse transcriptase polymerase-chain reaction (RT-PCR) assay by nasopharyngeal swabs or with >2 of the COVID-19 symptoms either in the absence of a confirmatory test or with negative RT-PCR assay.
MAbs = anti-CGRP monoclonal antibodies; SD = standard deviation; N/A = not applicable.

that they have not an increased risk on the basis of their migraine history. In our cohort, 13.7% of participants had confirmed or suspected COVID-19 in line with national epidemiological study in Spain conducted in the same period on the general population, showing COVID-19 prevalence around 13.6[9]–18.6%.[10]

Second, prevalence of COVID-19 is similar in migraine patients with and without MAbs, suggesting that anti-CGRP MAbs may be safe, not predisposing to COVID-19.

Third, patients with confirmed or suspected COVID-19 under MAb treatment do not seem to have a worse course of the disease compared with migraine patients under other preventive treatments.

Yet, what are the implications, in particular, of these latter findings?

Present implication: migraine

From the migraine standpoint, since new waves of the pandemic may be approaching, neurologists can keep prescribing anti-CGRP MAbs, reassuring migraine patients on their use in order to avoid unnecessary discontinuation, as it does not seem to be associated with an increased susceptibility to SARS-CoV-2 infection.

Future implication: COVID-19

Extremely interesting could be the COVID-19 perspective. In this context, new studies are hypothesizing that a neuro-immunological cross-talk within the lungs takes place through the activation of the vagus nerve during infections. CGRP may be involved in this pathway and could alter inflammatory responses.[11] In support to this concept, a study on animal models of *Staphylococcus aureus* pneumonia, for example, has observed that antagonizing CGRP improves survival in infected mice.[12] So far, the role of CGRP had not been studied in viral infections, but recently a clinical trial has started to investigate intranasal vazegepant, a new anti-CGRP molecule for migraine therapy, specifically to treat COVID-19.[13]

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Table 2 Characteristics of the COVID-19 subgroup.

	Total[b] (*n* = 41)	Without MAbs (*n* = 16)	With MAbs (*n* = 25)	Adj. *P*-value[a]
Demographic characteristics				
Sex, n (%)				
Female	37 (90.2%)	15 (93.8%)	22 (88.8%)	1.000
Mean ± SD, y	42.6 ± 13.1	41.3 ± 12.5	45.4 ± 11.6	0.282
Age, n (%)				
<30 y	9 (22.0%)	5 (31.3%)	4 (16.0%)	0.620
30—39 y	5 (12.2%)	1 (6.3%)	4 (16.0%)	
40—49 y	11 (26.8%)	3 (18.8%)	8 (32.0%)	
50—59 y	13 (31.7%)	7 (43.8%)	6 (24.0%)	
≥60 y	3 (7.3%)	0 (7.3%)	3 (12.0%)	
Migraine treatment				
Concomitant preventive treatment, n (%)	29 (70.7%)	11 (68.8%)	18 (72.0%)	1.000
Adherence to preventive treatment[c], n (%)	27/29 (93.1%)	11/11 (100.0%)	16/18 (88.9%)	0.512
Adherence to MAbs[c], n (%)	N/A	N/A	23/25 (92.0%)	N/A
Response to acute medication, n (%)	21 (51.2%)	6 (37.5%)	15 (60.0%)	0.208
COVID-19				
COVID-19 confirmed (RT-PCR), n (%)	12 (29.3%)	3 (18.8%)	9 (36.0%)	0.305
Previous history of pneumonia, n (%)	11 (26.8%)	3 (18.8%)	8 (32.0%)	0.478
Reported symptoms, n (%)				
Headache	34 (82.9%)	13 (81.3%)	21 (84.0%)	1.000
Cough	32 (78.0%)	13 (81.2%)	19 (76.0%)	1.000
Fever	25 (61.0%)	9 (56.3%)	16 (64.0%)	0.746
Myalgia	31 (75.6%)	14 (87.5%)	17 (68.0%)	0.265
Dyspnea	21 (51.2%)	9 (56.3%)	12 (48.0%)	0.757
Anosmia	25 (61.0%)	9 (56.3%)	16 (64.0%)	0.746
Diarrhea	18 (43.9%)	11 (68.8%)	7 (28.0%)	**0.022**
Odynophagia	17 (41.5%)	7 (43.8%)	10 (40.0%)	1.000
Expectoration	17 (41.5%)	6 (37.5%)	11 (44.0%)	0.753
Healthcare resource utilization in relation to COVID-19				
Outpatient visits, n (%)	37 (90.2%)	15 (93.8%)	22 (88.0%)	1.000
Telephonic visits, n (%)	25 (61.0%)	12 (75.0%)	13 (52.0%)	0.195
Emergency room, n (%)	20 (48.8%)	5 (31.3%)	15 (60.0%)	0.111
Hospitalization, n (%)	5 (12.2%)	1 (6.3%)	4 (16.0%)	0.632

In **bold** are marked statistically significant variables (*P* value ≤ 0.05).

[a] Adjusted *P* value with Benjamini-Hochberg procedure.

[b] Participants with confirmed SARS-CoV-2 real-time reverse transcriptase polymerase-chain reaction (RT-PCR) assay by nasopharyngeal swabs or with >2 of the COVID-19 symptoms either in the absence of a confirmatory test or with negative RT-PCR assay.

[c] Period starting from Feb 1st, 2020 until participants' submission of the survey.

MAbs = anti-CGRP monoclonal antibodies; SD = standard deviation; RT-PCR = reverse transcriptase polymerase-chain reaction; N/A = not applicable.

In our study, we have observed that anti-CGRP MAbs have no major negative effects during COVID-19, however according to this neuro-immunological hypothesis, they could even represent a new option to treat infectious diseases such as COVID-19. Therefore, further studies must be designed to specifically address this relevant question, while the results of the previously mentioned clinical trial are awaited.

As for the main limitations of our study, we are aware of the potential selection bias related to patient self-reported symptoms without possibility of a laboratory confirmation for SARS-CoV-2 in most of the cases. However, we have to consider that at the time of the peak of the pandemic, almost exclusively patients admitted at the hospital were tested to confirm COVID-19, being such bias inevitable in many online-based questionnaires. In addition, our findings should be examined carefully since the size of the COVID-19 group in our cohort is limited and participants had a mild-moderate course of the disease, being more severe patients unlikely to answer an online survey. Nevertheless, our work represents a useful exploratory study on the impact of anti-CGRP in patients with migraine. Our real-life data should be confirmed by further and ampler population-based studies with the objective of clarifying not only the absence of negative effects but also the potential therapeutic activity of these drugs in COVID-19.

Conclusions

The prevalence of COVID-19 cases in people with migraine is similar to the one of the general population. The use of monoclonal antibodies against CGRP may be safe in clinical practice, not being associated with an increased risk or worse prognosis of COVID-19.

Authors' contributions

PPR and EC made substantial contributions to conception and study design. EC, VJG, AA, MTF, NMSM, JVR, ACLV, ALB, MFR, MDGB, SMGS, MMB, MPA, CS, CV, BCC, CVE, PPJ, CTP, LGV, AGV, PIS, JPE, SSL, PPR worked for acquisition of data. VJG and EC contributed to analysis and interpretation of data. EC and VJG wrote first draft. PPR, MTF, AA critically revised and finally approved the version to be published. All authors fully comply with and approve the version to be published.

Consent to publish

All patients consented to publication of anonymous individual data.

Data availability statement

All data are available and any anonymized data will be shared by request from any qualified investigator.

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Conflict of interest

Dr. Caronna reports no disclosures relevant to the manuscript.

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Appendix A. Collaborators

Spanish CGRP-COVID Study Group: María Fernández-Recio; Maria Dolores García-Bargo; Sonia María García-Sánchez; María Martín Bujanda; Manuel Peinazo-Arias; Cristina Soriano; Carlos Vilar; Berta Claramonte-Clausell; Cecile Van Eendenburg; Patricia Perea-Justicia; Cristina Treviño-Peinado; Lidia Gómez-Vicente.

References

1. Goadsby P, Edvinsson L. The trigeminovascular system and migraine: studies characterizing cerebrovascular and neuropeptide changes seen in humans and cats. Ann Neurol. 1993;33:48–56, http://dx.doi.org/10.1002/ana.410330109.
2. Charles A, Pozo-Rosich P. Targeting calcitonin gene-related peptide: a new era in migraine therapy. Lancet. 2019;394(10210):1765–74, http://dx.doi.org/10.1016/S0140-6736(19)32504-8.
3. Russell FA, King R, Smillie SJ, Kodji X, Brain SD. Calcitonin gene-related peptide: physiology and pathophysiology. Physiol Rev. 2014;94:1099–142, http://dx.doi.org/10.1152/physrev.00034.2013.
4. Brain SD. Sensory neuropeptides: their role in inflammation and wound healing. Immunopharmacology. 1997;37:133–52, http://dx.doi.org/10.1016/S0162-3109(97)00055-6.
5. Holzmann B. Modulation of immune responses by the neuropeptide CGRP. Amino Acids. 2013;45:1–7, http://dx.doi.org/10.1007/s00726-011-1161-2.
6. Hong-min F, Chun-rong H, Rui Z, Li-na S, Ya-jun W, Li L. CGRP 8-37 enhances lipopolysaccharide-induced acute lung injury and regulating aquaporin 1 and 5 expressions in rats. J Physiol Biochem. 2016;73:381–6, http://dx.doi.org/10.1007/s13105-017-0563-3.
7. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 2020;395(10229):1033–4, http://dx.doi.org/10.1016/S0140-6736(20)30628-0.
8. World Health Organization. https://www.who.int/health-topics/coronavirus#tab=tab_3 [accessed 30.11.20].
9. Estudio ENE-COVID19: Primera Ronda Estudio Nacional de Sero-Epidemiología de la Infección por SARS-CoV-2 en España. Informe preliminar 13 de Mayo 2020. https://www.mscbs.gob.es/ciudadanos/ene-covid/docs/ESTUDIO_ENE-COVID19_PRIMERA_RONDA_INFORME_PRELIMINAR.pdf.

10. Estudio ENE-COVID19: Cuarta Ronda Estudio Nacional de Sero-Epidemiología de la Infección por SARS-CoV-2 en España. Informe 15 de Diciembre 2020. https://www.mscbs.gob.es/gabinetePrensa/notaPrensa/pdf/15.12151220163348113.pdf.
11. De Virgilis F, Di Giovanni S. Lung innervation in the eye of a cytokine storm: neuroimmune interactions and COVID-19. Nat Rev Neurol. 2020:1—8, http://dx.doi.org/10.1038/s41582-020-0402-y.
12. Baral P, Umans BD, Li L, Wallrapp A, Bist M, Kirschbaum T, et al. Nociceptor sensory neurons suppress neutrophil and γδ T cell responses in bacterial lung infections and lethal pneumonia. Nat Med. 2018;24:417—26, http://dx.doi.org/10.1038/nm.4501.
13. New Haven CBP. Biohaven achieves positive topline results in pivotal phase 2/3 study of & vazegepant, the first and only intranasal CGRP receptor antagonist in clinical development for the acute treatment of migraine. https://clinicaltrials.gov/ct2/show/NCT04346615 [accessed 30.11.20].

Appendix 3: Meta-analysis

Table 1. Search strategy performed on PubMed database search

Search Terms
((((((((((COVID-19[Title]) OR COVID19[Title]) OR coronavirus[Title]) OR nCoV[Title]) OR SARS-CoV-2[Title]) OR SARS-CoV2[Title])) AND ((((((((((((((((((((mortality[Title/Abstract]) OR deceased [Title/Abstract]) OR died [Title/Abstract]) OR recovered [Title/Abstract]) OR death [Title/Abstract]) OR fatility rate [Title/Abstract]) OR risk factor [Title/Abstract]) OR fatal [Title/Abstract]) OR hospitalization [Title/Abstract]) OR survived [Title/Abstract]) OR surviving [Title/Abstract]) OR hospitalized [Title/Abstract]) OR non-survivors [Title/Abstract]) OR admission [Title/Abstract]) OR in-hospital [Title/Abstract]) OR hospitalised [Title/Abstract]) OR dying [Title/Abstract]) OR prognosis [Title/Abstract]) OR c [Title/Abstract]) OR headache [Title/Abstract])) AND ("2020/04/01"[Publication Date] : "3000"[Publication Date]))

Table 2. Studies selected for the meta-analysis

Author	Research Timing	Design	Country	Region	All COVID19 Confirmation	Sample Size	≥50% Male	Median age ≥65 years old	REF
Aksel G et al.	Prospective	Cohort Study	Turkey	Europe	Yes	168	Y	59.6	(1)
Bellan M et al.	Retrospective	Cohort Study	Italy	Europe	Yes	407	N	Y	(2)
Berenguer J et al.	Retrospective	Cohort Study	Spain	Europe	Yes	4,035	Y	Y	(3)
Caronna E et al.	Prospective	Cohort Study	Spain	Europe	No	130	N	N	(4)
Chen F et al.	Prospective	Cohort Study	China	Asia	No	660	N	N	(5)
Chen L et al.	Retrospective	Cohort Study	China	Asia	Yes	1,859	N	N	(6)

Chen R et al.	Retrospective	Cohort Study	China	Asia	Yes	1,590	Y	N	(7)
Chen T et al.	Retrospective	Cohort Study	China	Asia	Yes	274	Y	N	(8)
Cheng A et al.	Retrospective	Cohort Study	China	Asia	Yes	305	Y	Y	(9)
Deng Y et al.	Retrospective	Cohort Study	China	Asia	Yes	225	Y	N	(10)
De Souza C et al.	Retrospective	Cross-Sectional	Brazil	South America	No	9,807	N	Y	(11)
Du RH et al.	Retrospective	Cohort Study	China	Asia	No	179	Y	N	(12)
Emara DM et al.	Retrospective	Cohort Study	Egypt	Africa	Yes	120	Y	N	(13)
Gao S et al.	Retrospective	Cohort Study	China	Asia	Yes	210	N	Y	(14)
Garibaldi BT et al.	Prospective	Cohort Study	US	North America	No	832	Y	N	(15)
Gil-Rodrigo A et al.	Prospective	Cohort Study	Spain	Europe	Yes	1,000	N	N	(16)
Homayounieh F et al.	Retrospective	Cohort Study	Iran	Africa	Yes	90	Y	N	(17)
Khalil K et al.	Prospective	Cohort Study	UK	Europe	Yes	220	Y	Y	(18)
Li J et al.	Retrospective	Cohort Study	China	Asia	Yes	161	Y	N	(19)
Li M et al.	Retrospective	Cohort Study	China	Asia	Yes	245	N	N	(20)
Li X et al.	Retrospective	Cohort Study	US	North America	Yes	1,022	Y	N	(21)

Ma X et al.	Retrospective	Cohort Study	China	Asia	No	523	Y	N	(22)
Marengoni A et al.	Retrospective	Cohort Study	Italy	Europe	No	165	Y	Y	(23)
Martin-Moro F et al.	Retrospective	Cohort Study	Spain	Europe	No	34	Y	Y	(24)
Mendes A et al.	Retrospective	Cohort Study	Switzerland	Europe	No	235	N	Y	(25)
Moon SS et al.	Retrospective	Cohort Study	Korea	Asia	Yes	348	N	N	(26)
Park JG et al.	Retrospective	Cohort Study	Korea	Asia	Yes	289	N	Y	(27)
Rana MS et al.	Retrospective	Cohort Study	Pakistan	Africa	Yes	100	Y	N	(28)
Rivera-Izquierdo M et al.	Retrospective	Cohort Study	Spain	Europe	Yes	131	Y	Y	(29)
Rodriguez-Molinero A et al.	Prospective	Cohort Study	Spain	Europe	Yes	418	Y	Y	(30)
Rodriguez-Nava G et al.	Retrospective	Cohort Study	US	North America	Yes	313	Y	Y	(31)
Rubio-Rivas M et al.	Retrospective	Cohort Study	Spain	Europe	Yes	12,066	Y	Y	(32)
Soares RDCM et al.	Retrospective	Cohort Study	Brazil	South America	No	1,152	Y	N	(33)
Tomlins J et al.	Retrospective	Cohort Study	UK	Europe	Yes	95	Y	Y	(34)
Trigo J et al.	Retrospective	Cohort Study	Spain	Europe	Yes	576	Y	Y	(35)

Van Halem K et al.	Retrospective	Cohort Study	Belgium	Europe	Yes	319	Y	Y	(36)
Varol Y et al.	Retrospective	Cohort Study	Turkey	Europe	Yes	383	Y	N	(37)
Vena A et al.	Retrospective	Cohort Study	Italy	Europe	Yes	317	Y	Y	(38)
Wang D et al.	Retrospective	Cohort Study	China	Asia	Yes	107	Y	N	(39)
Wang L et al.	Retrospective	Cohort Study	China	Asia	Yes	339	N	Y	(40)
Wang ZH et al.	Retrospective	Cohort Study	China	Asia	Yes	59	Y	Y	(41)
Yan Y et al.	Retrospective	Cohort Study	China	Asia	Yes	193	Y	Y	(42)
Yang X et al.	Retrospective	Cohort Study	China	Asia	Yes	52	Y	N	(43)
Yu Z et al.	Retrospective	Cohort Study	China	Asia	Yes	141	Y	Y	(44)
Zhang J et al.	Retrospective	Cohort Study	China	Asia	Yes	663	N	N	(45)
Zhang L et al.	Retrospective	Cohort Study	China	Asia	Yes	319	N	N	(46)
Zhao Z et al.	Retrospective	Cohort Study	US	North America	Yes	480	Y	N	(47)
Zhu J et al.	Retrospective	Cohort Study	US	North America	Yes	181	Y	N	(48)

Table 3. Pooled prevalence of symptoms and signs among COVID-19 inpatients from the sensitivity analysis

Symptoms	Symptom prevalence		Total number of	Between-study heterogeneity	Publication bias,

	[95% CI]	Number of studies included*	COVID-19 patients	I^2	*P* value	Egger's test (*P* value)
Anosmia	0.066 [0.041-0.104]	15/16	9,128	97%	**<0.001**	0.276
Cough	0.650 [0.612-0.687]	22/43	11,309	93%	**<0.001**	0.174
Diarrhea	0.113 [0.084-0.149]	23/35	22,121	97%	**<0.001**	0.802
Dyspnea	0.419 [0.339-0.503]	22/40	6,506	98%	**<0.001**	0.112
Fever (>37.3 ºC)	0.779 [0.720-0.829]	34/42	27,068	99%	**<0.001**	0.136
Headache	0.097 [0.078-0.120]	41/48	30,236	97%	**<0.001**	0.275
Myalgia	0.176 [0.146-0.211]	34/38	25,581	97%	**<0.001**	0.446
Nausea or Vomiting	0.103 [0.077-0.137]	27/30	14,191	97%	**<0.001**	0.109

*Studies with extreme effect sizes were discarded in order to obtain an unbiased publication effect (Egger's test).

In **bold** are marked *P values* < 0.05

Table 4. Sensitivity analysis of headache relative risk (RR) among COVID-19 inpatients

Strategy	Test for overall effect (random-model)	Headache-COVID19 RR [95% CI]	Between-study heterogeneity		Number of studies included	Publication bias, Egger's test (P value)
			I^2	P value		
Including all studies	t = 5.18; **p<0.001**	1.90 [1.46-2.47]	80.3%	**<0.001**	48/48	0.605
Excluding outliers and over-influencer studies*	t = 10.75; **p<0.001**	2.18 [1.88-2.52]	16.8%	0.175	41/48	0.733
Excluding retrospective studies	t = 3.55; **p=0.012**	3.38 [1.46-7.83]	53.5%	**0.050**	7/48	0.280
Excluding studies without COVID-19 confirmation (RT-PCR)	t = 5.18; **p<0.001**	1.92 [1.49-2.47]	76.0%	**<0.001**	38/48	0.457

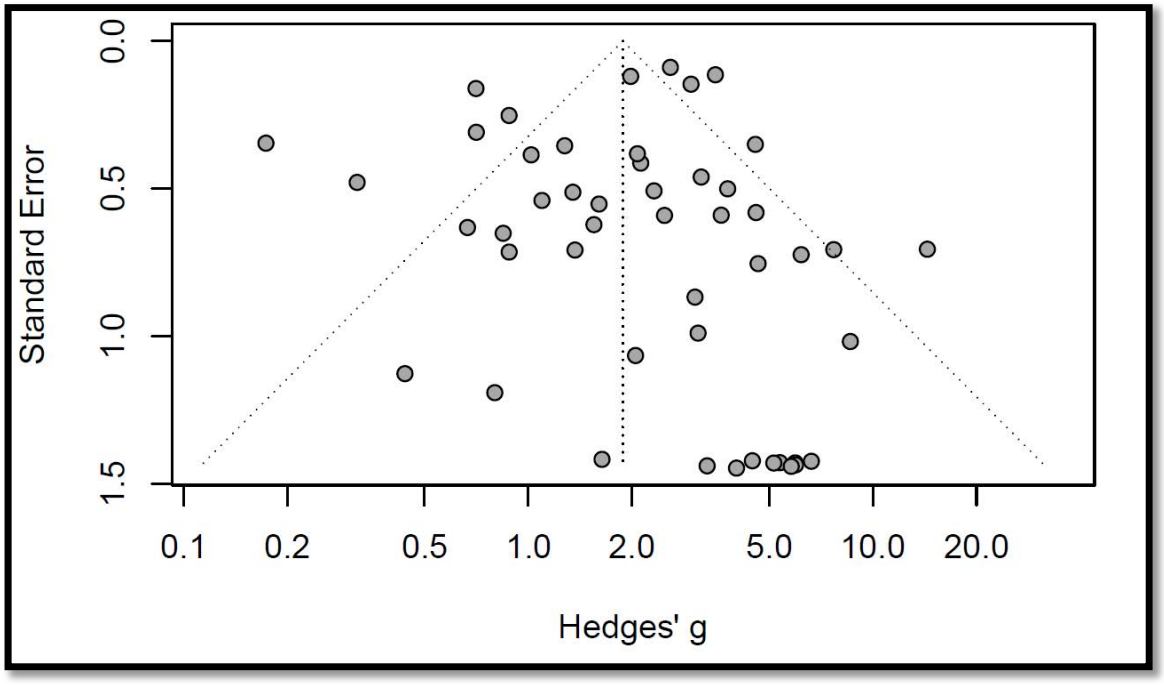
Excluding small studies (n < 250 patients)		t = 7.26; **p<0.001**	2.25 [1.79-2.83]	65.1%	**<0.001**	26/48	0.974
Excluding studies with lower quality (NOS score ≥ 7)		t = 8.24; **p<0.001**	2.60 [2.03-3.32]	23.6%	0.180	21/28	0.829
≥50% Male	N	Q (1) = 0.30; p = 0.586	2.10 [1.33 - 3.31]	25.8%	0.230	34/48	
	Y		1.82 [1.31 - 2.53]	85.1%	**<0.001**	14/48	
Median age ≥65 years old	N	Q (1) = 9.00; **p = 0.011**	1.61 [1.09-2.39]	86.4%	**<0.001**	27/48	
	Y		2.42 [1.78-3.31]	49.9%	0.059	21/48	

*Studies with extreme effect sizes (outliers) and studies with higher influence on overall effect (Leave-One-Out influence analysis) were discarded in order to obtain a homogenized between-studies effect

In **bold**, *P values* < 0.05

NOS: Newcastle-Ottawa Scale

Figure 1. Funnel plot on the risk of headache among COVID-19 inpatients



Egger's test significance was *P value* = 0.605

Meta-analysis references

1. Aksel G., İslam MM, Algın A, et al. Early predictors of mortality for moderate to severely ill patients with Covid-19. Am J Emerg Med. 2020;S0735-6757(20)30770-1.

2. Bellan M, Patti G, Hayden E, et al. Fatality rate and predictors of mortality in an Italian cohort of hospitalized COVID-19 patients. Sci Rep. 2020;10(1):20731.
3. Berenguer J, Ryan P, Rodríguez-Baño J, et al. Characteristics and predictors of death among 4035 consecutively hospitalized patients with COVID-19 in Spain. Clinical Microbiol Infect. 2020;26(11), 1525-1536.
4. Caronna E, Ballvé A, Llauradó A, et al. Headache: A striking prodromal and persistent symptom, predictive of COVID-19 clinical evolution. Cephalalgia 2020;40(13):1410-1421.
5. Chen F, sun W, Sun S, et al. Clinical characteristics and risk factors for mortality among inpatients with COVID-19 in Wuhan, China. Clinical Transl Med 2020;10(2):e40.
6. Chen L, Yu J, He W, et al. Risk factors for death in 1859 subjects with COVID-19. Leukemia 2020;34(8):2173-2183.
7. Chen R, Liang W, Jiang M, et al. Risk factors of fatal outcome in hospitalized subjects with coronavirus disease 2019 from a nationwide analysis in China. Chest 2020;158(1):97-105
8. Chen T, Wu D, Chen H, et al. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. BMJ 2020;368:m1091.
9. Cheng A, Hu L, Wang Y, et al. Diagnostic performance of initial blood urea nitrogen combined with D-dimer levels for predicting in-hospital mortality in COVID-19 patients. Int J Antimicrob Agents 2020;56(3):106110.
10. Deng Y, Liu W, Liu K, et al. Clinical characteristics of fatal and recovered cases of coronavirus disease 2019 in Wuhan, China: a retrospective study. Chin Med J (Engl) 2020;133(11):1261-1267.
11. de Souza CD, de Arruda Magalhães AJ, Lima AJ, et al. Clinical manifestations and factors associated with mortality from COVID-19 in older adults: Retrospective population-based study with 9807 older Brazilian COVID-19 patients. Geriatr Geront Int 2020 Dec;20(12):1177-1181
12. Du RH, Liang LR, Yang CQ, et al. Predictors of mortality for patients with COVID-19 pneumonia caused by SARS-CoV-2: a prospective cohort study. Eur Respir J 2020;55(5):2000524. 55(5).
13. Emara DM, Naguib NN, Moustafa MA, et al. Typical and atypical CT chest imaging findings of novel coronavirus 19 (COVID-19) in correlation with clinical data: impact on the need to ICU admission, ventilation and mortality. EJRNM 2020;5(1):1-9.
14. Gao S, Jiang F, Jin W, et al. Risk factors influencing the prognosis of elderly patients infected with COVID-19: a clinical retrospective study in Wuhan, China. Aging (Albany NY) 2020;12(13):12504-12516
15. Garibaldi BT, Fiksel J, Muschelli J, et al. Patient Trajectories Among Persons Hospitalized for COVID-19: A Cohort Study. Ann Intern Med 2021;174(1):33-41.
16. Gil-Rodrigo A, Miró Ò, Piñera P, et al. Evaluación de las características clínicas y evolución de pacientes con COVID-19 a partir de una serie de 1000 pacientes atendidos en servicios de urgencias españoles. Emergencias 2020;32(4):233-241.
17. Homayounieh F, Zhang EW, Babaei R, et al. Clinical and imaging features predict mortality in COVID-19 infection in Iran. PloS One 2020;15(9):e0239519.
18. Khalil K, Agbontaen K, McNally D, et al. Clinical characteristics and 28-day mortality of medical patients admitted with COVID-19 to a central London teaching hospital. J Infect 2020;81(3):e85-e89
19. Li J, Long X, Luo H, et al. Clinical characteristics of deceased patients infected with SARS-Cov-2 in Wuhan, China. (2/24/2020). Available at SSRN: https://ssrn.com/abstract=3546043 or http://dx.doi.org/10.2139/ssrn.3546043

20. Li M, Cheng B, Zeng W, et al. Analysis of the risk factors for mortality in adult COVID-19 patients in Wuhan: A multicenter study. Front Med (Lausanne) 2020;7:545. 7.
21. Li X, Ge P, Zhu J, et al. Deep learning prediction of likelihood of ICU admission and mortality in COVID-19 patients using clinical variables. PeerJ 2020;8:e10337.
22. Ma X, Li A, Jiao M, et al. Characteristic of 523 COVID-19 in Henan province and a death prediction model. Front Public Health 2020;8:475.
23. Marengoni A, Zucchelli A, Vetrano DL, et al. Beyond chronological age: Frailty and multimorbidity predict in-hospital mortality in patients with coronavirus disease 2019. J Gerontol A Biol Sci Med Sci 2020;glaa291.
24. Martín-Moro F, Marquet J, Piris M, et al. Survival study of hospitalized patients with concurrent Covid-19 and haematological malignancies. Br J Haematol 2020;190(1):e16-e20.
25. Mendes A, Serratrice C, Herrmann FR, et al. Predictors of in-hospital mortality in older patients with COVID-19: the COVIDAge study. J Am Med Dir Assoc 2020;21(11):1546-1554.
26. Moon SS, Lee K, Park J, et al. Clinical characteristics and mortality predictors of COVID-19 patients hospitalized at nationally-designated treatment hospitals. J Korean Med Sci 2020;35(36):e328.
27. Park JG, Kang MK, Lee YR, et al. Fibrosis-4 index as a predictor for mortality in hospitalised patients with COVID-19: a retrospective multicentre cohort study. BMJ Open 2020;10(11):e041989.
28. Rana MS. Clinical and laboratory characteristics of recovered versus deceased COVID-19 patients in Islamabad, Pakistan. J Infect 2020;S0163-4453(20)30705-2
29. Rivera-Izquierdo M, del Carmen Valero-Ubierna M, R-delAmo JL, et al. Sociodemographic, clinical and laboratory factors on admission associated with COVID-19 mortality in hospitalized patients: A retrospective observational study. PLoS One 2020;15(6):e0235107.
30. Rodríguez-Molinero A, Gálvez-Barrón C, Miñarro A, et al. Association between COVID-19 prognosis and disease presentation, comorbidities and chronic treatment of hospitalized patients. PloS One 2020;15(10):e0239571.
31. Rodriguez-Nava G, Yanez-Bello MA, Trelles-Garcia DP, et al. Clinical characteristics and risk factors for mortality of hospitalized patients with COVID-19 in a community hospital: A retrospective cohort study. Mayo Clinic Proc Innov Qual Outcomes 2020.
32. Rubio-Rivas M, Corbella X, Mora-Luján JM, et al. Predicting Clinical Outcome with Phenotypic Clusters in COVID-19 Pneumonia: An Analysis of 12,066 Hospitalized Patients from the Spanish Registry SEMI-COVID-19. J Clin Med 2020;9(11):3488.
33. Soares RDCM, Mattos LR, Raposo LM. Risk factors for hospitalization and mortality due to COVID-19 in Espírito Santo State, Brazil. Am J Trop Med Hyg 2020;103(3):1184-1190.
34. Tomlins J, Hamilton F, Gunning S, et al. Clinical features of 95 sequential hospitalised patients with novel coronavirus 2019 disease (COVID-19), the first UK cohort. J Infect 2020;81(2):e59-e61.
35. Trigo J, García-Azorín D, Planchuelo-Gómez Á, et al. Factors associated with the presence of headache in hospitalized COVID-19 patients and impact on prognosis: a retrospective cohort study. J Headache Pain 2020;21(1):94.
36. van Halem K, Bruyndonckx R, van der Hilst J, et al. Risk factors for mortality in hospitalized patients with COVID-19 at the start of the pandemic in Belgium: a retrospective cohort study. BMC Infect Dis 2020;20(1):897.
37. Varol Y, Hakoglu B, Cirak AK, et al. The Impact of Charlson Comorbidity Index on mortality from SARS-CoV-2 virus infection and A novel COVID 19 mortality index: CoLACD. Int J Clin Pract 2020;e13858.

38. Vena A, Giacobbe DR, Di Biagio A, et al. Clinical characteristics, management and in-hospital mortality of patients with coronavirus disease 2019 in Genoa, Italy. Clin Microbiol Infect 2020;26(11):1537-1544.
39. Wang D, Yin Y, Hu C, et al. Clinical course and outcome of 107 patients infected with the novel coronavirus, SARS-CoV-2, discharged from two hospitals in Wuhan, China. Crit Care 2020;24(1):188.
40. Wang L, He W, Yu X, et al. Coronavirus disease 2019 in elderly patients: Characteristics and prognostic factors based on 4-week follow-up. J Infect 2020; 80(6):639-645.
41. Wang ZH, Shu C, Ran X, et al. Critically Ill patients with coronavirus disease 2019 in a designated ICU: clinical Features and Predictors for mortality. Risk Manag Healthc Policy 2020;13:833-845.
42. Yan Y, Yang Y, Wang F, et al. Clinical characteristics and outcomes of patients with severe covid-19 with diabetes. BMJ Open Diabetes Res Care 2020;8(1):e001343.
43. Yang X, Yu Y, Xu J, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med 2020;8(5):475-481.
44. Yu Z, Ke Y, Xie J, et al. Clinical characteristics on admission predict in-hospital fatal outcome in patients aged≥ 75 years with novel coronavirus disease (COVID-19): a retrospective cohort study. BMC Geriatr 2020;20(1):514.
45. Zhang J, Wang X, Jia X, et al. Risk factors for disease severity, unimprovement, and mortality of COVID-19 patients in Wuhan, China. Clin Microbiol Infect 2020;26(6):767-772
46. Zhang L, Sun W, Chen L, et al. Clinical Features and a Simple Model for Predicting the Mortality of Coronavirus Disease 2019 Patients on Admission. (3/5/2020). Available at SSRN: https://ssrn.com/abstract=3551386 or http://dx.doi.org/10.2139/ssrn.3551386
47. Zhao Z, Chen A, Hou W, et al. Prediction model and risk scores of ICU admission and mortality in COVID-19. PloS One 2020;15(7):e0236618.
48. Zhu JS, Ge P, Jiang C, et al. Deep-learning artificial intelligence analysis of clinical variables predicts mortality in COVID-19 patients. J Am Coll Emerg Physicians Open 2020;1(6):1364-1373.

Appendix 4: Three cases of persistent headache with migraine-like features after mild COVID-19

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BRIEF COMMUNICATIONS

Toward a better understanding of persistent headache after mild COVID-19: Three migraine-like yet distinct scenarios

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Abstract

One year after the outbreak of coronavirus disease 2019 (COVID-19), referrals for persistent headache, often defined as "post-COVID headache," have become increasingly common in outpatient headache clinics. However, it is important to take into consideration that this term may include a spectrum of clinically different headache types. We describe three cases of migraine-like headaches in individuals with a history of mild COVID-19 infection to demonstrate some of the different phenotypes of persistent headaches seen. These cases highlight the importance of a careful evaluation when assessing the complexities of "post-COVID headache" as well as the need to further investigate the different, underlying, pathophysiological mechanisms.

KEYWORDS
headache, migraine, post-COVID-19, SARS-CoV-2

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel coronavirus, responsible for coronavirus disease 2019 (COVID-19),[1] that emerged in China at the end of 2019 causing the current global pandemic.[2] During the acute phase of infection,[3] COVID-19 can trigger a headache that is phenotypically similar to migraine or tension-type headache,[3,4] among other neurological symptoms.[5]

Some patients do not fully recover after the acute phase and experience persistent symptoms and/or delayed or long-term complications of COVID-19, generally referred to as "post-COVID syndrome."[6] These symptoms may include memory impairment, insomnia, fatigue, dizziness, etc.[7,8] Headache is also a post-COVID symptom[9] in some patients and consultations for persistent headache attributed to COVID-19, often referred to as "post-COVID headache," are presumably being seen more often in clinical practice.

Although data on persistent headache attributed to COVID-19 are still lacking, clinical observation by neurologists during their daily practice appears to indicate the presence of many different headache types. This would imply that the term "post-COVID headache" may be too broad to describe the complex spectrum seen. Different types of headaches may be a result of different pathophysiological mechanisms, even if they display similar characteristics, such as migraine-like features. Consequently, they may have different prognoses and responses to treatment.

Here, we describe the cases of three different patients, evaluated in our headache clinic, with "post-COVID headache." We focus on patients with a history of mild COVID-19 infection and migraine-like features to stress the existence of a complex scenario even within a group of patients with similar characteristics. We discuss our main findings and their implications below.

PATIENT 1: MIGRAINE CHRONIFICATION

A 56-year-old woman with a history of low-frequency episodic migraine (<1 day/month) and the absence of other comorbidities, who

Abbreviations: BTX, onabotulinumtoxinA; COVID-19, coronavirus disease 2019; RT-PCR, real-time reverse transcriptase polymerase-chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

TABLE 1 Main features of the three patients presented

	Patient 1	Patient 2	Patient 3
Demographics			
Age, years	56	55	44
Menopause	Yes	Yes	N/A
Sex	Female	Female	Male
Family migraine history	Yes	No	No
Personal migraine history	Yes	No	No
COVID-19 and headache characteristics			
COVID-19 severity	Mild	Mild	Mild
Headache in the acute phase	Yes	Yes	No
Anosmia/ageusia	Yes	Yes	No
Migraine-like features	Yes	Yes	Yes
Concomitant "post-COVID" symptoms	Fatigue, insomnia	Hyposmia, fatigue, insomnia	Fatigue, insomnia, mood disorder, loss of memory, dizziness
Treatment response			
Response to triptans	Yes	Yes	No
Response to preventive medications (AMT+BTX)	Yes	Yes	No

Abbreviations: AMT, amitryptiline; BTX, onabotulinumtoxinA; N/A, not applicable.

had menopause at the age of 49, had a mild COVID-19 infection. The patient exhibited symptoms of headache, anosmia/ageusia, and malaise at the end of May 2020. The presence of COVID-19 was confirmed using a positive real-time reverse transcriptase polymerase-chain reaction (RT-PCR) assay test. Headache was the first symptom of infection, starting a couple of days prior to the other symptoms. The patient's headache was characterized as being: frontally located, throbbing in quality, with a moderate–severe pain intensity, and associated with nausea, photophobia, and phonophobia. She reported that it differed from her usual migraine in that the pain since onset was constant. This constant pain was most likely the reason why the patient started overusing acute medication (paracetamol and nonsteroidal anti-inflammatory drugs) during the first 2 months, although later the lack of pain relief made her reduce her analgesic intake. The patient also started noticing allodynia while combing her hair.

The patient first visited our headache clinic at the end of October 2020 to discuss persistent headache. She also reported fatigue and insomnia. Results from a CT scan with contrast, lab work, and a neurological examination were within the norm and did not suggest the presence of secondary causes. The patient did not have hypertension or any new onset comorbidity. The patient was instructed on lifestyle changes and, given the disabling nature of her headaches and other symptomology, was offered combined preventive treatment with amitriptyline 25 mg QD, also beneficial in treating her insomnia, and onabotulinumtoxinA (BTX) 195U based on her chronic migraine-like phenotype. Because of the patient's initial reluctance toward BTX, we began with amitriptyline only. After 4 weeks, the patient's sleep quality had improved; however, she continued to experience headache almost daily, although with a reduction in intensity. We then added BTX to her treatment and after 3 months, the patient experienced a reduction in headache frequency to around 8–10 days/month and a return in character to her usual migraine. The patient's allodynia also disappeared. Her symptoms remained stable throughout the second cycle of BTX.

PATIENT 2: LONG-LASTING COVID HEADACHE

A 55-year-old woman, with no personal or family history of a primary headache disorder and no other comorbidities, who underwent menopause at age 51, had a mild COVID-19 infection. She exhibited symptoms of headache, anosmia/ageusia, cough, shortness of breath, malaise, and diarrhea at the end of March 2020. The presence of COVID-19 was confirmed by a positive RT-PCR. Pneumonia was ruled out with a chest X-ray. The patient's headache started a few days after the appearance of her other symptoms. Headache characteristics included holocranial location, moderate severity, and tightness/pressure in terms of type of pain although the patient also reported moments of more severe, throbbing pain. Headache was accompanied by photophobia, phonophobia, and nausea. The patient was unable to engage in her usual physical exercise due to worsening of pain with movement. She had constant pain following headache onset and was taking paracetamol daily.

The patient first visited our headache clinic at the beginning of July 2020 because of the presence of persistent headache. Furthermore, she was still experiencing hyposmia, fatigue, and insomnia. An MRI of the brain with MR angiography, blood tests, as well as a neurological examination were performed but did not yield any secondary causes. We discussed lifestyle changes with the

patient and, based on the same rationale as in Patient 1, a combined preventive treatment plan of amitriptyline 25 mg QD coupled with BTX 195U were initiated immediately. Three months later, the patient's sleep quality had improved alongside an important reduction in both headache frequency (1–2 headache days/week) and severity (50%–75% reduction). On headache days with migraine features, the patient displayed a good response to almotriptan 12.5 mg. The patient remained clinically stable with two subsequent BTX administrations, although she did report the effects of the treatment "wearing off" prior to each injection.

PATIENT 3: DELAYED-ONSET-COVID HEADACHE

A 44-year-old man with asthma and no personal or family history of a primary headache disorder had a mild COVID-19 infection. He exhibited symptoms including cough, shortness of breath, and malaise at the end of March 2020. The presence of COVID-19 was confirmed by a positive RT-PCR. Pneumonia was ruled out by a chest X-ray. The patient did not experience loss of smell/taste or headache in the acute phase of infection. In May 2020, when his respiratory symptoms began to improve, he started to report the presence of headaches with holocranial localization, throbbing pain, photophobia, phonophobia, nausea, and worsening of pain with movement. Over the course of the following weeks, his headaches increased to a constant, daily frequency although he could still distinguish severe migraine-like days from milder ones. He was not overusing acute medication given he did not experience any kind of pain relief from analgesic use. An MRI of the brain with MR angiography, blood tests, and neurological examinations were performed in that period and were not suggestive of secondary causes.

The patient first visited our headache clinic in mid-July 2020 because of the presence of persistent headache. He complained of fatigue and insomnia and was found to be newly hypertensive at 140/90 mm Hg. First, we attempted to address other possible factors related to headache through the use of a combined preventive treatment plan of amitriptyline 25 mg QD for insomnia and candesartan 16 mg QD for hypertension. The patient was also instructed to undertake changes in lifestyle. Five weeks later, hypertension was controlled, sleep quality had partially improved, but the patient did not report any significant clinical changes in his persistent headaches. BTX 195U was added to the patient's treatment plan but was discontinued after two cycles due to a lack of improvement. Rizatriptan 10 mg was ineffective in treating migraine-like days. During the follow-up, the patient started reporting other symptoms such as cognitive impairment, memory loss, heart palpitations, and dizziness, for which he had scheduled a visit at a memory unit and a cardiology clinic, respectively. On the patient's last visit in April 2021, escitalopram 10 mg QD was started due to changes in mood. He was still experiencing disabling, daily, and constant headache and had not returned to work or his usual activities.

DISCUSSION

Although we are aware of a potentially wide spectrum of persistent post-COVID headaches, we report these three cases as they (1) demonstrate the existence of phenotypically similar (i.e., all of these cases had migraine-like features and were found in patients having undergone a mild COVID-19 infection) but considerably different headache subtypes and (2) represent some of the patients we have observed in clinical practice (Table 1). Moreover, in light of the emerging complications post-COVID-19, they raise several questions of scientific and clinical interest in headache, based on the following aspects:

First, Patient 1 seems to suggest SARS-CoV-2 as a risk factor for the sudden worsening of a previous headache disorder, such as migraine, potentially leading to its chronification. Worsening of headache following acute SARS-CoV-2 infection has been reported by several patients with migraine.[10] Thus, the lingering headache observed, despite COVID-19 resolution, may reflect persistent changes in migraine characteristics as a result of COVID-19, rather than a new type of headache. Considering the high frequency of migraine in the general population, Patient 1 is a potential representation of one of the most common displays of post-COVID headache.

Second, migraine-like features are present in patients without a personal migraine history, as shown in Patients 2 and 3, pointing to an acquired activation of the trigeminovascular system by the SARS-CoV-2 infection.[3] In support of this hypothesis, a recent study has found SARS-CoV-2 proteins in trigeminal branches and the trigeminal ganglion.[11] The detection of these migraine characteristics may guide acute and preventive therapeutic strategies, although the response to specific migraine treatments may not be always effective (i.e., Patient 3) as a result of other concomitant factors or mechanisms.

Third, persistent headache attributed to COVID-19 may emerge despite not having experienced headache in the acute infection phase, as observed in Patient 3. Therefore, headache as a long-lasting symptom following the acute phase of COVID-19 infection may be pathophysiologically different from headache appearing as a delayed symptom when the infection is about to resolve. For example, factors such as altered levels of glutamate and hypoxic injury are believed to play a more prominent role in the post-COVID-19 phase.[12] This suggests that new daily persistent headache (Patients 2 and 3) may, in reality, harbor different subtypes with different clinical courses.

Fourth, headache can be associated with many other symptoms (insomnia, memory loss, dizziness, fatigue, etc.) that identify "post-COVID-19 syndrome," as seen in Patient 3. The presence of these comorbid conditions could (1) point to the involvement of different pathophysiological pathways (e.g., brainstem in insomnia,[13] neurotransmitter depletion in neuropsychiatric symptoms[12]) all leading to headache and (2) directly affect headache prognosis.[14,15]

Thus, future research studies should focus on studying how these aspects (i.e., migraine history, migraine-like features, headache in the acute phase, headache onset post-COVID, presence of associated symptoms) interact in determining specific clinical

phenotypes, to better understand their mechanisms and define the best therapeutic strategies.

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CONFLICT OF INTEREST

Dr. Caronna has received honoraria from Lundbeck, Novartis, and Chiesi. Dr. Alpuente has received honoraria from Allergan plc, Novartis, and Chiesi. Dr. Torres-Ferrus has received honoraria from Allergan plc, Novartis, and Chiesi. Dr. Pozo-Rosich has received honoraria as a consultant and speaker for: Allergan-AbbVie, Almirall, Biohaven, Chiesi, Eli Lilly, Medscape, Neurodiem, Novartis, and Teva. Her research group has received research grants from Novartis and funding for clinical trials from Alder, Amgen, Electrocore, Eli Lilly, Novartis, and Teva. She is an Elected Trustee of the board for the International Headache Society and a member of the Council for the European Headache Federation. She is a member of the editorial board for Revista de Neurologia. She is an editor for *Cephalalgia*, *Headache*, *Neurologia*, *Frontiers of Neurology* and an advisor for *The Journal of Headache and Pain*. She is a member of the Clinical Trials Standing Committee for the International Headache Society. She has edited the *Guidelines for the Diagnosis and Treatment of Headache* for the Spanish Society of Neurology. She is the founder of www.midolordecabeza.org. P.P.-R. does not own stocks from any pharmaceutical company. There are no conflicts of interest with regard to this manuscript.

AUTHOR CONTRIBUTIONS

Study concept and design: Edoardo Caronna, Patricia Pozo-Rosich. *Acquisition of data*: Edoardo Caronna. *Analysis and interpretation of data*: Edoardo Caronna, Patricia Pozo-Rosich. *Drafting of the manuscript*: Edoardo Caronna, Patricia Pozo-Rosich. *Revising it for intellectual content*: Edoardo Caronna, Alicia Alpuente, Marta Torres-Ferrus, Patricia Pozo-Rosich. *Final approval of the completed manuscript*: Edoardo Caronna, Alicia Alpuente, Marta Torres-Ferrus, Patricia Pozo-Rosich.

PATIENT CONSENT

All patients provided fully informed, voluntary and written consent to publish.

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REFERENCES

1. Hu B, Guo H, Zhou P, Shi ZL. Characteristics of SARS-CoV-2 and COVID-19. *Nat Rev Microbiol*. 2021;19(3):141-154. https://doi.org/10.1038/s41579-020-00459-7
2. World Health Organization. *Coronavirus Disease 2019 (COVID-19)*. Situation report—51. 2020. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200311-sitrep-51-covid-19.pdf?sfvrsn=1ba62e57_10
3. Caronna E, Ballvé A, Llauradó A, et al. Headache: a striking prodromal and persistent symptom, predictive of COVID-19 clinical evolution. *Cephalalgia*. 2020;40(13):1410-1421. https://doi.org/10.1177/0333102420965157
4. Trigo López J, García-Azorín D, Planchuelo-Gómez Á, García-Iglesias C, Dueñas-Gutiérrez C, Guerrero ÁL. Phenotypic characterization of acute headache attributed to SARS-CoV-2: an ICHD-3 validation study on 106 hospitalized patients. *Cephalalgia*. 2020;40(13):1432-1442. https://doi.org/10.1177/0333102420965146
5. Pezzini A, Padovani A. Lifting the mask on neurological manifestations of COVID-19. *Nat Rev Neurol*. 2020;16(11):636-644. https://doi.org/10.1038/s41582-020-0398-3
6. Silva Andrade B, Siqueira S, de Assis Soares WR, et al. Long-COVID and post-COVID health complications: an up-to-date review on clinical conditions and their possible molecular mechanisms. *Viruses*. 2021;13(4):700. https://doi.org/10.3390/v13040700
7. Nalbandian A, Sehgal K, Gupta A, et al. Post-acute COVID-19 syndrome. *Nat Med*. 2021;27(4):601-615. https://doi.org/10.1038/s41591-021-01283-z
8. Huang C, Huang L, Wang Y, et al. 6-month consequences of COVID-19 in patients discharged from hospital: a cohort study. *Lancet*. 2021;397(10270):220-232. https://doi.org/10.1016/S0140-6736(20)32656-8
9. Sampaio Rocha-Filho PA, Voss L. Persistent headache and persistent anosmia associated with COVID-19. *Headache*. 2020;60(8):1797-1799. https://doi.org/10.1111/head.13941
10. Al-Hashel JY, Ismail II. Impact of coronavirus disease 2019 (COVID-19) pandemic on patients with migraine: a web-based survey study. *J Headache Pain*. 2020;21(1):115. https://doi.org/10.1186/s10194-020-01183-6
11. Meinhardt J, Radke J, Dittmayer C, et al. Olfactory transmucosal SARS-CoV-2 invasion as a port of central nervous system entry in individuals with COVID-19. *Nat Neurosci*. 2021;24(2):168-175. https://doi.org/10.1038/s41593-020-00758-5
12. Boldrini M, Canoll PD, Klein RS. How COVID-19 affects the brain. *JAMA Psychiatry*. 2021;78(6):682-683. https://doi.org/10.1001/jamapsychiatry.2021.0500
13. Vgontzas A, Pavlović JM. Sleep disorders and migraine: review of literature and potential pathophysiology mechanisms. *Headache*. 2018;58(7):1030-1039. https://doi.org/10.1111/head.13358
14. Negro A, Seidel JL, Houben T, et al. Acute sleep deprivation enhances susceptibility to the migraine substrate cortical spreading depolarization. *J Headache Pain*. 2020;21(1):86. https://doi.org/10.1186/s10194-020-01155-w
15. Minen MT, Begasse De Dhaem O, Kroon Van Diest A, et al. Migraine and its psychiatric comorbidities. *J Neurol Neurosurg Psychiatry*. 2016;87(7):741-749. https://doi.org/10.1136/jnnp-2015-312233

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