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COVID-19 clinical and epidemiological profiles. Risk-group characterization in patients with chronic conditions and multimorbidity

Kevin Bliek Bueno

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COVID-19 clinical and epidemiological profiles. Risk-group characterization in patients with chronic conditions and multimorbidity.

Doctoral thesis dissertation presented by

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to apply for the degree of Doctor at the University of Barcelona.

Doctoral Program in Medicine and Translational Research

School of Medicine and Health Sciences. University of Barcelona

June 2024

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ACKNOWLEDGMENTS

I would like to start by thanking my tutor during my years of residency, Dr. Alexandra Prados Torres, for giving me the opportunity to take my first steps as a young researcher. She shared with me her passion for conceptualizing and dissecting the problems that our most complex patients face and taught me the significance of translating our findings into action, to try to answer the questions that can improve the healthcare system and the lives of those who need it most.

A most special thanks to my directors. To Dr. Antonio Gimeno Miguel, whom with I have shared a desk and many cups of coffee along the way, who has been a mentor and a driving force for my achievements in research, and who has selflessly offered his wisdom, his positivity, and his capacity to decisively capture and express what is important. And to Dr. Beatriz Poblador, for her reliability in all our work, and for sharing her vast experience and knowledge. Thank you both.

I am extremely fortunate to have found and nurtured friendships that have stood the tests of time and distance. Thank you to my dear friends from Ávila and Salamanca, who have helped shape the person and the professional that I am today. Our time together has been and continues to be invaluable, and I cannot wait for us to share many more achievements and adventures.

Thank you to my tutors, colleagues, and friends from Zaragoza, who made a home for me in a new city. We went through thunderstorms, the unending working hours and a global pandemic together, and my years among those windy streets and uneven bicycle paths have been some of the most fulfilling and gratifying of my life.

I would also like to thank my parents for their guidance, their support and their love. Thank you for the books you read to me, the places we have visited together, and for always having my back. I carry your teachings with me every day, and I strive to do them justice wherever I go. For this, I will be forever grateful.

Finally, thank you Carlos for your serenity, your unwavering support, and for being a steadfast presence in my life. I would not have made it here without you.

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LIST OF ARTICLES IN THE THESIS

Thesis in compendium of publications format. The thesis consists of 1 general objective, five specific objectives, and four articles:

1. Poblador-Plou B, Carmona-Pérez J, Ioakeim-Skoufa I, Poncel-Falcó A, **Bliek-Bueno K**, Cano-Del Pozo M, Gimeno-Feliú LA, González-Rubio F, Aza-Pascual-Salcedo M, Bandrés-Liso AC, Díez-Manglano J, Marta-Moreno J, Mucherino S, Gimeno-Miguel A, Prados-Torres A, EpiChron Group. Baseline Chronic Comorbidity and Mortality in Laboratory-Confirmed COVID-19 Cases: Results from the PRECOVID Study in Spain. *Int J Environ Res Public Health*. 2020 Jul 17;17(14):5171.
2. Gimeno-Miguel A, **Bliek-Bueno K**, Poblador-Plou B, Carmona-Pérez J, Poncel-Falcó A, González-Rubio F, Ioakeim-Skoufa I, Pico-Soler V, Aza-Pascual-Salcedo M, Prados-Torres A, Gimeno-Feliu LA; PRECOVID Group. Chronic diseases associated with increased likelihood of hospitalization and mortality in 68,913 COVID-19 confirmed cases in Spain: A population-based cohort study. *PLoS One*. 2021 Nov 12;16(11):e0259822.
3. **Bliek-Bueno K**, Mucherino S, Poblador-Plou B, González-Rubio F, Aza-Pascual-Salcedo M, Orlando V, Clerencia-Sierra M, Ioakeim-Skoufa I, Coscioni E, Carmona-Pérez J, Perrella A, Trama U, Prados-Torres A, Menditto E, Gimeno-Miguel A. Baseline Drug Treatments as Indicators of Increased Risk of COVID-19 Mortality in Spain and Italy. *Int J Environ Res Public Health*. 2021 Nov 10;18(22):11786.
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ABSTRACT IN ENGLISH

INTRODUCTION

In December 2019 an outbreak of pneumonia cases of unknown etiology was reported in Wuhan, China. The disease, eventually known as COVID-19, was caused by the novel Severe Acute Respiratory Syndrome Coronavirus 2, or SARS-CoV-2. The virus quickly crossed borders and spread through other countries turning into a global pandemic, and in March 2020 the World Health Organization declared it a Public Health Emergency of International Concern. The number of cases of a new and highly contagious virus to which the whole population was susceptible forced governments and communities worldwide to take drastic public health measures, reshaping and disrupting the daily lives of thousands of millions of people.

COVID-19 proved to be a markedly heterogeneous disease, ranging from asymptomatic or pauci-symptomatic to severe or even fatal infections. The wide range of clinical presentations and the severity and lethality in some cases called for the timely identification of risk factors and of especially vulnerable populations to target and prioritize public health prevention measures, access to healthcare and medical assistance, as well as access to vaccination when available.

The impact of age or the differences due to sex on COVID-19 prognosis were some of the first characteristics that stood out as determining factors for disease severity. Aside from sociodemographic characteristics, however, a wide variety of chronic diseases were considered as risk factors for severe presentations of COVID-19. Though some subgroups of diseases such as cardiovascular and respiratory conditions were identified early on, there was still room for improvement in the understanding of how different kinds of chronic conditions co-exist, interact with each other, and affect the outcomes of COVID-19 infections. Furthermore, multimorbidity, which associates more risks than could be expected from the sum of the individual chronic conditions that conform it, showed a variability and complexity in the derived clinical presentations that warranted further research for the development of personalized approaches for prevention and the clinical management of patients during and after COVID-19 infection.

HYPOTHESIS

Analyzing the clinical and demographic characteristics of patients affected by COVID-19, especially those with more severe presentations of the infections, can help identify the profiles with a higher risk of potential complications and unfavorable health outcomes, as well as to elaborate clinical and public health strategies that can anticipate their occurrence or even prevent them, reducing the impact of SARS-CoV-2 infection.

OBJECTIVES

The general objective was to analyze, from a clinical, epidemiological, and population-based perspective, the clinical and demographic characteristics of COVID-19 disease and identify which conditions can predict greater severity or less favorable prognoses.

The specific objectives were: i) To exhaustively describe the demographic and clinical characteristics of patients affected by COVID-19 through population-based studies in the Autonomous Community of Aragón; ii) To identify and characterize the demographic and clinical risk factors of poor health outcomes, especially considering the underlying chronic conditions and treatments of the population; iii) To identify and characterize the existing multimorbidity profiles in patients with COVID-19 and analyze their impact on the disease's severity; iv) to compare the results from Aragón regarding the clinical and epidemiological characterization of COVID-19 with other affected areas.

METHODS AND MAIN FINDINGS

We conducted a series of retrospective cohort studies including all individuals with laboratory-confirmed SARS-CoV-2 infection in the Spanish region of Aragon, as well as the Italian Region of Campania in Manuscript 3, in various stages of the pandemic. We exhaustively identified all chronic conditions and medications present in the electronic health registries of each patient and followed them to determine the occurrence of selected unfavorable health outcomes including all-cause mortality, hospitalization or both. In Manuscript 4, we used network analysis to characterize the multimorbidity profiles present within our population, stratified by age and sex. Finally, we performed multivariate logistic regression to analyze the association between each chronic disease or multimorbidity pattern and infection severity.

One of the main findings of our work is that old age was the most influencing factor for severe COVID-19 infection, increasing the odds of severity up to eight-fold in patients over the age of 80 years. Women and men shared similar but not identical chronic disease risk profiles. Congestive heart failure, diabetes, chronic renal failure and obesity increased the likelihood of severe infection between 21% and 39% in both sexes. Chronic skin ulcers, acute cerebrovascular disease, chronic obstructive pulmonary disease, urinary incontinence and neoplasms in men, and infertility, obstructive sleep apnea, hepatic steatosis, rheumatoid arthritis and menstrual disorders in women were also associated with more severe outcomes, with higher odds of between 18% and 87%.

We also found that the presence at baseline of the treatments intended for these conditions such as potassium-sparing agents, antipsychotics, vasodilators, high-ceiling diuretics or antithrombotic agents were also systematically associated with mortality in COVID-19 patients. These treatments could therefore be used as proxy for them, serving as timely indicators of an increased likelihood of mortality after the infection, and the assessment of pharmacological profiles could be an additional approach to the identification of at-risk individuals in clinical practice.

Regarding the results of our network analyses, we found that multimorbidity was more frequent than single non-communicable diseases in chronic patients affected by COVID-19. Furthermore, not all multimorbidity patterns showed the same risk of COVID-19 hospitalization or mortality, and this risk depended on the components of the pattern itself, apart from age and sex. Poor health outcomes were mainly associated with cardiovascular and metabolic patterns in which diabetes and obesity were most prominent conditions, especially in older women and men.

CONCLUSIONS

Comorbidity and medication burden at the time of infection can be predictors of the risk of poor COVID-19 health outcomes, with women and men sharing similar but not identical chronic disease risk profiles, and advanced age being by far the most influencing factor. Specific cardiovascular and metabolic conditions such as heart

failure, diabetes mellitus, obesity and chronic renal disease consistently increased the risk of severe SARS-CoV-2 infections.

Baseline chronic treatments for COVID-19 chronic risk factors are systematically associated with mortality in SARS-CoV-2-infected patients, and the assessment of pharmacological profiles in COVID-19 patients as proxy of the chronic diseases they are intended to treat can be an alternative approach to a prompt identification of at-risk individuals in clinical practice.

The characterization of multimorbidity profiles is necessary to better identify vulnerable individuals. Patterns that include principally cardiovascular and metabolic diseases are the main aggravating chronic factors that affect COVID-19 outcomes, with diabetes mellitus in women and obesity in men acting as key disease attractors for other chronic conditions within their respective sex-specific multimorbidity patterns.

ABSTRACT EN ESPAÑOL

INTRODUCCIÓN

En diciembre de 2019 se notificó un brote de casos de neumonía de etiología desconocida en Wuhan (China). La enfermedad, finalmente denominada COVID-19, estaba causada por un nuevo coronavirus del Síndrome Respiratorio Agudo Severo, o SARS-CoV-2. El virus cruzó fronteras rápidamente y se propagó por otros países convirtiéndose en una pandemia mundial, declarada en marzo de 2020 por la Organización Mundial de la Salud como una Emergencia de Salud Pública de Importancia Internacional. El número de casos de un virus nuevo y altamente contagioso al que era susceptible toda la población obligó a gobiernos y comunidades de todo el mundo a tomar medidas drásticas de salud pública, remodelando y alterando la vida diaria de miles de millones de personas.

La COVID-19 demostró ser una enfermedad marcadamente heterogénea, que abarcaba desde infecciones asintomáticas o paucisintomáticas hasta infecciones graves o incluso mortales. El amplio abanico de presentaciones clínicas y su gravedad y letalidad en algunos casos requerían de una identificación temprana de los factores de riesgo y de las poblaciones especialmente vulnerables para dirigir y priorizar las medidas de prevención de salud pública, el acceso a la asistencia sanitaria y médica, y el acceso a la vacunación.

El impacto de la edad o las diferencias atribuibles al sexo en el pronóstico de la COVID-19 fueron algunas de las primeras características que destacaron como factores determinantes de la gravedad de la enfermedad. Sin embargo, aparte de las características sociodemográficas, una amplia variedad de enfermedades crónicas se consideraron factores de riesgo para las presentaciones graves de la COVID-19. Aunque desde el inicio se identificaron algunos subgrupos de enfermedades, como las cardiovasculares y respiratorias, existía margen de mejora en cuanto al entendimiento de cómo coexisten los distintos tipos de enfermedades crónicas, cómo interactúan entre sí y cómo afectan a los resultados de las infecciones COVID-19. Además, la multimorbilidad, que asocia más riesgos de los que cabría esperar de la suma de las afecciones crónicas individuales que la conforman, mostraba una variabilidad y

complejidad en sus presentaciones clínicas que justificaban una investigación más profunda que permitiera el desarrollo de enfoques personalizados para la prevención y el manejo clínico de estos pacientes durante y después de la infección.

HIPÓTESIS

Analizar las características clínicas y demográficas de los pacientes COVID-19, especialmente de aquellos con presentaciones más graves de la infección, puede ayudar a identificar los perfiles con mayor riesgo de complicaciones potenciales y resultados desfavorables para la salud, así como a elaborar estrategias clínicas y de salud pública que puedan anticipar su aparición o incluso prevenirlas, reduciendo el impacto de la infección por SARS-CoV-2.

OBJETIVOS

El objetivo general fue analizar, desde una perspectiva clínica, epidemiológica y poblacional, las características clínicas y demográficas de la COVID-19 e identificar qué patologías pueden predecir una mayor gravedad o un pronóstico menos favorable.

Los objetivos específicos fueron: i) Describir exhaustivamente las características demográficas y clínicas de los pacientes afectados por COVID-19 a través de estudios de base poblacional en la Comunidad Autónoma de Aragón; ii) Identificar y caracterizar los factores de riesgo demográficos y clínicos de peores resultados en salud, especialmente considerando las enfermedades crónicas subyacentes y los tratamientos de la población; iii) Identificar y caracterizar los perfiles de multimorbilidad existentes en pacientes COVID-19 y analizar su impacto en la gravedad de la infección; iv) Comparar los resultados de Aragón en cuanto a la caracterización clínica y epidemiológica de la COVID-19 con otras áreas afectadas.

MÉTODOS Y HALLAZGOS PRINCIPALES

Se realizaron una serie de estudios de cohortes retrospectivos que incluyeron a todos los individuos con infección por SARS-CoV-2 confirmada mediante pruebas de laboratorio en la región española de Aragón, así como en la región italiana de Campania en el Manuscrito 3, en diversas fases de la pandemia. Se identificaron exhaustivamente todas las enfermedades crónicas y medicaciones presentes en los

registros electrónicos de salud de cada paciente y se les hizo un seguimiento para determinar la aparición de resultados de salud desfavorables, incluyendo mortalidad por todas las causas, hospitalización o ambas. En el Manuscrito 4, utilizamos análisis de redes para caracterizar los perfiles de multimorbilidad presentes en nuestra población, estratificados por edad y sexo. Por último, realizamos una regresión logística multivariante para analizar la asociación entre cada enfermedad crónica o patrón de multimorbilidad y la gravedad de la infección.

Uno de los principales hallazgos de nuestro trabajo fue que la edad avanzada fue el factor que más influyó en la gravedad de la infección COVID-19, aumentando la probabilidad de gravedad en hasta ocho veces en pacientes mayores de 80 años. Mujeres y hombres comparten perfiles de riesgo de enfermedades crónicas similares, pero no idénticos. La insuficiencia cardíaca congestiva, la diabetes, la insuficiencia renal crónica y la obesidad aumentaron la probabilidad de infección grave entre un 21% y un 39% en ambos sexos. Las úlceras crónicas de la piel, enfermedad cerebrovascular aguda, enfermedad pulmonar obstructiva crónica, incontinencia urinaria y las neoplasias en los hombres, y la infertilidad, apnea obstructiva del sueño, esteatosis hepática, artritis reumatoide y trastornos menstruales en las mujeres también se asociaron a peores resultados en salud, con aumentos en la probabilidad de infección grave de entre el 18% y el 87%.

Además, se observó que la presencia basal de tratamientos destinados a tratar estas enfermedades, como los fármacos ahorradores de potasio, antipsicóticos, vasodilatadores, diuréticos del asa o agentes antitrombóticos, también se asoció sistemáticamente con un aumento de la mortalidad en pacientes COVID-19. Por ello, estos tratamientos podrían utilizarse como proxy de dichas patologías, sirviendo como indicadores de una mayor probabilidad de mortalidad tras la infección, y la evaluación de los perfiles farmacológicos podría considerarse como un enfoque adicional para la identificación de individuos en riesgo en la práctica clínica.

En cuanto a los resultados de nuestros análisis de redes, encontramos que la multimorbilidad fue más frecuente que las enfermedades crónicas aisladas en los pacientes crónicos afectados por COVID-19. Además, no todos los patrones de multimorbilidad mostraron el mismo riesgo de hospitalización o mortalidad por COVID-

19, que dependía de los componentes del patrón en sí, aparte de la edad y el sexo. Los peores resultados en salud se asociaron principalmente a patrones cardiovasculares y metabólicos donde la diabetes y la obesidad eran las patologías más relevantes, especialmente en mujeres y hombres de edad avanzada.

CONCLUSIONES

Las cargas de comorbilidad y de medicación en el momento de la infección pueden ser factores predictivos del riesgo de resultados desfavorables en salud tras la infección COVID-19. Las mujeres y los hombres comparten perfiles de riesgo de enfermedades crónicas similares, pero no idénticos, y la edad avanzada es el factor más influyente. Determinadas enfermedades cardiovasculares y metabólicas, como la insuficiencia cardíaca, la diabetes mellitus, la obesidad y la enfermedad renal crónica, aumentaron sistemáticamente el riesgo de infecciones graves por SARS-CoV-2.

Los tratamientos crónicos basales para factores de riesgo crónicos para la COVID-19 se asociaron sistemáticamente con una mayor probabilidad de mortalidad en pacientes infectados, y la evaluación de los perfiles farmacológicos en pacientes COVID-19 como proxy de las enfermedades crónicas que están destinados a tratar puede ser un enfoque alternativo para una pronta identificación de los individuos de mayor riesgo en la práctica clínica.

La caracterización de los perfiles de multimorbilidad es necesaria para identificar a los individuos más vulnerables. Los patrones que incluyen fundamentalmente enfermedades cardiovasculares y metabólicas son los principales factores crónicos agravantes para la COVID-19, con la diabetes mellitus en mujeres y la obesidad en hombres actuando como atractores clave de otras enfermedades crónicas dentro de sus respectivos patrones de multimorbilidad.

1. INTRODUCTION

1.1. EPIDEMIOLOGICAL CONTEXT OF THE COVID-19 PANDEMIC

1.1.1. Initial outbreak

In December 2019, the Municipal Health Commission of Wuhan, the capital city of the province of Hubei, China, reported an outbreak of pneumonia cases of unknown etiology (1). A few days later, in January 2020, the Chinese Center for Disease Control and Prevention (CDC China) reported that a novel coronavirus was responsible for the increase in cases of respiratory disease with symptoms ranging from dry cough and fatigue to more severe presentations with fever, myalgia, dyspnea, and bilateral lung infiltrates and other radiological signs of pneumonia (2). This novel coronavirus would eventually come to be known as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and was classified as such by the International Committee on Taxonomy of Viruses (ICTV) in February 2020. The disease itself was called COVID-19 (3).

SARS-CoV-2 is not the first coronavirus to cause a large-scale outbreak. Other examples are those caused by Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-1) in 2002, with over 8000 cases and 11% mortality spanning 30 countries (4), or the Middle East Respiratory Syndrome (MERS-CoV) in 2012, with over 2500 cases in 27 Member States and 35% mortality (5). However, the magnitude and consequences of COVID-19 have been unprecedented in recorded history, affecting the practical totality of countries in the world, barring the most isolated nations, and severely impacting the health of populations, the economy, travel, and the lives of millions of people worldwide (6).

1.1.2. The global threat of COVID-19

Though detected initially in China, COVID-19 quickly crossed borders and started spreading through other countries in Asia, Europe, Africa, and America. This was favored by the limited information that was available in the initial stages regarding the nature, mutation capacity, incubation period, transmission mechanisms and transmissibility of the virus, as well as due to social conditions such as large gatherings and massive national and international travel worldwide, which allowed COVID-19 to appear rapidly throughout the globe (7–9). The magnitude of the epidemic led the

World Health Organization (WHO) to eventually declare it a global pandemic and a Public Health Emergency of International Concern in March 2020 (10).

The very initial estimates with data from China suggested that the virus could spread exponentially with around a 0.1/day growth rate, epidemic doubling times of 6 to 7 days approximately and a basic reproductive number (R_0) of up to 2.7 (11). Predictive models developed soon after including data from several European countries and the United States estimated that the growth rate was much higher, of up to 0.2-0.3/day depending on the country, with a doubling time of up to 3.7 days (12). As new variants started to appear, and although the quality of the data and the heterogeneity of the situations across countries made many findings unreliable (13), the COVID-19 R_0 was estimated to reach up to an average of 8.2 based on information from studies conducted in different parts of the world, and grew with the constant progression of the virus' characteristics to more contagious sub-lineages (14,15).

The sheer number of cases of a completely new and highly contagious virus to which the whole population was susceptible, before the development of effective targeted treatments and vaccines, forced governments and communities worldwide to take drastic public health measures (16). It soon became clear that detecting, quarantining, and contact-tracing symptomatic cases alone was not enough to control the epidemic, and that asymptomatic and presymptomatic transmission was possible and contributing to the rapid spread of the virus (17,18). Many countries initially enforced restrictive measures on the general population of varying intensities, going from recommended or compulsory testing, physical distancing, limitations on travel, use of masks and hand hygiene measures, to full population-based or "hard" lockdowns, barring essential workers and activities (19). Due to the long-term unsustainability of the more severe isolation measures and their effects on population health, social activities, and global economies (20–23), strategies evolved from mass confinements towards contain-delay-mitigate approaches. These methods aimed at developing and implementing targeted actions to contain the virus through case detection and isolation, delaying transmission to flatten epidemic curves allowing healthcare systems to cope with new cases without collapsing or, in worst-case scenarios, mitigating the

damages caused by the virus by preserving the health and safety of as many people as possible (24).

1.1.3. Surveillance systems, diagnostics, and treatments

The need for action and the implementation of public health measures brought along a need for information, which led to the establishment of an unparalleled surveillance apparatus (25). Most countries were unprepared and suffered from technical and material constraints for COVID-19 diagnosis in the initial stages of the pandemic, making surveillance extremely challenging and greatly understating the virus' incidence and prevalence (26,27). Health systems were forced to build up testing and diagnostic capacities on the go combining different approaches, from clinical surveys to laboratory tests for molecular diagnosis, such as real-time polymerase chain reaction (RT-PCR) and serological assays (28,29). The later introduction of affordable, equipment-free antigen tests based on lateral flow immunoassays, purchasable in pharmacies and available at point of use in hospitals and primary care, despite varying sensitivities and specificities, revolutionized detection once again making diagnosis simpler, faster, and more deliverable to end users (30–32).

Increased diagnostic capacity and data contributions from countries all over the world led to complex information dashboards, such as the ones offered by Johns Hopkins University or WHO, which allowed governments, but also media and the public, to follow up on the state of the art of the pandemic with an unprecedented level of scrutiny using free open-access epidemiological data (33). In its later stages, when such an exhaustive level of detection and notification ceased to be as relevant, surveillance shifted towards less costly methods with alternatives such as the detection of the virus in wastewater, or the inclusion of COVID-19 in traditional sentinel acute respiratory infection systems used for other viruses such as the flu and the respiratory syncytial virus (RSV) (34,35). Notwithstanding the changes in surveillance requirements and intensity, as of November 2023 the WHO COVID-19 dashboard reports there have been a total 771.820.937 confirmed and notified cases of COVID-19, including 6.978.175 deaths, and a total of 13.534.474.309 vaccine doses have been administered around the world (36).

The demand for large-scale and timely detection tools was called for, especially considering the COVID-19 pandemic quickly led to millions of infections and casualties (37), with more and more people requiring medical assistance and straining healthcare systems worldwide as the virus spread (38). Like with other respiratory viruses, the cornerstone of COVID-19 treatment consisted of supportive care and respiratory support, including conventional oxygen therapy, high-flow oxygen, or even mechanical ventilation (39). Though some traditional pharmacological options were considered and recommended in the initial stages of the epidemic, such as hydroxychloroquine, or antibiotics like azithromycin, their lack of effectiveness and inability to improve respiratory symptoms and reduce mortality forced researchers and clinicians to explore other therapeutic options (40). Further research led to include different categories of targeted antiviral agents such as the polymerase inhibitor remdesivir, novel antivirals such as molnupiravir, fluvoxamine and paxlovid, and complimentary anti-inflammatory drugs such as steroids, which have become staples of the therapeutic arsenal when dealing with COVID-19 infections (41,42).

1.1.4. Developments in immunization

Despite improvements in the clinical management of the infection and the wider variety of available treatments, the single most impactful element that allowed to completely turn the tide of the pandemic at a population-based level and prevent millions of deaths in the world was the development of effective COVID-19 vaccines (43). The pressing need for a solution to the ongoing crisis drove governments and not-for profit entities to invest in vaccine-related actions, with a 9-billion-euro investment in research, development and manufacturing of the vaccines authorized in the European Union alone, and a 21-billion-euro investment for companies in the form of advance purchase agreements (44,45).

The race for the development and commercialization of an effective COVID-19 vaccine led researchers and the pharmaceutical industry to test and develop different vaccine platforms, from the more traditional inactivated, live attenuated or subunit, to the more innovative viral vector, and DNA- or mRNA-based vaccines (46). According to the phase III trial results reported by manufactures on vaccine efficacy, and though effectiveness varied due to population-dependent factors, some vaccines reduced

COVID-19 severity in up to 100% of patients in controlled settings (47). Specifically, mRNA vaccines revolutionized the vaccine development landscape through the sequencing of the viral genome and nucleoside base modifications, showing the capacity to prevent hospitalization or death in up to 96% of patients after a full immunization, and were the first to obtain marketing authorization, (48,49). These advances, which enabled the development of rapid and highly effective vaccines against COVID-19, would eventually lead to a Nobel prize in Medicine in 2023 (50).

Vaccine development and massive vaccination campaigns finally allowed governments to scale back on other preventive measures and to gradually return to a pre-pandemic lifestyle (51,52), notwithstanding the differences in acceptance and hesitancy across different countries, and the unequal distribution of vaccine availability in developed versus developing countries (53–55). However, sustained COVID-19 transmission and its capacity to mutate led to the emergence of different variants and lineages, in some cases with sufficient fundamental variations to be able to escape predeveloped immunity and cause breakthrough infections (56). These changes, often granting increased transmissibility or severity, have continued to generate public health challenges for the scientific community, governments, and other stakeholders over the past years (57–59). Waning immunity or breakthrough infections caused by new variants has called for the adaptation of vaccines to current variants and for revaccination with periodical variant-specific or pan-coronavirus boosters (60). The inclusion of COVID-19 vaccination in seasonal flu immunization programs has led to updated coverages, though how often this will be necessary remains to be seen in the long term (61).

1.1.5. The aftermath of the pandemic

As of 5 May 2023, the WHO Emergency Committee on the COVID-19 pandemic declared that SARS-CoV-2 constitutes an ongoing health issue but no longer warrants the status of Public Health Emergency of International Concern. In its statement, WHO Director General did however recommend several key points that must be considered for all State Parties such as sustaining national capacity gains and response for future events, integrate COVID-19 vaccination into regular vaccination schedules, accrue

holistic information for circulating respiratory pathogens, solidify the supply and availability of medical countermeasures, and continue to support related research (62).

SARS-CoV-2 and the COVID-19 pandemic were capable of reshaping and disrupting the daily lives of thousands of millions of people for months, while also completely upsetting and revolutionizing scientific research and the production of the scientific community from 2020 onwards (63). From December 2019 to December 2023, a total of 2.261.569 COVID-19-related publications have been published, 1.781.657 (78,8%) of which are research articles, and 180.613 (8,0%) of which are dedicated chapters in books (64,65). Keeping up with this unfathomable amount of information would equate to reading 1549 articles per day during the past four years since the beginning of the pandemic, a never-before-seen torrent of information with any other topic. With a majority of research articles lacking original data, and many of them leaning towards expert opinions exclusively or showing high risk of bias, an equilibrium must be found going forward between timely and deliverable information versus quality and rigor to push forward evidence-based knowledge and data (66).

1.2. SARS-CoV-2

1.2.1. Origin

SARS-CoV-2 is an enveloped single strand RNA β -coronavirus with a crown-like structure (67). Like other coronaviruses, the origin of SARS-CoV-2 was traced back to bats and was found to be extremely close genomically to several other bat-related SARS-like coronavirus strains (68). Bats would therefore serve as reservoir hosts, with other mammals like civets as thought initially, and upon further investigation more likely pangolins, acting only as intermediate hosts (69). At some point, similarly to how SARS-CoV and MERS-CoV developed the capacity to infect human beings, a spillover effect occurred with SARS-CoV-2 towards humans with subsequent human-to-human transmission, most likely due to direct or indirect contact, animal handling or even the consumption of infected animals (70,71). The reality under which this qualitative inter-species jump occurred is probably multifaceted, and can be attributed to a variety of coexisting factors, mainly the conditions of the animals kept and handled in wet

markets, with substantial evidence linking the initial cases to such a place in Wuhan, but also to the illegal trading of wildlife and environmental destruction, which can lead to closer and less controlled contact with infectious animals (72).

1.2.2. Transmission

SARS-CoV-2 human-to-human transmission occurs mainly through respiratory droplets produced by infected individual while talking, coughing, or sneezing (67,73). When in proximity, these droplets contact mucosal surfaces of susceptible individuals such as those of the eyes, nose, and mouth, and through them gain access to the upper respiratory tract (74). The droplets have varying sizes, and in some cases are small enough to allow aerosolization, enabling virus suspension, thus increasing its contagiousness, and facilitating the direct contraction of the virus (75).

Elimination is also possible through other bodily fluids such as feces or urine, though the virus has a special tropism towards the respiratory tract, and this constitutes the main transmission pathway (75,76). Furthermore, after setting on different surfaces and materials, infectious droplets containing SARS-CoV-2 allow it to survive for hours or even days depending on the characteristics and conditions of the location and the environment. Though less impactful than direct transmission, this capacity to remain viable on surfaces and commonly used materials enables indirect transmission through fomites, especially after repeated exposure to frequently touched surfaces (74,77). After the acquisition of the virus, as stated mainly through the contact with infectious respiratory droplets, it binds to the nasal multiciliated epithelial cells of the new host, from where it can progress to the conducting airways, and subsequently the upper and lower respiratory tract, as well as disseminate throughout the body to affect other organs and systems (74,76,78).

1.2.3. Variants

Like other respiratory viruses, SARS-CoV-2 evolves while spreading among people and over time and, with sufficient changes, can become a new subtype of the virus known as a variant (59). While some variants can appear and disappear more quickly, others remain predominant for extended periods of time and can persist until becoming the main strand in transmission (79). WHO establishes different terms based on the characteristics of these variants and considers three categories (80). The first

encompasses variants under monitoring (VUM), which refer to those that should be closely monitored from a public health perspective to identify potential threats for global health compared to other variants. The second comprises variants of interest (VOI), which already possess significant changes that are known to modify the virus' behavior or how it can affect human health. And finally, variants of concern (VOC) are VOIs that can cause a detrimental variation in disease severity, hinder the ability of health systems to provide healthcare services and therefore require larger-scale public health interventions, and/or decrease the effectiveness of available vaccines (80,81).

To facilitate identification and communication, VOCs have been named following the Greek alphabet and five of them have been notified so far with different characteristics: Alpha, with increased severity and low immune evasion; Beta, more severe and with higher transmissibility; Gamma, with stronger humoral immune evasion and infectivity; Delta, causing less severe disease but with far greater transmissibility; and Omicron, the most recently identified, competitive and prevalent variant, which is more transmissible than any of the previous variants though is not particularly associated with more severe presentations of the disease (82). As of its initial identification in November 2021, several Omicron sub-lineages have been identified such as BA.1 to BA.5, and the BA.5-derived BQ1.1 and XBB.1, but without the substantial differences required to be classified as a new VOC (83,84).

1.2.4. Structure

Irrespective of the variant, the virus possesses a crown-like structure to which it owes its name composed of surface glycoproteins called spike (S) proteins. The S protein is fundamental in the virus' pathogenesis as it contains specific domains with subunits such as the S1 subunit, which acts as a receptor-binding site giving the virus the capacity to enter targeted host cells (85). Barring S proteins, SARS-CoV-2 genetically encodes three other key structural proteins namely the envelope (E), nucleocapsid (N) and membrane (M) proteins, as well as non-structural proteins necessary for processes such as assembly and replication (86). The N protein, generated in large quantities during the infection, packages the viral genome and aids in modifying cellular processes and viral replication, the M protein participates in membrane budding and

the release of new virions after replication, while the E protein is the smallest of the four and helps the virus assemble and escape infected cells (87).

1.2.5. Pathogenesis

The S protein binds through S1 to a ubiquitous host cell receptor called the angiotensin-converting enzyme 2 receptor (ACE-2), which can be found on the cell surface of many tissues across the human body, most notably in the respiratory tract, heart, blood vessels, liver, kidneys, small intestine, colon, adipose tissue, or thyroid, among others (88). In addition to being one of the most critical elements of the virus' pathophysiology, the S protein is one of the major targets of the host immune response, vaccines and treatments, and its molecular variability have allowed the virus through different mutations to increase its binding affinity to ACE-2 receptors over time, or even to elude the immunity generated after an exposure to previous variants (89,90). The inclusion in surveillance of viral whole genome sequencing along the course of the pandemic has warranted a more in depth understanding of the main mutational targets of SARS-CoV-2 that contribute to its virulence and differentiation (71).

The ACE-2 receptor, with the participation of host type II transmembrane serine proteases (TMPRSS2), allows the virus to enter the cells through receptor-mediated endocytosis and generate the infection, causing cell invasion, damage, and destruction (76,91). Once the virus has attached and penetrated within the cell, it frees its genetic RNA-based material and uses cell ribosomes to generate viral polyproteins, which are then cleaved into effector proteins, and an RNA polymerase to synthesize new viral genomic RNA and thus allow for viral replication within the host cell (92). Finally, the new virions are assembled within the Golgi body and endoplasmic reticulum and released through exocytosis thus completing the virus' full multiplication cycle (93).

1.3. IMMUNE RESPONSE TO SARS-CoV-2

Many viruses, including SARS-CoV-2, display an intrinsic capacity to directly damage their hosts (94). The process of viral replication within host cells causes direct tissue and cell destruction, which constitutes the first phase of aggression by the virus during its pathogenesis. However, this is not the only mechanism that can cause harm or

aggravate the course of viral infections, as the subsequent immune response from the host can act as a friend and foe, with the potential to cause excessive collateral damage towards the host (95). Though infections can activate both innate and adaptive responses intended to contain the aggression, direct viral toxicity can lead to an excessive activation with secondary damage, both of which appear to modulate disease severity (95–97).

1.3.1. Innate immune response

The innate immune system constitutes the first barrier of protection when facing viral infections, removing infected cells, and recruiting a range of components that initialize a cascade of reactions that eventually kickstart an adaptive response, all of which, when functioning correctly, generate a coordinated defense that can contain the infection and ultimately lead to its resolution (95,98). Specifically, macrophages, neutrophils, dendritic cells, natural killer (NK) cells, eosinophils, basophils, and innate lymphoid cells (ILCs) are all different types of cells pertaining to the innate immunity that are activated when infected by SARS-CoV-2. The virus' specific viral patterns can be detected by a variety of host pattern recognition receptors present in these cells, including membrane receptors, such as the toll-like receptors, and cytosolic receptors, such as NOD-like receptors, absent in melanoma 2-like receptors, and retinoic acid-inducible gene-I-like receptors (98,99).

Responding cells become activated after recognizing viral patterns and undergo a series of modifications in their location, form, activation status, metabolism, and the production of molecules, impacting other cellular types and continuing the immune activation chain (94). Antiviral innate immune response actors against the virus also include humoral elements such as natural antibodies, mainly IgM and IgA, the complement and coagulation-fibrinolysis systems, interferons, cytokines, or chemokines (97). All these elements engage in complex interactions that contribute to identifying the virus, stimulating phagocytosis, and modulating inflammation, creating a state of viral resistance, and activating the adaptive response (97,100).

1.3.2. Adaptive immune response

An adaptive response after exposure to the virus is necessary for the control of the infection and the development of protective immunity, with both humoral and cellular

elements, mainly B cells, antibodies, CD4+ T cells, and CD8+ T cells (101). Through different signaling pathways, the stimulus of the infection leads B cells to differentiate into two subtypes, long-lasting high-affinity antibody-producing plasma cells and germinal center-dependent memory B cells that will be able to respond faster in future exposures to the same antigen (102). The S protein is the most antigenic component of the virus, allowing for the development of targeted anti-SARS-CoV-2 antibodies of three classes: IgA, which peak 20-22 days after the infection, IgM, peaking at 10-12 days after the infection and disappearing after around 18 days, and the longer-lasting IgG antibodies, which are responsible for long-term immunity (97,103). The activation of CD4+ T helper cells also leads to differentiation, mostly mediated by cytokines, into subpopulations such as Th2 and follicular subtypes, which aid in signaling and maturation of B cells, a memory subtype, and a Th1 subtype, which are critical for the activation of CD8+ T cells, with cytotoxic activity and the capacity to differentiate into memory cytotoxic T cells (102). Although humoral immunity is much easier to measure and quantify, the adaptive cellular response is one of the most critical elements of the defense against viruses such as SARS-CoV-2, and robust T cell immune reactions have been demonstrated after COVID-19 infection, with deficits in CD4+ and CD8+ T cell counts correlating with more severe forms of the disease and its complications (97,104). The initial activation of innate mechanisms and a healthy coordinated response of B, and CD4+ and CD8+ T cells are all necessary to successfully overcome the infection and, among other factors, influence the gravity of the clinical presentations and health outcomes (102).

1.3.3. Immune hyperactivation

Under normal circumstances, a functional and balanced immune response can impede the progression of the infection to achieve full recovery while limiting secondary damage to the host (71). However, in the presence of a dysregulated immune system, SARS-CoV-2 can induce a potentially life-threatening hyperinflammatory response that is disproportionate to the threat of the virus and that contributes to disease severity (105). Though inflammation is one of the cornerstones of the immune response, when uncontrolled in the context of an impaired immune response, the mass liberation of proinflammatory mediators can prove fatal (106). This exacerbated systemic

inflammatory response marked by high levels of circulating cytokines and chemokines and the hyperactivation of host immune cells is known as the cytokine release syndrome or cytokine storm (107). Patients that develop this condition are prone to constitutional symptoms, severe damage of pulmonary tissue, and eventual multiorgan dysfunction that can evolve to multiorgan failure or even death if not appropriately treated (108,109).

1.4. COVID-19 CLINICAL PRESENTATIONS

COVID-19 is a markedly heterogeneous disease in its incubation period, clinical characteristics, severity, and health outcomes, ranging from asymptomatic or paucisymptomatic to severe or even fatal infections. The presentations can be targeted and localized to specific organs or systems, which especially affect the lungs and respiratory system, or appear as systemic infections with multi-organ affectation (110).

1.4.1. Incubation period

The infection's incubation period, the lapse of time between the moment of exposure to the virus and symptom onset, is variable and fluctuates depending on disease severity, the characteristics of the patient and the variant of the virus that causes it (111). Broadly, WHO sets a wide range for incubation, from 1-14 days, with an average of 5 to 6 (80), while the European Center for Disease Prevention and Control establishes the lower range at 2 days with the same maximum and average limits (112). Clinical presentation appears to influence incubation times, with some studies suggesting that patients with more severe infections have shorter intervals and patients with milder presentations have longer incubation periods, while other studies report that these differences are inconclusive (111). Age is another factor to consider regarding mean incubation, with elderly adults showing prolonged incubation compared to younger cases, possibly due to less responsive immune systems (113). In children, it is generally considered that incubation periods can also be longer, with a mean of up to around 8 days (111). The predominant variant at each given time is yet another factor that affects incubation, with Omicron being one of the fastest to translate into symptomatic infection in around 2-3 days on average, but as short as 1 day in some cases, compared to the longer 4,5-median of Delta (114).

1.4.2. Asymptomatic infection

Asymptomatic COVID-19 cases were initially extremely underdiagnosed due to a lack of a basic understanding of the virus and limited testing capacities (18). Though initial estimates were low, the accrual of data and scientific evidence led to estimate the prevalence of asymptomatic infection in up to 45% (115). The direct impact of the infection on asymptomatic individuals themselves is negligible but, as previously stated, a lack of symptoms does not equate to an interruption in transmission and can therefore contribute to perpetuating viral circulation within the community, making the upscaling of preventive measures more challenging, and posing a threat to more vulnerable patients who might encounter infectious cases unknowingly (116,117). Little is known regarding the exact mechanisms that allow some patients to overcome the infection without developing any symptoms whatsoever, and although some structural mutations at the genomic level have been postulated as potential influencing factors in asymptomatic infections, and patient-related factors play a role as well, more evidence in this field is called for (118).

1.4.3. Mild infection

The typical clinical presentation of mild COVID-19 is that of a common cold or flu-like syndrome, with symptoms that may include sore throat, cough, fever, malaise and myalgias (119). The duration of these symptoms is variable, with a median of 15 days and a range of between 8 and 24 days, with up to 25% of patients reporting durations of over three weeks (120). Aside from general and respiratory symptoms, digestive symptoms such as vomiting and diarrhea were also common, though their duration was shorter than that of coughing and expectoration (121). A more characteristic finding in COVID-19 cases that was reported from the very initial phases of the pandemic was the loss of smell or anosmia, and loss of taste or ageusia, which are considered neurological complications and can be as common as in 85% of patients (122). The mechanisms behind anosmia and ageusia appear to be the dysfunction of olfactory sensory neurons and taste buds secondary to the infection itself as well as due to local inflammation, nasal obstruction and rhinorrhea, and the subsequent loss of function of other supporting non-neuronal sustentacular cells in the mucosa (122,123). These conditions seem to be more common than with other respiratory viral

infections and can sometimes last for months on end, and while some therapeutic options have been evaluated such as pentoxifylline, caffeine, theophylline, or intranasal vitamins or corticosteroids, there is still limited information regarding its potential treatments (122).

Another typical symptom that can be present during or after the infection even in mild cases of COVID-19 is mental fatigue, also known as postinfectious fatigue syndrome, often attributed to dysfunction of the immune system and neuroinflammation (124). Patients can experience difficulties in attention, planning, memory, and decision-making, with an overall decrease in their functional status and associating prolonged negative effects on cognition and concentration (124–126). Physical fatigue or asthenia are also common after the infection, where patients report signs of tiredness, low energy, muscular fatigue, muscular weakness, and malaise (127). Though sometimes subjective, electrophysiological evidence has been found at a motor level, supporting questionnaire and patient-reported evidence (124).

1.4.4. Severe infection

Most COVID-19 patients experience mild to moderate infections (120). However, around 15-20% of cases are at risk of severe presentations with poor health outcomes, risk of hospitalization, intensive care unit (ICU) admissions and even death (128,129). In severe infections patients can become critically ill, developing acute respiratory distress syndrome (ARDS), typically about seven days after initial disease onset (130). ARDS is a non-cardiogenic pulmonary affection that results in respiratory failure and hypoxemia and can be present in up to 40% of COVID-19-related hospitalizations (131). Though present in other respiratory viruses, its etiology in SAR-CoV-2 infected patients seems to be more complex and responds to a combination of dysregulation in pulmonary perfusion, inflammation of the vascular system, loss of hypoxic pulmonary vasoconstriction, neo-angiogenesis, and intravascular thrombosis, among others (132,133). From an epidemiological standpoint, these infections are considered severe when patients experience dyspnea, more than 30 breaths per minute, blood oxygen saturation under 94% on room air, arterial oxygen partial pressure to inspired oxygen ($\text{PaO}_2/\text{FiO}_2$) under 300 mmHg, or lung infiltrates affecting more than 50% of the lungs' territory (130).

SARS-CoV-2 has a special tropism for the respiratory system and therefore, the involvement of the upper and lower respiratory tracts is common, with COVID-19-associated pneumonia being one of the most challenging and frequent presentations in severe cases (133). The severity of this presentation is conditioned by the gravity of the underlying hypoxemia, with an increasingly inefficient gas-exchange and a progression of the infiltration in the lungs, where patients can experience a growing deterioration of their respiratory distress, eventually leading to ARDS (134).

Respiratory dysfunction can motivate the need for a varying range of supportive treatments that can be more or less invasive (129). Managing these cases relies on early oxygen supplementation through different support methods to improve oxygenation and contribute to the patients' respiratory efforts (135). The use of high-flow oxygen through a nasal cannula is more accessible procedure than mechanical ventilation and can be an alternative to conventional low-flow options, though in patients additional respiratory support is indispensable (136). In cases where these options are insufficient, such as patients with hypotension or altered mentation, patients with underlying conditions such as chronic obstructive pulmonary disease (COPD) or chronic heart failure, or those that continue with work of breathing and low PaO₂/FiO₂ ratios where the use of high-flow nasal cannulas is unsatisfactory, non-invasive or invasive mechanical ventilation results crucial for the survival and recovery of patients after severe COVID-19 (132–134). Once the oxygen supply is guaranteed, other complementary treatments are warranted to improve the respiratory function, such as antivirals, targeted monoclonal antibodies, or corticosteroids (41,42,137).

1.4.5. Post COVID-19

Apart from anosmia, ageusia, and mental and physical fatigue, some patients can continue experiencing a variety of other symptoms after the initial acute phase of the infection has passed and can occur in up to 35% of patients lasting sometimes weeks or even months (138). These range from unspecific general discomfort, dizziness, headaches, sleep disorders or memory loss, to more specific symptoms like persistent cough, dyspnea, atypical chest pain and palpitations (139). Seeing as an increasing number of patients was experiencing prolonged or persistent symptomatology, and several different terms had been used to refer to the condition such as long COVID,

long-haul COVID or post-acute COVID, a WHO expert committee set out to establish the criteria to unify and define what came to be known as post COVID-19. The final definition comprised all individuals with a history of COVID-19, usually three months after initial onset, lasting for at least two months and that cannot be explained by an alternative diagnosis (140). This includes new onset symptoms following initial recovery, but also those that persist from the initial presentation or even those that fluctuate or relapse over time (127,138,140).

1.4.6. System-specific repercussions

As mentioned, the affectation of the respiratory system is generally the main component of COVID-19, however, extra-respiratory manifestations are also frequent, and can sometimes precede or be the only sign or symptom of the infection (141). As mentioned, the ACE-2 receptor to which SARS-CoV-2 binds to enter host cells is physiologically present in various systems across the human body, and can therefore facilitate symptomatology at the cardiovascular, gastrointestinal, renal, neurological, and hematological levels, among others (142).

From a cardiovascular standpoint, SARS-CoV-2 has both direct and indirect consequences. The former are secondary to direct viral cardiotoxicity, dysregulation of the renin–angiotensin–aldosterone system, endothelial damage, and thrombo-inflammation, while the latter respond to immune dysregulation and a demand/supply mismatch (143). Chest pain and arrhythmias are among the most prevalent symptoms and elevated D-dimer, troponin, and lactate dehydrogenase levels are some of the most relevant clinical findings, which correlate with the appearance of acute cardiac failure, venous thromboembolisms, or pulmonary embolisms (143,144). In fact, the risk of thrombotic and embolic events in selected patients can last even up to six months after COVID-19, especially in severe cases, with an excess risk in patients admitted to hospital and more so in those admitted to ICUs, which could even warrant the use of thromboprophylaxis in some instances. The impact of COVID-19 on coagulation can also entail decreased platelet and lymphocyte counts as evidenced by prolonged prothrombin levels, which can be caused by the virus itself and due to the subsequent endothelial damage, coagulopathy and disseminated intravascular coagulation that follow (145).

The repercussions of COVID-19 on the digestive system have also been widely reported, sometimes appearing alongside or even before the more frequent respiratory presentations (146). The exact mechanisms behind this phenomenon remain unclear. As mentioned, ACE-2 receptors are present within areas of the digestive tract and related organs such as in the duodenum, jejunum, and liver (147). Intestinal damage in SARS-CoV-2 has been proven through autopsy and biopsy, and the presence of the virus within gastric and intestinal epithelial cells have been verified under electron microscopy, which could explain the ensuing inflammatory findings and related symptomatology (146,147).

Aside from direct viral toxicity and ACE-2 dysregulation, the general inflammatory environment created by the virus alongside gut dysbiosis of the microbiota are further contributors to these outcomes (148,149). Nausea, vomiting, diarrhea, and abdominal pain are among the most common reported digestive symptoms and have been reported in around a third of patients (150). These clinical conditions seem to be associated with more severe presentations of the infection, and patients with digestive symptoms are more likely to require vasopressor drugs and non-invasive ventilation, with higher probability of ICU admissions and mortality (151). The monitoring of patients with digestive affectation, especially in more serious cases, is warranted, and the elimination of the virus in fecal samples is an additional tool both for clinical and for epidemiological surveillance purposes (35,146).

Neurological issues are commonplace during and after COVID-19, and like other respiratory viruses, SARS-CoV-2 can plausibly penetrate the central nervous system, accessing via the blood-brain barrier or trans-neuronal pathways (152,153). Barring the above symptoms regarding mental fatigue, smell and taste, neurological complications can manifest in more severe forms such as altered conscience, especially in adults, or seizures in children (154). Less common but potentially critical consequences of the infection, among others, involve demyelination, which generally occur in a pro-inflammatory context through cytokine production and T-cell stimulation (155). Furthermore, this potential inflammatory response induced by the infection can increase the risk of thromboses and stroke, leaving long lasting or even irreversible damage on affected patients (156).

1.5. COVID-19 RISK FACTORS FOR MORBIDITY AND MORTALITY

The wide range of clinical presentations and the severity of some cases that led to fatal outcomes called for the timely identification of risk factors and of especially vulnerable populations to target and prioritize non-pharmacological public health prevention measures, access to healthcare and medical assistance, and vaccination when available (157). Previous experiences with other coronaviruses such as SARS-CoV and MERS-CoV were the only reference points when trying to identify at-risk groups to an emerging pathogen such as SARS-CoV-2 (158). Even so, the magnitude of the pandemic as the virus continued to spread in terms of number of cases, severe presentations and fatalities evidenced that COVID-19 was its own clinical and epidemiological entity, and that the learnings and preparations that should have been derived from these previous pandemics were insufficient (159).

The relentless speed at which information related to COVID-19 continuously unfolded from the very beginning of the pandemic posed a challenge for newly published research to sustain scientific relevance. In the initial stages, many of the risk factors for morbidity and mortality that are common knowledge today were still unknown or based on low-quality data from swiftly published articles that on occasion even motivated withdrawals and retractions (160). The publication of timely information based on reliable sources such as clinical health registries with real-world data were pivotal at each phase of the pandemic, building on the understanding of the infection and its repercussions in every stage and, in retrospect, facilitating the publication of the high-quality meta-analyses and systematic reviews that are available today (161).

1.5.1. Sociodemographic risk factors

1.5.1.1. Age

The impact of old age on COVID-19 prognosis was one of the first characteristics that stood out as a determining factor for disease severity and, aside from its implications in clinical management, highlighted that demographic characteristics, the prevalence of 65-year-olds and over, or the presence of nursing homes in certain areas, were critical for the initial targeting of epidemiological surveillance programs, outbreak

management strategies and the implementation of preventive actions (162,163). The connection between age and severity can respond to various underlying mechanisms.

For one, older patients are prone to frailty and to having a higher number of and more serious comorbidities (164,165). The conjunction of older age, frailty and comorbidity reduce the tolerance of patients to withstand disease, limiting the defensive mechanisms capable of minimizing tissue damage (166). One of the common denominators for these underlying factors is the impact of aging on the immune system, mainly through the combination of two intertwined mechanisms, immunosenescence and inflammation (167). Immunosenescence is the process by which both the adaptive and innate immune responses are modified with age, with a progressive depletion and functional deterioration of monocytes/macrophages, dendritic cells, B cells, T CD4⁺ and CD8⁺ cells, impairing the response to infectious agents such as SARS-CoV-2 (168,169). With time, senescent cells acquire detrimental secretory phenotypes that enable them to produce cytokines that contribute to the pro-inflammatory state known as inflammaging, which is also induced by concatenating factors such as the accumulation of cellular products, the release of mitochondrial DNA or gut dysbiosis, among others (170).

Additionally, the expression levels of ACE-2 receptors and TMPRSS2, which have a direct link to SARS-CoV-2 pathogenesis, can also fluctuate based on age. Children, for instance, show lower ACE-2 protein activity compared to adults, which can partly explain the generally milder presentations of COVID-19 at younger ages (171). In contrast, activity is increased in older adults, affecting target cells with high ACE-2 expression such as type 2 alveolar cells, which are key in repairing lung tissue of the alveolar surface after injury and have been shown to be downregulated in COVID-19 patients with acute respiratory distress syndrome (172). Therefore, in the context of alveolar damage, these changes can be partially responsible for the increase in the proportion of ICU admissions and mortality in these patients (173).

1.5.1.2. Sex

Sex is another conditioning factor for COVID-19 prognosis, with men having a significantly increased risk of more serious presentations over women (174). In fact, apart from age and among other factors, ACE-2 receptors and TMPRSS2 are also

influenced by sex. Higher rates of SARS-CoV-2-positive saliva samples in male patients led to further explorations through different biological testing methods, with results showing that oral epithelial cells have higher TMPRSS2 expression in male patients compared to those of women of the same age, facilitating cell invasion and infection (175).

Other biological causes may also play a role in these differences. For instance, sexual dimorphisms in the immune transcriptome have pointed to a protective quality of the X chromosome, reducing susceptibility to viral infections (168,176). Hormonal factors are also modulators of the immune response. A mounting body of evidence suggests that higher levels of serum testosterone can have immunosuppressive repercussions, on the one side leading to fewer autoimmune diseases compared to women, while on the other negatively impacting the defenses against infections promoting viral entry into host cells (168,177).

Social determinants and factors regarding lifestyle choices that can be influenced by sex have also been considered, with smoking as the prime example and smoking rates having been traditionally higher in men. The association of COVID-19 and smoking, however, is quite controversial. Some studies demonstrated that active smoking could associate an increased risk of both infection and disease severity, linking it in some instances as well to an upregulation in ACE-2 expression. Other studies, by contrast did not find a significant association between smoking and COVID-19 severity or mortality, with some of them even reporting a protective effect of tobacco at an individual level but also on a larger scale, where countries with higher smoking rates evidenced lower incidence rates and mortality due to COVID-19 after adjusting for different confounding elements. Therefore, more research is still needed in this regard (178).

1.5.1.3. Socioeconomic status

Socioeconomic status is another determinant for clinical outcomes. The basal clinical condition of low-income COVID-19 patients is generally poorer, with higher rates of predisposing comorbidities for severe outcomes such as asthma, diabetes, obesity, peripheral vascular disease, or congestive heart failure. Furthermore, patients in high poverty settings are more likely to be admitted to hospital due to SARS-CoV-2 infection at a much younger age than those with a more favorable socioeconomic situation (179).

In fact, lower income has even been described as a predictor for more serious outcomes such as the need for mechanical ventilation or admission to an intensive care unit (180).

The structural and habitational circumstances of low-income populations can also have a direct impact on the risk of COVID-19. Poverty, poor housing conditions and living in small and overcrowded households were all challenges for the introduction of transmission-prevention measures in these groups, making social distancing, isolation, or abiding by stay-at-home policies often very difficult and sometimes impossible (181). Another hinderance in this sense was the unequal access to healthcare services and testing facilities in different areas and groups during the initial stages of the pandemic. Notwithstanding, case definitions regarding all the socioeconomic variables are inconsistent, and the quality and characteristics of the studies are heterogeneous, leading to conflicting results among them (180).

1.5.1.4. Migrant status

Migrants were an especially vulnerable collective during the COVID-19 pandemic. Regarding information quality and availability, migrant status indicators suffered from the same problems as socioeconomic factors, with non-existent or poorly structured registries, a lack of quantitative and qualitative data, and inconsistent terms and definitions, making public health research and surveillance in these communities more challenging (182). However, it was clear that the precarity faced by many migrant communities in our context was only exacerbated during the international health crisis. Difficulties to self-isolate and restricted access to healthcare were commonplace, with linguistic barriers only widening the gap with healthcare services, vaccination programs, information, and resources, added to the direct impact of the infection on physical health (182–184). Racialized medical perceptions and legal and cultural barriers were relevant factors while often being gender-based, with migrant women facing greater difficulties due to their generally more vulnerable positions and due to the stigma associated with seeking care (184). Moreover, mental health was also a concern, where worry, anxiety and depression secondary to a plethora of issues such as food and job insecurity, higher workloads, poor health or the inability to care of oneself, or the

COVID-19 situation in the country of origin, among others, were widely reported and poorly tackled (185).

1.5.2. Clinical risk factors

1.5.2.1. *Chronicity*

The Centers for Disease Prevention and Control (CDC) define chronic or non-communicable diseases (NCDs) as those lasting for more than one year, and that require the continued use of medical or healthcare services and/or that limit the activities of daily living (186). WHO estimates that chronic conditions, mainly cardiovascular diseases, cancer, chronic respiratory diseases, and diabetes, are responsible for 41 million deaths worldwide every year, which equates to 74% of all deaths (187). However, this has not always been the case. Communicable conditions have been responsible throughout history for the larger portion of disease burden, morbidity, and mortality around the world. In just a few decades, progress in sanitation and immunization strategies implemented during the 20th century led to a paradigm shift with a drastic reduction of the infections that had decimated populations for centuries. Although this was especially so in developed countries, low-income countries have also seen a slower though steady decline in the impact of communicable diseases when compared with chronic conditions in recent years, where NCDs have already started to take the lead as the main cause of death (188).

The incidence and prevalence of NCDs is strongly impacted by a few inherently modifiable behaviors (189). The use of tobacco, for instance, is one of the risk factors most clearly and directly associated to the development of NCDs such as COPD or lung cancer, and it accounts for over 8 million deaths worldwide every year when including individuals exposed to second-hand smoke, while the NCDs related to the consumption of alcohol, including cancer, are responsible for 1,5 million deaths. Barring the use or exposure to detrimental substances, lifestyle conditions such as physical inactivity and unhealthy diets, low in fruits and vegetables and high in sodium and saturated fats, are also major drivers of NCD incidence, increasing the risk of heart disease, obesity, type 2 diabetes, and certain types of cancer (186,187). Furthermore, the incidence and prevalence of chronic conditions increases with age and, with the growth of life expectancy, which has almost doubled in over the past century and a half in many

countries, the trend of increasing numbers of NCDs and longer periods of time of living in poor health will continue to rise moving forward (190).

The distribution of NCDs is global though not homogenous and, regardless of the still heavy toll of infectious diseases in low- and middle-income countries, these areas are disproportionately affected by NCDs, concentrating over three fourths of all chronic conditions in the world (187). Despite this, chronic conditions had not been prioritized as part of the sustainable development goals at a global level by United Nations until recently, and the question as to how the lacking health infrastructures and resources in developing countries are going to address the epidemiological and economic burden of chronic conditions remains unanswered (191). Notwithstanding, high-income countries also face the challenges of chronicity due to poor lifestyle habits and aging populations. In fact, in some developed countries like United States, even younger people are gravely affected by NCDs, with over half of adults aged 18-34 years reporting having at least one chronic condition, and almost a quarter reporting having more than one (192).

While non-communicable diseases are one of the most important public health challenges of our time in themselves, they can also become a risk factor for infectious diseases, with adverse and synergistic bidirectional associations between them (193). In this sense, a wide variety of chronic diseases are key risk factors for severe presentations of COVID-19. Respiratory comorbidities are the obvious candidates for poor health outcomes due to the virus' tropism for the respiratory system, but chronic patients with affectation of practically any organ or system are at higher risk (194). The increased risk is consistent when analyzing different health outcomes, be it hospitalization, ICU admission or mortality, however, the impact of each condition and its consequences varies. For instance, COPD is a robust predictor for all three outcomes; obese patients are at a greater risk of severe symptoms rather than fatal infections, while cerebrovascular disease or cancer patients faced higher ICU admission and mortality risk. All in all, it has been estimated that over 35% of patients who died due to COVID-19 had at least one chronic condition, while this figure rises to over 70% of ICU-admitted COVID-19 patients (195). Therefore, present and future public health

programs will need to mind the associations of chronic and infectious diseases and develop holistic solutions for these transversal challenges (193).

Nevertheless, the course of the pandemic has borne witness to changes in the insights regarding chronicity and its effects on COVID-19 infection. Though some subgroups of diseases such as cardiovascular and respiratory conditions were identified early on as potential aggravating factors, the prevalence of practically any type of chronic disease has been shown to increase in patients diagnosed with severe infection compared to non-severe presentations, and a variety of conditions have been suggested as aggravating factors, including hypertension, diabetes, asthma, COPD and other respiratory disorders, coronary heart disease and other cardiovascular conditions, cerebrovascular disease, obesity, chronic liver disease, chronic renal disease, or cancer, among others (194–196). In spite of all this, there is still room for improvement in the understanding of how different kinds of chronic conditions co-exist, interact with each other, and affect the outcomes of COVID-19 infections, with isolated chronic conditions being less and less common in favor of more complex chronic profiles with combinations of different types of NCDs (197).

1.5.2.2. Multimorbidity

Multimorbidity is defined as the coexistence of two or more chronic conditions in a single individual and has become the norm rather than the exception in patients suffering from chronic diseases, with an increasing global burden of disease, and with larger proportions of the population affected every year (198). Multimorbidity refers to concomitant long-term conditions that can be both of physical or mental and, depending on the consulted guideline, can also include more complex entities such as frailty, ongoing disabilities, or the impairment of the senses. Like individual chronic conditions, multimorbidity increases significantly with age, reaching prevalences of 30% in people aged between 45 and 64 years, 65% among 65-84-year-olds, and of over 80% among those aged 85 years and over (199).

Developing multiple chronic conditions associates more risks than could be expected from the sum of the individual diseases that conform each multimorbidity profile, ultimately, leading patients to having poorer prospective health, major health decline, increased mortality, and to be more vulnerable in general than healthy individuals or

those with isolated NCDs (200). One of the fundamental hazards associated to multimorbidity is the intake of multiple drugs and their potential interactions, also known as polypharmacy, when the number of different treatments reaches five or above. The negative effects of multiple drug intake for the patient are varied, including decreased quality of life, increased morbidity and mortality, and a higher risk of adverse drug events, disability, falls, frailty, or non-adherence, among others (201). Multimorbidity often makes the use of several drugs inevitable, though mindful prescription, deprescribing, and medication reconciliation can help reduce the risk of adverse medical events, improve adherence and limit treatment costs (202). Even so, in a positive light and from a research perspective, drug treatments and polypharmacy studies using prescription or dispensation registries can be an additional tool to evaluate health statuses and underlying risk factors, either considering the direct effects and repercussions of the use of medicines on the health of patients, or using them as proxy for the diseases they are meant to treat in order to characterize different populations (203).

Another risk for patients suffering from multimorbidity is their more complex healthcare needs and management regimens as well as their more frequent and complicated interactions with healthcare providers (204). This often demands the coordinated participation of multiple healthcare professional at different levels, from hospital to primary care, mental health, and social care, which can easily lead to fragmented care, increasing the risk of negative outcomes (205). There have been many initiatives to develop integrated care programs to face these issues, though their variability and the need to push for a fundamental and costly reshaping of healthcare systems from acute-oriented care structures to more holistic, patient-centered approaches have made their implementation a global challenge (206).

In this regard, the consequences of the COVID-19 pandemic were two-pronged, impacting the continuity of healthcare delivery and the follow-up capacity of care providers for chronic patients, with the ensuing effects on late diagnosis, treatment, and control over the patients' conditions and wellbeing, while constituting a direct health hazard due to the risk of severe infections, complications, and mortality after SARS-CoV-2 (207). During the first waves of the pandemic, individuals affected by

multimorbidity were shown to be more likely to develop COVID-19, especially those affected by cardiometabolic conditions and polypharmacy (208). Moreover, the underlying frailty and comorbidities in these patients set them at a higher risk of unfavorable health outcomes, increasing the risk of hospitalization, ICU admission and mortality (197,209).

The understanding of multimorbidity profiles and the impact of COVID-19 on complex patients has advanced from the initial low-quality studies to more robust population-based analyses, offering a wider range of references for comparability among studies regarding case definitions and outcomes, as well as different options for statistical analysis and research methodologies (197,208). However, the variability of these profiles and the complexity of the derived clinical presentations, superior to those of individual chronic conditions, still warrants further research for the development of personalized approaches for prevention, for the clinical management of patients during and after COVID-19 infection, and to ideate effective preparedness and response programs for potential future health crises of a similar nature (210).

2. HYPOTHESIS

Analyzing the clinical and demographic characteristics of patients affected by COVID-19, especially those with more severe presentations of the infections, can help identify the profiles with a higher risk of potential complications and unfavorable health outcomes, as well as to elaborate clinical and public health strategies that can anticipate their occurrence or even prevent them, reducing the impact of SARS-CoV-2 infection.

3. OBJECTIVES

General:

- To analyze, from a clinical, epidemiological, and population-based perspective, the clinical and demographic characteristics of COVID-19 disease and identify which ones can predict greater severity or less favorable prognoses.

Specific:

1. To exhaustively describe the demographic and clinical characteristics of patients affected by COVID-19 through population-based studies in the Autonomous Community of Aragón.
2. To identify and characterize the demographic and clinical risk factors of poor health outcomes in patients with COVID-19, especially considering the underlying chronic conditions and treatments of the population.
3. To identify and characterize the existing multimorbidity profiles in patients with COVID-19 and analyze their impact on the disease's severity.
4. To compare the results from Aragón regarding the clinical and epidemiological characterization of COVID-19 with other affected areas.

4. MATERIALS, METHODS, AND RESULTS

4.1. Manuscript 1: Baseline chronic comorbidity and mortality in laboratory-confirmed COVID-19 cases: Results from the PRECOVID Study in Spain.

Poblador-Plou B, Carmona-Pírez J, Ioakeim-Skoufa I, Poncel-Falcó A, Bliek-Bueno K, Cano-Del Pozo M, Gimeno-Feliú LA, González-Rubio F, Aza-Pascual-Salcedo M, Bandrés-Liso AC, Díez-Manglano J, Marta-Moreno J, Mucherino S, Gimeno-Miguel A, Prados-Torres A, EpiChron Group. **Baseline chronic comorbidity and mortality in laboratory-confirmed COVID-19 cases: Results from the PRECOVID Study in Spain.** *Int J Environ Res Public Health*. 2020 Jul 17;17(14):5171. Doi: 10.3390/ijerph17145171

Journal: International Journal of Environmental Research and Public Health

Impact Factor: 3.390, Q1 (PUBLIC, ENVIRONMENTAL & OCCUPATIONAL HEALTH-SSCI), Q2 (PUBLIC, ENVIRONMENTAL & OCCUPATIONAL HEALTH-SCIE)

Status: Published



Article

Baseline Chronic Comorbidity and Mortality in Laboratory-Confirmed COVID-19 Cases: Results from the PRECOVID Study in Spain

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Received: 18 June 2020; Accepted: 15 July 2020; Published: 17 July 2020



Abstract: We aimed to analyze baseline socio-demographic and clinical factors associated with an increased likelihood of mortality in men and women with coronavirus disease (COVID-19). We conducted a retrospective cohort study (PRECOVID Study) on all 4412 individuals with laboratory-confirmed COVID-19 in Aragon, Spain, and followed them for at least 30 days from cohort entry. We described the socio-demographic and clinical characteristics of all patients of the cohort. Age-adjusted logistic regressions models were performed to analyze the likelihood of mortality based on demographic and clinical variables. All analyses were stratified by sex. Old age, specific diseases such as diabetes, acute myocardial infarction, or congestive heart failure, and dispensation of drugs like vasodilators, antipsychotics, and potassium-sparing agents were associated with an increased likelihood of mortality. Our findings suggest that specific comorbidities, mainly of cardiovascular nature, and medications at the time of infection could explain around one quarter of

the mortality in COVID-19 disease, and that women and men probably share similar but not identical risk factors. Nonetheless, the great part of mortality seems to be explained by other patient- and/or health-system-related factors. More research is needed in this field to provide the necessary evidence for the development of early identification strategies for patients at higher risk of adverse outcomes.

Keywords: chronic diseases; cohort; comorbidity; COVID-19; drugs; gender; mortality; multimorbidity; Spain

1. Introduction

Six months after the first case of infection by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) reported in Wuhan, China, in December 2019, the virus has spread worldwide [1,2]. The World Health Organization has declared that the ongoing pandemic of coronavirus disease 2019 (COVID-19) represents a public health emergency of international concern. Even though governments all around the world have adopted control measures, mainly through social distancing and isolation of patients, the coronavirus outbreak has affected over 200 countries with approximately 8 million confirmed cases and over 445,000 deaths worldwide as of 1 June 2020 [3]. Spain was the second country in Europe after Italy to suffer the COVID-19 pandemic, with the first case reported in January 2020, and is one of the countries with the highest number of attributable deaths after the United Kingdom, Italy and France at the moment of writing this study [3].

The impact of the disease has varied among countries, sub-populations and ethnicities in terms of fatality rates and other health-related outcomes, which could be explained not only by genetic differences but also by diverse cultural, political, and socio-behavioral factors [4,5]. Its rapid geographic spread across countries requires large-scale epidemiological studies to ascertain and develop effective health policies considering the general context in which the outbreak has taken place.

The first published reports of COVID-19 were mostly case series of hospitalized patients, describing the more severe end of the clinical spectrum of the disease [6], mainly life-threatening pneumonia. Later observational studies reported a wide range of symptoms, from asymptomatic infection to respiratory failure and death [7]. It has been estimated that eight out of every ten patients infected with SARS-CoV-2 will develop mild symptoms, while the remaining two can evolve to severe or critical conditions [8].

As with other acute respiratory infections, certain comorbidity profiles could predispose COVID-19 patients to more frequent adverse outcomes. Several chronic conditions, such as diabetes, hypertension, cardiovascular disease, cerebrovascular disease, or chronic obstructive pulmonary disease have already been identified [9,10], while other factors, such as older age or d-dimer levels on admission over 1 µg/mL in hospitalized patients, have also been found to entail poorer health outcomes [8,11]. However, previous reports on the role that other baseline chronic conditions and medications play in the evolution and outcomes of infected patients are contradictory, and the relevance of these factors remains unclear.

Longitudinal, population-based epidemiological studies, in both inpatient and outpatient settings, including baseline information on every possible disease and medication, are necessary to shed light on the risk factors behind the most severe cases. The results of such an approach are relevant for the early identification of patients susceptible to poorer health outcomes and, therefore, potential targets of specific prevention strategies and/or of closer clinical monitoring.

This exploratory study aims to analyze which socio-demographic and clinical factors are associated with an increased risk of mortality in a Spanish cohort of individuals infected by COVID-19, with a particular focus on baseline chronic diseases and treatments present at the time of infection.

2. Materials and Methods

2.1. Design and Study Population

We conducted a retrospective cohort study (PRECOVID Study), including all individuals with laboratory-confirmed infection by SARS-CoV-2 in the Spanish region of Aragon (reference population, 1.3 million inhabitants), from a total of 14,724 laboratory-tested individuals. In Aragon, the national health system provides free-of-charge healthcare with universal coverage to approximately 95% of the population. The PRECOVID Study was created to serve as the basis for future observational studies on the epidemiology and trajectory of the disease through the linkage of real-world data from patients' electronic health records (EHRs) and clinical-administrative databases.

For this study, we included all the individuals of the cohort from 4 March 2020 (i.e., date of the first confirmed infection in the region) to 17 April 2020 (enrolment period). We followed patients from the date of inclusion in the cohort to 17 May 2020, or to the date of death, to ensure a minimum follow-up period of 30 days for all analyzed patients and allow sufficient time for the studied event (i.e., death) to occur. Laboratory tests were conducted in the region to confirm the infection in hospitalized patients with compatible symptoms, exposed healthcare workers, residents and workers from social care institutions where an outbreak was taking place (e.g., residential homes for the elderly; physical or mental disability care centers), and individuals at a community level associated to family or work-related clusters of cases.

This study was conducted in line with the Declaration of Helsinki, as revised in 2013. The Clinical Research Ethics Committee of Aragón (CEICA) approved the research protocol (PI20/226). CEICA waived the requirement to obtain informed consent from patients given the epidemiological nature of the project, which used anonymized data.

2.2. Variables of Study and Data Sources

For each individual of the cohort, we analyzed the following baseline variables at the time of inclusion: (i) socio-demographic characteristics: Sex, age (0–14, 15–44, 45–64, 65–79, ≥80 years), country of birth (native vs. non-native), administrative health area (urban vs. rural), and deprivation index of the area measured at an aggregated level and divided into four quartiles from least (Q1) to most (Q4) deprived [12]; and (ii) clinical information: All chronic disease diagnoses present at the date of infection and registered in primary care EHRs, all drugs dispensed during the month prior to infection in community and hospital pharmacies, presence of multimorbidity (i.e., the coexistence of two or more chronic conditions) and polypharmacy (i.e., the dispensation of five or more drugs), Anticholinergic Drug Scale (ADS) score [13] based on an updated version [14], and flu vaccination. During the follow-up period, we analyzed all-cause mortality. Hospitalizations and Intensive Care Unit admissions were not analyzed due to potential biases influenced by the healthcare system's organization, amongst others.

Primary care diagnoses were initially coded using the International Classification of Primary Care, First Edition (ICPC-1). These codes were mapped to ICD-9-CM using a mapping system developed to codify temporary disability [15]. Diagnoses were subsequently sorted into 226 clinically relevant categories using the Clinical Classifications Software (CCS) for ICD-9-CM [16]. Diagnoses were classified as chronic or not chronic using the Chronic Condition Indicator software tool [17], with a total of 153 conditions categorized as chronic. Some of the diagnostic labels were renamed to facilitate their clinical interpretation. Chronic conditions were defined as those present during the last 12 months at least, and meeting one or both of the following criteria: (i) Entails limitations on self-care, independent living, and social interactions; (ii) requires of ongoing interventions using medical products, services, and special equipment. We analyzed all drugs using their Anatomical-Therapeutic-Chemical (ATC) classification system code at the third level (i.e., pharmacological subgroup). The ADS score was rated from level 0 (absence of known anticholinergic properties) to level 3 (markedly anticholinergic), as previously described [13,14].

Patient data was obtained from the Aragon Health System linking, at a patient level and in a pseudo-anonymized form, the information contained in the users' database, primary care EHRs, primary care and hospital pharmaceutical billing records, and an *ad hoc* registry developed for monitoring the evolution of the COVID-19 disease pandemic in the region of Aragon.

2.3. Statistical Analysis

We described the socio-demographic and clinical characteristics of all patients from the cohort (alive and deceased at the end of follow-up) as means accompanied by their respective standard deviations or as absolute and relative frequencies and proportions, by sex. Differences in means between alive and deceased men and women were analyzed using age-adjusted logistic regression analysis.

We performed logistic regression models to analyze the association between the presence of each chronic comorbidity and drug studied at baseline and the likelihood of mortality during the follow-up period, by sex. We used this technique instead of Cox regression since we had the date of registry but not the exact date of infection and/or of confirmatory test for all patients. We calculated age-adjusted odds ratios (ORs) accompanied by their respective 95% confidence intervals (CI). For this analysis, only chronic diseases and medications with at least 10 cases in deceased men and/or women were considered.

Finally, we performed a multivariate logistic regression analysis to identify which of the studied variables were significantly associated with higher mortality when studied in combination. In doing so, we included as potential explanatory variables all socio-demographic characteristics, chronic diseases and medications that showed significant association at baseline. Multivariate analysis was performed using a stepwise method with an exit *p*-value for analyzed variables of 0.05. We calculated the pseudo R squared statistics to estimate the proportion of the variation in mortality that was explained by the variables included in each model for men and women. Statistical analyses were conducted using Stata software (Version 12.0, StataCorp LLC, College Station, TX, USA), and statistical significance was set at $p < 0.05$.

3. Results

A total of 4412 individuals (58.8% women, mean age of 67.7 (standard deviation, s.d., 20.7) years) were included in the study (Table 1). Of them, 771 (47.2% women, mean age of 84.2 (s.d. 10.0) years) died during the follow-up period. The crude overall mortality rate during the studied period was 3.84 deaths per 100,000 persons per day. Three in four deceased patients were older than 80 years, compared to one in four in those alive. Men died at an earlier age than women (81.5 vs. 87.2 years). The mean numbers of chronic diseases (5.22 vs. 3.03; 6.66 vs. 3.67) and dispensed medications (5.41 vs. 3.00; 5.57 vs. 2.75) were higher in deceased men and women compared to their alive counterparts. Deceased individuals also showed a higher burden of medications with anticholinergic activity, especially women. Although the proportion of influenza-vaccinated individuals was higher in deceased compared to alive men (64.1% vs. 36.3%) and women (68.4% vs. 31.9%), these differences were not significant after controlling for age.

The most prevalent chronic conditions in the individuals of the cohort who died during the follow-up period were hypertension (49.4%), obesity and other nutritional, endocrine and metabolic disorders (28.7%), cataract (28.0%), diabetes (27.5%), and urinary incontinence (25.3%) in men, and hypertension (62.6%), urinary incontinence (50.5%), obesity and other nutritional, endocrine and metabolic disorders (37.9%), mood disorders (36.3%), and osteoarthritis (33.2%) in women (Table 2). These figures were slightly different from those observed in the entire population (see Supplementary Table S1). In men, nine conditions with at least 10 cases amongst deceased individuals were significantly associated with an increased likelihood of death, including (OR (95% CI)) epilepsy (2.56 (1.09–5.99)), inflammatory conditions of genital organs (2.56 (1.15–5.70)), and congestive heart failure (2.15 (1.30–3.54)), among others. Rheumatoid arthritis was also highly associated (5.15 (1.13–23.5)), but only five cases were observed (see Supplementary Table S1). In women, acute myocardial infarction (2.98 (1.25–7.11)), coagulation and hemorrhagic disorders (1.85 (1.15–2.97)), and degenerative nervous system conditions

(1.56 (1.05–2.33)) could be highlighted amongst the eight conditions significantly associated with higher mortality. Diabetes (1.65 (1.23–2.21); 1.40 (1.02–1.91)) and chronic ulcer of the skin (2.24 (1.26–3.96); 2.04 (1.35–3.10)) were the only two conditions associated with higher mortality in both sexes.

Some of the most frequently dispensed drugs among individuals who died of both sexes were (men vs. women) drugs for peptic ulcer and gastro-esophageal reflux disease (GORD) (49.6% vs. 58.5%), antithrombotic agents (36.9% vs. 39.0%), other analgesics and antipyretics (31.0% vs. 37.4%), high-ceiling diuretics (24.6% vs. 34.6%), and antidepressants (20.4% vs. 38.7%), among others (Table 3).

The dispensation of 12 drugs in men and 9 drugs in women was associated with an increased likelihood of death during the study period. Out of all of them (OR (95% CI)), drugs for peptic ulcer and GORD (1.45 (1.13–1.86); 1.32 (1.02–1.71)), high-ceiling diuretics (1.79 (1.30–2.49); 1.71 (1.29–2.27)), antipsychotics (1.71 (1.18–2.48); 1.96 (1.41–2.73)), and potassium-sparing agents (2.52 (1.27–4.97); 2.86 (1.50–5.44)) were associated with higher risk of mortality in both men and women, respectively. Women treated with other beta-lactam antibacterials, antigout preparations, and antiepileptics presented 2.17, 2.06, and 1.94 times greater mortality risk than those not receiving these medications, respectively. Men treated with corticosteroids, vasodilators for cardiac diseases, and immunosuppressants showed 3.83, 3.15, and 2.49 times higher risk of death. Plain angiotensin-converting enzyme (ACE) inhibitors were not associated with a higher risk of death in men; however, patients in treatment with medications of ACE inhibitors in combination with other active ingredients were less likely to die (0.46 (0.23–0.93)). Supplementary Table S2 displays the complete list of drugs analyzed in the study population.

The models developed to assess mortality risk revealed that age was the most influencing factor, being the risk of death between 10 and 40 times higher in men and women above 80 years of age compared with their younger 45–64-year-old counterparts (Table 4). Three diseases and six drugs in men, and three diseases and five drugs in women, remained significant in the models. In men, the likelihood of mortality was 2.04, 1.48, and 1.46 times higher when chronic ulcer of skin, cataract, and diabetes were present, respectively. In women, acute myocardial infarction, chronic ulcer of the skin and urinary incontinence were associated with 3.42, 1.65, and 1.35 times higher mortality. Corticosteroids, vasodilators, immunosuppressants, and antipsychotics were amongst the medications associated the most with an increased likelihood of mortality. The models explained 22.8% and 25.9% of the variance of mortality in men and women, respectively.

Table 1. Socio-demographic and clinical characteristics at cohort entry of the individuals with laboratory-confirmed infection by COVID-19 in Aragón, Spain, March to May 2020 ($n = 4412$), by sex.

Characteristics	Alive ^a ($n = 3641$)				Dead ($n = 771$)				p Value ^b	
	Men		Women		Men		Women		Men	Women
	n	%	n	%	n	%	n	%		
	1414	38.8	2229	61.2	407	52.8	364	47.2		
Age, years										
0–14	6	0.42	0	0	0	0	0	0	-	-
15–44	205	14.5	516	23.1	1	0.25	0	0	0.019	-
45–64	540	38.2	844	37.9	29	7.13	7	1.92	Reference	Reference
65–79	352	24.9	318	14.3	121	29.7	51	14.0	<0.001	<0.001
≥80	309	21.9	551	24.7	256	62.9	306	84.1	<0.001	<0.001
Mean (mean, sd ^c)	63.3	18.1	60.8	20.2	81.5	10.7	87.2	8.20	<0.001	<0.001
Non-native ^d	163	11.5	299	13.4	8	1.97	7	1.92	0.369	0.649
Rural area ^e	510	36.1	768	34.5	168	41.3	161	44.2	0.121	0.473
Deprivation index ^f										
Q1	474	33.6	743	33.3	130	31.9	142	39.0	Reference	Reference
Q2	339	24	513	23.0	96	23.6	78	21.4	0.156	0.657
Q3	275	19.5	423	19.0	87	21.4	70	19.2	0.729	0.078
Q4	324	22.9	550	24.7	94	23.1	74	20.3	0.458	0.849
Flu vaccination, yes	512	36.3	710	31.9	261	64.1	249	68.4	0.110	0.126
No. of chronic diseases ^g (mean, sd)	3.03	3.35	3.67	3.65	5.22	4.02	6.66	4.14	0.012	0.003
Multimorbidity ^h	793	56.2	1410	63.3	307	75.4	311	85.4	0.398	0.022
No. of drugs ⁱ (mean, sd)	3.00	3.14	2.75	3.11	5.41	3.73	5.57	3.42	<0.001	0.006
Polypharmacy ^j	402	28.5	568	25.5	242	59.5	217	59.6	<0.001	0.125
ADS score ^k										
0	1106	78.3	1680	75.4	234	57.5	168	46.2	Reference	Reference
1	152	10.8	270	12.1	76	18.7	85	23.4	0.088	0.044
2	79	5.59	128	5.74	47	11.5	60	16.5	0.091	0.003
≥3	75	5.31	151	6.77	50	12.3	51	14.0	0.010	0.070

^a Alive or dead at the end of the follow-up period; ^b p -value of the comparison between alive and dead individuals by sex, using age-adjusted logistic regression analysis; ^c standard deviation; ^d Born out of Spain; ^e Based on the type of administrative health area (urban, if 80% or more of the population of the area is concentrated in one of the municipalities, or rural for the rest); ^f Calculated at aggregated level per administrative health area and divided into quartiles from less (Q1) to more (Q4) deprived; ^g Primary care diagnoses from a list of 153 chronic conditions, using the Clinical Classification System and the Chronic Condition Indicator; ^h Defined as the presence of two or more chronic diseases; ⁱ Dispensed during the month prior to cohort entry, using the Anatomical-Therapeutic-Chemical classification system at the third level (i.e., pharmacological subgroup); ^j Defined as the dispensation of five or more drugs; ^k Anticholinergic Drug Scale score.

Table 2. Prevalence of chronic diseases at cohort entry of individuals that died during the follow-up period, and association with all-cause mortality, by sex.

Chronic Conditions	Men (n = 407)			95% CI ^a		Women (n = 364)			95% CI	
	n	%	OR ^c	Lower Limit	Upper Limit	n	%	OR	Lower Limit	Upper Limit
CCS Code ^b										
Hypertension	201	49.4	0.96	0.75	1.23	228	62.6	1.20	0.92	1.56
Urinary incontinence	103	25.3	1.33	0.97	1.83	184	50.5	1.50	1.16	1.94
Obesity and other nutritional, endocrine, metabolic dis. ^d	117	28.7	1.02	0.78	1.34	138	37.9	1.26	0.97	1.63
Cataract	114	28.0	1.62	1.20	2.18	99	27.2	1.19	0.89	1.59
Mood disorders	70	17.2	1.38	0.98	1.95	132	36.3	1.46	1.12	1.91
Osteoarthritis	74	18.2	0.93	0.67	1.29	121	33.2	1.00	0.77	1.31
Diabetes Mellitus	112	27.5	1.65	1.23	2.21	80	22.0	1.40	1.02	1.91
Delirium, dementia	60	14.7	1.04	0.72	1.49	101	27.7	1.20	0.90	1.61
Dis. of lipid metabolism	70	17.2	1.22	0.88	1.70	61	16.8	0.92	0.66	1.28
Anxiety dis.	38	9.34	0.80	0.53	1.21	83	22.8	1.21	0.90	1.64
Chronic renal failure	55	13.5	1.32	0.89	1.94	61	16.8	1.24	0.87	1.76
Cardiac dysrhythmias	58	14.3	1.01	0.70	1.46	58	15.9	1.47	1.02	2.12
Osteoporosis	9	2.21	1.16	0.48	2.82	95	26.1	1.27	0.95	1.70
Other ear/sense dis.	54	13.3	0.85	0.59	1.22	53	14.6	0.90	0.63	1.28
Spondylosis	45	11.1	1.27	0.84	1.92	54	14.8	1.10	0.77	1.57
Hyperplasia of prostate	97	23.8	1.15	0.86	1.56	0	0	-	-	-
Congestive heart failure	41	10.1	2.15	1.30	3.54	40	11.0	1.40	0.91	2.15
Chronic ulcer of skin	30	7.37	2.24	1.26	3.96	50	13.7	2.04	1.35	3.10
Thyroid dis.	26	6.39	1.18	0.70	1.97	53	14.6	0.82	0.58	1.16
Glaucoma	39	9.58	0.69	0.46	1.05	41	11.3	1.05	0.70	1.57
Hereditary/degenerative nervous system conditions	23	5.65	1.43	0.79	2.60	49	13.5	1.56	1.05	2.33
Blindness and vision defects	48	11.8	1.70	1.12	2.57	19	5.22	0.78	0.46	1.35
Coagulation/hemorrhagic dis.	29	7.13	0.84	0.52	1.36	35	9.62	1.85	1.15	2.97
Headache, including migraine	20	4.91	0.64	0.38	1.09	43	11.8	0.94	0.64	1.38
Acute cerebrovascular dis.	34	8.35	1.83	1.09	3.07	29	7.97	1.26	0.77	2.06
Transient cerebral ischemia	30	7.37	1.60	0.93	2.73	26	7.14	0.86	0.53	1.41
Neoplasms	31	7.62	1.22	0.75	1.98	18	4.95	1.41	0.77	2.58
COPD ^e and bronchiectasis	38	9.34	0.99	0.64	1.52	10	2.75	1.17	0.54	2.55
Upper respiratory disease	23	5.65	1.29	0.75	2.21	19	5.22	1.04	0.60	1.81
Coronary atherosclerosis	24	5.90	1.05	0.62	1.81	17	4.67	1.07	0.59	1.97
Diverticulosis/diverticulitis	18	4.42	1.25	0.67	2.35	22	6.04	1.25	0.72	2.16
Gout	30	7.37	0.85	0.54	1.35	10	2.75	1.57	0.68	3.61
Acute myocardial infarction	22	5.41	1.53	0.85	2.73	13	3.57	2.98	1.25	7.11
Miscellaneous mental health dis.	14	3.44	0.64	0.34	1.19	19	5.22	1.38	0.77	2.46
Other nervous system dis.	16	3.93	0.87	0.46	1.62	15	4.12	0.79	0.43	1.44
Acquired foot deformities	5	1.23	0.91	0.30	2.74	24	6.59	0.82	0.50	1.34
Developmental dis.	11	2.70	1.32	0.60	2.92	16	4.40	1.22	0.65	2.27
Parkinson's disease	13	3.19	1.63	0.73	3.67	13	3.57	2.10	0.96	4.60
Asthma	6	1.47	0.45	0.18	1.11	19	5.22	0.68	0.40	1.17
Prolapse of genital organs	0	0	-	-	-	24	6.59	1.53	0.89	2.61
Other non-traumatic joint dis.	10	2.46	0.90	0.40	2.03	15	4.12	0.89	0.48	1.66
Retinal detachments, vascular occlusion, retinopathy	12	2.95	1.89	0.84	4.25	13	3.57	1.15	0.58	2.29
Residual codes; unclassified	18	4.42	1.44	0.78	2.66	5	1.37	1.84	0.58	5.84
Menopausal dis.	0	0	-	-	-	20	5.49	0.97	0.57	1.66
Other gastrointestinal dis.	11	2.70	1.66	0.71	3.88	11	3.02	1.04	0.50	2.14
Allergic reactions	14	3.44	2.02	0.93	4.39	8	2.20	0.72	0.32	1.64
Other inflammatory condition skin	15	3.69	1.74	0.86	3.53	7	1.92	0.75	0.31	1.79
Esophageal dis.	9	2.21	0.80	0.37	1.73	11	3.02	0.85	0.42	1.72
Other endocrine dis.	9	2.21	1.23	0.50	3.05	11	3.02	1.92	0.84	4.38
Epilepsy, convulsions	12	2.95	2.56	1.09	5.99	8	2.20	0.96	0.41	2.25
Adjustment dis.	6	1.47	0.72	0.27	1.92	13	3.57	0.98	0.50	1.92
Peripheral, visceral atherosclerosis	16	3.93	1.79	0.86	3.73	2	0.55	0.96	0.18	5.15
Menstrual dis.	0	0	-	-	-	14	3.85	1.31	0.68	2.53
Inflammatory conditions of male genital organs	13	3.19	2.56	1.15	5.70	0	0	-	-	-
Other bone disease and musculoskeletal deformities	1	0.25	0.38	0.04	3.40	10	2.75	1.47	0.65	3.31
Other male genital dis.	10	2.46	1.41	0.65	3.06	0	0	-	-	-

^a Confidence interval; ^b Primary care diagnoses from a list of 153 chronic conditions, using the Clinical Classification System (CCS) and the Chronic Condition Indicator. Diseases are listed in order of descending overall prevalence; only those with at least 10 cases in men and/or women are presented. The complete list of diseases is presented in Supplementary Table S1; ^c Odds ratio; the OR of mortality for each basal chronic disease was estimated using logistic regression models. The ORs were adjusted by age, included in the model as a categorical variable. Conditions with statistically significant ORs in men and/or women are highlighted in bold. All the individuals of the cohort were followed for at least 30 days after cohort entry; ^d Disorders; ^e Chronic obstructive pulmonary disease.

Table 3. Prevalence of medications dispensed during the month prior to cohort entry of individuals that died during the follow-up period, and association with all-cause mortality, by sex.

Medications ATC Code ^b	Men (n = 407)			95% CI ^a		Women (n = 364)			95% CI	
	n	%	OR ^c	Lower Limit	Upper Limit	n	%	OR	Lower Limit	Upper Limit
Drugs for peptic ulcer, GORD ^d (A02B)	202	49.6	1.45	1.13	1.86	213	58.5	1.32	1.02	1.71
Antithrombotic agents (B01A)	150	36.9	1.25	0.96	1.63	142	39.0	1.51	1.16	1.97
Other analgesics and antipyretics (N02B)	126	31.0	1.04	0.80	1.36	136	37.4	0.93	0.72	1.21
High-ceiling diuretics (C03C)	100	24.6	1.79	1.30	2.49	126	34.6	1.71	1.29	2.27
Antidepressants (N06A)	83	20.4	1.30	0.94	1.79	141	38.7	1.33	1.02	1.73
Lipid modifying agents, plain (C10A)	93	22.9	0.93	0.69	1.24	70	19.2	0.83	0.60	1.13
Beta blocking agents (C07A)	85	20.9	1.53	1.11	2.11	68	18.7	1.28	0.92	1.79
Antipsychotics (N05A)	70	17.2	1.71	1.18	2.48	82	22.5	1.96	1.41	2.73
Anxiolytics (N05B)	57	14.0	1.52	1.05	2.22	89	24.5	1.32	0.98	1.78
Blood glucose lowering drugs (A10B)	73	17.9	1.14	0.82	1.58	48	13.2	1.28	0.88	1.88
Drugs for prostatic hypertrophy (G04C)	108	26.5	0.96	0.72	1.28	0	0	-	-	-
Opioids (N02A)	42	10.3	1.13	0.75	1.71	62	17.0	1.30	0.92	1.83
Antiepileptics (N03A)	37	9.09	1.00	0.65	1.55	52	14.3	1.94	1.31	2.88
ARBs ^e , plain (C09C)	45	11.1	1.16	0.78	1.74	42	11.5	0.99	0.67	1.46
ARBs, comb. ^f (C09D)	49	12.0	1.00	0.69	1.46	32	8.79	0.68	0.44	1.03
ACE ^g inhibitors, plain (C09A)	40	9.83	0.90	0.60	1.36	38	10.4	1.14	0.74	1.73
Iron preparations (B03A)	35	8.60	1.44	0.88	2.35	38	10.4	1.06	0.70	1.62
Vitamin B12 and folic acid (B03B)	45	11.1	1.88	1.20	2.97	28	7.69	1.16	0.71	1.88
Adrenergics, inhalants (R03A)	45	11.1	1.35	0.90	2.02	24	6.59	0.98	0.60	1.62
Selective calcium channel blockers with mainly vascular effects (C08C)	39	9.58	1.10	0.72	1.69	27	7.42	0.82	0.51	1.31
Hypnotics and sedatives (N05C)	30	7.37	1.04	0.64	1.69	36	9.89	0.92	0.60	1.39
Antiglaucoma preparations (S01E)	33	8.11	1.02	0.64	1.61	25	6.87	1.07	0.65	1.76
Antigout preparations (M04A)	35	8.60	1.29	0.82	2.04	17	4.67	2.06	1.05	4.04
Anti-dementia drugs (N06D)	18	4.42	0.73	0.40	1.32	33	9.07	0.92	0.60	1.42
Macrolides, lincosamides (J01F)	26	6.39	0.84	0.51	1.36	23	6.32	1.19	0.71	2.01
Other drugs for obstructive airway diseases, inhalants (R03B)	33	8.11	1.83	1.10	3.05	16	4.40	0.96	0.52	1.76
B-lactam antibacterials, penicillins (J01C)	22	5.41	0.84	0.49	1.42	24	6.59	1.62	0.94	2.79
Quinolone antibacterials (J01M)	31	7.62	1.55	0.94	2.56	15	4.12	1.37	0.70	2.66
Potassium-sparing agents (C03D)	23	5.65	2.52	1.27	4.97	23	6.32	2.86	1.50	5.44
Corticosteroids systemic use, plain (H02A)	23	5.65	1.44	0.82	2.54	21	5.77	1.13	0.65	1.94
Insulins and analogues (A10A)	21	5.16	2.33	1.21	4.48	16	4.40	1.40	0.73	2.68
Vasodilators for cardiac diseases (C01D)	24	5.90	3.15	1.55	6.41	12	3.30	1.15	0.55	2.40
Calcium (A12A)	12	2.95	1.69	0.73	3.88	23	6.32	0.70	0.43	1.15
Other systemic drugs for obstructive airway diseases (R03D)	20	4.91	1.18	0.66	2.11	10	2.75	0.78	0.37	1.62
Vitamin A and D (A11C)	7	1.72	0.90	0.35	2.30	21	5.77	1.73	0.97	3.10
Other beta-lactam antibacterials (J01D)	15	3.69	2.02	0.95	4.30	13	3.57	2.17	1.00	4.71
Anti-inflammatory/ antirheumatic products, non-steroids (M01A)	13	3.19	0.58	0.31	1.10	13	3.57	0.70	0.37	1.32
ACE inhibitors, comb. (C09B)	11	2.70	0.46	0.23	0.93	15	4.12	1.00	0.53	1.87
Other antibacterials (J01X)	8	1.97	0.80	0.34	1.91	18	4.95	1.17	0.64	2.13
Antihistamines for systemic use (R06A)	15	3.69	1.46	0.74	2.88	10	2.75	1.54	0.70	3.40
Dopaminergic agents (N04B)	17	4.18	1.15	0.60	2.21	8	2.20	1.17	0.48	2.81
Urologicals (G04B)	17	4.18	1.34	0.69	2.62	7	1.92	0.80	0.34	1.92
Psychostimulants, agents used for ADHD ^h and nootropics (N06B)	14	3.44	2.01	0.89	4.54	9	2.47	1.24	0.54	2.89
Thyroid preparations (H03B)	8	1.97	1.22	0.48	3.09	14	3.85	0.98	0.52	1.86
Drugs affecting bone structure and mineralization (M05B)	7	1.72	2.42	0.71	8.22	14	3.85	0.82	0.43	1.56
Selective calcium channel blockers with direct cardiac effects (C08D)	11	2.70	1.09	0.50	2.37	10	2.75	1.46	0.65	3.31
Immunosuppressants (L04A)	11	2.70	2.49	1.08	5.76	3	0.82	0.89	0.24	3.27
Corticosteroids, plain (D07A)	11	2.70	3.83	1.43	10.3	3	0.82	0.61	0.16	2.25
Lipid modifying agents, comb. (C10B)	10	2.46	0.95	0.44	2.02	2	0.55	0.29	0.07	1.27

^a Confidence interval; ^b Drugs were coded using the Anatomical-Therapeutic-Chemical (ATC) classification system at the third level (i.e., pharmacological subgroup). Drugs are listed in descending order of overall frequency of dispensation; only those with at least 10 cases in men and/or women are presented. The complete list of drugs is presented in Supplementary Table S2; ^c Odds ratio; the OR of mortality for each dispensed drug was estimated using logistic regression models. The ORs were adjusted by age, included in the model as a categorical variable. Drugs with statistically significant ORs in men and/or women are highlighted in bold (OR > 1) or in italics (OR < 1). All the individuals of the cohort were followed for at least 30 days after cohort entry; ^d Gastro-esophageal reflux disease; ^e Angiotensin II receptor blockers; ^f Combinations; ^g Angiotensin-converting enzyme; ^h Attention-deficit hyperactivity disorder.

Table 4. Models of likelihood of mortality according to baseline demographic and clinical variables of the individuals with laboratory-confirmed infection by COVID-19, by sex.

Variables ^a	OR ^b	95% CI ^c Lower Limit	95% CI Upper Limit	p-Value
Men (n = 407)				
<i>Age (years)</i>				
0–14	-			
15–44	0.10	0.01	0.73	0.024
45–64	Reference			
65–79	5.18	3.35	8.03	<0.001
≥80	10.4	6.75	16.1	<0.001
<i>Chronic diseases</i>				
Chronic ulcer of skin	2.04	1.14	3.65	0.017
Cataract	1.48	1.08	2.01	0.014
Diabetes Mellitus	1.46	1.08	1.98	0.014
<i>Drugs</i>				
Corticosteroids, single drugs	3.32	1.25	8.80	0.016
Vasodilators used in cardiac diseases	2.77	1.33	5.75	0.006
Immunosuppressive agents	2.54	1.08	5.98	0.033
Potassium-sparing agents	2.45	1.23	4.88	0.011
Antipsychotics	1.66	1.13	2.43	0.009
Vitamin B12 and folic acid	1.69	1.05	2.70	0.029
Women (n = 364)				
<i>Age (years)</i>				
0–14	-			
15–44	-			
45–64	Reference			
65–79	15.7	6.99	35.0	0.000
≥80	40.7	18.8	88.3	0.000
<i>Chronic diseases</i>				
Acute myocardial infarction	3.42	1.41	8.32	0.007
Chronic ulcer of skin	1.65	1.07	2.54	0.024
Urinary incontinence	1.35	1.04	1.77	0.027
<i>Drugs</i>				
Peripheral vasodilators	5.49	1.29	23.3	0.021
Potassium-sparing agents	2.17	1.10	4.29	0.026
Antipsychotics	1.81	1.29	2.53	0.001
Antiepileptics	1.63	1.08	2.45	0.019
High-ceiling diuretics	1.41	1.04	1.90	0.028

^a Demographic (age) and clinical (chronic diseases and drugs) variables that remained significant of all the analyzed ones in each of the models after performing multivariate stepwise logistic regression with an exit *p*-value for variables of 0.05; ^b Odds ratio; ^c Confidence interval.

4. Discussion

In this study, we described the main socio-demographic and baseline clinical characteristics of the first 771 deceased individuals with laboratory-confirmed SARS-CoV-2 infection in Aragon, Spain. We analyzed which factors were associated with an increased likelihood of mortality in order to identify potential profiles of men and women to whom prevention and control strategies should be directed during the present and possible future outbreaks of the COVID-19 disease. Our findings suggest that women and men probably share similar but not identical mortality risk factors, and that the focus should be set on older individuals with cardiovascular comorbidity and under treatment with high anticholinergic activity medications, like antipsychotics.

Advanced age is associated with a higher risk of mortality in COVID-19 patients [11,18], which concurs with our findings (more markedly in women). We believe that the higher mortality rates reported in our study, especially among 80-year-old patients and over, could be partially explained by the severity of their clinical status (laboratory testing for COVID-19 infection was initially implemented only in patients at high risk of adverse outcomes), and the large follow-up period (up to ten weeks and at least one month from cohort entry) which allowed for a higher number of definite outcomes to occur. A recent study in 244 hospitalized COVID-19 patients aged 60 and over, with definite outcomes (discharged or deceased), reported 121 deaths (mortality rate of approximately 50%), identifying older

age as one of the most important risk factors [19]. In Spain, the pandemic hit hardest in residential care homes for the elderly, at least during the first weeks of the crisis when less was known about the disease's transmissibility and pathophysiology. This could explain, in part, the older age of infected patients in our study when compared to those of other regions. Individuals living in residential care homes are amongst the most susceptible to poorer health outcomes from COVID-19, mainly due to the higher number of underlying chronic conditions, immunosenescence, and the potential for rapid transmissibility from asymptomatic and pre-symptomatic patients [20–22]. Although the infection was more prevalent in women, we found that COVID-19 mortality was more common in men. In female adults, innate and adaptive immunity is generally stronger than in men, and males are more susceptible to infectious diseases [23]. A closer look at the deceased population in our study reveals that men were younger than women, with a lower mean number of comorbidities and medication profile with lower anticholinergic activity. A higher mean number of medications appears to be associated with poorer outcomes, especially in drugs with anticholinergic activity.

During seasonal influenza outbreaks, the reported incidence of acute cardiovascular events in elders traditionally grows, and emergency-visits for influenza-like disease have been associated with cardiovascular morbidity and mortality [24]. Previous studies reported that some patients with COVID-19 develop acute cardiac injury and that this comorbidity may result in a poorer prognosis [9,25]. It has also been suggested that patients with underlying cardiovascular conditions might also have an increased risk of adverse outcomes [26,27]. Our results show that chronic cardiovascular comorbidity in COVID-19 patients is associated with an increased likelihood of mortality. More precisely, male COVID-19 patients with non-hypertensive congestive heart failure are more likely to die than men without this condition. In female COVID-19 patients, we observe similar results for clinical history of acute myocardial infarction or cardiac dysrhythmias. These findings underline the need to understand how SARS-CoV-2 interacts with cardiovascular comorbidities and medications. Regarding the recent controversy over a possible adverse effect of seasonal flu shot on COVID-19 prognosis, we must highlight that no association between influenza vaccination and risk of mortality was found in our study.

Viral respiratory infections could result in the activation of pathways leading to vascular complications [24]. Various mechanisms could be contributing to cardiac injury by SARS-CoV-2, including, amongst others, direct viral infection of myocardial cells mediated by angiotensin-converting enzyme 2 (ACE2) receptors [25–27]. Activation of coagulation and inflammatory pathways could lead to vascular complications, theoretically both in the form of newly presented acute cardiovascular diseases or the deterioration of underlying chronic conditions. Various authors have recommended caution when prescribing ACE inhibitors or angiotensin-receptor blockers (ARBs) in COVID-19 patients with cardiovascular comorbidity, as this medication can increase ACE2 levels, which could potentially lead to a more severe health outcomes [28,29]. However, in our study, we did not observe any association of plain ACE inhibitors or ARBs with mortality. This concurs with other authors, who did not find evidence of an increased risk of adverse outcomes in patients treated with ACE2 and ARBs in their studies [30]; thus, we believe that more research is needed on this aspect. Our findings underscore the importance of studying the role of ACE2 receptors in the pathophysiology of COVID-19 and of conducting longitudinal population-based epidemiological studies in order to design and validate proper preventive strategies for patients at high risk of adverse outcomes.

Multimorbidity and polypharmacy are very common among the elderly, making it challenging to identify and interpret all the possible interactions among diseases and/or drugs, especially in the presence of an emerging disease such as this one. An interesting finding in our study was that the association of specific pharmacological subgroups with a higher likelihood of mortality does not necessarily constitute a therapeutic indication for chronic diseases that were also identified as potential risk factors. This observation should be interpreted with caution, as there are many epidemiological, clinical, and pathophysiological factors that could contribute to this finding. Possible drug-related side effects and adverse reactions, and drug–drug or drug–disease interactions, could result in a substantially higher risk of poorer prognosis, particularly in multimorbid patients. Our findings may

provide useful information for future studies on this aspect. An interesting observation was the higher likelihood of mortality in patients who were in treatment with high anticholinergic activity medications. Potential adverse consequences of such treatments in the elderly include cognitive and functional decline, cardiovascular complications, and other adverse effects that could lead to institutionalization or other serious outcomes [31–33].

By analyzing all statistically significant associations with an increased likelihood of mortality, we identified different models for men and women. Although these models might describe patients with different clinical profiles, a common and constant characteristic is the presence of diseases that could originate/cause vascular complications, or of medications prescribed for such conditions. Nonetheless, we must highlight that these models explained up to 23% and 26% of the variance of mortality in men and women, respectively. This means that approximately 75% of the mortality would be explained by other factors probably related to genetics, other clinical variables, or the management of the patient. Furthermore, statistical associations do not necessarily translate into the existence of causal relationships, and these findings should be interpreted with caution. Future large-scale population studies based on real-world data in different European settings are encouraged to provide the necessary evidence for the development of early identification strategies for patients at higher risk of adverse outcomes.

The main strength of this study lies in the fact that we analyzed all the individuals of the reference population with a laboratory-confirmed infection by COVID-19 who died during the study period. Baseline comorbidity was individually and exhaustively studied by analyzing a list of 153 chronic conditions, and not only the most common or severe ones. The medication burden was also exhaustively analyzed, however, we only focused on medications dispensed during the month prior to the infection, and we might have missed some of the patients' treatments. Another limitation lies in the unavailability of certain variables that would have been of interest for the aim of our study, such as socio-economic variables, genetics, laboratory tests, and inpatient treatments, amongst others.

5. Conclusions

Our findings suggest that the comorbidity and medication burden present at infection with SARS-CoV-2 could partially affect the outcome of the COVID-19 disease, and that women and men probably share similar but not identical risk factors. Advanced age seems to be the most influencing factor, followed by the presence of various underlying conditions and medications, particularly those related to the cardiovascular system. Nonetheless, much of the mortality seems to be explained by other factors probably related to patients' clinical management, genetics, and other clinical variables. More research is needed in this field, in different populations and clinical settings, to provide the necessary evidence for the development of early identification strategies for patients at higher risk of adverse outcomes.

Supplementary Materials: The following are available online at <http://www.mdpi.com/1660-4601/17/14/5171/s1>, Table S1: Prevalence of chronic diseases at cohort entry of all the individuals of the COVID-19 Aragon cohort and of those died during the follow-up period, and association with mortality, by sex; Table S2: Prevalence of drugs dispensed during the month prior to cohort entry of all the individuals of the COVID-19 Aragon cohort and of those died during the follow-up period, and association with mortality, by sex.

Author Contributions: Conceptualization, A.P.-T. and A.G.-M.; methodology, B.P.-P., A.P.-F., J.C.-P. and S.M.; formal analysis, B.P.-P.; resources, A.P.-F., A.C.B.-L. and M.C.-d.P.; data curation, A.P.-F.; writing—original draft preparation, I.I.-S. and A.G.M.; writing—review and editing, F.G.-R., L.A.G.-F., M.A.-P.-S., J.D.-M., J.M.-M., K.B.-B., M.C.-d.P., A.C.B.-L., A.P.-T. and all members of the EpiChron Group; supervision, A.P.-T.; funding acquisition, A.P.-T., A.G.-M. and J.C.-P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Gobierno de Aragón (Grant numbers B01_20R and PI20/226) and the European Regional Development Fund "Construyendo Europa desde Aragón". J.C.-P. was awarded a Río Hortega research grant from Instituto de Salud Carlos III and IIS Aragón (Grant number CM19/00164).

Acknowledgments: The authors sincerely thank all professionals from Dirección General de Asistencia Sanitaria, Dirección General de Salud Pública, and Servicio Aragonés de Salud (SALUD), from Departamento de Salud del Gobierno de Aragón, for their essential collaboration to advance this research line in the midst of such challenging times. This research was funded by Gobierno de Aragón (Grant numbers B01_20R and PI20/226) and the European Regional Development Fund “Construyendo Europa desde Aragón”.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Appendix A

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4.2. Manuscript 2: Chronic diseases associated with increased likelihood of hospitalization and mortality in 68,913 COVID-19 confirmed cases in Spain: A population-based cohort study.

Gimeno-Miguel A, Bliek-Bueno K, Poblador-Plou B, Carmona-Pérez J, Poncel-Falcó A, González-Rubio F, Ioakeim-Skoufa I, Pico-Soler V, Aza-Pascual-Salcedo M, Prados-Torres A, Gimeno-Feliu LA; PRECOVID Group. **Chronic diseases associated with increased likelihood of hospitalization and mortality in 68,913 COVID-19 confirmed cases in Spain: A population-based cohort study.** *PLoS One*. 2021 Nov 12;16(11):e0259822. Doi: 10.1371/journal.pone.0259822

Journal: PLoS One

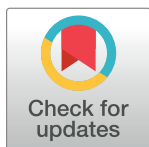
Impact Factor: 3.752, Q2 (MULTIDISCIPLINARY SCIENCES-SCIE)

Status: Published

RESEARCH ARTICLE

Chronic diseases associated with increased likelihood of hospitalization and mortality in 68,913 COVID-19 confirmed cases in Spain: A population-based cohort study

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OPEN ACCESS

Citation: Gimeno-Miguel A, Bliek-Bueno K, Poblador-Plou B, Carmona-Pérez J, Poncel-Falcó A, González-Rubio F, et al. (2021) Chronic diseases associated with increased likelihood of hospitalization and mortality in 68,913 COVID-19 confirmed cases in Spain: A population-based cohort study. PLoS ONE 16(11): e0259822. <https://doi.org/10.1371/journal.pone.0259822>

Editor: Alessandro de Sire, University of Catanzaro: Università degli Studi Magna Graecia di Catanzaro, ITALY

Received: August 11, 2021

Accepted: October 26, 2021

Published: November 12, 2021

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Data Availability Statement: Data used in this study cannot be publicly shared because of restrictions imposed by the Aragon Health Sciences Institute (IACS) and asserted by the Clinical Research Ethics Committee of Aragon (CEICA, ceica@aragon.es). Authors who accessed the data belong to the EpiChron Research Group of IACS and received permission from IACS for its use in this specific study. Data can be made

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Abstract

Background

Clinical outcomes among COVID-19 patients vary greatly with age and underlying comorbidities. We aimed to determine the demographic and clinical factors, particularly baseline chronic conditions, associated with an increased risk of severity in COVID-19 patients from a population-based perspective and using data from electronic health records (EHR).

Methods

Retrospective, observational study in an open cohort analyzing all 68,913 individuals (mean age 44.4 years, 53.2% women) with SARS-CoV-2 infection between 15 June and 19 December 2020 using exhaustive electronic health registries. Patients were followed for 30 days from inclusion or until the date of death within that period. We performed multivariate logistic regression to analyze the association between each chronic disease and severe infection, based on hospitalization and all-cause mortality.

available for potential collaborations upon reasonable request to the Principal Investigator of the EpiChron Research Group, Alexandra Prados-Torres (sprados.iaes@aragon.es).

Funding: This research was funded by Gobierno de Aragón (Grant numbers B01_20R and DECRETO-LEY 3/2020, de 3 de junio, del Gobierno de Aragón), which provided financial support to the EpiChron Research Group and financed the article processing charge. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Results

5885 (8.5%) individuals showed severe infection and old age was the most influencing factor. Congestive heart failure (odds ratio -OR- men: 1.28, OR women: 1.39), diabetes (1.37, 1.24), chronic renal failure (1.31, 1.22) and obesity (1.21, 1.26) increased the likelihood of severe infection in both sexes. Chronic skin ulcers (1.32), acute cerebrovascular disease (1.34), chronic obstructive pulmonary disease (1.21), urinary incontinence (1.17) and neoplasms (1.26) in men, and infertility (1.87), obstructive sleep apnea (1.43), hepatic steatosis (1.43), rheumatoid arthritis (1.39) and menstrual disorders (1.18) in women were also associated with more severe outcomes.

Conclusions

Age and specific cardiovascular and metabolic diseases increased the risk of severe SARS-CoV-2 infections in men and women, whereas the effects of certain comorbidities are sex specific. Future studies in different settings are encouraged to analyze which profiles of chronic patients are at higher risk of poor prognosis and should therefore be the targets of prevention and shielding strategies.

Introduction

The coronavirus disease 2019 (COVID-19) pandemic was declared a Public Health Emergency of International Concern by the World Health Organization in March 2020 and, since then, SARS-CoV-2 has affected more than 150 million people and has caused over 3.2 million deaths worldwide [1].

Clinical characteristics among COVID-19 patients vary greatly, especially with age [2]. Most cases, in particular in children and younger adults, manifest as asymptomatic or mild infections, while others, typically older adults or patients with comorbidities, can develop severe to critical presentations with acute respiratory distress syndrome (ARDS) and even death [2, 3]. Apart from age, the concurrence of specific chronic conditions has been amply linked through metanalytical data to increased risk of severe infection, with chronic lung and kidney diseases, obesity, diabetes, hypertension, or malignancy as some of the main examples [4, 5]. COVID-19 has also been associated with intense multisystemic inflammation and an uncontrolled immune response, leading to cardiovascular complications and poorer outcomes, more so in patients with underlying comorbidities [6, 7].

The definition of severity for COVID-19 infection differs across the existing literature, as evidenced by the systematic reviews conducted by Flook et al. and Li et al., where some authors followed the international guidelines for community acquired pneumonia or clinical criteria to classify severe cases (acute respiratory distress syndrome, tachypnea, hypoxemia, pulmonary imaging, multi-organ failure), while others considered outcomes such as intensive care unit (ICU) admission, invasive ventilation support or death, among others [5, 8]. Clinical factors, along with other individual features such as functional capacity and autonomy, are key in determining COVID-19 severity in routine clinical practice [9]. However, indicators such as mortality and hospitalization can serve as proxy, and aid in decision-making for the allocation of expected resources and the development of mitigation and prevention strategies at a health system level, which also have an influence on health results in infected patients [10].

In our previous study on comorbidity in COVID-19 patients within the PRECOVID cohort we used all-cause mortality as the only outcome to measure severity [11]. The epidemiological situation at the time, with an overburdened healthcare system that could not assume all the necessary hospitalizations, and a lack of overall testing capacity, impeded the use of other relevant indicators like hospital and ICU admissions during follow-up. With the improvement of testing capacity, allowing for microbiological confirmation of all COVID-19 cases, and less pressure in hospitals thanks to large-scale preventive measures (i.e., use of facemasks, confinements, quarantines, limited social reunions and contact tracing), current data on admission can be reliably studied along with mortality as an indicator of severity. While many studies focus on in-hospital mortality alone, often with self-reported diagnoses of specific chronic conditions [12], PRECOVID is a population-based cohort that offers a holistic view of baseline comorbidity profiles and health outcomes, diagnosed and recorded in electronic health records by healthcare professionals in both in- and out-patient settings.

The objective of the present study is to determine the demographic and clinical factors, particularly baseline chronic conditions, associated with an increased risk of severity in COVID-19 patients, operationalized through a combined outcome of hospitalization and all-cause mortality.

Methods

Design and study population

We conducted a retrospective, observational study in the PRECOVID cohort [10], an open cohort of all the individuals with confirmed SARS-CoV-2 infection in the Spanish region of Aragon (reference population, 1.3 million inhabitants). Cases were confirmed using PCR and antigen-detection tests following the national protocol from the Ministry of Health on early detection, surveillance and control of COVID-19. We analyzed all 68,913 cases that were confirmed in Aragon during enrollment, which spanned from 15 June 2020 to 19 December 2020 for this study. We excluded patients diagnosed during the first three months of the pandemic (March through May 2020) as testing capacity in our region was limited at the time.

Each patient was followed for up to 30 days from the date of inclusion in the cohort (index date) or until the date of death within that period in order to analyze the effect of patients' baseline characteristics on infection severity. The index date was defined by the date of the confirmatory test result. Infections were categorized as mild or moderate-to-severe, referred to from now on as severity, based on the need for hospital admission (including admissions to the Intensive Care Unit—ICU) and 30-day mortality.

The Clinical Research Ethics Committee of Aragón approved the research protocol for this study (CEICA; PI20/226) and waived the requirement to obtain informed consent from participants due to the epidemiological nature of the project and the use of anonymized data, presented only at an aggregated level.

Variables of study and data sources

The PRECOVID study cohort analyzes COVID-19 clinical and epidemiological characteristics through the linkage of real-world data from electronic health records (EHRs) and clinical-administrative databases. Patient data was obtained from the Aragon Health System by associating information from the users' health database, EHRs, and an ad hoc COVID-19 registry, at a person level and in a pseudo-anonymized form.

We studied the following baseline demographic and clinical characteristics for each participant at the time of inclusion in the cohort: sex, age, type of residence area (rural vs. urban), and all baseline chronic diseases registered in EHRs. Primary care diagnoses were coded using

the International Classification of Primary Care, First Edition (ICPC-1), and mapped to ICD-9-CM using a system developed to codify temporary disability [13]. The codes were subsequently sorted into 226 clinically relevant categories using the Clinical Classifications Software (CCS) [14], and 153 of them were classified as chronic via the Chronic Condition Indicator Software [15]. The group's clinical experts recoded the final list into 156 diagnostic categories with minor adjustments to facilitate their clinical interpretation. Conditions present at least during the last 12 months and meeting one or both of the following criteria were included in the analysis: a) entails limitations on self-care, independent living, and social interactions; b) requires of ongoing interventions using medical products, services, and special equipment.

During follow-up, we analyzed whether the patient experienced severe infection, defined as hospitalization (including admission to the ICU) or all-cause mortality, or mild infection (rest of the cases). Patients were followed for a maximum of 30 days from their inclusion in the cohort to give enough time for the studied outcomes to occur. Events taking place after the set follow-up period were considered difficult to attribute to COVID-19 infection seeing as the exact cause of death was not available in our registries. Regarding hospitalizations, only admissions occurring within 15 days of the index date were considered a consequence of the infection. Since some patients were diagnosed only after admission, we also accounted for hospitalizations occurring up to 15 days before the index date.

Statistical analysis

We described the sociodemographic and clinical characteristics of all patients from the study as means accompanied by their respective standard deviations or as absolute and relative frequencies and proportions, stratified by sex and by severity of infection.

We performed logistic regression models to analyze the association between the presence of each baseline chronic disease and the occurrence of severe infection. We used logistic regression instead of Cox regression since we had the date of inclusion in the cohort (date of the first confirmatory test result), but not the exact date of infection; moreover, follow-up was truncated at 30 days after the index date for all individuals. We calculated age-adjusted odds ratios (ORs) accompanied by their respective 95% confidence intervals (CI). Analysis was stratified by sex. Only chronic diseases with a prevalence equal to or higher than 0.5% in each sex and with a significant association with infection severity were presented in tables; the rest can be found as Supporting information.

Finally, a multivariate logistic regression analysis was performed to identify which chronic comorbidities were significantly associated with higher infection severity when studied in combination, with age as an added explanatory variable. The multivariate analysis was performed using a stepwise method via the STEP function from the stats package, and was based on the Akaike Information Criterion (AIC) with an exit p-value of 0.05. AIC is preferable as a criterion for model selection in situations where the model is intended for predictive purposes. All statistical analyses were conducted in R 3.6.3 and Stata 14. Statistical significance was set at $p < 0.05$.

Results

A total of 68,913 individuals were included in the study, with a mean age of 44.4 years. 53.2% were women, 39.1% lived in rural areas and 58.6% presented multimorbidity, with an average of 2.89 chronic diseases at infection (Table 1). During follow-up, 5885 (8.5%) individuals showed severe infection as defined in our study (88.6% of them were hospitalized, 5.52% were admitted to the ICU, and 27.2% died). The risk of severe infection was 71.1% higher in men compared to women and consistently increased with age in both sexes, with patients aged 80

Table 1. Demographic and clinical characteristics of individuals with confirmed COVID-19 infection and effect on disease severity (defined as mild or moderate-to-severe according to hospital admission and mortality).

Characteristics	All (n = 68,913)			P value	Women (n = 36,682)			P value	Men (n = 32,231)			P value
	Mild ^a (x = 63,028)	Moderate-to-severe ^b (n = 5885)	OR ^c (95% CI ^d)		Mild (n = 33,904)	Moderate-to-severe (n = 2778)	OR (95% CI)		Mild (n = 29,124)	Moderate-to-severe (n = 3107)	OR (95% CI)	
Sex, men (n, %)	29,124 (46.2)	3107 (52.8)	1.71 (1.61–1.82)	<0.001	-	-	-	-	-	-	-	-
Age (mean, sd ^e)	41.9 (22.7)	71.6 (18.7)	1.07 (1.06–1.07)	<0.001	43.3 (23.3)	73.9 (19.2)	1.06 (1.06–1.06)	<0.001	40.3 (21.9)	69.6 (18.0)	1.07 (1.07–1.07)	<0.001
Age intervals (n, %)												
≤14	8075 (12.8)	47 (0.80)	0.07 (0.06–0.10)	<0.001	4020 (11.9)	16 (0.58)	0.07 (0.04–0.12)	<0.001	4055 (13.9)	31 (1.00)	0.07 (0.05–0.10)	<0.001
15–44	26,367 (41.8)	469 (7.97)	0.23 (0.21–0.25)	<0.001	13,902 (41.0)	234 (8.42)	0.31 (0.27–0.37)	<0.001	12,465 (42.8)	235 (7.56)	0.18 (0.16–0.21)	<0.001
45–64	18,358 (29.1)	1422 (24.2)	Reference	-	9844 (29.0)	530 (19.1)	Reference	-	8514 (29.2)	892 (28.7)	Reference	-
65–79	5905 (9.37)	1427 (24.2)	3.11 (2.88–3.37)	<0.001	3118 (9.20)	579 (20.8)	3.45 (3.05–3.91)	<0.001	2787 (9.57)	848 (27.3)	2.90 (2.62–3.22)	<0.001
≥80	4323 (6.86)	2520 (42.8)	8.23 (7.64–8.87)	<0.001	3020 (8.91)	1419 (51.1)	8.73 (7.84–9.72)	<0.001	1303 (4.47)	1101 (35.4)	8.07 (7.26–8.97)	<0.001
Rural area^f (n, %)	24,561 (39.0)	2415 (41.0)	0.87 (0.82–0.92)	<0.001	12,822 (37.8)	1097 (39.5)	0.82 (0.75–0.89)	<0.001	11,739 (40.3)	1318 (42.4)	0.91 (0.84–0.99)	0.033
Chronic diseases^g (mean, sd)	2.7 (2.8)	5.2 (3.8)	1.04 (1.03–1.05)	<0.001	3.1 (3.0)	5.8 (3.9)	1.03 (1.02–1.04)	<0.001	2.2 (2.4)	4.6 (3.5)	1.05 (1.04–1.07)	<0.001
Follow-up outcome												
Hospital adm.^h (n, %)	-	5214 (88.6%)	-	-	-	2441 (87.9%)	-	-	-	2773 (89.3%)	-	-
ICUⁱ adm. (n, %)	-	325 (5.52%)	-	-	-	107 (3.85%)	-	-	-	218 (7.02%)	-	-
Death^j (n, %)	-	1598 (27.2%)	-	-	-	791 (28.5%)	-	-	-	807 (26.0%)	-	-

^aDefined as individuals without hospital admission or mortality during the follow-up period;^bDefined as individuals admitted to hospital or dead during the follow-up period;^cOdds ratio of moderate-to-severe infection adjusted by categorical age;^d95% confidence interval;^estandard deviation;^fVersus urban area, according to patients' administrative health area;^gFrom a list of 153 chronic conditions;^hAdmission to hospital during 15 days before/after the index date;ⁱAdmission to the Intensive Care Unit;^j30-day all-cause mortality.<https://doi.org/10.1371/journal.pone.0259822.t001>

years and over showing 8.23 (7.64–8.87) times higher risk than those in the 45–64 years age group.

Baseline chronic diseases associated the most with severe infection in COVID-19 infected women when studied separately and after controlling by age were (OR [95% CI]) female infertility (1.91 [1.25–2.91]), obstructive sleep apnea (OSA; 1.65 [1.19–2.29]), hepatic steatosis and

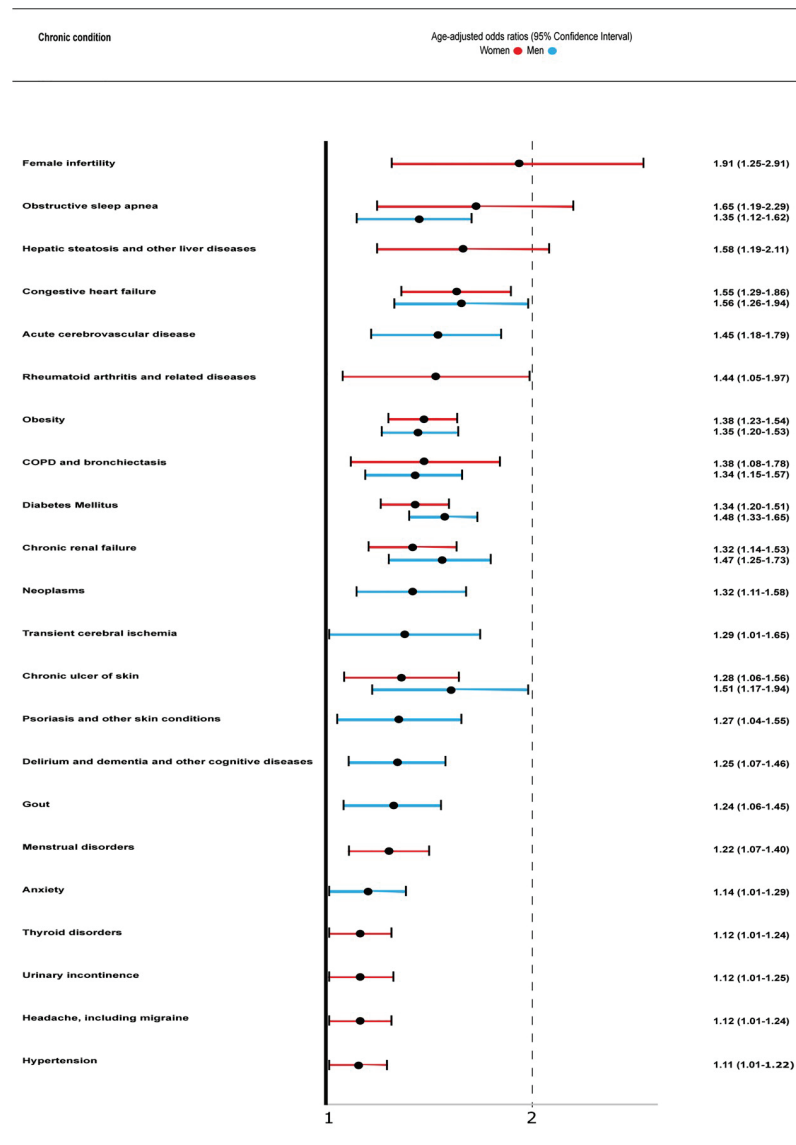


Fig 1. Baseline chronic comorbidities associated with moderate-to-severe infection, defined as the combined outcome of hospital admission and 30-day all-cause mortality, in women (red) and men (blue) with COVID-19 infection. COPD, chronic obstructive pulmonary disease.

<https://doi.org/10.1371/journal.pone.0259822.g001>

other liver diseases (1.58 [1.19–2.11]), congestive heart failure (1.55 [1.29–1.86]), rheumatoid arthritis (1.44 [1.05–1.97]), obesity (1.38 [1.23–1.54]), chronic obstructive pulmonary disease (COPD; 1.38 [1.08–1.78]), diabetes mellitus (1.34 [1.20–1.51]), and chronic renal failure (1.32 [1.14–1.53]), among others (Fig 1). The complete list of basal chronic conditions in infected women along with their prevalence and association with infection severity is presented as [S1 Table](#).

In men, baseline chronic conditions associated the most with higher infection severity when studied separately were (OR [95% CI]) congestive heart failure (1.56 [1.26–1.94]), chronic ulcers of skin, (1.51 [1.17–1.94]), diabetes mellitus (1.48 [1.33–1.65]), chronic renal failure (1.47 [1.25–1.73]), acute cerebrovascular disease (1.45 [1.18–1.79]), obesity (1.35 [1.20–

Table 2. Models of likelihood of moderate-to-severe COVID-19 infection according to baseline demographic and clinical variables of the individuals with laboratory-confirmed infection, by sex.

Sex	Women (n = 36,682)				Men (n = 32,231)			
Variables	OR ^b	95% CI ^a Lower limit	95% CI Upper limit	p value	OR	95% CI Lower limit	95% CI Upper limit	p value
(Intercept)	0.05	0.04	0.05	<0.001	0.09	0.09	0.10	<0.001
Age, 0–14 years	0.08	0.05	0.14	<0.001	0.08	0.06	0.12	<0.001
Age, 15–44 years	0.32	0.28	0.38	<0.001	0.20	0.17	0.23	<0.001
Age, 45–64 years	3.33	2.92	3.81	<0.001	2.45	2.20	2.73	<0.001
Age, ≥65 years	8.19	7.24	9.26	<0.001	6.19	5.47	6.99	<0.001
Congestive heart failure	1.39	1.16	1.68	<0.001	1.28	1.03	1.60	0.028
Diabetes Mellitus	1.24	1.10	1.40	<0.001	1.37	1.23	1.53	<0.001
Chronic renal failure	1.22	1.05	1.42	0.011	1.31	1.11	1.55	0.002
Obesity	1.26	1.13	1.42	<0.001	1.21	1.06	1.37	0.003
Female infertility	1.87	1.22	2.85	0.004	-	-	-	-
Obstructive sleep apnea	1.43	1.03	2.00	0.035	-	-	-	-
Hepatic steatosis and other liver diseases	1.43	1.07	1.91	0.016	-	-	-	-
Rheumatoid arthritis and related disease	1.39	1.01	1.92	0.042	-	-	-	-
Menstrual disorders	1.18	1.03	1.35	0.016	-	-	-	-
Chronic ulcer of skin	-	-	-	-	1.32	1.02	1.71	0.032
Acute cerebrovascular disease	-	-	-	-	1.34	1.09	1.65	0.006
COPD ^c and bronchiectasis	-	-	-	-	1.21	1.03	1.42	0.022
Urinary incontinence	-	-	-	-	1.17	1.00	1.36	0.044
Neoplasms	-	-	-	-	1.26	1.06	1.51	0.010

^a95% confidence interval;^bOdds ratio;^cChronic obstructive pulmonary disease.<https://doi.org/10.1371/journal.pone.0259822.t002>

1.53]), obstructive sleep apnea (1.35 [1.12–1.62]), COPD (1.34 [1.15–1.57]), urinary incontinence (1.33 [1.15–1.54]), and neoplasms (1.32 [1.11–1.58]), among others (Fig 1). The complete list of chronic diseases and their association with infection severity in men is presented as S2 Table.

The models assessing the likelihood of severe COVID-19 infection based on sex, age and comorbidity at baseline showed that age was the most influencing factor, with an increased average risk of 6.19 in men and 8.19 in women aged 80 and over compared to their 45–64-year-old counterparts (Table 2). Nine conditions in men and nine in women were significant in the models (OR men, OR women), with congestive heart failure (1.28, 1.39), diabetes mellitus (1.37, 1.24), chronic renal failure (1.31, 1.22) and obesity (1.21, 1.26) increasing the likelihood of severe infection in both sexes. Additionally, chronic ulcers of the skin (1.32), acute cerebrovascular disease (1.34), COPD (1.21), urinary incontinence (1.17) and neoplasms (1.26) in men, and female infertility (1.87), OSA (1.43), hepatic steatosis (1.43), rheumatoid arthritis (1.39) and menstrual disorders (1.18) in women were also associated with higher risk of severity.

Discussion

This large-scale population-based study describes the clinical and demographic characteristics associated with an increased risk of severity in 68,913 individuals with confirmed SARS-CoV-2 infection. Our findings show that, apart from old age and male sex, specific baseline

cardiovascular and metabolic conditions are common risk factors for severe infection in men and women, while other comorbidities are sex-specific.

Our analyses reasserted that age is by far the most influencing factor on the vulnerability of older people to develop more severe forms of COVID-19, most notoriously when crossing the threshold of the 80s, highlighting the need for targeted prevention and early detection strategies in older age groups [16, 17]. Men were at higher risk of severity than women, a fact that has been amply reported and attributed to various causes such as lifestyle differences (e.g. smoking) or hormone differences in inflammatory processes [18].

Cardiovascular and metabolic comorbidities (i.e. obesity, diabetes, congestive heart failure and chronic renal failure) increased the risk of severe infection in both sexes even when adjusting by age and the remaining significant comorbidities, as described in previous studies [19–24]. Patients with these conditions seem to be predisposed to potentially serious COVID-19 infections through common pathophysiological pathways, with chronic systemic inflammation at their center [7]. Key aspects include ACE-2-mediated endothelial injury, blood flow stasis due to immobilization, and a state of hypercoagulability induced by prothrombotic circulating factors, which contribute to the occurrence of coagulopathies and thromboembolic events, markedly increasing the risk of severe outcomes [25–29]. Arterial hypertension was abundantly associated with poor health outcomes after COVID-19 infection in early studies, but was usually reported along with potentially confounding effects such as age and other chronic conditions [30]. In our study, hypertension was not a risk factor for severity in men, and lost its significance when adjusting by the rest of comorbidities in women, supporting the theory that it entails higher risk of severity only in association with other diseases but not as an isolated condition [31].

Vulnerable ACE2-expressing tissues include the endothelium, heart, kidneys, lungs, liver, brain, thyroid, testis, and small intestine. ACE2 expression levels and immune signatures depends on the organ or tissue, and is also sex- and age-dependent, partly explaining distinct symptoms and severity [32]. Chronic respiratory diseases like COPD and OSA have been previously identified as risk factors for severe outcomes in COVID-19 patients, and have also been associated with higher risk of thrombosis, coagulopathy and cardiovascular morbidity [22, 33–40]. In our study we found similar results but, when adjusting by the rest of comorbidities, COPD only remained significant in men, and OSA only in women.

In addition to COPD and OSA, some chronic conditions were independent predictors of severity only in one sex. In men, neoplasms and acute cerebrovascular disease were independently associated with COVID-19 severity. Both entities had been previously identified as risk factors for poor prognosis [20–23]. Chronic ulcers of the skin and urinary incontinence, usually linked to serious states of functional impairment, were also significantly associated with higher risk in our age-adjusted analysis, concurring with our previous study [11]. Anxiety, as well as dementia, delirium and other cognitive disorders were additional risk factors for severity, also only in men in our case, and further research could shed light on the benefits of including neuropsychological evaluation and rehabilitation during and after COVID-19 infection, as has been stated in the literature [41, 42]. In women, hepatic steatosis and rheumatoid arthritis were risk factors for severe outcomes. Although the association of liver disease with a more severe progression of COVID-19 has been described [43], the role of rheumatoid arthritis remains unclear in women, highlighting the importance of age, comorbidity, disease activity and pharmacological treatments in future studies [44, 45].

An unexpected finding was the increased risk of severe infection in women with clinical history of infertility or menstrual disorders. It is well known that assisted reproduction techniques, especially ovarian stimulation, are associated with a higher risk of arterial and venous thromboembolic complications [27]. Further studies are encouraged in the future to

specifically address the potential mid- and/or long-term effects of these conditions and its treatments on COVID-19 infection severity. The interpretation of the association of menstrual disorders with severity is challenging, especially considering how varied the pathophysiology of the processes included under this denomination is (i.e., polycystic ovary, dysmenorrhea, metrorrhagia), and would also require more targeted and exhaustive studies.

Regarding thyroid disorders, we found higher risk of severe outcomes in women, though the effect was lost when adjusting by the rest of comorbidities. To date, there is no evidence suggesting that these disorders increase the risk of poor prognosis in COVID-19 patients [46]. Notwithstanding, thyroid dysfunction does influence the regulation of immunity, particularly if it is cell-based. Low concentrations of thyroid hormones have been associated with increased mortality in patients with serious infectious diseases [46, 47] and could have been partly responsible for some of the more severe COVID-19 infections, seeing as 80% of those diagnosed with thyroid disorders had hypothyroidism. As far as we know, only two previous studies have analysed this hypothesis, and although both of them ruled out any significant association between hypothyroidism and COVID-19 infection severity, further studies are warranted [46, 48].

Due to their low prevalence (between 0.1–0.5%), some chronic conditions were not presented in the [Results](#) section of the manuscript despite statistical significance (available as supplementary tables), and their potential impact on infection severity may have not been sufficiently analyzed. In the case of men, two inflammatory conditions were associated with higher severity: rheumatoid arthritis (in line with what was observed in women) and polymyalgia. It has been reported that autoimmune diseases are not significantly associated with severe COVID-19 progression [49], and poor outcomes could simply be linked to older age and comorbidity [45], though one study did find a 39% increase in the adjusted risk of hospitalization and mortality in a sample of mostly male patients with COVID-19 infection and rheumatoid arthritis [50]. In women, we found a high risk of severe COVID-19 outcomes in patients with HIV infection and, although similar findings have been published in the literature, further studies are needed to better assess this risk taking into account disease control, treatment, analytical blood parameters, and comorbidity [51–53].

Strengths and limitations

The main strength of our study is its population-based nature. It includes all the confirmed COVID-19 cases in the region, with a sample size of nearly 70,000 individuals. Another key feature of our study is that baseline comorbidities were not limited to the most prevalent or to those self-reported by patients, but were based on a comprehensive analysis of all the chronic diagnoses registered by physicians in their EHRs instead. Furthermore, we used open-access software with an open algorithm to manage chronic conditions in the analyses, facilitating reproducibility and comparability. Our study, however, is not free of limitations. Some socio-demographic variables, such as socioeconomic status and ethnicity, or clinical variables like laboratory tests results, acute respiratory distress, multi-organ failure, pharmacological treatments, or genetic profiles could have influenced infection severity or aided in the interpretation of the results, but were not available in our cohort. Moreover, the causes of hospitalization and/or death were also unavailable, and we were therefore unable to directly attribute the observed outcomes to the infection. To minimize these limitation, we assessed both outcomes in a limited time window based on clinical criteria, and excluded COVID-19 patients from the first three months of the pandemic where access to diagnostic tests and in some cases medical care was more limited. Finally, though we conducted comorbidity-adjusted multivariate logistic regression analyses, each chronic condition contributed independently to estimate the

associated risk of severity. We know, however, that patients with more severe forms of infection are usually affected by more than one chronic disease, as was the case in our study (81% were multimorbid, with an average of 5.2 chronic conditions). Considering that chronic diseases tend to cluster into multimorbidity patterns, future studies should focus on the combined burden of coexisting conditions and the effects of their interactions on COVID-19 outcomes, as well as on the consequences of COVID-19 on patients and the healthcare system.

Conclusions

This population-based study reasserts that old age is the most influencing factor on COVID-19 severity and contributes to the ongoing discussion on the effects of comorbidity on the evolution of the infection. The presence of specific cardiovascular and metabolic conditions (i.e. heart failure, diabetes mellitus, obesity and chronic renal failure) consistently increased the risk of severe SARS-CoV-2 infections in men and women. Results also reveal sex differences in certain disorders, such as neoplasms and acute cerebrovascular disease in men or thyroid disorders and hepatic steatosis in women, whose effects on COVID-19 severity have been less studied to date. More in-depth research on the impact of each disease and their combinations is encouraged to ascertain whether COVID-19 could act as a trigger for negative health outcomes in patients with specific multimorbidity profiles.

Supporting information

S1 Table. Complete list of baseline chronic comorbidities of women with COVID-19 infection and association with moderate-to-severe infection, defined as the combined outcome of hospital admission and 30-day all-cause mortality.

(XLSX)

S2 Table. Complete list of baseline chronic comorbidities of men with COVID-19 infection and association with moderate-to-severe infection, defined as the combined outcome of hospital admission and 30-day all-cause mortality.

(XLSX)

Acknowledgments

The authors sincerely thank Dirección General de Asistencia Sanitaria, Dirección General de Salud Pública, and Servicio Aragonés de Salud, from Departamento de Salud del Gobierno de Aragón, for their essential collaboration in developing the COVID-19 registry.

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4.3. Manuscript 3: Baseline drug treatments as indicators of increased risk of COVID-19 mortality in Spain and Italy.

Bliek-Bueno K, Mucherino S, Poblador-Plou B, González-Rubio F, Aza-Pascual-Salcedo M, Orlando V, Clerencia-Sierra M, Ioakeim-Skoufa I, Coscioni E, Carmona-Pírez J, Perrella A, Trama U, Prados-Torres A, Menditto E, Gimeno-Miguel A. **Baseline drug treatments as indicators of increased risk of COVID-19 mortality in Spain and Italy.** *Int J Environ Res Public Health*. 2021 Nov 10;18(22):11786. Doi: <https://doi.org/10.3390/ijerph182211786>

Journal: International Journal of Environmental Research and Public Health

Impact Factor: 4.614, Q1 (PUBLIC, ENVIRONMENTAL & OCCUPATIONAL HEALTH-SSCI), Q2 (PUBLIC, ENVIRONMENTAL & OCCUPATIONAL HEALTH-SCIE)

Status: Published



Article

Baseline Drug Treatments as Indicators of Increased Risk of COVID-19 Mortality in Spain and Italy

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Citation: Blik-Bueno, K.; Mucherino, S.; Poblador-Plou, B.; González-Rubio, F.; Aza-Pascual-Salcedo, M.; Orlando, V.; Clerencia-Sierra, M.; Ioakeim-Skoufa, I.; Coscioni, E.; Carmona-Pírez, J.; et al. Baseline Drug Treatments as Indicators of Increased Risk of COVID-19 Mortality in Spain and Italy. *Int. J. Environ. Res. Public Health* **2021**, *18*, 11786. <https://doi.org/10.3390/ijerph182211786>

Academic Editor: Paul B. Tchounwou

Received: 7 September 2021

Accepted: 6 November 2021

Published: 10 November 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



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Abstract: This study aims to identify baseline medications that, as a proxy for the diseases they are dispensed for, are associated with increased risk of mortality in COVID-19 patients from two regions in Spain and Italy using real-world data. We conducted a cross-country, retrospective, observational study including 8570 individuals from both regions with confirmed SARS-CoV-2 infection between 4 March and 17 April 2020, and followed them for a minimum of 30 days to allow sufficient time for the studied event, in this case death, to occur. Baseline demographic variables and all drugs dispensed in community pharmacies three months prior to infection were extracted from the PRECOVID Study cohort (Aragon, Spain) and the Campania Region Database (Campania, Italy) and analyzed using logistic regression models. Results show that the presence at baseline of potassium-sparing agents, antipsychotics, vasodilators, high-ceiling diuretics, antithrombotic agents, vitamin B12, folic acid, and antiepileptics were systematically associated with mortality in COVID-19 patients from both countries. Treatments for chronic cardiovascular and metabolic diseases, systemic inflammation, and processes with increased risk of thrombosis as proxies for the conditions they are intended for can serve as timely indicators of an increased likelihood of mortality after the infection, and the assessment of pharmacological profiles can be an additional approach to the identification of at-risk individuals in clinical practice.

Keywords: COVID-19; medications; drugs; mortality; real-world data

1. Introduction

Around 182 million COVID-19 cases, including almost 4 million deaths, have been reported to the World Health Organization (WHO) worldwide since the beginning of the pandemic in 2020 [1]. The wide range of clinical manifestations and possible outcomes of the infection, from asymptomatic to critical or even deadly, has made identifying underlying conditions that affect COVID-19 mortality a top priority.

Age is widely considered the most influential factor in infection mortality [2,3]. Others include male sex, and cardiovascular and metabolic comorbidities such as diabetes, obesity, chronic renal failure and chronic heart disease [4]. A fundamental aspect common to these conditions and COVID-19 pathophysiology is its repercussion on the body's inflammatory response and immune and coagulation systems [5].

Given their critical role on health outcomes, population-based studies using pharmacological information have set their focus on heavily age-related factors such as anticholinergic risk and polypharmacy, mainly based on anticholinergic scales or the number of prescribed or dispensed drugs [6]. However, and apart from the effects of the drugs themselves on COVID-19 prognosis, the use of dispensation data prior to the infection can be an additional tool for risk stratification in infected patients, especially when information on the underlying comorbidities is unavailable or when the pressure on the healthcare system demands expeditious risk evaluation and decision making. Though less common, studying medication profiles as a proxy of disease and their association with COVID-19 severity can be a swift and objective approach to the characterization of vulnerable patient profiles for the improvement of their clinical management and the development of targeted prevention strategies for at-risk individuals [7–9].

The objective of this study was to identify baseline medications associated with an increased risk of mortality that can serve as timely indicators of severity in COVID-19 patients from two regions in Spain and Italy, two of the hardest hit countries during the initial SARS-CoV-2 outbreak in Europe.

2. Materials and Methods

2.1. Design and Study Population

We conducted a cross-country, retrospective observational study using real-world data. Our study population was obtained from two cohorts comprising users of the public health services of two European regions: the PRECOVID Study cohort in Aragon (Spain) and the Campania Region Database (CaReDB) in Campania (Italy). From this point on, the cohorts will be referred to simply as Aragon and Campania.

For this study, we included all the individuals from both regions with confirmed SARS-CoV-2 infection between 4 March 2020 and 17 April 2020 (enrollment period). We followed patients for a minimum of 30 days from the date of inclusion in the cohort to allow sufficient time for the studied event, in this case death, to occur. Follow-up was stopped invariably on 17 May 2020.

2.2. Variables and Data Sources

The following baseline variables were analyzed for each individual at the date of entry: sex, age (0–14, 15–44, 45–64, 65–79, ≥ 80 years), and all drugs dispensed in community pharmacies during the three months prior to infection. Drugs were coded using the Anatomical Therapeutic Chemical (ATC) classification system at its third level (i.e., pharmacological subgroup). To facilitate clinical interpretation, we used the Rx-Risk Index, a validated comorbidity index that maps the dispensed ATC pharmacological and therapeutic groups to their corresponding comorbidity categories [10]. Patient data were obtained from healthcare databases with routinely collected information, which are detailed below.

The CaReDB includes demographic information on the approximately 6 million residents living in Campania (roughly 10% of the Italian population) and links with their pharmaceutical dispensation electronic records. Data in CaReDB were validated in previous drug utilization studies [11–13]. COVID-19 information in the region was collected via a specific surveillance system, which was developed during the initial stages of the pandemic to detect all cases identified by reverse transcription-polymerase chain reaction (RT-PCR). The diagnostic algorithm was based on the protocol released by the WHO and consisted of the collection of nasopharyngeal swab samples tested with at least two real-time RT-PCT assays targeting different genes of SARS-CoV-2 [14]. A unique identifier, later encrypted for anonymization purposes, served to connect all the aforementioned data sources. The governance board of Unità del Farmaco della Regione Campania granted permission to the researchers of the Centro di Ricerca in Farmacoeconomia e Farmacoutilizzazione (CIRFF) to use anonymized data for this study.

The PRECOVID Study cohort in Aragon included all individuals with laboratory-confirmed infection by SARS-CoV-2 in the region (reference population, 1.3 million inhabitants). The regional public health service provides free-of-charge healthcare to approximately 95% of the population of Aragon. Patient data were obtained from the Aragon Health System linking, at a patient level and in a pseudo-anonymized form, the information contained in the health system users' database, primary care and hospital pharmaceutical billing records, and an ad hoc registry implemented for COVID-19 surveillance in the region. The protocol of this study was approved by the Clinical Research Ethics Committee of Aragon (CEICA; protocol number PI20/226). CEICA waived the requirement to obtain informed consent from patients given the epidemiological nature of the project with anonymized data. The research protocol adheres to the tenets of the Declaration of Helsinki 1975 and its later amendments.

2.3. Statistical Analysis

Demographic and drug utilization patterns of all patients who tested positive for SARS-CoV-2 (alive and deceased at the end of follow-up) were reported as means and standard deviations or as absolute and relative frequencies and proportions, stratified by sex.

Logistic regression models were performed to analyze the association between the presence of each drug dispensed at baseline and the likelihood of mortality during the 30-day follow-up period in each region, stratified by sex. Age-adjusted odds ratios (ORs) were calculated together with their respective 95% confidence intervals (CI). Only medications with an overall prevalence equal to or greater than 1% were considered for the analysis in order to avoid spurious findings. Statistical significance was set at $p < 0.05$.

Data management was performed with Microsoft SQL server (version 2018). All analyses were performed in Stata software (Version 12.0, StataCorp LLC, College Station, TX, USA), SPSS software for Windows (version 17.1, SPSS Inc., Chicago, IL, USA) and platform R (version 3.6, The R Foundation for Statistical Computing, Vienna, Austria).

3. Results

A total of 8570 individuals were included in this study (Table 1). Of these, 4412 belonged to Aragon (58.8% women, mean age of 67.7 (standard deviation, SD, 20.7)) and 4158 (44.3% women, mean age 63.0 (SD 17.9)) to Campania. 771 (47.2% women, mean age of 84.2 (SD 10.0)) and 455 (35.4% women, mean age 74.3 (SD 13.5)) individuals died during follow-up in Aragon and Campania, respectively. Three in four deceased patients were 80 years of age and over in Aragon, as opposed to Campania where only one in three were above that threshold. Men died at an earlier age than women in both regions. The mean number of dispensed medications was higher in deceased individuals than in those alive by the end of follow-up in both regions (7.6 vs. 4.0 drugs in Aragon and 6.6 vs. 3.0 in Campania) (Table 2).

Table 1. Demographic characteristics of the study population, by region and follow-up status (i.e., exitus no/yes).

Region	Aragon, Spain						Campania, Italy					
Sex	All (N = 4412)		Women (N = 2593)		Men (N = 1819)		All (N = 4158)		Women (N = 1843)		Men (N = 2315)	
Exitus	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
Age (mean, sd)	(N = 3641)	(N = 771)	(N = 2229)	(N = 364)	(N = 1412)	(N = 407)	(N = 3703)	(N = 455)	(N = 1682)	(N = 161)	(N = 2021)	(N = 294)
Age (n, %)	61.8 (19.5)	84.2 (10.0)	60.8 (20.2)	87.2 (8.2)	63.3 (18.1)	81.5 (10.7)	60.7 (17.8)	74.3 (13.5)	61.8 (18.8)	76.2 (14.9)	59.8 (16.8)	73.0 (12.3)
≤14	6 (0.16)	0 (0)	0 (0)	0 (0)	6 (0.42)	0 (0)	156 (4.2)	2 (0.4)	67 (4.0)	1 (1.2)	89 (4.4)	0 (0)
15–44	721 (19.8)	1 (0.13)	516 (23.1)	0 (0)	205 (14.5)	1 (0.25)	1145 (30.9)	45 (9.9)	531 (31.6)	16 (9.9)	614 (30.4)	29 (9.9)
45–64	1384 (38.0)	36 (4.67)	844 (37.9)	7 (1.92)	540 (38.2)	29 (7.13)	1578 (42.6)	106 (23.3)	674 (40.1)	36 (22.4)	904 (44.7)	70 (23.8)
65–79	670 (18.4)	172 (22.3)	318 (14.3)	51 (14.0)	352 (24.9)	121 (29.7)	575 (15.5)	169 (37.1)	257 (15.3)	43 (26.7)	318 (15.7)	126 (42.9)
≥80	860 (23.6)	562 (72.9)	551 (24.7)	306 (84.1)	309 (21.9)	256 (62.9)	249 (6.7)	133 (29.2)	153 (9.1)	64 (39.8)	96 (4.8)	69 (23.5)

Table 2. Number of drugs dispensed of the study population, by region and follow-up status (i.e., exitus no/yes).

Region	Aragon, Spain						Campania, Italy					
Sex	All (N = 4412)		Women (N = 2593)		Men (N = 1819)		All (N = 4158)		Women (N = 1843)		Men (N = 2315)	
Exitus	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
Drugs (mean, sd)	(N = 3641)	(N = 771)	(N = 2229)	(N = 364)	(N = 1412)	(N = 407)	(N = 3703)	(N = 455)	(N = 1682)	(N = 161)	(N = 2021)	(N = 294)
	4.0 (3.9)	7.6 (4.2)	4.0 (3.9)	7.6 (4.0)	4.0 (4.0)	7.7 (4.3)	3.0 (2.9)	6.6 (4.2)	3.3 (3.5)	6.8 (5.1)	3.2 (3.6)	6.4 (5.2)

Drug families with the highest dispensation prevalence during the three months prior to COVID-19 infection in both regions were (Aragon, Campania) drugs for peptic ulcer and gastro-esophageal reflux disease (GORD) (35.3%, 35.1%), antithrombotic agents (22.8%, 24.2%), beta-blocking agents (11.7%, 16.4%), high-ceiling diuretics (14.4%, 6.3%), and blood-glucose-lowering drugs (11.2%, 7.7%) (Table 3). Drug dispensation was higher in Aragon than in Campania in all pharmacological groups except for antithrombotic agents and drugs for peptic ulcer and GORD, with notable differences in antidepressants (21.2%, 5.6%) and antipsychotics (10.9%, 3.6%).

The following drug families were significantly associated with 30-day mortality in patients with COVID-19 in both regions (OR Aragon, OR Campania): potassium-sparing agents (1.98, 3.01), antipsychotics (1.92, 3.01), vasodilators used in cardiac diseases (1.76, 3.23), high-ceiling diuretics (1.87, 2.54), antithrombotic agents (1.58, 2.54), vitamin B12, folic acid (1.59, 2.70), and antiepileptics (1.61, 2.72).

The dispensation of some drug families increased the risk of mortality in COVID-19 patients in only one of the two regions (OR (95% CI)), or data for them were unavailable at the time. Angiotensin-converting enzyme inhibitors (ACE) (2.25 (1.69–3.00)), lipid-modifying agents (2.06 (1.63–2.59)), peripherally acting antiadrenergic agents (1.88 (1.10–3.21)) and corticosteroids for systemic use (1.58 (1.23–2.02)), among others, were associated with a higher risk of mortality in Campania, while anxiolytics (1.41 (1.15–1.72)), immunosuppressants (1.89 (1.01–3.54)), and anti-infectives (1.95 (1.03–3.69)) showed increased risk only in Aragon. These results are presented as Supporting Information (see Supplementary Table S1).

Table 3. Drug families in which dispensation within the three months prior to COVID-19 infection was consistently associated with 30-day mortality in patients from both regions studied.

Region		Aragon, Spain				Campania, Italy			
Sex		All	All	Women	Men	All	All	Women	Men
ATC Code	Drug Family	Prev. (%)	OR (95% CI)	OR (95% CI)	OR (95% CI)	Prev. (%)	OR (95% CI)	OR (95% CI)	OR (95% CI)
C03D	Potassium-sparing agents	3.0	1.98 (1.36–2.89)	1.70 (1.03–2.81)	2.32 (1.32–4.07)	2.4	3.01 (1.91–4.77)	2.32 (1.06–5.08)	3.60 (2.01–6.42)
N05A	Antipsychotics	10.9	1.92 (1.55–2.39)	2.05 (1.54–2.74)	1.76 (1.26–2.45)	3.6	3.01 (2.06–4.39)	4.44 (2.76–7.14)	2.16 (1.12–4.18)
C01D	Vasodilators used in cardiac diseases	2.1	1.76 (1.14–2.72)	1.27 (0.66–2.44)	2.30 (1.27–4.16)	1.2	3.23 (1.73–6.04)	3.58 (1.40–9.15)	3.11 (1.34–7.22)
C03C	High-ceiling diuretics	14.4	1.87 (1.53–2.28)	1.65 (1.26–2.16)	2.11 (1.57–2.85)	6.3	2.54 (1.87–3.46)	2.48 (1.53–4.02)	2.71 (1.81–4.05)
M04A	Antigout preparations	5.5	1.48 (1.10–1.99)	2.22 (1.30–3.81)	1.26 (0.88–1.79)	4.6	3.45 (2.48–4.81)	4.86 (2.87–8.24)	2.75 (1.80–4.21)
B01A	Antithrombotic agents	22.8	1.58 (1.32–1.90)	1.60 (1.23–2.07)	1.56 (1.21–2.02)	24.2	2.54 (2.08–3.11)	3.11 (2.24–4.33)	2.25 (1.74–2.91)
B03B	Vitamin B12 and folic acid	7.6	1.59 (1.21–2.04)	1.59 (1.10–2.31)	1.59 (1.11–2.28)	3.6	2.70 (1.83–3.99)	3.13 (1.83–5.35)	2.69 (1.52–4.77)
N03A	Antiepileptics	8.8	1.61 (1.26–2.07)	1.86 (1.33–2.60)	1.37 (0.95–1.99)	4.4	2.72 (1.91–3.88)	3.47 (2.11–5.71)	2.39 (1.44–3.98)
C07A	Beta blocking agents	11.7	1.52 (1.23–1.89)	1.33 (0.98–1.82)	1.70 (1.25–2.30)	16.4	2.28 (1.83–2.85)	2.18 (1.50–3.17)	2.31 (1.75–3.05)
A02B	Drugs for peptic ulcer and GORD	35.3	1.54 (1.28–1.84)	1.28 (0.99–1.67)	1.78 (1.38–2.29)	35.1	2.27 (1.87–2.77)	2.70 (1.94–3.75)	2.11 (1.65–2.70)
G04C	Drugs used in benign prostatic hypertrophy	8.5	n.a.	n.a.	1.10 (0.84–1.45)	6.3	n.a.	n.a.	2.07 (1.50–2.87)
A10B	Blood glucose lowering drugs	11.2	1.27 (1.02–1.59)	1.33 (0.96–1.85)	1.23 (0.91–1.66)	7.7	2.19 (1.64–2.94)	2.48 (1.48–4.15)	1.97 (1.38–2.81)
N06A	Antidepressants	21.2	1.44 (1.20–1.76)	1.44 (1.12–1.86)	1.44 (1.07–1.93)	5.6	2.36 (1.70–3.28)	2.58 (1.65–4.03)	2.79 (1.68–4.63)

ATC: Anatomical-Therapeutic-Chemical code at the third level; Prev.: prevalence; OR: odds ratio; CI: confidence interval; GORD: Gastro-oesophageal reflux disease; n.a.: not applicable. Only drugs with an overall prevalence equal to or greater than 1% and with a statistically significant association with mortality ($p < 0.05$) in both regions are presented. The complete list of drugs from both regions is available as Supplementary Material.

4. Discussion

In this comparative study we identified the main pharmacological groups that, as a proxy for the baseline comorbidities they are dispensed for, were associated with an increased risk of mortality in COVID-19 patients from two regions in Spain and Italy, evidencing common results. We found that the dispensation of drugs related to the treatment of chronic cardiovascular and metabolic diseases such as β -blocking agents, vasodilators, antithrombotic agents, diuretics, and blood glucose-lowering drugs dispensed three months prior to infection were associated with higher mortality risks in these patients. Dispensed vitamin B12 and folic acid supplementations, often linked to deficits during systemic inflammation and processes with increased risk of thrombosis, were also found to increase the risk of mortality after the infection.

The first weeks of the pandemic already underscored the now notorious effects of advanced age on the risk of COVID-19-related mortality [15]. Almost three in four deceased individuals in Aragon, a region where residential care homes for the elderly were hit hardest during the first months of the pandemic, were 80 years of age or older, while in Campania, a region with one of the youngest populations in Italy, this proportion was much lower, approximately one in three. Chronic diseases and multimorbidity, which are more frequent in older patients, as well as immune-senescence, are some of the added factors that could contribute to the higher morbidity and mortality risks in these age groups after COVID-19 infection [16].

Different methodological approaches (for example, in the selection of chronic diseases and data sources), settings (in-hospital and out-hospital patients, special treatment during admission, healthcare system's capacity), and regions (sociodemographic characteristics, varying social-distancing measures and health policies) have been used for the characterization of the population at risk of poor prognosis. These discrepancies cumbered the comparability and interpretation of the different findings, especially during the first stages of the pandemic. Despite these limitations, certain underlying conditions were identified as risk factors of adverse outcomes in COVID-19 patients across the board; amongst them diabetes, obesity, chronic cardiovascular diseases, and chronic renal failure [17]. Consequently, pharmacological treatments as proxies for these chronic conditions can also be expected to be associated with a higher risk of mortality. Our study identified the chronic dispensation at baseline of β -blocking agents, vasodilators used in cardiac diseases, antithrombotic agents, high-ceiling diuretics, potassium-sparing agents, and blood glucose-lowering drugs as indicators of increased risk in both regions of Spain and Italy. Though previous studies have shown that the treatments themselves are safe during COVID-19 infection and should not be discontinued, real-world data on their dispensation can be an objective and prompt tool to detect patients at risk and anticipate severe outcomes [18–20].

According to our findings, the predominant pharmacological profile of a high-risk individual in Aragon was that of a geriatric patient with heart failure and dysrhythmias, while in Campania, it was a hypertensive patient with cardiometabolic diseases. The influence of pharmacological profiles on the clinical evolution of COVID-19 patients may be significant for both decision-making and prognosis. Many patients, especially the elderly, have multimorbidity and are in treatment with several drugs (polypharmacy) and thus are susceptible to potentially inappropriate medication, drug-drug and drug-disease interactions, adverse drug reactions, and therapeutic cascades [21]. Swift identification of disease patterns with a higher risk of severe outcomes, both directly or through the medications used to treat them, is vital when designing prevention strategies in the changing landscape of the pandemic, especially considering the pathophysiological characteristics of the infection remain unclear and considering new drugs can be developed or implemented in future guidelines.

In addition to cardiovascular and cardiometabolic medication, we found associations between other drug categories and COVID-19 mortality. The use of antigout preparations could be linked to treatments with antidiuretics and heart failure, which are also associated with a higher risk of negative outcomes [22]. Vitamin B12 and folic acid supplementation

are prescribed in situations of vitamin B12 deficiency, and were the only drugs that could not be mapped to a corresponding comorbidity category using the Rx-Risk Index. However, it has been well established that elevated homocysteine levels during vitamin B12 deficiency are associated with higher cardiovascular risk, and could be related to antithrombotic treatment implementation in these patients [23]. Systemic inflammation and conditions with an increased risk of thrombosis are important risk factors of adverse outcomes in infected patients [5]. One of the most critical findings in our study was the identification of antidepressants, antipsychotics, and antiepileptics as high-risk medications of COVID-19 mortality in both Aragon and Campania. The use of these drugs with high anticholinergic activity seems to be higher in Aragon, including anxiolytics, probably due to the older mean age of the population, but the role of these drug groups and the conditions they are indicative of still require further research.

The core strength of this study is its population-based approach. We used real-world data to include all confirmed COVID-19 cases and all the drugs dispensed during the three months prior to infection in the regions of Campania and Aragon. Another strength is its comparative nature, allowing the contrasting of results from two settings with unique characteristics from two of the countries that suffered the most during the initial stages of the pandemic in Europe. Its main limitation is that it only included drugs dispensed from community pharmacies under prescription, and not over-the-counter medications or in-hospital treatments. Another limitation is that we lacked the information on the chronic diagnoses that justified the dispensation of each drug. Though we mapped each pharmacological code to the corresponding comorbidity group based on their most frequent indications, some drugs can be used for a wider number of pathologies. Furthermore, some of the information for certain drug groups of interest, such as hypnotics, sedatives, and anxiolytics, were only available in one of the two regions and could not be compared. Future studies, including all relevant pharmacological groups and their combinations, are called for to better understand their role as potential mortality risk indicators in COVID-19 patients.

5. Conclusions

Results show that baseline chronic treatments for known COVID-19 risk factors such as chronic cardiovascular and metabolic diseases, systemic inflammation, and processes with increased risk of thrombosis, are systematically associated with mortality in infected patients. When chronic diagnoses are unavailable or complex, the assessment of pharmacological profiles as proxies can be an alternative approach to a swift identification of at-risk individuals in clinical practice.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/ijerph182211786/s1>. Table S1: Dispensation rates of the complete list of drugs studied and adjusted odds ratios of 30-day mortality in each region.

Author Contributions: Conceptualization, A.P.-T. and E.M.; methodology, B.P.-P.; formal analysis, B.P.-P. and S.M.; investigation, F.G.-R., M.A.-P.-S., M.C.-S., I.I.-S., K.B.-B. and J.C.-P.; data curation, B.P.-P. and V.O.; writing—original draft preparation, K.B.-B., A.G.-M. and I.I.-S.; writing—review and editing, E.C., A.P., U.T., A.P.-T. and E.M.; supervision, A.G.-M.; funding acquisition, A.P.-T., E.M. and A.G.-M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Government of Aragon through the Recognized Groups Grant reference B01_20R and Decree-Law 3/2020, published on 3 June.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Clinical Research Ethics Committee of Aragón (CEICA; Protocol number PI20/226). CEICA waived the requirement to obtain informed consent from Aragon participants due to the epidemiological nature of the project and the use of anonymized data that was presented at an aggregated level. The governance board of Unità del Farmaco della Regione Campania granted permission to the researchers of the CIRFF to use anonymized data for this study.

Informed Consent Statement: Patient consent was waived due to the epidemiological nature of the project and the use of anonymized data that was presented at an aggregated level.

Data Availability Statement: Data used in this study cannot be publicly shared because of restrictions imposed by the Aragon Health Sciences Institute (IACS) and asserted by the CEICA. Nonetheless, data can be made available for potential collaborations upon reasonable request to the Principal Investigators Alexandra Prados-Torres (sprados.iacs@aragon.es) and Enrica Menditto (enrica.menditto@unina.it).

Acknowledgments: The authors sincerely thank Dirección General de Asistencia Sanitaria, Dirección General de Salud Pública, and Servicio Aragonés de Salud, from Departamento de Salud of the Government of Aragon for their essential collaboration in developing the COVID-19 registry. This work was funded by the Government of Aragon through the Recognized Groups Grant reference B01_20R and Decree-Law 3/2020, published on 3 June. This study was also supported by grants from the Italian Medicine Agency (AIFA), funding on Pharmacovigilance of the Campania Region (Progetto per la valutazione e l'analisi della prescrizione farmaceutica in Regione Campania; Osservatorio sull'uso appropriato dei farmaci).

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data, in the writing of the manuscript or in the decision to publish the results.

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4.4. Manuscript 4: Identifying multimorbidity profiles associated with COVID-19 severity in chronic patients using network analysis in the PRECOVID Study.

Carmona-Pérez J, Gimeno-Miguel A, Bliek-Bueno K, Poblador-Plou B, Díez-Manglano J, Ioakeim-Skoufa I, González-Rubio F, Poncel-Falcó A, Prados-Torres A, Gimeno-Feliu LA; PRECOVID Group. **Identifying multimorbidity profiles associated with COVID-19 severity in chronic patients using network analysis in the PRECOVID Study.** *Sci Rep.* 2022 Feb 18;12(1):2831. Doi: <https://doi.org/10.1038/s41598-022-06838-9>

Journal: Scientific Reports

Impact Factor: 4.6, Q2 (MULTIDISCIPLINARY SCIENCES-SCIE)

Status: Published



OPEN

Identifying multimorbidity profiles associated with COVID-19 severity in chronic patients using network analysis in the PRECOVID Study

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A major risk factor of COVID-19 severity is the patient's health status at the time of the infection. Numerous studies focused on specific chronic diseases and identified conditions, mainly cardiovascular ones, associated with poor prognosis. However, chronic diseases tend to cluster into patterns, each with its particular repercussions on the clinical outcome of infected patients. Network analysis in our population revealed that not all cardiovascular patterns have the same risk of COVID-19 hospitalization or mortality and that this risk depends on the pattern of multimorbidity, besides age and sex. We evidenced that negative outcomes were strongly related to patterns in which diabetes and obesity stood out in older women and men, respectively. In younger adults, anxiety was another disease that increased the risk of severity, most notably when combined with menstrual disorders in women or atopic dermatitis in men. These results have relevant implications for organizational, preventive, and clinical actions to help meet the needs of COVID-19 patients.

Immediately after the coronavirus disease (COVID-19) outbreak in December 2019, the clinical and research communities worldwide set their focus on identifying aspects that influence the disparity of symptoms and outcomes in infected individuals. More than a year and a half later, and even though several factors related to poor prognosis have already been identified, the ongoing COVID-19 pandemic still represents a challenge for clinicians, healthcare systems and society. Severe outcomes and complications can occur at any age, even in previously healthy individuals¹. Nevertheless, we now know that male sex is a risk factor for poor prognosis^{2–4} and that older patients, especially those over the age of 80, are more likely to develop more severe outcomes^{5,6}.

The presence of certain chronic diseases at the time of infection seems to be another determining factor of COVID-19 severity. Cardiovascular and metabolic diseases such as obesity, heart failure, chronic renal failure, and diabetes mellitus^{1,7} have been consistently associated with a higher risk of severity, while the role of other conditions such as asthma remains unclear⁸. To date, most studies on comorbidity and COVID-19 severity have analyzed the impact of some diseases individually. However, multimorbidity (i.e., the presence of two or more chronic diseases) is the norm rather than the exception in chronic patients, especially in the elderly⁹. It is well known that chronic diseases tend to cluster into non-random patterns, usually referred to as multimorbidity

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Characteristics	Total (N = 48,415)	Men (N = 20,936)	Women (N = 27,479)
Mean age (sd)	52.0 (21.10)	51.7 (20.47)	52.3 (21.56)
Age interval (N, %)			
15–64 years	35,106 (72.5%)	15,279 (73.0%)	19,827 (72.2%)
65–79 years	6,961 (14.4%)	3,431 (16.4%)	3,530 (12.8%)
≥ 80 years	6,348 (13.1%)	2,226 (10.6%)	4,122 (15.0%)
Mean number of chronic diseases (sd)	3.94 (2.88)	3.50 (2.61)	4.29 (3.02)
Multimorbidity † (N, %)			
Total	38,323 (79.2%)	15,659 (74.8%)	22,664 (82.5%)
15–64 years	25,605 (72.9%)	10,306 (67.5%)	15,299 (77.2%)
65–79 years	6,568 (94.4%)	3,190 (93.0%)	3,378 (95.7%)
≥ 80 years	6,150 (96.9%)	2,163 (97.2%)	3,987 (96.7%)
Outcomes			
Hospitalization (N, %)	4,586 (9.5%)	2,400 (11.5%)	2,186 (8.0%)
30-day-mortality (N, %)	1,311 (2.7%)	683 (3.3%)	628 (2.3%)
Severity †† (N, %)			
Total	5,117 (10.6%)	2,671 (12.8%)	2,446 (8.9%)
15–64 years	1,572 (4.5%)	903 (5.9%)	669 (3.4%)
65–79 years	1,338 (19.2%)	790 (23.0%)	548 (15.5%)
≥ 80 years	2,207 (34.8%)	978 (43.9%)	1,229 (29.8%)

Table 1. Demographic and clinical characteristics of individuals aged 15 years or older with COVID-19 infection in the PRECOVID Cohort. † Multimorbidity, defined as the simultaneous co-existence of two or more chronic diseases, excluding COVID-19; sd, standard deviation; †† Severity, defined as a composite outcome based on the need for hospital admission (including in Intensive Care Units) or 30-day all-cause mortality.

patterns¹⁰. It is expected that some patterns could affect COVID-19 prognosis in a more significant way than others, depending on the disease composition of each pattern and also on various factors such as sex and age. In this sense, some studies have already suggested that patterns linked to low-grade systemic inflammation could be decisive in COVID-19 prognosis¹¹, though only a few have focused on identifying disease patterns with an impact on COVID-19 patients^{12–15}.

The continuously changing landscape of the COVID-19 pandemic with the appearance of new variants of the virus with high transmission rates and unpredictable severity calls scientists to identify vulnerable chronic patients. Characterization of the clinical profiles of the population at higher risk of hospitalization or mortality may help us effectively design and develop preventive strategies targeting people with specific chronic diseases (for both primary and secondary prevention). It can also give us valuable information to better understand the etiopathological characteristics of the infection and its complications, and potentially generate hypotheses for a more appropriate clinical management of patients with multiple chronic conditions. The use of electronic health records and COVID-19 registries can bring potential improvements in population health, increasing research efficiency and data quality and management^{16,17}, among other benefits. Real-world data can generate solid scientific evidence for important questions that challenge healthcare professionals and public health systems. In this context, innovative data-mapping tools, such as network analysis, could help detect patterns from within the wide range of conditions and their potential combinations, discriminating between different high-risk profiles of infected individuals based on their underlying comorbidities. Network analysis applies community detection methods and has been used to study multimorbidity patterns through the correlations among diseases^{18,19}. However, this is the first time to the extent of our knowledge that this method is applied to study the correlations between individuals based on common comorbidities, and more specifically in COVID-19 patients, in order to detect higher-risk severity profiles.

This population-based study aims to (1) identify clinical profiles of COVID-19 patients based on their baseline morbidity using network analysis, (2) clinically describe the multimorbidity patterns obtained, and (3) assess their impact on infection severity (compared with chronic patients without multimorbidity).

Results

Characteristics of the study population: 48,415 patients, 80% had multimorbidity. The study population comprised all patients aged 15 years or older infected and with chronic diseases from June 15, 2020, to December 19, 2020, from the Spanish region of Aragon. A total of 48,415 individuals (mean age 52.0 years) with laboratory-confirmed COVID-19 infection (approximately 4% of the reference population) were included. Eight in every ten of them had multimorbidity, with a mean number of 3.9 chronic diseases per patient (Table 1), slightly higher in women than in men. During follow-up, 9.6% of the individuals developed severe infection; of those, 9.5% were admitted to the hospital, and 2.7% died.

The most frequently observed chronic diseases/conditions were cardio-metabolic (i.e., hypertension, dyslipidemia, diabetes, and obesity), cardiovascular (i.e., ischemic heart disease, cardiac dysrhythmia, and heart failure),

chronic respiratory (i.e., asthma, rhinitis, chronic obstructive pulmonary disease -COPD-, and obstructive sleep apnea-OA), and mental health disorders (i.e., anxiety and mood disorders) (Fig. 1). In the 38,823 patients with two or more chronic diseases included in the network analysis, these conditions were combined into up to fourteen different multimorbidity patterns with certain sex- and age-specificities and a differential impact on COVID-19 severity (Table 2). The complete output of the pattern analysis is available as supplementary material in which we detailed the complete disease analysis with their prevalence and observed/expected prevalence ratios.

Multimorbidity patterns in women and impact on infection severity: menstrual-dysphoric and cardiometabolic patterns highlight. We identified eight multimorbidity patterns in women, which were classified as cardiometabolic, thyroid-cardiometabolic, osteoporotic-cardiovascular, upper respiratory, mental health, psychogeriatric, menstrual-dysphoric, and non-specific. Their disease composition, specificities according to age, and impact on infection severity are described below and in Fig. 1 and Table 2.

In women aged 15–64, we found five patterns. The cardiometabolic pattern, which included 15 diseases such as hypertension, dyslipidemia, diabetes, and menopausal disorders amongst others, was associated with an 86% increase in severity (age-adjusted odds ratio OR 1.86, 95% confidence interval CI 1.43–2.41) compared with individuals with only one chronic condition. The menstrual-dysphoric pattern included anxiety, menstrual disorders, and headaches, and associated (OR [95% CI]) the second highest severity risk (1.48 [1.08–2.02]). A less-specific pattern with menstrual disorders as its most prevalent chronic conditions was also described to increase severity by 35% (1.35 [1.02–1.77]). The two remaining patterns were an upper respiratory pattern and a mental health pattern, none of which was associated with higher COVID-19 infection severity compared to the reference group that included chronic patients without multimorbidity.

Five other patterns were identified in women aged 65–79 years. An advanced cardiometabolic pattern including 13 diseases such as diabetes (present in 99% of women), obesity, hypertension, retinopathy, heart failure, acute myocardial infarction, and gout, was associated (OR [95% CI]) with severity (1.97 [1.12–3.49]). Four other patterns in which the prevalence of diabetes did not exceed 1% and tagged as thyroid-cardiometabolic, osteoporotic-cardiovascular, mental health, and non-specific, did not associate higher severity risk.

In women aged 80 and older, we identified four multimorbidity patterns, none of which associated higher severity risk compared with the chronic reference group. Most cases were grouped into a non-specific pattern. The cardiometabolic pattern in this group included cardiac dysrhythmias, coagulation/ hemorrhagic disorders, heart failure, and acute myocardial infarction, while the mental health pattern included mood disorders, anxiety, and cognitive disorders. We also found a psychogeriatric group with urinary incontinence and dementia.

Multimorbidity patterns in men and impact on infection severity: mental health, cardiometabolic and dementia patterns highlight. A total of ten multimorbidity patterns were identified in men, which we referred to as cardiometabolic, hypertension-obesity, arthrosis-cardiometabolic, prostatic-cardiometabolic, prostatic, mental health, upper respiratory, headache, dementia, and non-specific. Their composition, age-specificities and effects on COVID-19 severity are described below and in Fig. 1.

In men aged 15–64 years, six patterns were identified. A hypertension-obesity pattern including hypertension, obesity, and transient cerebral ischemia was associated (OR [95% CI]) the most with infection severity (1.60 [1.25–2.05]). We also found a cardiometabolic group, mainly including hypertension and dyslipidemia, was also associated with a 43% increase in severity (1.43 [1.14–1.80]). A mental health group with anxiety disorders and atopic dermatitis was also associated with a 36% higher risk of severity (1.36 [1.06–1.74]). The non-specific pattern, with dyslipidemia as the most prevalent chronic condition, was also described to increase severity by 28% (1.28 [1.03–1.60]), in addition to two other patterns unrelated to severity named (upper-tract) respiratory pattern and headache pattern.

Six multimorbidity patterns were described in men aged 65–79. The only one associated with higher severity risk (OR [95% CI]) was a cardiometabolic pattern (1.76 [1.18–2.62]) that included seven diseases: obesity (present in 99% of men), hypertension, dyslipidemia, diabetes, osteoarthritis, microcrystalline arthritis, and diseases of veins and lymphatics. Five other patterns unrelated to severity were also described: two other presentations of the cardiometabolic pattern (cardiometabolic II and III), two patterns also with these kinds of conditions (prostatic-cardiometabolic and arthrosis-cardiometabolic), and a non-specific multimorbidity pattern. In these groups, the prevalence of obesity did not exceed 1%, except in the prostatic-cardiometabolic one (18%).

In men aged 80 years and older, we detected five patterns. A psychogeriatric pattern comprising urinary incontinence, cognitive disorders, and behavioral and mental disorders among other diseases, was the only one associated (OR [95% CI]) with increased infection severity risk (1.42 [1.04–1.94]). The four other patterns unrelated to severity were defined as prostatic, cardiometabolic I, cardiometabolic II, and arthrosis-cardiometabolic.

Discussion

In this large-scale study, we explored network analysis as a new approach to identify the characteristics behind different profiles of COVID-19 individuals that could explain the disparities observed in infection severity based on their underlying morbidity. Our results evidenced the presence of up to fourteen multimorbidity patterns, most of which included cardiometabolic and vascular diseases as the most frequent chronic conditions, with varying effects on infection severity depending on sex, age, and comorbidity. Our findings can contribute to the improvement of the healthcare organization of COVID-19 patients and help direct health interventions and shielding strategies towards the most vulnerable chronic patients.

Our study revealed that multimorbidity patterns comprising diseases of predominantly cardiometabolic nature were the ones most consistently associated with infection severity. These findings align with previous studies that have identified cardiometabolic (i.e., diabetes, obesity, hypertension, dyslipidemia)⁷ and cardiorespiratory

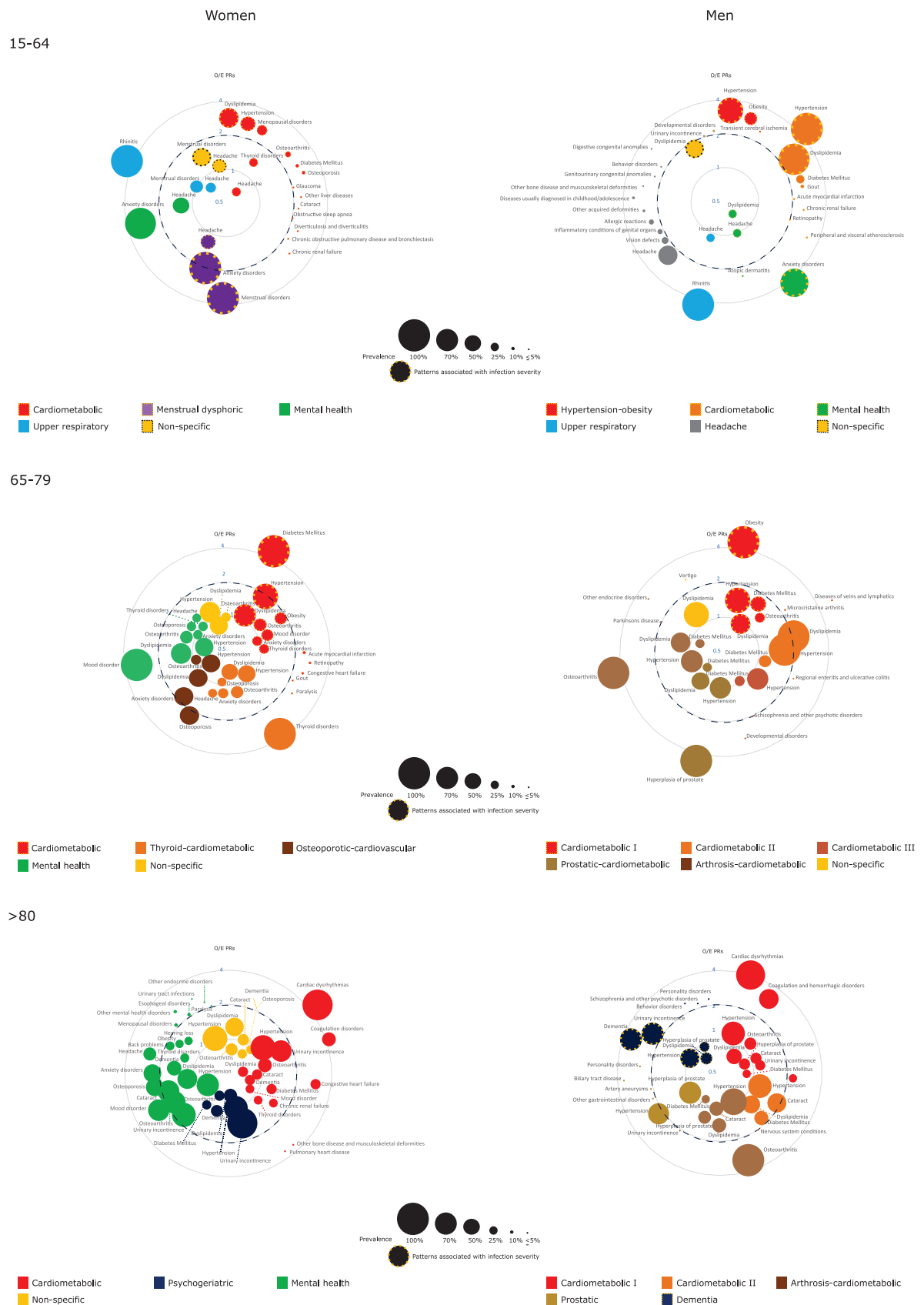


Figure 1. Disease composition of the multimorbidity patterns identified in the study population based on sex and age, according to prevalence (proportional to node size) and observed/expected prevalence ratios (O/E PRs). A disease was included as a part of a pattern when presented a prevalence greater than 25% and/or a O/E PR ≥ 2 (with a prevalence of at least 1%). In broken lines patterns with statistically significant age-adjusted odds ratios of infection severity.

	15–64 years		65–79 years		≥ 80 years	
	Pattern name (N, prevalence %)	Severity aOR (95% CI)	Pattern name (N, prevalence %)	Severity aOR (95% CI)	Pattern name (N, prevalence %)	Severity aOR (95% CI)
Women	Cardiometabolic (4,205, 21.4%)	1.85 (1.43–2.41)	Cardiometabolic (673, 19.1%) (99% diabetes mellitus)	1.97 (1.15–3.49)	Cardiometabolic (541, 13.1%)	1.29 (0.85–1.96)
	Menstrual-dysphoric (2,204, 11.1%)	1.48 (1.08–2.02)	Thyroid-cardiometabolic (515, 14.6%) (no diabetes mellitus)	1.55 (0.86–2.79)	Psychogeriatric (1,011, 24.5%)	1.06 (0.71–1.58)
	Mental health (2,046, 10.3%)	0.90 (0.63–1.29)	Osteoporotic-cardiovascular (70, 19.9%) (no diabetes mellitus)	1.20 (0.67–2.14)	Mental health (753, 18.3%)	1.32 (0.87–1.98)
	Upper respiratory (1,987, 10.0%)	1.13 (0.79–1.62)	Mental health (823, 23.3%) (no diabetes mellitus)	1.58 (0.90–2.80)	Non-specific (1,679, 40.8%)	1.05 (0.71–1.55)
	Non-specific (4,810, 24.3%)	1.35 (1.02–1.77)	Non-specific (663, 18.8%) (no diabetes mellitus)	1.22 (0.68–2.18)		
	Control group (4,528, 22.8%)		Control group (152, 4.3%)		Control group (135, 3.3%)	
Men	Hypertension-obesity (1,585, 10.4%)	1.60 (1.25–2.05)	Cardiometabolic I (421, 12.3%) (99% obesity)	1.76 (1.18–2.62)	Cardiometabolic I (546, 24.6%)	1.28 (0.96–1.70)
	Cardiometabolic (1,136, 7.4%)	1.43 (1.14–1.80)	Cardiometabolic II (619, 18.1%) (no obesity)	1.23 (0.84–1.81)	Cardiometabolic II (590, 26.5%)	1.28 (0.96–1.70)
	Mental Health (1,911, 12.5%)	1.35 (1.06–1.74)	Cardiometabolic III (637, 18.6%) (no obesity)	1.41 (0.96–2.06)	Arthrosis-cardiometabolic (444, 20.0%)	1.28 (0.96–1.71)
	Upper respiratory (1,446, 9.5%)	1.30 (0.97–1.75)	Prostatic-cardiometabolic (699, 20.4%) (18% obesity)	1.30 (0.89–1.89)	Prostatic (233, 10.5%)	1.30 (0.92–1.84)
	Headache (1,949, 12.8%)	1.24 (0.93–1.64)	Arthrosis-cardiometabolic (394, 11.5%) (no obesity)	1.20 (0.80–1.82)	Dementia (348, 15.6%)	1.42 (1.04–1.94)
	Non-specific (2,274, 14.9%)	1.18 (1.03–1.60)	Non-specific (416, 12.1%) (no obesity)	1.28 (0.85–1.93)		
	Control group (4,973, 32.6%)		Control group (241, 7.0%)		Control group (63, 2.8%)	

Table 2. Denomination and prevalence of multimorbidity patterns found in COVID-19 patients based on sex and age and their age-adjusted odds ratios (aOR) of infection severity compared with chronic patients without multimorbidity. In bold letters, patterns with statistically significant age-adjusted odds ratios of infection severity; Control group: chronic patients without multimorbidity; CI: confidence interval.

diseases (i.e., ischemic heart disease, cardiac dysrhythmia, heart failure, COPD, OSA)¹ as severity risk factors. However, not all cardiometabolic patterns seemed to be related with increased severity in our population.; obesity in men and diabetes in women were differential diseases in high-risk cardiometabolic patterns, which supports the low-grade systemic inflammation pattern hypothesis in COVID-19¹¹. This study identified combinations of diseases responsible for the most severe cases of infection that should be considered in effective prevention strategies. Below we discuss such combinations considering sex and age group, focusing on the most severe and novel ones.

Cardiometabolic patterns were strongly associated with a high risk of severity. In patients aged 65–79, we observed important sex differences in the composition of the cardiometabolic pattern: diabetes was the differential disease in women, present in 99% of patients, while obesity was in men, present in 99% of individuals. Surprisingly, although these two diseases are very common in the general population, we observed a low prevalence (below 1%) in the rest of the patterns identified in this age group in both sexes. This suggests that diabetes in women and obesity in men could act as differential factors or triggers that attract other diseases in this stratum and configure the most severe patterns in both men and women.

A medical history of metabolic and vascular diseases is a risk factor for additional comorbidity and potential complications. Many of those patients can present a cardiometabolic and/or respiratory pattern. Thus, underlying chronic conditions must be taken into consideration for the clinical management of infected patients. Severity, in general, seems to be linked to more advanced stages of the cardiometabolic pattern, which include diseases such as COPD, OSA, heart failure, chronic renal failure, or retinopathy, where target organs can sustain significant damage over time.

Low-grade chronic systemic inflammation is characterized by a proinflammatory state with increased macrophage infiltration in peripheral cells. Such state does not involve loss of function but is strongly related to the appearance of multiple cardiovascular diseases. It starts with an inflammation of the visceral adipose tissue that leads to insulin resistance, connecting obesity, diabetes, and cardiovascular diseases²⁰. The results of our work are consistent with previous studies highlighting the importance of chronic systemic inflammation on COVID-19 severity¹¹. The association between chronic inflammation status and infection severity has also been described at molecular level, especially in aging^{21,22}. The existence of low levels of HMGA1 gene may play a key role as a risk factor for COVID-19 patients by triggering inflammatory pathways and atherosclerosis^{22,23}. Low levels of HMGA1 in airway tissues are also associated with smoking and COPD²⁴, connecting cardio and respiratory diseases with COVID-19 severity.

In women, patterns including menstrual and menopausal disorders were associated with higher infection severity. Menopausal disorders were part of an advanced cardiometabolic pattern that had hypertension and dyslipidemia at its core, but which also included conditions related to organ failure such as heart failure, chronic renal failure or COPD.

In young and adult population of up to 64 years of age, patterns with anxiety disorders were associated with severity, in men with atopic dermatitis and in women with menstrual disorders. The role of chronic anxiety in severity risk is still unclear. It seems that anxiety affects general health, but can also have an impact on protective factors for COVID-19 perceived risk such as the attitudes towards relationships or self-esteem²⁵. A chronically anxious population with even higher anxiety levels would deteriorate general health and protective factors, including risk perception. The dysphoric-menstrual pattern and its impact on severity also deserve further investigation, not just related to anxiety, but also to understand its connection with menstrual disorders, which has not been related to severity risk before.

Age is one of the most influencing factors in COVID-19 prognosis²⁶. In advanced ages, it seems that the effect of multimorbidity on severity is, in general, not significant when compared with the presence of just one chronic condition. A dementia pattern in men was the only one associated with severity in the population of 65 years of age and over. Previous studies have also linked dementia, male sex, and age with higher clinical severity²⁷. Patients with dementia have multiple comorbidities such as diabetes and pneumonia, higher baseline inflammation and limited capacity to follow protective recommendations, factors that could explain this result²⁸. Our results are consistent with the importance of age and sex in COVID-19 infection^{3,4,22,29}; the study of such repeatable and straightforward variables that are accessible to health professionals and policy-makers could help to develop sex-balance and age-adaptive guidelines²⁹.

One of the main strengths of our study is its large-scale population-based nature, including all individuals with laboratory-confirmed infection in the region. In relation to this real-world dataset, another strength of this study is its innovative approach to detect communities of COVID-19 patients based on their multimorbidity patterns using network analysis. This is the first time to the extent of our knowledge that this methodology is applied for this purpose. Network science studies the collective behavior of interconnected elements and how patterns emerge from them. It has developed several tools to visualize and exhaustively analyze big real-world data sets to predict their behavior and improve the objectivity of the analysis. Network analysis and this approach in particular facilitates the automation and replicability of this analysis compared to other classical cluster techniques that allow analyzing patients as the grouping unit, but present relevant computational limitations (e.g., agglomerative hierarchical methods) or higher subjectivity (e.g., k-means clustering)³⁰. This paper shows the potential to apply this method to the study of patient associations based on their common underlying chronic conditions. In connection to this network approach, another principal strength of our research is that we exhaustively analyzed virtually all chronic diseases (operationalized into 153 chronic conditions) obtained from primary sources of information (i.e., patients' electronic health records -EHRs), and not only those most prevalent, relevant, or self-reported by the patients. The most important limitation is that our database only included information on all-cause mortality and not on the cause of death, so we could not assess the direct association between COVID-19 infection and death. On the other hand, some variables that were not available in our cohort could have been relevant in the interpretation of the results, such as socio-economic variables, genetics, laboratory tests, and inpatient treatments, amongst others.

Conclusions

In conclusion, network analysis could help us to discern which specific combinations of chronic diseases are behind most severe cases of COVID-19 infection. Its application to our large-scale population-based cohort reveals the presence of multimorbidity patterns with a differential impact on infection severity based on age, sex, and disease composition. Our results support the importance of cardiovascular and metabolic disease patterns as aggravating factors of the infection as well as other disease combinations, mainly including anxiety, which had not been described as risk factors, thus deserving future investigation. We hypothesize that diabetes mellitus in women and obesity in men play an important role as disease attractors within their respective sex-specific multimorbidity patterns, which are associated with severity in patients over the age of 65 years. This new approach could be especially relevant for young-adult individuals in which different multimorbidity patterns are associated with increased severity risk, whereas age seems to be the most influencing factor of severity in older patients, regardless of their type of multimorbidity. Our findings can be helpful in the identification of at-risk chronic patients that should be the priority targets of shielding and close monitoring strategies during the pandemic.

Methods

Design and study population. We performed a retrospective, observational study in the PRECOVID cohort³¹, which includes demographic and clinical information of all the users of the public health system with laboratory-confirmed infection by SARS-CoV-2 in Aragon, a region of northeastern Spain with a reference population of 1.3 million inhabitants. Aragon's Health System provides universal health coverage for all citizens at no cost, and is used by approximately 98% of the reference population in the region³². For this study, we included all 48,415 individuals aged 15 years or older, infected, and with chronic diseases from June 15, 2020 to December 19, 2020. We excluded patients below the age of 15 years due to the low prevalence of chronic conditions, multimorbidity and severe COVID-19 cases in this group. Patients were followed for a maximum of 30 days from the index date (i.e., date in which the confirmatory test sample was taken) or until the date of hospitalization and/or death within this period to analyze infection severity. Severity was measured as a composite outcome based on the need for hospital admission (including in Intensive Care Units) or 30-day all-cause mortality. We operationalized severity in this way to distinguish between mild and moderate-to-severe cases.

The Clinical Research Ethics Committee of Aragón (CEICA) approved the research protocol for this study (PI20/226). CEICA waived the requirement to obtain informed consent from the participants included in this study due to its epidemiological nature and the use of anonymized data. We performed this study following the Declaration of Helsinki and the Spanish Law on the protection of personal data (LOPD 15/1999 of December 14).

Study variables and data sources. For each individual, we analyzed sex, age (15–64, 65–79, ≥ 80 years), and all baseline chronic diseases from patients' EHRs present at the time of inclusion in the cohort. Diagnoses were classified using the International Classification of Primary Care, First Edition (ICPC-1), which was later mapped to the International Classification of Diseases, 9th revision, Clinical Modification (ICD-9-CM) codes³³. These codes were then assigned to 226 clinical categories based on the Clinical Classification Software³⁴, 153 of which were classified as chronic according to the Chronic Condition Indicator software³⁵. The software considers as such those present at least during the last 12 months and that meet one or both of the following criteria: (a) entail limitations on self-care, independent living, and social interactions; (b) require of ongoing interventions using medical products, services, and special equipment. To facilitate their clinical interpretation, some diagnostic labels were renamed by the clinicians of the group. Multimorbidity was defined as the presence of two or more chronic conditions meeting the aforementioned criteria.

During follow-up, we analyzed patient hospitalization (including admission to the ICU) and mortality. Patients were followed for a maximum of 30 days, considering difficult to attribute to COVID-19 infection any events occurring afterwards, seeing as the exact cause of death was not available. Regarding hospital admissions, only those occurring within 15 days of the index date were considered a consequence of the infection. Since some patients were diagnosed only after hospitalization, we also accounted for those occurring up to 15 days before the index date.

The study variables were obtained from patient EHRs, the user's health database, and an ad hoc COVID-19 registry developed by the Aragonese Health System that links all data at a person level and in a pseudo-anonymized form.

Statistical analysis. This multistep study aims to (1) identify clinical profiles of COVID-19 patients based on their baseline morbidity using network analysis, (2) to clinically describe the multimorbidity patterns obtained, and (3) to assess their impact on infection severity (compared with chronic patients without multimorbidity).

First, we described the population's demographic and clinical characteristics as means and/or frequencies.

Then, to describe the multimorbidity patterns, we first applied network analysis in the population with multimorbidity, stratifying by sex and age, to identify communities of similar patients in each subpopulation based on all their chronic conditions. Network science studies maps of linked components to understand complex systems, capturing that it is challenging to infer their collective behavior based on the knowledge of the system's elements³⁶.

Due to the dichotomous nature of the diagnostic variables (i.e., presence/absence), the Jaccard index (JI) was used to measure the similarity between patients, just as in previous multimorbidity pattern studies^{30,37}. This coefficient measures the distance between patients based on individual and shared characteristics (chronic conditions in our case), ignoring diagnoses that none of them has. Its formula is the intersection over the union of the datasets analyzed, and we applied it to each pair of patients based on their disease datasets. A JI = 1 means that two individuals are identical, and a JI = 0 means that they share no values. We considered a link between patients if the JI between them was ≥ 0.33 to analyze patients who share with another at least half of their chronic conditions. Thus, a node in each network represents a different patient, and a link means a $\text{JI} \geq 0.33$ between patients. Patients aged 15–64 had, on average, four diseases, so if two patients have four diseases each and share two of them, their JI is 0.33 ($\text{intersection/union} = 2/6 = 0.33$). This threshold is particularly determinant in patients with two diseases, a very frequent case, especially in young patients; if these individuals share one disease, their JI is 0.33 ($1/3 = 0.33$). A higher threshold deletes them unless they have an identical pair or share the same diseases with other patients, which is rare, hyper-fragmenting the networks and creating tiny communities. The stratum aged 65–79 had six chronic conditions on average; two patients with such diseases pass the threshold only if they share at least three of them ($3/9 = 0.33$). Patients aged 80 and older had, on average, seven diseases, and two patients with such characteristics have to share at least four diseases ($4/10 = 0.4$) to pass the threshold.

This cut-off for building the patient networks allowed for the inclusion of almost all the patients with multimorbidity (38,303 out of 38,323 patients). At the same time, it only included 7.58% of all possible combinations between patients (14,501,518 out of 191,198,218 possible combinations), saving computation memory.

The network's modularity was used to search for communities of patients within each network using the Louvain method³⁸, as previously used in comorbidity and multimorbidity pattern studies^{18,19}. Modularity calculates the density of links inside communities compared to the links between them³⁸. By optimizing modularity in an iterated procedure, we detected communities, also called groups or clusters of patients, until modularity ceased to increase³⁹. Community detection methods like this one allow the number and size of the groups to be determined by the network's structure⁴⁰ based on the number of links between individuals and their weight (measured by the JI) and not by the researcher. This step allowed us to assign each individual to a community or cluster of patients.

Once the groups of patients were identified for each subpopulation, and in order to characterize multimorbidity patterns within each group, we calculated the prevalence of each chronic condition together with their observed/expected (O/E) prevalence ratio (i.e., the disease prevalence observed in a specific group divided by the disease prevalence observed in the subpopulation of reference). A chronic condition was included in a pattern if (1) the prevalence was higher than 25%; or (2) the O/E prevalence ratio was ≥ 2 ^{41,42} and the prevalence was higher than 1%. The patterns were then named by all clinicians by consensus, taking into consideration the

most relevant diseases within each pattern according to their prevalence and O/E prevalence ratio, and in line with the denominations used in the literature.

Finally, to analyze the impact on infection severity of each cluster of patients characterized by a multimorbidity pattern, we performed age-adjusted logistic regression models in each subpopulation. In doing so, we used the group of patients with one chronic condition as the reference group, and calculated age-adjusted ORs accompanied by their respective 95% CI, which represented the likelihood of infection severity for each multimorbidity pattern compared with having only one chronic disease.

All the analyses were conducted using RStudio software (version 1.4.1106, RStudio, Boston, MA, US) and STATA software (Version 14.0, StataCorp LLC, College Station, TX, US).

Data availability

The data used in this study cannot be publicly shared, because of restrictions imposed by the Aragon Health Sciences Institute (IACS) and asserted by the Clinical Research Ethics Committee of Aragon (CEICA, ceica@aragon.es). The authors who accessed the data belong to the EpiChron Research Group of IACS, and received permission from IACS to utilize the data for this specific study, thus implying its exclusive use by the researchers appearing in the project protocol approved by CEICA. The EpiChron Group can establish future collaborations with other groups based on the same data. However, each new project based on these data has to be previously submitted to the CEICA to obtain the respective mandatory approval. Potential collaborations should be addressed to the Principal Investigator of the EpiChron Research Group, Alexandra Prados-Torres at sprados.iacs@aragon.es.

Received: 10 September 2021; Accepted: 7 February 2022

Published online: 18 February 2022

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Acknowledgements

The authors sincerely thank Dirección General de Asistencia Sanitaria, Dirección General de Salud Pública, and Servicio Aragonés de Salud, from Departamento de Salud del Gobierno de Aragón, for their essential collaboration in developing the COVID-19 registry. This work was cofunded by Instituto de Investigación Sanitaria Aragón and Instituto de Salud Carlos III [Río Hortega Program, grant number CM19/00164; RICAPPS, grant number RD21/0016/0019], Gobierno de Aragón (Grant numbers B01_20R and DECRETO-LEY 3/2020, de 3 de junio, del Gobierno de Aragón) and the European Union – NextGenerationEU.

Author contributions

Conceptualization, J.C.P., A.P.T., A.G.M. and L.A.G.F.; methodology, J.C.P.; formal analysis, J.C.P.; data curation, B.P.P.; writing—original draft preparation, J.C.P.; writing—review and editing, A.G.M., K.B.B., I.I.S., F.G.R., J.D.M., L.A.G.F., A.P.T. and all members of the PRECOVID Group; visualization, J.C.P.; supervision, A.P.T. and L.A.G.F.; funding acquisition, A.P.T., A.G.M., J.C.P. All authors have read and agreed to the published version of the manuscript.

Funding

This work was cofunded by Instituto de Investigación Sanitaria Aragón and Instituto de Salud Carlos III [Río Hortega Program, grant number CM19/00164; RICAPPS, grant number RD21/0016/0019], Gobierno de Aragón (Grant numbers B01_20R and DECRETO-LEY 3/2020, de 3 de junio, del Gobierno de Aragón) and the European Union – NextGenerationEU.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-022-06838-9>.

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on behalf of the PRECOVID Group

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5. DISCUSSION

The results from this doctoral dissertation contribute to the analysis of the clinical and epidemiological characteristics of patients with COVID-19 and how these factors relate to the severity of the infection, with a focus on chronic diseases, multimorbidity and polypharmacy as underlying risk conditions. We used real-world data from large-scale, population-based electronic health information systems, where all the baseline conditions and clinical data from each patient had been previously diagnosed and registered by healthcare professionals. All COVID-19 diagnoses were laboratory-confirmed, identified through the detection of SARS-CoV-2 using RT-PCR in nasopharyngeal swab samples.

The analyses conducted across the studies included in this thesis described the presence and distribution of chronic conditions or chronic medications, and were stratified by age and sex, two additional key variables for COVID-19. We also conducted analyses on the interactions among different chronic conditions, how they associate with each other to conform contrasting multimorbidity profiles, and their impact on the outcome of the infection, separately and in combination. Mortality, hospitalization, or a combination of both, were the main events used to define severity and the basis for our results.

The findings presented here were published throughout the course of the Public Health Emergency of International Concern caused by the COVID-19 pandemic. The prompt publication of population-based studies during that period of demanding public health and clinical necessities was undertaken with the will to contribute to the ongoing crisis and help not only clinicians, but also policy- and decision-makers, by providing information for the development of targeted strategies to mitigate the impact of the epidemic on higher risk groups, the allocation of healthcare resources, and to better prepare for the succession of waves that followed. The aim of this work is to recapitulate and offer a cohesive view of the learnings extracted from the published articles, which can be relevant when preparing public health interventions for new epidemic waves of the disease as well as for potential future outbreaks of similar pathogens.

5.1. Main findings

5.1.1. Associations between baseline chronic conditions and COVID-19 severity

The first two manuscripts were aimed at analyzing the socio-demographic and clinical factors that were associated with an increased risk of poor outcomes in a population-based cohort of individuals affected by COVID-19, with a special focus on chronicity. In both studies, men were at a higher risk of severe infection than women. Differences based on sex are multifaceted, from changes in ACE-2 receptor expression, hormonal factors, social determinants, and factors regarding lifestyle choices (168,175,178). However, despite the effects of sex, both studies showed that advanced age was decidedly the most important factor associated with severity, especially for patients over 80, a fact that would be amply confirmed across the literature (162–164). In older age groups, immunosenescence, inflammaging, an increased chronic disease burden and a greater number of coexisting comorbidities, polypharmacy and frailty are the main contenders behind the increased incidence of poor health outcomes after SARS-CoV-2 infection (164–170).

The data shown in Manuscript 1 correspond exclusively to the first wave of the pandemic, which in Spain globally, and in the region of study particularly, affected more notably the most elderly and vulnerable populations, such as institutionalized patients in care homes. This made it so that the age average of infected patients in our study was above that of other similar works in other settings. Furthermore, the strict clinical conditions that patients had to meet to be eligible for testing due to the limited access to RT-PCR in the first wave made it so that most of the diagnosed cases were among the most severe. Gatekeeping the access to healthcare services and allocating testing resources was necessary to avoid a collapse of the system, but lead to a potential overestimation of the proportion of adverse outcomes in this group. These circumstances were shared with most studies conducted during this period and made a certain degree of inaccuracy in mortality results inevitable at the time (211).

The progress of the pandemic, however, was accompanied by an improvement in detection standards, a better understanding of the disease with advances in clinical management, a reinforcement of response capacities, and a more measured approach to the implementation of public health interventions based on epidemiological data,

which allowed for a smoother access to healthcare services to those who required it in the successive epidemic waves of COVID-19 transmission (212). To accommodate to this new context, where hospitalization was more accessible and mortality decreased despite the ongoing diagnoses of severe infections, we included a composite result variable that considered both hospitalization and mortality for Manuscript 2, therefore analyzing the underlying comorbidities of all moderate-to-severe COVID-19 cases with a more robust sample size.

In spite of these differences, we found common patterns in both studies, with an association of a history of cardiovascular comorbidities, such as congestive heart failure, acute myocardial infarction or cardiac dysrhythmias, or to an increased risk of severe COVID-19, even in age-adjusted models. As shown in different studies and reviews, the effects of SARS-CoV-2 on the cardiovascular system can be attributable to direct viral damage on cardiac and endothelial tissues, shown by elevated D-dimer, troponin, and lactate dehydrogenase levels, and the interference of the virus' activity on the renin–angiotensin–aldosterone system, mainly via its interactions with ACE-2 receptors, which can explain this association (76,143). Acute cerebrovascular disease and other coagulation-related and hemorrhagic disorders were also associated with an increased risk of severe COVID-19. SARS-CoV-2 has been shown to elevate the risk of thrombotic and embolic events in some patients, especially in patients who required hospitalization and more so in those who were admitted to ICU. Therefore, patients with previous complications of the same nature, where endothelial tissue can be weaker or more susceptible to further damage, can be prone to coagulopathy and disseminated intravascular coagulation (145).

The virus' tropism for the respiratory tract is key in its pathogenesis. In Manuscript 2 we found that chronic respiratory conditions such as COPD and obstructive sleep apnea (OSA) were associated with poor COVID-19 infection prognosis, though significance was sex-specific when considering the rest of potentially confounding comorbidities, with COPD remaining relevant only in men and OSA only in women after adjusting the model. These effects were not observed in Manuscript 1 when studying respiratory conditions directly, though patients of both sexes receiving inhalant treatments directed towards obstructive airway diseases were at a significantly higher risk of

mortality. These findings underpin that conditions that entail chronic and often irreversible damage to the airways and lungs, along with an overexpression of ACE-2 in epithelial cells as is the case of COPD, can be hazardous for patients infected by SARS-CoV-2, which in turn can also exacerbate baseline chronic respiratory diseases like other respiratory viral infections (91). Apart from its clear association with obesity, an independent risk factor on its own, the recurrent episodes of airway obstruction present in OSA patients lead to hypoxia, hypoxemia, bronchoconstriction, airway inflammation, oxidative stress and ultimately immune dysfunction, which can explain its negative impact in conjunction with COVID-19 (165,213). Like in other studies, we found no significant association of other potentially aggravating chronic respiratory conditions like asthma with COVID-19 severity (214).

Similarly to what was observed with COPD and OSA, men and women in our studies presented other discrepancies in their risk profiles, which were similar but not identical. Specific risk factors of severe infection in men in age-adjusted analyses were neoplasms, often associated to immunosuppressive states and treatments, and cognitive disorders such as dementia, anxiety, or delirium, likely due to poorer functional capacity, pro-inflammatory statuses, along with the aggravating factor of stress due to disruptions in daily living, hospitalizations, and the pandemic itself, explaining already well-known associations with infection severity (195,215). Chronic ulcers of the skin were also a factor in men, usually linked to vulnerable states with prolonged hospitalizations, other chronic illnesses, the use of medical devices, and poor physical activity, all potentially leading to poorer health outcomes due to infection, as well as making them more likely to develop new ulcers or worsen already existing ones after admission (216,217). Likewise, male patients with a diagnosis of urinary incontinence were at higher risk of severe COVID-19. This condition in men is often associated with prostate cancer and its treatments, including radiotherapy and chemotherapy, as well as poor general health, severe physical limitations, cognitive impairment, stroke or diabetes, which could all be linked to the increase in risk (168,195,218).

In women, we found that rheumatoid arthritis and related conditions were sex-specific risk factors. While the prevalence of rheumatoid arthritis is of up to three times higher

in women than in men, which could explain the differences in significance along with possible unmeasured confounders, the association of these and other immune-mediated inflammatory diseases to severe COVID-19 have been previously identified. The factors that lead to adverse outcomes are probably multifactorial and warrant further studies, though alterations in the inflammatory and immune responses in these patients could be a potential explanation (107,219,220). Another finding in Manuscript 2 was the link between hepatic steatosis and poor outcomes after SARS-CoV-2 infection in women. Non-alcoholic fatty liver disease is the most common chronic liver disease, and it has been widely associated with poor COVID-19 outcomes potentially due to its association with pernicious metabolic profiles that also include obesity, diabetes mellitus, or cardiovascular disease. Some authors suggest that this relationship can also be attributable to an associated exacerbation of the inflammatory response, an upregulation of ACE-2 and TMPRSS2, increased viral replication capacity via lipid droplets, or dysregulations of the gut microbiota (221,222).

A more challenging risk factor to analyze was the association between menstrual disorders and female infertility with COVID-19 severity found in our population. Many studies conducted during the SARS-CoV-2 period have reported that women experienced changes in their menstrual cycles due to the pandemic. Some authors have attributed these changes to large-scale public health strategies like lockdowns and social distancing, which have led to psychological stress, depression, and anxiety, generally affecting the well-being of the population, and particularly in young adults and women (223). This, however, does not explain why these patients showed more severe infections, a fact that was also evident when studying menstrual and menopausal disorders as part of more complex multimorbidity patterns in Manuscript 4, and though chronic stress could be a possible aggravating factor, when analyzed in different studies, infection status itself did not affect outcomes such as cycle regularity, bleeding duration, or fertility, and thus, the potential underlying associations are still unclear (224,225). Others suggest that the link between menstrual irregularities or female infertility could be of a biological nature linked to ACE-2 expression, wherein could lie the answer to our results, though the mechanisms that could respond to this

theory and the connections to severity have yet to be fully explained, and further research would be warranted (225,226).

As stated, metabolic conditions such as obesity and diabetes mellitus were shown to be linked to severe presentations of COVID-19 in both sexes in our population. As proven by other authors, being overweight has shown a linear association with COVID-19-related health outcomes, with increasing levels of obesity translating to mounting risks of hospital admission, ICU admission, invasive mechanical ventilation and death, and can be attributable to the viral reservoir effect of adipose tissue, increase ACE-2 expression, and inflammatory and immune dysfunction, as well as mechanical limitations such as decreased lung capacity (227). Diabetes has also been shown to increase the risk of severe COVID-19 through the virus' capacity to directly bind to receptors in the already vulnerable beta pancreatic cells as well as by impairing glycemic control through similar inflammatory and immunity-related principles as in obesity (228–230). The interaction is therefore bidirectional, with diabetic patients having higher risk of complications due to SARS-CoV-2 infection, and the virus acting as a diabetogenic agent, binding to receptors in pancreatic beta cells and leading to acute dysfunction and changes in glucose levels (231).

Similarly to other risk factors like the aforementioned cardiovascular conditions, many of these metabolic diseases have a common pathophysiological denominator in low-grade chronic systemic inflammation (232). Patients with a history of these conditions, all of which share low-grade chronic inflammation with increased levels of pro-inflammatory mediators and cytokines such as tumor necrosis factor (TNF) or interleukin-1 (IL), IL-6, IL-8, and IL-12, have been amply reported to condition more severe presentations of SARS-CoV-2 infection (233). Inflammatory mediator levels can increase during COVID-19 infection due to an exacerbated and dysfunctional response of the immune system, leading to a serious and potentially lethal presentation known as a cytokine storm. Though not measured specifically in our study groups, the expected alterations of inflammatory components at baseline could be partly responsible for the unfavorable outcomes in patients with these NCDs (107).

A noteworthy exception is hypertension. Though thoroughly studied as one of the most prevalent chronic comorbidities, its role as an independent risk factor for severe

COVID-19 has remained controversial over the past years. Some authors have suggested that hypertension increases the risk of severe COVID-19 (234,235). Concurring with other studies, however, we did not find hypertension to be an independent risk factor for severe COVID-19 after adjusting for other comorbidities, especially in terms of mortality (236–238). The pathophysiological association between ACE-2 receptors and COVID-19 was one of the findings that drew attention to hypertension from the first months of 2020, to the point of considering interrupting antihypertensive treatment in COVID-19 patients. Notwithstanding, it was quickly apparent that treatments using angiotensin receptor blockers or ACE inhibitors were not only safe but also fundamental to ensure adequate blood pressure management and reduce mortality during COVID-19, with no specific negative effects on infection severity (239,240).

Some authors have considered that the different results across studies could be explained by the quality of blood pressure control. Patients with hypertension have been reported to have altered proportions of neutrophil-to-lymphocyte ratios, which could impact severity, though these changes can at least partially revert with adequate blood pressure treatment, therefore leading to postulate that only uncontrolled hypertension could be a potential risk factor for poor health outcomes during infection (240–242). In Manuscripts 1 and 2 we did find a significant increase in mortality in COVID-19 patients receiving two groups of drugs with antihypertensive capacity, namely high-ceiling or loop diuretics and potassium sparing agents, that can sometimes be used to treat hypertension, though rarely as first-line therapy, and which are more commonly used to treat congestive heart failure or chronic kidney disease, which can explain the association (243).

5.1.2. The use of chronic treatments as predictors of COVID-19 severity

Manuscript 1 included both chronic medications and chronic conditions in the analysis with the aim to obtain as much information as possible for potential underlying risk factors during the first wave in Europe, where quality information was still scarce. By contrast, in Manuscript 2 we chose to exclude chronic treatments to focus on a more in-depth analysis of comorbidities and diagnoses after the experience of our first study, considering that the associations of most treatments could be linked or explained by

the conditions they were intended to treat. Notwithstanding, this was the first step that led us to develop a separate study in Manuscript 3, where we used information regarding drug dispensation to characterize and compare two separate population-based cohorts from two regions in two European countries, Spain and Italy, during the same time period.

The characteristics of the populations in our study were nuanced, with Campania as one of the areas with the youngest population of all of Italy, and Aragon with an older population on average, and where the epidemic wave subject of our study, before the introduction of vaccination, especially affected residential care homes for the elderly, which could explain the higher mortality rates associated to COVID-19 that were found in the Spanish region, as well as higher dispensation overall (166,170). Patients at higher risk of poor prognosis in Campania were receiving treatments intended for hypertension and cardiometabolic diseases, while in Aragon they were predominantly geriatric patients treated for heart failure and dysrhythmias.

Despite these differences, baseline chronic dispensation of vasodilators used in cardiac diseases, β -blocking agents, high-ceiling diuretics, potassium-sparing agents, antithrombotic agents, and blood glucose-lowering drugs were identified as common risk factors for COVID-19 mortality in both Spain and Italy. These drugs are primarily used to treat conditions such as chronic kidney disease, prothrombotic states, congestive heart failure and other cardiac diseases, or diabetes, among others, all of which are known risk factors for severe COVID-19 (76,165,228). Moreover, other treatments such as antigout preparations, strongly correlating with the presence of cardiovascular diseases or often used in onco-hematological, or B12 supplementation, linked to elevated homocysteine levels, were also associated with poor prognosis after infection, potentially relating to the increase in risk due to malignancies, cardiovascular disease, or prothrombotic states, concurring with existing literature (174).

The association of the use of certain treatments with COVID-19 mortality could reasonably lead to attribute the increase in risk to the presence of the drugs themselves. However, it has been made abundantly evident that discontinuing usual chronic medications during SARS-CoV-2 infection is unwarranted and should be discouraged as it does not entail specific risks and could lead to further complications

(239,244). Furthermore, the results from the pharmacological analysis were consistent with our findings from Manuscript 1 on chronic diseases, and lead to similar conclusions in both geographical areas, which supports that information that can be extracted from population-based drug dispensation data can be used as proxy for the conditions they are meant to treat when other clinical data might be unavailable or more difficult to extract, and therefore can help identify and target collectives with underlying comorbidities with potential impact on COVID-19 mortality (203).

5.1.3. Multimorbidity patterns and their impact on COVID-19 severity

Multimorbidity is more frequent than being affected by isolated chronic conditions and has a greater impact on health than the sum of its parts (200). Having identified the main chronic conditions that are a hazard during COVID-19, we set the focus on chronic patients specifically and addressed how these risk factors relate to each other and how they impact SARS-CoV-2 infection severity when combined. In Manuscript 4 we identified up to fourteen unique multimorbidity patterns with an impact on COVID-19 severity among our chronic patients using network analysis. Concurring with our previous studies and with the other reported experiences from different authors, cardiometabolic patterns were the most prominent among the different risk profiles (197,245).

In line with our findings in Manuscripts 1 and 2, diabetes and obesity had an important role in the configuration of high-risk multimorbidity patterns and in this case were definitory traits of the main risk profiles in women and men, respectively, seemingly serving as attractors of other chronic conditions, and rarely occurring in other patterns despite their high prevalence in the general population, sustaining existing evidence (246,247). Both conditions are interrelated, with common predisposing risk factors and have shared pathophysiological pathways, which further adds on to the hypothesis of the detrimental effects of low-grade chronic inflammation (232). Moreover, both conditions have been shown to increase the risk of COVID-19 severity independently (227,230,231). This evidence on their key role as drivers for severity both as standalone conditions and as catalyzers within complex multimorbidity profiles highlights the importance of targeting patients with these conditions for priority

vaccination and shielding strategies, as well as of anticipating their development with early detection, and primary and secondary preventive strategies.

Another interesting multimorbidity pattern linked to increased COVID-19 severity was related to mental health issues, and mainly anxiety disorders. These results aligned with our findings when studying pharmacological groups intended to treat mental health conditions, such as antipsychotics and antidepressants, both of which were associated with higher mortality in SARS-CoV-2-infected patients in the study regions of both Spain and Italy. Other authors have found similar associations, and the reasoning behind them is multifaceted, ranging from and exacerbation of respiratory conditions, higher prevalence and severity of other morbidities, or immune dysregulation, to the use of psychotropic medications or combinations of all the above (248).

As we analyzed further into more advanced age groups, the significance of many of the multimorbidity patterns on COVID-19 severity was lost, leading again to the conclusion of our previous studies and existing literature that the impact of age on health outcomes surpasses the expected effects of other risk-inducing variables (165,166,170). Notwithstanding, the study of multimorbidity patterns instead of isolated chronic diseases adds another layer on to the understanding behind the chronic risk factors for COVID-19 severity and is necessary to characterize at-risk populations more consistently with real world settings, where the prevalence of single NCDs in chronic patients is steadily decreasing in favor of multimorbidity.

5.2. Strengths and limitations

The main strengths of our studies are their large-scale population-based approach, by which we included all the individuals of the reference populations in the studied areas, as well as the exhaustive analysis of virtually all the chronic conditions identified for each individual patient, where all the diagnoses were extracted from primary sources in the form of electronic health records, registered by authorized healthcare professionals, that did not require less objective sources such as self-reported data, and that were not limited only to the most prevalent or relevant conditions. Additionally, the software utilized to identify, categorize, and manage chronic conditions for our studies was based on open access technologies and algorithms, facilitating reproducibility and comparability. Regarding SARS-Cov-2 infection, all

COVID-19 cases were laboratory-confirmed by RT-PCR, ensuring strict case definitions were met for all individuals.

Another major strength of our studies was their timely publication. Our work was developed and made available through open access during the most demanding periods of the pandemic from a public health standpoint and allowed to shed light on key risk factors for severe COVID-19 early on, when high quality epidemiological data was scarce, especially large-scale data from electronic health records with sample sizes large enough to be representative of a specific population. Additionally, we included data from two European regions with their own singular characteristics and applied a structured methodology to obtain comparable results, giving the delivered health outcomes more solid basis for clinical and public health significance.

Finally, from a methodological standpoint, we used two different sets of variables across our studies, chronic condition data on the one hand and dispensed pharmacological data on the other, making reproducibility more accessible based on the data that could be available for other research groups. Furthermore, we applied not only conventional statistical analyses such as logistic regression models and multivariate logistic regression analysis, with age-adjusted odds ratios, but also more innovative techniques such as network analysis. Through network science, we were able to detect and characterize complex multimorbidity patterns by computing millions of connections that would be otherwise more challenging to automate with other cluster-type analyses.

The main limitation is that some variables of interest for their potential impact on health outcomes, such as socio-economic variables, genetic test results, clinical laboratory tests, and inpatient treatments, among others, were unavailable in our studies. Moreover, our outcome analyses were based on information related to all-cause mortality and not the cause of death, and therefore a direct association between COVID-19 and mortality could not be established. The specific causes for other outcomes such as hospital admission or ICU admission were also unavailable for these studies. An additional and final limitation to the thesis is external to the works themselves but nevertheless meaningful and is related to the speed at which COVID-19 related information was published. Fast-paced research, the frenetic delivery of

scientific evidence in a completely new and unexplored field in clinical, biological and public health sciences, and a completely new disease that aroused unprecedented interest on the scientific community and general public, made staying up to date and maintaining relevance for new publications extremely challenging in the long term.

5.3. Future perspectives

The research results presented in these studies complement existing work on chronic risk factors for COVID-19 severity, with population-based data and exhaustive diagnostic categories, innovative methodological approaches such as network analysis, and a detailed analysis of multimorbidity patterns in chronic patients affected by SARS-CoV-2. Some of the risk factors presented in this body of work have been clearly linked to COVID-19 over the years since the beginning of the COVID-19 pandemic, though fewer studies have conducted more holistic patient-centered approaches, where the object of study is not the disease itself, but the complete configuration of diagnosed chronic conditions in each individual, how they relate to each other and how they define specific multimorbidity profiles.

Our ongoing work in this field is primarily focused on further exploring this line of research to better understand how chronic conditions interact with each other, the intricacies of multimorbidity, its patterns, and their impact on COVID-19 severity. Ideally, the intention is to expand the analyses using baseline characteristics up to now unavailable to us such as clinical disease markers, which can offer more information regarding the inflammatory and immune characteristics of these patients. Future goals include studying the consequences of COVID-19 in our chronic populations in the form of sequelae, or the debut of new chronic diseases and other adverse outcomes in the follow-up after the infection has subsided. If possible, data regarding cause of death, hospitalization and ICU admission will be included in future studies, in order to better associate infection and adverse outcomes.

Finally, post-COVID-19 is also a cause for concern, especially among chronic patients, with long-term and very limiting consequences for daily living for individuals that survive SARS-CoV-2 infection. Prospective studies should aim to better understand the mechanisms and impact of post-COVID-19 in our populations as well as its repercussions specifically on chronic patients. The final goal of all our research is to

provide health systems, care providers, clinicians and researchers with the tools to develop and improve effective and targeted preventive strategies that can protect our most vulnerable from current and future threats.

6. CONCLUSIONS

The conclusions that can be extracted from the present thesis are the following:

1. Comorbidity and medication burden at the time of infection can be predictors of the risk of poor COVID-19 health outcomes, with women and men sharing similar but not identical chronic disease risk profiles, and men generally being at a higher risk than women.
2. Advanced age is by far the most influencing factor for poor COVID-19 outcomes and can increase the odds of severe infection up to eight-fold in patients over the age of 80 years.
3. Specific cardiovascular and metabolic conditions such as heart failure, diabetes mellitus, obesity and chronic renal disease consistently increased the risk of severe SARS-CoV-2 infections in age- and sex-adjusted models.
4. Baseline chronic treatments for COVID-19 risk factors such as chronic cardiovascular and metabolic diseases, systemic inflammation, and processes with increased risk of thrombosis, are systematically associated with mortality in SARS-CoV-2-infected patients. Therefore, the assessment of pharmacological profiles in COVID-19 patients as proxy of the chronic diseases they are intended to treat when diagnoses are unavailable can be an alternative approach to a prompt identification of at-risk individuals in clinical practice.
5. Multimorbidity is more frequent than single non-communicable diseases in chronic patients affected by COVID-19, and the characterization of these complex profiles is necessary to better identify vulnerable individuals. In this sense, multimorbidity patterns that include principally cardiovascular and metabolic diseases are the main aggravating chronic factors that affect COVID-19 outcomes, increasing the likelihood of severe infection by up to 86% compared with patients with only one chronic condition.
6. Diabetes mellitus in women and obesity in men are key disease attractors for other chronic conditions within their respective sex-specific multimorbidity patterns and are associated with COVID-19 severity in patients over the age of 65 years.

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