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PERSPECTIVES

COVID-19 pandemic, coronaviruses, and diabetes mellitus

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Muniyappa R, Gubbi S. COVID-19 pandemic, coronaviruses, and diabetes mellitus. *Am J Physiol Endocrinol Metab* 318: E736–E741, 2020. First published March 31, 2020; doi:10.1152/ajpendo.00124.2020.—The pandemic of coronavirus disease (COVID-19), a disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is causing substantial morbidity and mortality. Older age and presence of diabetes mellitus, hypertension, and obesity significantly increases the risk for hospitalization and death in COVID-19 patients. In this Perspective, informed by the studies on SARS-CoV-2, Middle East respiratory syndrome (MERS-CoV), and the current literature on SARS-CoV-2, we discuss potential mechanisms by which diabetes modulates the host-viral interactions and host-immune responses. We hope to highlight gaps in knowledge that require further studies pertinent to COVID-19 in patients with diabetes.

diabetes mellitus; coronavirus; COVID-19

INTRODUCTION

Coronaviruses (CoV) are enveloped viruses with a single-stranded, positive-sense RNA genome known to cause respiratory infections in humans (7, 38). In general, in most immunocompetent individuals, human CoV infection leads to mild upper respiratory infection. However, two highly pathogenic CoV have resulted in outbreaks of severe acute respiratory syndrome (SARS) in 2003 in Guangdong province, China and Middle East respiratory syndrome (MERS) in Middle Eastern countries a decade later. SARS-CoV and MERS-CoV were identified to cause SARS and MERS, respectively (11, 51, 55). In December 2019, a novel coronavirus, SARS-CoV-2, was identified as the pathogen causing coronavirus disease (COVID-19) in Wuhan, China (11, 51, 55). On March 11, 2020, COVID-19 was declared a pandemic by the World Health Organization. As of March 27, 2020, there have been a total of 103,942 confirmed cases with 1689 deaths in the United States (19a). Globally, 27,324 deaths have been reported among 595,800 confirmed cases (19a).

Individuals with diabetes mellitus (DM), hypertension, and severe obesity (BMI $\geq$ 40 kg/m^2) are more likely to be infected and are at a higher risk for complications and death from COVID-19 (3a, 16, 30, 48, 50, 52, 56). Interestingly, there was a similar increased risk for SARS and MERS in individuals with DM. In the United States, 34.2 million or 10.5% of the total population have DM (3a). Among those aged 65 years or older, a population at higher risk for death from COVID-19, 26.8% have DM (3a). Hypertension and severe obesity are present in 68.4% and 15.5% of individuals diagnosed with DM, respectively. Over a period of months, a substantial portion of the US population will be infected by SARS-CoV-2 (12). Although a significant number will remain asymptomatic and be able to transmit the virus, the estimated proportion of symptomatic individuals requiring hospitalization increases with age (12). In individuals older than 60 years, that proportion ranges from 17 to 27%. Furthermore, in this older group, the percentage of hospitalized patients requiring care in an intensive care unit (ICU) is 27–71% with an infection fatality rate (IFR) ranging from 2.2 to 9.3% (12). Although these estimates are preliminary and likely to change, considering the prevalence of DM, hypertension, and severe obesity in the United States and the substantial increased risk for COVID-19 and its complications in patients with these conditions, it is likely the pandemic has the potential to cause significant mortality and morbidity. Specialists and health care providers will be providing clinical care to many patients with COVID-19 in inpatient, outpatient, and telehealth settings. Increased awareness of the clinical features, pathophysiology, and potential mechanisms that increase the risk is needed to provide better care and spur new investigations, both basic and clinical, to better understand COVID-19 in patients with diabetes.

Clinical features and natural course of COVID-19. The median age of patients infected with SARS-CoV-2 is in the range of 47–56 years, men comprise more than half of the cases, the average incubation period is 5.2 days, and 98% of those who develop symptoms will do so within 11.5 days (5, 16, 19, 22, 42). The clinical manifestations of COVID-19 vary and include the asymptomatic carrier status, acute respiratory disease (ARD), and pneumonia (16, 42). The prevalence of asymptomatic cases is significant (20–86% of all infections) and are defined as individuals with positive viral nucleic acid tests, but without any COVID-19 symptoms (3, 4, 23, 29, 57). Transmission rates and respiratory viral load in asymptomatic carriers are similar to symptomatic patients (23, 57), partially explaining the rapid spread of SARS-CoV-2. In addition to a laboratory-confirmed COVID-19 diagnosis, patients with ARD manifest with fever, fatigue, respiratory (cough, dyspnea) or gastrointestinal (nausea, diarrhea, vomiting) symptoms, and no significant abnormalities on chest imaging (16, 42). Patients with pneumonia have respiratory symptoms and positive findings in chest imaging. Severe pneumonia can present as acute respiratory distress syndrome (ARDS), leading to severe hypoxia, respiratory failure, multiorgan failure, shock, and death (16, 37, 42).

The pathophysiology of SARS-CoV-2 infection. The genetic sequence of SARS-CoV-2 showed more than 80% shared identity to SARS-CoV and 50% to the MERS-CoV, and both

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SARS-CoV and MERS-CoV originate in bats and infect humans and wild animals (1, 7, 26, 38). Cellular CoV entry is a complex process that involves receptor binding and proteolysis leading to virus-cell fusion. CoV is made up of four structural proteins: spike (S), membrane (M), nucleocapsid (N), and envelope (E) proteins. The S protein mediates receptor binding on the host cell membrane through the receptor-binding domain (RBD) in the S1 domain and membrane fusion through the S2 subunit (18, 40). Angiotensin-converting enzyme 2 (ACE2) is the cellular receptor for SARS-CoV and SARS-CoV-2, in contrast to MERS-CoV, which utilizes dipeptidyl peptidase 4 (DPP4) as its cellular receptor (24, 33) (Fig. 1). This interaction thus determines host tropism and ultimately clearance of the virus. ACE2 is expressed in the upper respiratory system, type I and II alveolar epithelial cells in the lungs, the heart, endothelial cells, kidney tubular epithelium, enterocytes, and the pancreas (10, 24, 25, 54). After binding to ACE2, proximal serine proteases such as TMPRSS2 are involved in S protein priming and cleavage of the spike (Fig. 1). Proteases such as Furin subsequently release the spike fusion peptide, and the cellular virus enters through an endosomal pathway (18, 40). The low pH and presence of proteases such as cathepsin-L characteristic of the endosomal microenvironment favor the delivery of SARS-CoV-2 genome into the cytosol where further viral replication leads to the formation of mature virions and subsequent spread.

Infected cells undergo apoptosis or necrosis and trigger inflammatory responses marked by the activation of proinflammatory cytokines or chemokines, which leads to the recruitment of inflammatory cells. CD4+ T helper (Th1) cells regulate antigen presentation and immunity against intracellular pathogens such as CoV through interferon gamma (IFN-γ) production. Th17 cells induce the recruitment of neutrophils and macrophages by producing interleukin-17 (IL-17), IL-21, and IL-22 (9). SARS-CoV-2 infects circulating immune cells and increases apoptosis of lymphocytes (CD3, CD4, and CD8 T cells), leading to lymphocytopenia. Indeed, the degree of lymphocytopenia is associated with the severity of SARS-CoV-2 infection (16, 45, 50, 52). Lower T cell function relieves the inhibition on innate immune system leading to secretion of high amounts of inflammatory cytokines in what is known as a "cytokine storm" (31). In fact, circulating levels of cytokines/chemokines [IL-6, tumor necrosis factor-α (TNF)] and chemokines [CXC-chemokine ligand 10 (CXCL10) and CC-chemokine ligand 2 (CCL2)] involved in the cytokine storm syndrome are elevated and may play a role in SARS-CoV-2-driven hyperinflammation leading to multiorgan failure (15, 28, 41).

Potential mechanisms that increase the risk of COVID-19 in diabetes. It is now well recognized that older age and the presence of DM, hypertension, and severe obesity (BMI ≥ 40 kg/m^2) increase morbidity and mortality in patients with COVID-19 (3a, 16, 30, 48, 50, 52, 56). Considering the high prevalence of cardiovascular disease (CVD), obesity, and hypertension in patients with DM, it is unknown whether DM independently contributes to this increased risk. However, plasma glucose levels and DM are independent predictors for mortality and morbidity in patients with SARS (49). Potential mechanisms that may increase the susceptibility for COVID-19 in patients with DM include: *1*) higher affinity cellular binding and efficient virus entry, *2*) decreased viral clearance, *3*) diminished T cell function, *4*) increased susceptibility to hyperinflammation and cytokine storm syndrome, and *5*) presence of CVD (Fig. 2).

Augmented ACE2 expression in alveolar AT2 cells, myocardium, kidney, and pancreas may favor increased cellular binding of SARS-CoV-2 (25, 27, 58). Increased expression of ACE2 has been demonstrated in the lung, kidney, heart, and pancreas in rodent models of DM (35, 46). Insulin administration attenuates ACE2 expression (35, 46), while hypoglycemic agents such as glucagon-like peptide-1 (GLP-1) agonists (liraglutide) and thiazolidinediones (TZDs; pioglitazone), antihypertensives such as ACE inhibitors, and statins upregulate ACE2 (14, 36, 39, 44, 53). Until recently, whether DM was causally linked to ACE2 expression levels in the lung in humans was unknown. Using a phenome-wide Mendelian

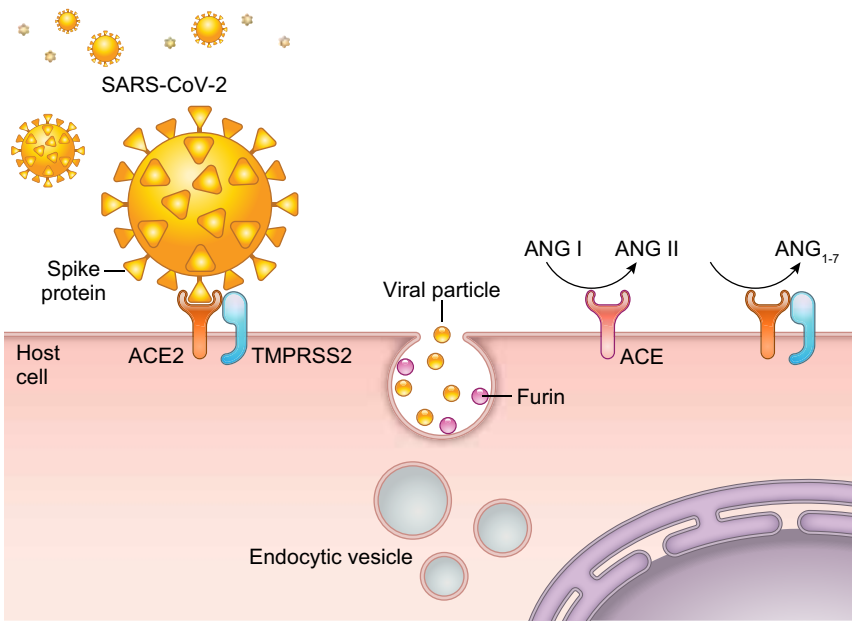

Fig. 1. Cellular entry of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The initial step in cellular entry of the virus is the binding of SARS-CoV-2 spike protein to cell surface angiotensin converting enzyme 2 (ACE2). Cellular proteases such as TMPRSS2 and furin are involved in priming of the S protein, which involves cleavage at the S1/S2 domains. This allows the fusion of the virus to the cell surface. Virions are taken up into endosomes, where SARS-CoV-2-S is cleaved and possibly activated by the pH-dependent cysteine protease cathepsin L. Once inside the cell, SARS-CoV-2 uses the endogenous cellular machinery to replicate itself. ACE catalyzes the conversion of angiotensin (Ang) I to the octapeptide AngII, whereas ACE2 converts AngII to Ang$_{1–7}$. AngII through the activation of Ang II type 1a receptors induces vasoconstriction and proliferation, whereas Ang$_{1–7}$ stimulates vasodilatation and suppresses cell growth.

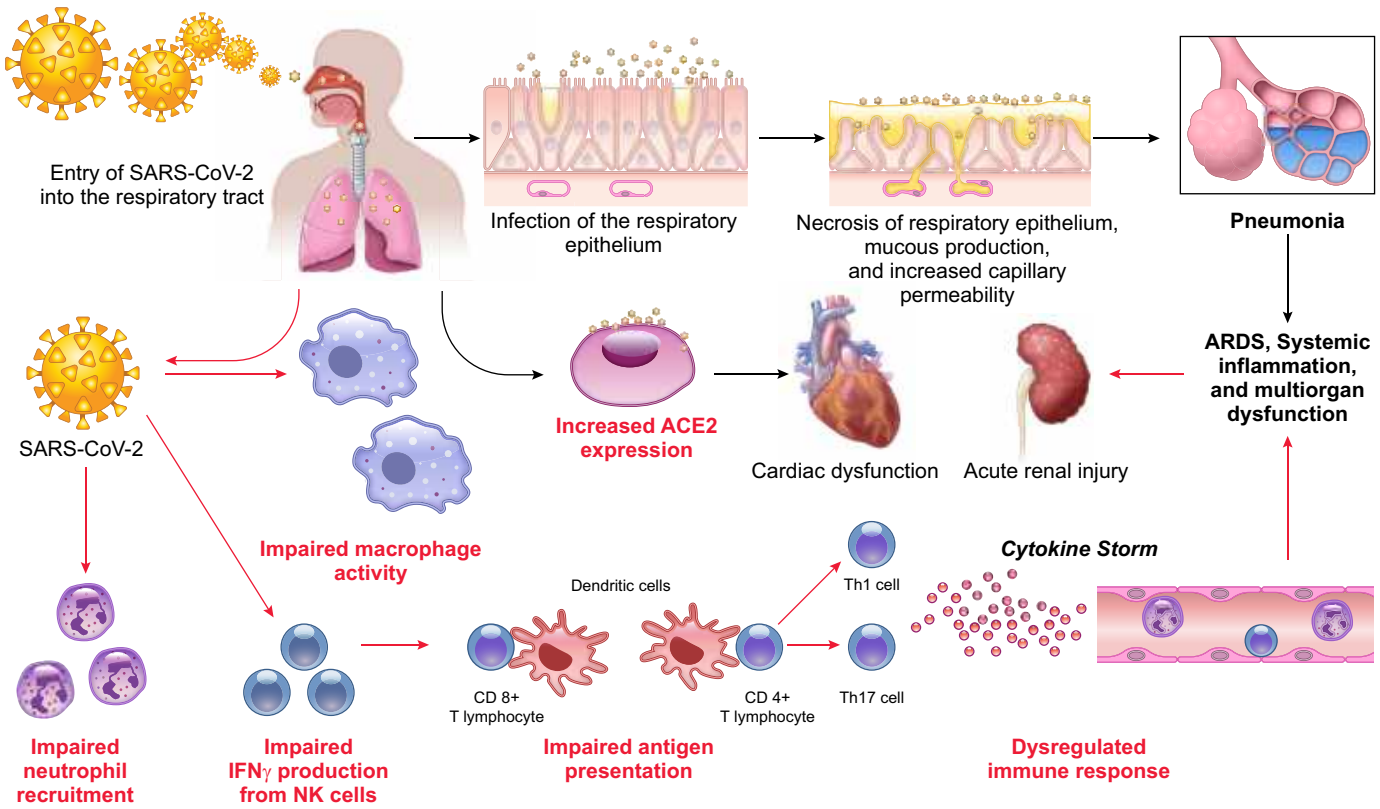

Fig. 2. Putative mechanisms contributing to increased susceptibility for coronavirus disease (COVID-19) in patients with diabetes mellitus (DM). Following aerosolized uptake of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), invasion of the respiratory epithelium and other target cells by SARS-CoV-2 involves binding to cell surface angiotensin converting enzyme 2 (ACE2). Increased expression of ACE2 may favor more efficient cell binding and entry into cells. Early recruitment and function of neutrophils and macrophages are impaired in DM. Delay in the initiation of adaptive immunity and dysregulation of the cytokine response in DM may lead to the initiation of cytokine storm.

randomization study, Rao et al. (34) explored diseases or traits that may be causally linked to increased ACE2 expression in the lung. Interestingly, they found that DM was causally associated with increased lung ACE2 expression. Circulating levels of furin, a cellular protease involved in facilitating viral entry by cleaving the S1 and S2 domain of the spike protein, are elevated in patients with DM (13). These studies support the hypothesis that patients with DM are susceptible to SARS-CoV-2 infection. Indeed, a recent study reported that clearance of SARS-CoV-2 was delayed in patients with DM, a finding that needs to be confirmed in larger studies (6) (Fig. 2).

ACE catalyzes the conversion of the prohormone, angiotensin (Ang) I to the octapeptide, AngII), whereas ACE2 converts AngII to $Ang_{1–7}$. AngII, through the activation of Ang II type 1a receptors induces vasoconstriction and proliferation, whereas $Ang_{1–7}$ stimulates vasodilatation and suppresses cell growth (Fig. 1). Increased ratio of pulmonary ACE/ACE2 activity as observed in patients with ARDS (43) favors AngII generation. Once bound to ACE2, SARS-CoV downregulates cellular expression of ACE2, and the unopposed action of AngII contributes to acute lung injury (20). Binding to ACE2 alone does not lead to severe lung injury as is observed with other CoVs (NL63) (7, 38). Whether SARS-CoV-2 causes downregulation of pulmonary ACE2 is unknown. Nevertheless, there exists a potential for salutary, if not therapeutic, effects of Ang II receptor blockers, ACE inhibitors, TZDs, GLP-1 agonists, and statins in the setting of low ACE2 expression. Lacking further evidence of risk or benefit, the American College of Cardiology, the American Heart Association, and the American Society of Hypertension have recommended that patients should continue treatment with their usual antihypertensive therapy (8).

DM inhibits neutrophil chemotaxis, phagocytosis, and intracellular killing of microbes. Impairments in adaptive immunity characterized by an initial delay in the activation of Th1 cell-mediated immunity and a late hyperinflammatory response is often observed in patients with diabetes (17). In an elegant study, Kulcsar et al. (21) examined the effects of DM in a humanized mouse model of MERS-CoV infection on a high-fat diet. Following MERS-CoV infection, the disease was more severe and prolonged in diabetic male mice and was characterized by alterations in CD4+ T cell counts and abnormal cytokine responses (such as elevated IL17a). Consistent with this finding, in patients with COVID-19, peripheral counts of CD4+ and CD8+ T cells are low, but with a higher proportion of highly proinflammatory Th17 CD4+ T cells, as well as elevated cytokine levels (16, 45, 47, 50, 52). Thus, it is likely that patients with DM may have blunted anti-viral IFN responses, and the delayed activation of Th1/Th17 may contribute to accentuated inflammatory responses (Fig. 2).

Conclusion

There is a paucity of data in the United States regarding comorbidities and COVID-19 outcomes and mechanisms that modulate viral pathogenesis. Certain racial groups such as African Americans, Hispanics, Asians, and Native Americans are highly prone to develop DM, and disparities in health care make these groups more vulnerable. Identification of clinical and biochemical parameters using multi-omics approaches that predict severity of the COVID-19 in DM using large data sets is urgently needed. Studies in humanized ACE2 (hACE2) mice and non-human primates aimed at understanding how hyperglycemia, hyperinsulinemia, and hypoglycemic agents affect pathogenesis of COVID-19 and how DM affects the efficacy of vaccines and antiviral investigational agents currently in trials are warranted. Finally, we need to develop novel ways to deliver care to our patients with DM using telehealth, remote patient monitoring, and wearable technologies. As the global pandemic unfolds and rapidly spreads across the United States, social isolation measures will enable the transition, but there is an urgent need for basic and clinical investigations to address the many important and unanswered questions.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

R.M. and S.G. prepared figures; R.M. and S.G. drafted manuscript; R.M. and S.G. edited and revised manuscript; R.M. and S.G. approved final version of manuscript.

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Review
OBESITY BIOLOGY AND INTEGRATED PHYSIOLOGY

Obesity

Is Adipose Tissue a Reservoir for Viral Spread, Immune Activation, and Cytokine Amplification in Coronavirus Disease 2019?

Paul MacDaragh Ryan and *Noel M. Caplice*

Coronavirus disease 2019 (COVID-19), the worst pandemic in more than a century, has claimed >125,000 lives worldwide to date. Emerging predictors for poor outcomes include advanced age, male sex, preexisting cardiovascular disease, and risk factors including hypertension, diabetes, and, more recently, obesity. This article posits new obesity-driven predictors of poor COVID-19 outcomes, over and above the more obvious extant risks associated with obesity, including cardiometabolic disease and hypoventilation syndrome in intensive care patients. This article also outlines a theoretical mechanistic framework whereby adipose tissue in individuals with obesity may act as a reservoir for more extensive viral spread, with increased shedding, immune activation, and cytokine amplification. This paper proposes studies to test this reservoir concept with a focus on specific cytokine pathways that might be amplified in individuals with obesity and COVID-19. Finally, this paper underscores emerging therapeutic strategies that might benefit subsets of patients in which cytokine amplification is excessive and potentially fatal.

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Introduction

Coronavirus disease 2019 (COVID-19) has now infected over 2 million people worldwide, with a death toll of more than 125,000 people. Emerging risk factors for poor outcomes in this disease include age, male sex, and cardiovascular comorbidities, including hypertension, prior cardiovascular disease, diabetes, and, more recently, obesity (1). The Centers for Disease Control and Prevention (Atlanta, Georgia) has now reported a threefold increase in death in New Orleans compared with New York, and speculation has grown as to whether these worrying mortality statistics might in part be attributable to higher levels of morbid obesity (2). In this paper, we develop a theoretical framework that describes why individuals with obesity may be at increased risk of poor outcomes compared with counterparts without obesity (3). We propose a mechanism for adverse consequences of virus seeding to adipose tissue (AT), with potential for prolonged viral shedding and extended cytokine activation in a voluminous and richly vascularized organ that is already perturbed in a metabolic and inflammatory sense in human individuals with obesity (4). We present a rationale for testing this concept in patients with COVID-19 through prospective studies of individuals with and without obesity with accessible AT and plasma to determine whether or not an inflammatory and cytokine signature presages a systemic cytokine storm and clinical decline.

Evidence for Association of Obesity with Worse COVID-19 Outcome

Obesity was not specifically reported in the initial cohort studies of COVID-19 from Wuhan, China (5), but regional epidemiological data from the United States suggests that at least 25% of patients who die of this disease have obesity, which is similar to reported rates of cardiovascular disease in the same high-risk group (21%) (6). More recently, a small retrospective study of 85 individuals with COVID-19 identified obesity as a risk factor for admission to the intensive care unit, with patients requiring increased medical attention (3). Moreover, in the influenza A subtype H1N1 pandemic, obesity was also strongly associated with a worse disease outcome and death (7). Together, these data raise the questions of whether there is a mechanistic link between obesity and disease survival and whether obesity over and above its endocrine or cardiometabolic associations might independently contribute to COVID-19 risk.

Likely Mechanisms Involved in Poor Outcomes in Individuals with Obesity

It is clear that obesity could contribute to both diabetic and cardiovascular COVID-19 risk, and these elements of risk, in addition to thrombosis more recently, have been well described in the scientific literature (8-12). Moreover, obesity is an independent risk factor for hypoventilation syndrome in patients in the intensive care unit (13) and could thus contribute to respiratory failure in patients with acute respiratory distress syndrome (14). Here, we propose additional unheralded pathophysiologic aspects of increased AT burden in morbid obesity that may amplify the pro-inflammatory response to extensive viral infection. AT should be viewed as a highly active organ interfacing immune, endocrine, and metabolic homeostasis throughout the body (15). In individuals with obesity, there is marked

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dysregulation of myeloid and lymphoid responses within AT, with associated dysregulation of cytokine profiles (15). Intrinsically bound to this are endocrine and metabolic derangements, including insulin resistance and adipokine dysregulation with dysfunctional lipid and fatty acid metabolism (16). In highly vascularized AT, endothelial and smooth-muscle cells, as well as resident macrophages, exhibit additional perturbations in response to an activated renin angiotensin system at a local level, with attendant depletion and dysfunction of the counterregulatory angiotensin converting enzyme 2 Mas receptor system (17,18). This makes AT, particularly in visceral distributions, pro-immunogenic, metabolically active, and highly integrated into the cardiovascular system, with the capability to drive acute disease through augmented inflammation at an organ level in the heart, vasculature, pancreas, liver, and kidneys (19). This "preactivation state" of AT in obesity makes this organ a potential target for further immune amplification by external pathogens such as viruses.

Viral Spread to AT and Potential for Activation of Resident Inflammation and Cytokine Pathways

Currently, there is no evidence for direct severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection of AT, although angiotensin converting enzyme 2 receptor expression represents a basis for viral tropism in several cells within this tissue (20), including adipocytes, smooth-muscle cells, and endothelial cells (21). Moreover, many AT-resident cells are proven targets for multiple viruses, including adipocytes [H1N1, type A influenza, and adenovirus 36 (7,22,23)], adipo-stromal cells [adenovirus 36 (24), cytomegalovirus (25)], endothelial cells [SARS-CoV (26)], macrophages [influenza A, SARS-CoV, adenovirus36, human immunodeficiency virus (26,27)], and lymphocytes [SARS-CoV, human immunodeficiency virus (25,26)]. Although SARS-CoV-2 was detected only at low levels in blood in a small human study (28), we cannot exclude hematogenous spread to AT, given the very high virus affinity for its target cell receptor. Alternative routes of SARS-CoV-2 spread to AT include local egress of the virus from organs known to be infected to adjacent visceral fat deposits, such as intrathoracic fat (lungs), epicardial fat (heart), perirenal fat (kidneys), and mesenteric fat (intestines). Finally, shared viral tropism for lung epithelium and AT has already been shown for H5N1 virus infection (29), and AT significantly prolongs the duration of viral shedding in humans with obesity infected with influenza (22). Were similar tropism of SARS-CoV-2 to occur within AT of individuals with COVID-19 and obesity, there exists the potential for prolonged viral shedding in this organ, with extended activation of local "preactivated" immune systems and resident cytokine signaling pathways.

Resident myeloid and lymphoid cells are plentiful in AT, and obesity is associated with macrophage (30) and lymphocyte dysfunction (31). Expansion of distinct memory T lymphocytes within AT can also activate aberrant immune responses with wider tissue damage on viral challenge (31). A recent report from Wuhan suggests that SARS-CoV-2 induces a dysregulated immune response in severely ill individuals with COVID-19 (32), characterized by reduced numbers of circulating memory T lymphocytes, as well as reduced helper/suppressor and regulatory T-cell subtypes. It is tempting to speculate whether already dysfunctional immune responses in individuals with obesity may accentuate this SARS-CoV-2 effect on T-cell function.

Specific inflammatory cytokine programs such tumor necrosis factor (TNF)-α, interleukin (IL)-1, and IL-6 are known to be preactivated in AT in the context of obesity (33), and, thus, viral infection may similarly amplify the already primed organ cytokine response in AT. The cytokine storm identified in multiple respiratory viral infections, including COVID-19, exhibits diverse interferon, IL, chemokine, TNF, and colony-stimulating factor responses, which are beyond the scope of this paper but are comprehensively reviewed elsewhere (34). The intensity of inflammatory lung responses reflect the imbalance between pro-inflammatory cytokines (such as TNF and Il1β) and their soluble cognate receptors that inhibit cytokine effects in aqueous phase (35). IL-10 produced by macrophages and T lymphocytes (T helper 2 and regulatory T cells) acts as a negative regulator of inflammation, whereas IL-6 and its soluble receptor enhance activity of IL-6 on target cells, providing a mechanism for enhancement of TNF and IL-1β activity when these soluble cognate receptors are particularly high (35). Thus, a balance between pro- and anti-inflammatory mechanisms is critical in maintaining lung-tissue homeostasis. It is conceivable that if one or more of these regulatory elements were absent or dysfunctional, then it might contribute to a cytokine storm in the lung or in other tissues such as AT, where aberrant cytokine activation exists. Temporal studies of cytokine dynamics in human "cytokine storm" models show that IL-6 sustains activation of multiple cytokine pathways for many days after the initial immune insult (36). Interestingly, in early COVID-19 studies, IL-6 was a strong independent predictor of mortality (37). Human AT is a major source of IL-6 and its receptor IL-6R (38), and, thus, AT may provide a reservoir for IL-6 activation and cascade signaling in viral infection (Figure 1). Viral spread from affected organs to adjacent AT may take days, with subsequent prolonged viral shedding contributing to the delayed cytokine storm and consequences for tissue injury in patients with COVID-19.

Testing AT Inflammatory Cytokine Reservoir Concept in COVID-19

Initial studies should aim to detect SARS-CoV-2 in AT on autopsy of individuals who have died of COVID-19. Focus should be on analysis of specific cells that have evidence of infection by immunocytopathic and *in situ* viral detection techniques. Parallel studies should identify whether specific cell types in AT can support SARS-CoV-2 infection and replication *ex vivo*. With respect to the cytokine storm, an integrated-systems biology approach would enable multiple pathways to be assessed simultaneously. In this regard, cytokine and chemokine genomic data analysis in blood and AT would be an important first step. Moreover a weighted gene correlation network analysis of SARS-CoV-2–mediated transcriptional response in infected cells could also be used as a model for human AT analysis downstream (39). Interesting aspects of transcriptional network analysis could then be tested in appropriate animal models to determine pivotal components of the cytokine storm, including key cytokine and chemokine genes that are conserved across species (40). These insights may allow rational diagnostics and therapeutic strategies to be developed. In line with this, IL-6 inhibition has already been proposed as a treatment in COVID-19, and the results of trials of tocilizumab are awaited (41). It would be interesting to examine whether individuals with obesity, who are expected to have higher circulating IL-6 levels compared with lean counterparts, respond more favorably to IL-6 inhibition strategies in COVID-19 in a post hoc analysis of

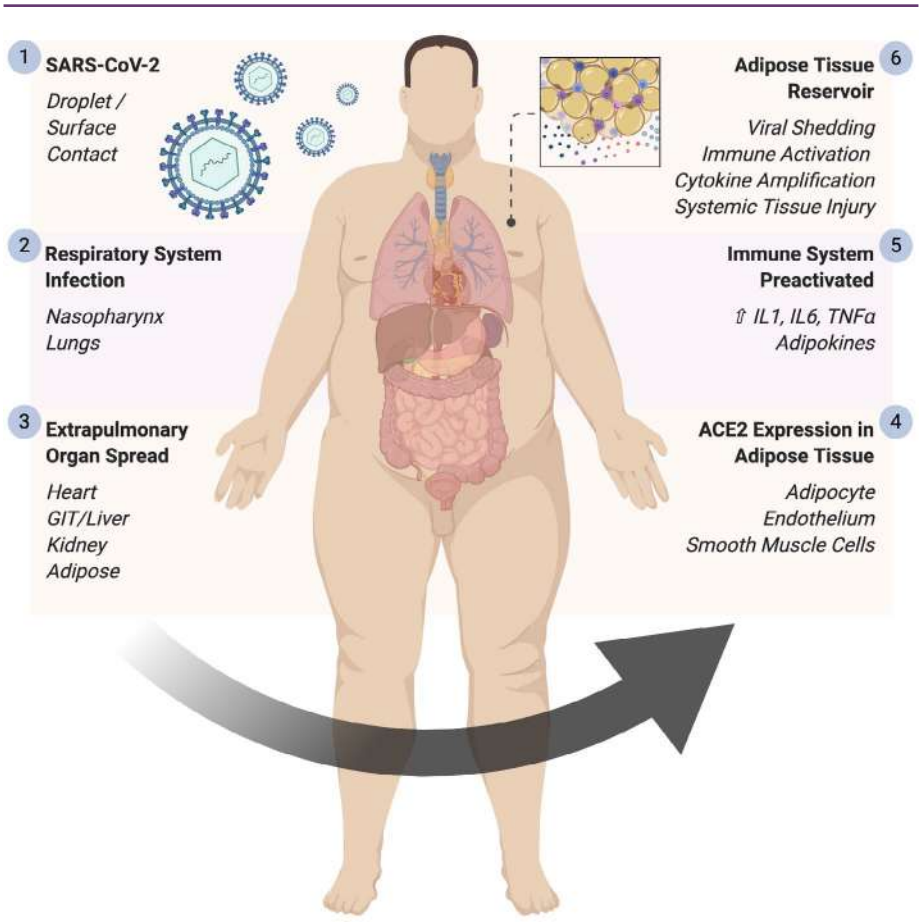

Figure 1 Adipose tissue as a reservoir for SARS-CoV-2 spread, viral shedding, immune activation, and cytokine amplification. Schematic demonstrating the proposed centrality of adipose tissue in the dissemination of SARS-CoV-2 and the ensuing systemic immune activation. Created with Biorender.com. GIT, gastrointestinal tract; IL, interleukin; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TNF, tumor necrosis factor.

this randomized controlled trial. Similarly, tissue and systemic analysis of cytokine dynamics may identify likely responders and nonresponders to such therapy.

In summary, we present a rationale for studying the relationship between obesity and COVID-19 disease severity. We provide a theoretical framework whereby viral systemic spread, entry, and prolonged viral shedding in already "inflamed" AT may augment immune responses with consequences for cytokine cascade amplification. We highlight AT as an abundant source for local and systemic enrichment of cytokines, some already independently associated with increased COVID-19 mortality. Finally, we suggest a series of research studies to identify whether a mechanistic link exists among AT, SARS-CoV-2 infection, organ seeding of infection, immune activation, and the delayed cytokine storm known to presage rapid clinical decline in high-risk patients with COVID-19.O

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Obesity and COVID-19: A Virchow's Triad for the 21st Century

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Thromb Haemost

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a single-strand β-RNA virus which causes coronavirus disease-2019 (COVID-19).[1] The majority of infected subjects remain asymptomatic or experience mild disease but many require hospitalization including intensive care admission and have substantial morbidity and mortality.[2]

Early COVID-19 characterization identified significant numbers of (often younger) patients without significant pre-existing medical disorders that developed a severe clinical phenotype characterized by rapid cardiorespiratory deterioration that was attributed to a "cytokine storm."[3] This hyperinflammatory syndrome with high mortality and apparent stochastic nature has been particularly alarming to clinicians and remains incompletely understood.

Recent reports have also highlighted that many younger subjects hospitalized with COVID-19 are overweight. In a large series of 5,700 patients hospitalized in New York, the rate of mechanical ventilation was 12.2% and the death rate of those ventilated was 88%. The overall rate of obesity in this series was 41%.[4] Obesity is associated with well-recognized deleterious effects on pulmonary mechanics and ventilation including lower lung volumes, lower respiratory muscle strength, and impaired gas exchange.[5] Strategies that have been adopted to overcome these anomalies and improve oxygenation include prone ventilation. In other types of pneumonia, including acute respiratory distress syndrome (ARDS), an "obesity paradox" has been previously observed where the risk of death is decreased in those with higher body mass index.[6] The mechanisms responsible for this paradox are speculative but its existence contrasts with the severity of COVID-19 respiratory failure where precipitous decline in clinical course of a subset of critically ill patients with COVID-19 cannot be explained by deterioration in pulmonary parameters alone.

A minority of COVID-19 patients evolve a rapid inflammatory syndrome with an ARDS-like clinical phenotype (~30%)[7] and multiorgan failure approximately 8 to 9 days after symptom onset.[8] In a second shunt phenotype (>60%) patients show well-preserved lung mechanics but severe hypoxia, high respiratory compliance, and high shunt fraction.[7] While direct lung injury due to SARS-CoV-2 infection plays a central role in the pathophysiology of all COVID-19 patients, it does not explain the differential severity of the disease between lean and overweight subjects, nor the profound mismatch between hypoxia and ventilatory compromise in some patients. Disease severity is significantly mediated by multifaceted host responses and obesity may be one "hidden driver" of the heterogeneous host response and hyperinflammatory COVID-19. We posit that adipose tissue acts as a powerful inflammatory reservoir for SARS-CoV-2 viral replication in overweight subjects with more adipose tissue generating a larger inflammatory response than in lean subjects.[9] This inflammatory disorder in subjects with obesity may drive heterogeneity in vascular injury,[10] in situ thrombosis events, and thromboembolic complications in the systemic[11] and pulmonary vascular system (►**Fig. 1**).[12]

SARS-CoV-2 enters human cells by binding to the angiotensin-converting enzyme 2 (ACE2) on the plasma membrane,[13] which is widely expressed in lung alveolar cells, cardiomyocytes, and vascular endothelium.[14] ACE2 is also expressed in adipocytes, smooth muscle cells, and myofibroblasts, and its expression has been found to be significantly upregulated in obesity.[15] Enhanced ACE2-mediated viral access and replication in local organ adipose tissue very likely leads to significant paracrine/endocrine elaboration of proinflammatory cytokines and adipokines that mediate inflammation in COVID-19.[9] This activation may include inter alia elements of the complement system[16,17] and an unbalanced renin–angiotensin system (RAS), whereby RAS is activated and the counterregulatory ACE2/MAS receptor system is downregulated.[18] Patients with severe COVID-19 show higher plasma levels of interleukin (IL)-2, IL-6, and IL-7, with IL-6 being an independent predictor

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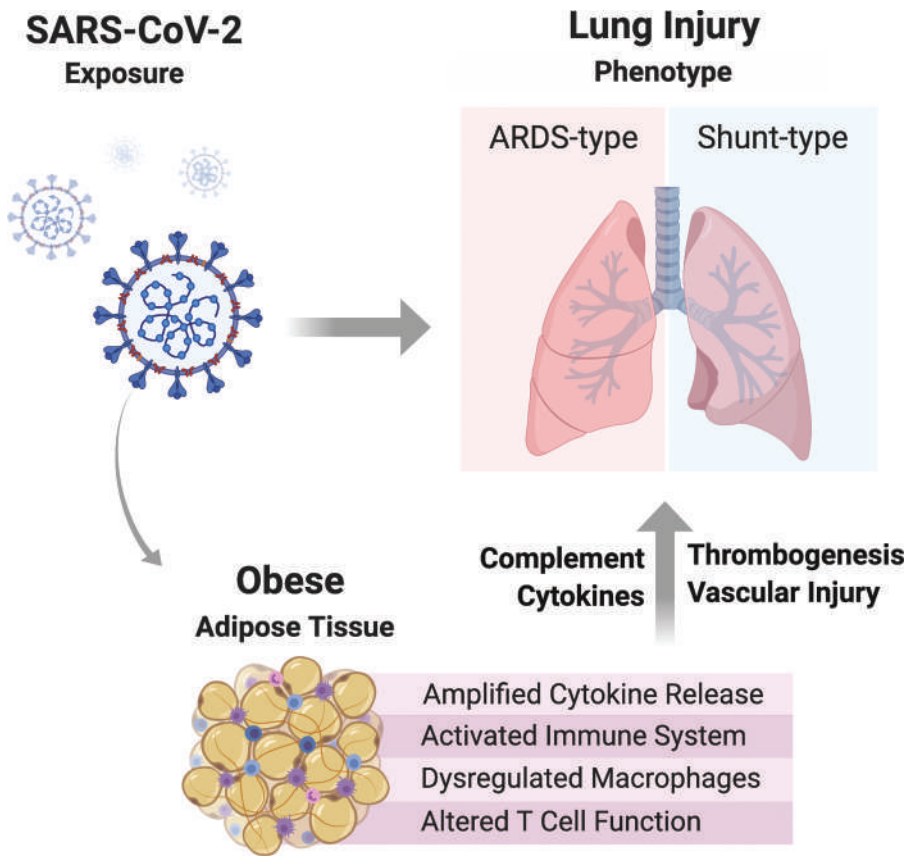

Fig. 1 Schematic of direct and indirect effects of SARS-CoV-2 manifesting two phenotypes of lung injury.[1,2,7,8] Hyperimmune adipose tissue in subjects with obesity may contribute to increased activation of inflammatory cells, cytokines,[3] complement,[17] local and systemic coagulation,[11,12] and vascular injury.[19] ARDS, acute respiratory distress syndrome.

of mortality.[2,3] Inflammatory cytokines released from visceral and perivascular adipose tissue include IL-6, IL-2, granulocyte colony-stimulating factor, interferon-γ, monocyte chemotactic protein-1, and tumor necrosis factor-α, and these same cytokines are primarily responsible for recruitment of monocytes and T-lymphocytes to infected and inflamed organs.[9] Magro et al recently reported COVID-19 pneumonitis without classic ARDS features including complement activation, septal capillary injury, and mural and luminal fibrin deposition, in addition to neutrophil infiltration.[17] Moreover numerous emerging studies indicate COVID-19 association with elements of disseminated intravascular coagulation[17] and regional pulmonary intravascular coagulopathy.[12] Activation of the immune,[19] complement,[16] and coagulation[20] systems already exists in obesity and may contribute to augmented tissue injury seen in organs directly impacted by SARS-CoV-2 tissue damage, such as the lungs (►**Fig. 1**).

Furthermore, SARS-CoV-2-mediated inflammation via these pathways may occur in other "pockets" of adipose tissue in the heart, kidney, liver, and vasculature, explaining some of the more unexpected clinical phenomena seen in younger subjects with COVID-19, such as stroke, acute kidney injury, and apparent myocardial infarction.[21] Cases of ST-elevation myocardial infarction (STEMI) without demonstrable coronary occlusion have been widely reported and remain unexplained.[22] These "STEMIs" have been speculated to represent SARS-CoV-2 myocarditis or acute coronary syndrome secondary to sepsis or hypoxia. It is also conceivable that lung-associated SARS-CoV-2-mediated inflammation of epicardial white adipose tissue may masquerade as a STEMI with the epicardium and adjacent adipose tissue known to share a common microcirculation.

Beyond local effects, systemic release of inflammatory cytokines/adipokines from adipose tissue may promote a Virchow's triad of events including vascular thrombosis, endothelial dysfunction, and blood flow stasis through reactive oxygen species and vasoconstriction (►**Fig. 2**). Such an inflammatory thrombogenic vasculopathy may in part explain the precipitous multiorgan failure and also the variability of clinical course and treatment response in some patients.

The role of obesity and the potentially deleterious impact of adipose tissue in COVID-19 warrant significant worldwide attention. Recognition of enhanced risk in overweight subjects should be at the forefront of clinical decision-making in COVID-19. Stratification based on traditional risk scores (APACHE, SAPS, SOFA, and MPM), lung injury phenotype, and circulating inflammatory markers, such as soluble urokinase plasminogen activator receptor (suPAR), known to be elevated in obesity at baseline and to predict poorer clinical course in COVID-19, may guide earlier intervention in these groups.[10] In parallel we need to gain a deeper understanding of the biology of inflammation and thrombotic vasculopathy in these patients, in particular how adipose tissue plays a role in this aspect of disease amplification. While awaiting successful vaccines, preemptive strategies to lessen the severity of the

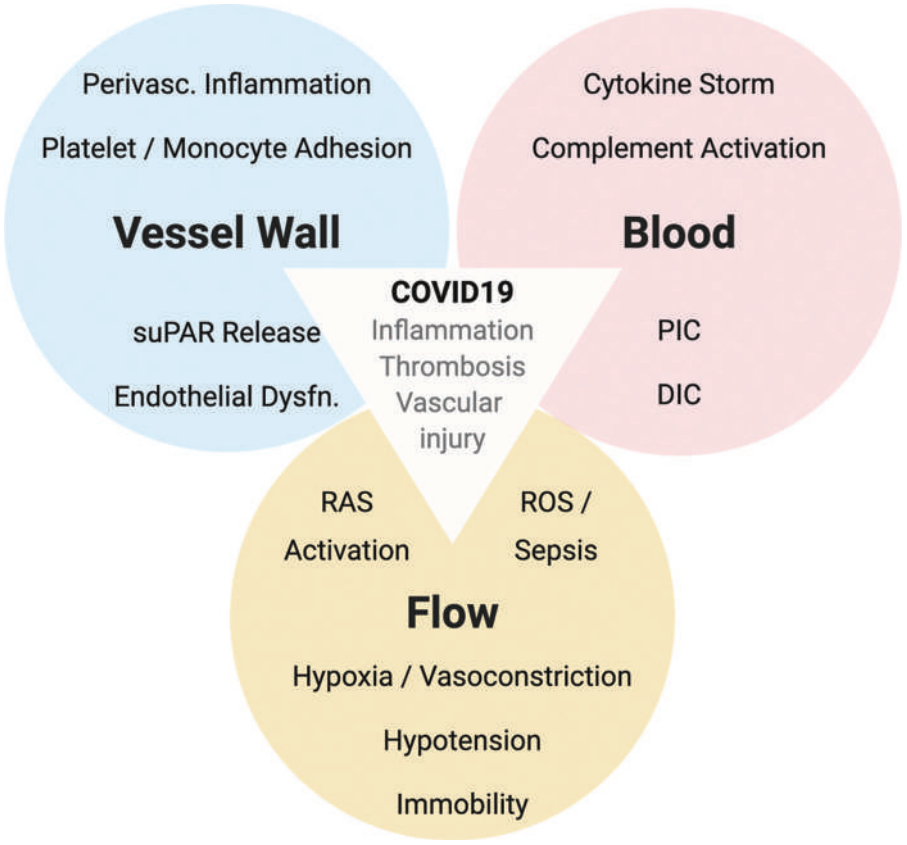

Fig. 2 Virchow's triad revisited. Potential for multiorgan involvement in COVID-19 with heterogeneous presentation depending on predominance of sepsis, inflammation,[3] coagulation,[11,12,17] hypoperfusion,[9,10] or vasculopathy.[10,17,21] DIC, disseminated intravascular coagulation; PIC, pulmonary intravascular coagulation; RAS, renin–angiotensin system; ROS, reactive oxygen species; suPAR, soluble urokinase plasminogen activator receptor.

inflammatory, thrombogenic, and vasculopathic phenotypes in COVID-19 will prove important. These may involve monoclonal antibodies against pivotal cytokines in the inflammatory cascade.[23] In addition, prospective targeting of downstream elements of complement activation (monoclonal antibodies) and thrombophilia (thrombin inhibitors) in obese subjects from a preventive and therapeutic aspect may be useful with clinical trial results of such approaches eagerly awaited.

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Conflict of Interest
None declared.

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Estradiol, progesterone, immunomodulation and COVID-19 outcomes

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Abstract

Severe outcomes and death from the novel coronavirus disease 2019 (COVID-19) appear to be characterized by an exaggerated immune response with hypercytokinemia leading to inflammatory infiltration of the lungs and acute respiratory distress syndrome. Risk of severe COVID-19 outcomes is consistently lower in women than men worldwide, suggesting that female biological sex is instrumental in protection. This mini-review discusses the immunomodulatory and anti-inflammatory actions of high physiological concentrations of the steroids, 17β-estradiol (E2) and progesterone (P4). We review how E2 and P4 favor a state of decreased innate immune inflammatory response while enhancing immune tolerance and antibody production. We discuss how the combination of E2 and P4 may improve the immune dysregulation that leads to the COVID-19 cytokine storm. It is intended to stimulate novel consideration of the biological forces that are protective in women compared to men, and to therapeutically harness these factors to mitigate COVID-19 morbidity and mortality.

SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) is causing the novel coronavirus disease 2019 (COVID-19) pandemic that has infected over 5 million people and killed more than 350,000 worldwide. The systematic investigation of clinically approved drugs is a priority in order to improve disease outcomes and invest resources to go to full-scale production. The search for an effective therapy is ongoing actively but is currently limited in success. Perhaps we should look outside the box and consider what is hidden in plain sight such as *the biological reasons why women are relatively protected from COVID-19 compared to men.* This review highlight experimental evidence that the steroid hormones, 17β-estradiol and progesterone, at high physiological concentrations, are powerful immunomodulators and argues that acute steroid therapy with the combination of E2 and P4 may represent a safe and viable therapeutic option that needs to be tested in clinical trials to mitigate severe COVID-19 outcomes.

COVID-19 mortality is lower in women compared to men

Since the beginning of the 21st century, two previous deadly zoonotic βcoronavirus outbreaks have crossed the species barriers to infect humans and exhibited the same apparent female protection from severe outcomes. The first SARS-CoV outbreak emerged in 2002 in Guangdong province, China and among 1755 hospitalized patients in Hong Kong the case fatality rates (CFR) was 13% in women compared to 22% in men (1). During the ongoing Middle East respiratory syndrome coronavirus (MERS-CoV) outbreak that began in 2012 in Saudi Arabia, among 425 reported cases, disease occurrence was lower among women (38% of cases) and the CFR was 23% for women compared to 52% for men (2). Today in China, Europe and the United States (U.S.), COVID-19 severity and mortality is consistently lower in women than in men (3-8).

Taking the most representative series to date, in the cohort of 1,099 COVID-19 hospitalized patients in Wuhan, China, only 42% of subjects were women (4). Among severe case (i.e., admitted to an intensive care unit (ICU), requiring mechanical ventilation, or fatal), women accounted for 32% of patients (4). Similarly, women represented only 18% of all COVID-19 admissions in intensive care unit (ICU) in the Lombardy region of Italy (9). In New York City, among 5700 hospitalized patients, women represented 33% of cases and 39% of deaths (7). The International Severe Acute Respiratory and Emerging Infections Consortium (ISARIC) in a prospective observational cohort study of over 17,000 patients in the United Kingdom reported that among hospitalized patients, women accounted for only 40%, with a 20% lower mortality than in men (10). Although advancing age is associated with greater risk of mortality in both sexes, the female protection remains evident (11). An analysis of COVID-19 data from Italy, Spain, Germany, Switzerland, Belgium, and Norway reveals that among all age groups over the age of 20, fatality rates are greater for males than females (12). In contrast, male-female differences in the rate of confirmed SARS-CoV-2 infections are age-dependent in all countries, being greater among females 10-50 years old and greater among males before the age of 10 and after the age of 50 (12). We interpret these data to suggest that biological sex differences contribute to female-biased protection against death, but gender-associated risk of exposure may affect rates of infection differently for males and females at differential ages. A question then arises as to what biological factors are protective in women compared to men, and how can we harness these modifiable factors to mitigate COVID-19 morbidity and mortality?

Role of the pro-inflammatory cytokine storm in COVID-19 outcomes

Severe COVID-19 outcomes are associated with delayed and exaggerated innate immune responses, including hypercytokinemia and inflammatory cell infiltration in the lungs. Our current understanding of the disease, which is rapidly evolving as we write this review, is that patients with COVID-19 do not die from damage caused by virus replication, they die from the consequences of a so called "cytokine storm" (13-16). In an attempt to protect the body from SARS-CoV2, immune cells infiltrate the lungs, cause hyperactivation of monocytes and macrophages, and elevated production of proinflammatory cytokines [e.g., interleukin-6 (IL-6), interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α)] and chemokines [e.g., monocyte chemoattractant protein-1 (MCP-1/CCL2)] (15). The cytokine storm is also associated with lymphopenia, and a study in 21 patients from Wuhan reported a decrease in CD4+ and CD8+ T cells, as well as suppressed interferon-γ (IFN-γ) production by CD4+ T cells, which was associated with COVID-19 severity (15). The local outpour of chemokines and cytokines attracts more inflammatory cells, such as neutrophils and monocytes, into lung tissue resulting lung injury. Ironically, the cytokine storm is a result of the immune system responding to infection in an effort to protect the host, but results in acute respiratory distress syndrome (ARDS) and multi-organ failure (13,14). Increased production and elevated local and systemic IL-6 is hypothesized to be central to the development of the cytokine storm (17,18). Accordingly, therapeutic strategies targeting the inflammatory response such as IL-6 blockade (19) or the transplantation of mesenchymal stem cells to restore immune tolerance (20) are showing promising preliminary results in mitigating the cytokine storm. Here we discuss a paradigm in which therapy with the steroid hormones 17β-estradiol and progesterone could mitigate this virally induced innate immune inflammatory response.

Females generally exhibit greater immune responses to viruses

Females generally develop heightened immune responses compared to males. In 1967, Butterworth et al. reported that women produce higher levels of circulating immunoglobulins, IgG and IgM than men (21) which was subsequently confirmed by multiple studies. Accordingly, following vaccination against influenza, yellow fever, rubella, measles, mumps, hepatitis, herpes simplex 2, rabies, smallpox, and dengue viruses, protective antibody responses are twice as high in women than in men (22). Women also have higher frequencies of CD4+ T helper cells than men (23). The biological reasons why females develop a more robust immune response against pathogens, including viruses, than males likely explain the observed female protection from COVID-19 fatal outcomes. First, females enjoy the genetic benefit of two X chromosomes and being a mosaic of X-linked genes (i.e., randomly expressing alleles inherited from their mother or father), including over 60 immune-response genes (24). By contrast, males have only one X chromosome inherited from their mother. Several studies show that genetic diseases associated with deleterious X-linked alleles are more frequently observed in males (25). Generally, there should be no dosage effect associated with position of two X chromosomes in females. Incomplete inactivation of immunoregulatory genes on the X chromosome in females, however, can cause a gene dosage imbalance between sexes (26,27), which is implicated in female-biased autoimmune diseases (28) and vaccine efficacy (29). The Y chromosome also has immunoregulatory functions (30) that are linked to influenza outcomes, at least in mice (31).

Sex steroids are potent immune-modulators and the different concentrations of estrogens, progesterone and androgens between women and men, in addition to genetics described above, are likely to influence COVID-19 immune responses and inflammatory outcomes. This is especially important because acute and severe illnesses, such as COVID-19, may alter the function of the hypothalamo-pituitary gonadal axis and decrease the endogenous production of estrogens and progesterone. Hormones are also amenable to therapeutic intervention. Below, we discuss

immunomodulation provided by high physiological serum concentrations of estrogens and progesterone as it relates to SARS-CoV-2 infection. This background knowledge is paramount to appreciate the potential benefits that E2 and P4 treatment could provide in the context of SARS-CoV2-mediated hyper-inflammation and ARDS.

Estrogens, progesterone and immune function

Estrogen receptors (ERs) are expressed in all immune cells serving as transcriptional regulators of cellular function. In human peripheral blood mononuclear cells (PBMCs), CD4+ T lymphocytes express higher levels of ERα mRNA than ERβ, whereas B cells express higher levels of ERβ than ERα mRNA (32). Peripheral blood CD8+ T cells and monocytes express low but comparable levels of both ERs (32). Therapy with E2, leading to serum concentrations equivalent to ovulation or pregnancy, possess beneficial immuno-modulatory and anti-inflammatory actions in mice and humans [Reviewed in (24,33)]. In most experimental human or rodent models, the anti-inflammatory actions of E2 on innate immunity includes the suppression of the production of pro-inflammatory cytokines, e.g., IL-6, IL-1β and TNF-α, by monocytes and macrophages (a major factor in COVID-19 cytokine storm) and a strong inhibition of CCL2, thus preventing innate immune cells migration into inflamed areas, particularly neutrophils and monocytes. E2 stimulates CD4+ T helper cells production of anti-inflammatory cytokines, e.g., IL-4, IL-10, and IFN-γ. Generally, high E2 concentrations favor Th2-type anti-inflammatory responses. E2 decreases IL-17 production by pro-inflammatory T_H17 helper cells. E2 enhances the expansion of regulatory T cells (Treg) thus promoting immune tolerance. E2 also stimulates antibody production by B cells (Figure 1).

There is strong evidence in metabolic bone disease and virus-induced liver disease that estrogens inhibit disease pathogenesis through suppression of IL-6 production. For example, estrogens inhibit osteoclast development and resorptive function in bone by inhibiting IL-6 gene transcription and production (34).

Additionally, the incidence of chronic hepatitis B-induced hepatocellular carcinoma (HCC) in humans shows a strong male predominance. IL-6 is believed to be a key component in inflammation-associated tumorigenesis of HCC (35). In a retrospective study of postmenopausal women with chronic hepatitis C, progression to liver fibrosis was decreased in women who took menopausal estrogen therapy, compared to women who did not (36). In a rat model of chemically-induced HCC, males produced more IL-6 from liver Kupffer cells and were more prone to HCC than females (37). Estrogens protected males from HCC via inhibition of IL-6 production by Kupffer cells.

In a mouse model of acute lung inflammation by instillation of bacterial lipopolysaccharide, males and ovariectomized females exhibited increased lung infiltration of polymorphonuclear cells with elevated production of IL-6, IL-1β and intercellular adhesion molecule-1 (ICAM-1), which was reduced by E2 treatment of males and ovariectomized females (38). In preclinical models of Influenza infection, estrogens exhibit powerful immune-modulatory actions leading to a more appropriate innate immune response in the lungs which is associated with decrease pro-inflammatory cytokines and chemokine responses before the clinical disease develops (39-41). In primary human nasal epithelial cell cultures, estrogenic compounds, including E2, signaling through ERβ significantly reduce influenza virus replication (42). Further, SARS-CoV2 and SARS-CoV both produce deadly pneumonias with the same apparent female protection. In a mouse model of SARS-CoV infection, female mice developed lower virus titers, lower infiltration with inflammatory monocyte, macrophages and neutrophils producing fewer inflammatory cytokines (IL-6, IL-1β and TNF-α) and chemokines (CCL2) resulting in milder pulmonary damage and a lower female mortality (20%) compared to males (80%) (43), a sex distribution similar to that observed in patients with SARS. Importantly, the endogenous production of E2 in female mice was instrumental in this protection. Castration of males had no effect on the disease, while surgical removal of the ovaries or treatment with the ER antagonist fulvestrant in female mice infected with SARS-CoV resulted in the same pulmonary damages and mortality rate as in males. Further, treatment of

ovariectomized mice with the selective estrogen receptor modulator (SERM), tamoxifen —a mixed estrogen receptor (ER) agonist and antagonist prescribed for the treatment of breast cancer— restored the female protection. This study indicates that in a murine model of SARS-CoV infection, ovarian hormones and especially estrogens protected females from lethal pneumonia, and the FDA-approved SERM, tamoxifen, mimicked the female-biased protection. A screening of multiple FDA-approved compounds for anti-coronavirus activity identified tamoxifen and toremifene (another SERM) among the top ten most effective and safe drugs at inhibiting MERS-CoV and SARS-CoV infections *in vitro* (44). Toremifene also inhibits Ebola virus infection *in vitro* and *in vivo* in mice (45). The mechanism of toremifene action seems related to the multiple cationic amphiphile structure of the molecule that impairs late step of virus entry or fusion. Taken together, these findings suggest that E2 and related SERMs have two potential protective mechanisms of action against SARS-CoV mediated pneumonias in mice: 1) an estrogen-dependent decrease in the deadly innate immune response and cytokine storm in the lungs, thus preventing respiratory failure, and 2) specific to SERMs, an off-target direct inhibition of SARS-CoV replication and cytopathic effects.

Progesterone (P4) is another important immuno-modulatory and anti-inflammatory hormone that is produced at high levels by the placenta during pregnancy. Progesterone receptors (PRs) are expressed in most immune cells including epithelial cells, macrophages, DCs, lymphocytes, mast cells and eosinophils (24). However, P4 can also signal via glucocorticoid and mineralocorticoid receptors. P4 inhibits proinflammatory cytokines IL-1β and IL-12 production by human and rodent macrophages and dendritic cells. Progesterone favors the skewing of CD4+ T helper cell responses from T_H1-type towards T_H2-type and the production of anti-inflammatory IL-4 and IL-10 cytokines (24,46,47). Treatment of cord blood cells with P4 increases percentage of FOXP3+ Treg cells (thus promoting immune tolerance), while decreasing the frequencies of pro-inflammatory T_H17 cells (Figure 1). Administration of progesterone at concentrations mimicking the luteal phase to progesterone-depleted adult female mice conferred protection from lethal influenza A virus (IAV)

pneumonia (48). In these mice, progesterone treatment decreased the inflammatory environment of the lungs, improved pulmonary function, and promoted cell proliferation and pulmonary repair, which resulted in an earlier recovery, without effects on viral load. Interestingly, in this case, progesterone treatment promoted faster recovery by increasing TGF-β, IL-6, IL-22, and the numbers of regulatory Th17 cells expressing CD39. Importantly, progesterone promoted pulmonary tissue repair by upregulating the epidermal growth factor amphiregulin in the lungs (48). Although IAV infection is different and produces a different immune reaction than that induced by SARS-CoV-2 (for example the beneficial effect of IL-6), this study provides important insight into the immunomodulatory and healing effects of progesterone. Further, progesterone also seem to exhibit antiviral activity in VeroE6 cells infected with SARS-CoV2 (49).

Pregnancy and COVID-19

During pregnancy, the innate and adaptive immune responses shift from an inflammatory to an anti-inflammatory phenotype to avoid fetal rejection and favor passive transfer of maternal antibodies to the fetus [reviewed in (50)]. These effects, which are relevant to COVID-19 protection are largely mediated by E2 and P4. During pregnancy, increased levels of E2 suppress many cytotoxic and innate immune inflammatory responses but stimulate antibody production by B cells (33,51). In fact, one of the most important immunological features of pregnancy is the increase in B cell responses with enhanced antibody production due to dual stimulation by estrogens and P4, the production of which is maximal in the third trimester (33,51). Progesterone also stimulates the synthesis of progesterone-induced binding factor (PIBF) by lymphocytes which promotes the differentiation of CD4+ T cells into helper T cell type 2 (Th2) cells secreting anti-inflammatory cytokines, including IL-4, IL-5, and IL-10 (52). This explains why during pregnancy B cell/antibody-driven diseases, like systemic lupus erythematous, exacerbate;

whereas T cell-driven diseases with cytotoxic and innate immune responses, like rheumatoid arthritis or multiple sclerosis, improve (53,54).

Pregnant women are not protected from SARS-CoV2 infection but seem to be relatively protected from the severe outcomes of SARS-CoV2. Currently, studies evaluating COVID-19 outcomes during pregnancy have not yet separated outcomes occurring during pregnancy (i.e., when E2 and P4 concentrations are high) from those in the immediate post-partum period (i.e., when E2 and P4 concentrations are undetectable). In a Chinese retrospective series of 82 women (28 pregnant women, 54 reproductive-aged non-pregnant women) hospitalized in Wuhan with confirmed COVID-19, pregnant women exhibited comparable severity of disease, virus clearance time, and length of hospital stay compared with reproductive-aged non-pregnant women (55). The authors concluded that pregnant women infected with SARS-CoV-2 have comparable clinical course and outcomes compared with control women. However, in this study, the non-pregnant women received more anti-viral, corticosteroid and immunoglobulin therapies than pregnant women and therefore the groups were not comparable in terms of treatments and related outcomes. A larger retrospective review of 118 pregnant women admitted for COVID-19 pneumonia in China reported only 9 cases (8%) of severe pneumonia with hypoxemia. Notably, in 6 of these women, including the one requiring mechanical ventilation, the exacerbation of pneumonia occurred during the postpartum period, after serum concentrations of E2 and P4 had already dropped (56). Therefore, the actual number of severe cases in this study was 3 (2.5% of the pregnant population), which is less than the severity of COVID-19 in Chinese women in a similar age range (around 6%) (2). In fact in the only published series of 9 pregnant women with fatal COVID-19, a detailed analysis of the cases reveals that 7 of these women deteriorated and died in the hours or days following delivery (57). Therefore, larger studies addressing COVID-19 mortality during pregnancy compared to early post-partum as a primary endpoint are needed to determine if the hormonal environment of the third trimester is protective.

Immunomodulation by hormone-based therapies in women

Treatment of postmenopausal women with menopausal hormone therapy (MHT) and use of oral contraceptives by women in their reproductive ages are accompanied by concomitant physiological changes associated with increased concentrations of estrogens and progestins. Thus, effects of these two hormones cannot be separated. Most studies assessing the effect of MHT using E2 alone or in combination with progestins showed that MHT inhibits the production of pro-inflammatory cytokines (e.g., TNF-α, IL-1β and IL-6) by PBMC *ex vivo* or *in vivo* in the serum of MHT-treated women (58-61). In addition, transdermal E2 blunted the pro-inflammatory cytokine responses to an inflammatory challenge (60). The anti-inflammatory effect of E2 therapy in menopausal women with regard to low grade systemic inflammation seemed to be observed mostly following transdermal rather than oral E2 administration and was not reproduced by conjugated equine estrogens. Bazedoxifene belong to a new generation of SERMs used in combination with estrogens in oral menopausal hormone therapy. In obese female mice, treatment with bazedoxifene decreases IL-6 and multiple markers of systemic inflammation (62). However, this effect was not observed in a pilot randomized trial of 8-week treatment with oral estrogens and bazedoxifene in obese postmenopausal women (63). Likely, the absence of beneficial effect of orally-administered estrogens on systemic inflammation is related to the first-pass liver metabolism following oral estrogens administration, which increase C-reactive protein (CRP) production and markers of inflammation (64).

Transdermal and oral estrogen therapy with or without progestin increases CD19+ B-cell numbers and activity (65). Accordingly, the stimulating effects of menopausal therapy with estrogens and progestins on B cells promotes the progression of SLE in postmenopausal women (33).

Classical ERα, ERβ and PRs are present both in extra-nuclear and nuclear pools in most cells (66). To what extent each receptor cellular pool has collaborative or unique effects on immune function has not

been determined and will be of interest for the design of future studies assessing the effect of sex steroid receptor ligands in modulating immune functions.

Repurposing estrogens and progesterone to mitigate COVID-19 mortality?

High physiological concentrations of E2 and P4 possibly synergize to mitigate innate immune cells production of proinflammatory cytokines, promote T cells anti-inflammatory responses and immune tolerance and stimulate antibody production by B cells (Figure 1). In individuals with confirmed COVID-19, acute hormone therapy with E2 and P4 could mitigate the cytokine storm while increasing antibody production. Pandemics such as SARS-CoV-2 give little time for drug development. Repurposing existing and approved drugs that have already been tested in humans—and for which detailed information is available on their pharmacology, formulation, dose, and potential toxicity—provides an expedited and safe approach for off-label use of potentially life-saving therapeutics. As discussed above acute E2 and P4 treatment would be expected to blunt innate immune inflammatory responses and at the same time stimulate B cell responses and antibody production (33,51) without noticeable side effects. A critical advantage of estrogen, SERMs and progestin compounds is the depth of knowledge regarding their clinical efficacy and toxicity that has accumulated from decades of clinical and basic studies. Hormone therapy is used by millions of women for contraception, and prevention of menopausal symptoms. It is widely available in hospitals, inexpensive, manufacturable to scale, and can be prescribed immediately. As this review is being written, two clinical trials are testing E2 (ClinicalTrials.gov identifier NCT04359329) or P4 (ClinicalTrials.gov identifier NCT04365127) individually in COVID-19 patients. It is worth considering the potential benefit of hormone therapy alone or in combination therapy with anti-viral drugs or IL-6 blockade as an immune modulation in single-center off-label clinical trials. In an outbreak like this, and while we are waiting for a safe and efficient vaccine to be developed, the systematic investigation of clinically approved drugs is a priority to determine which compounds may mitigate the disease and invest resources to begin full-scale production.

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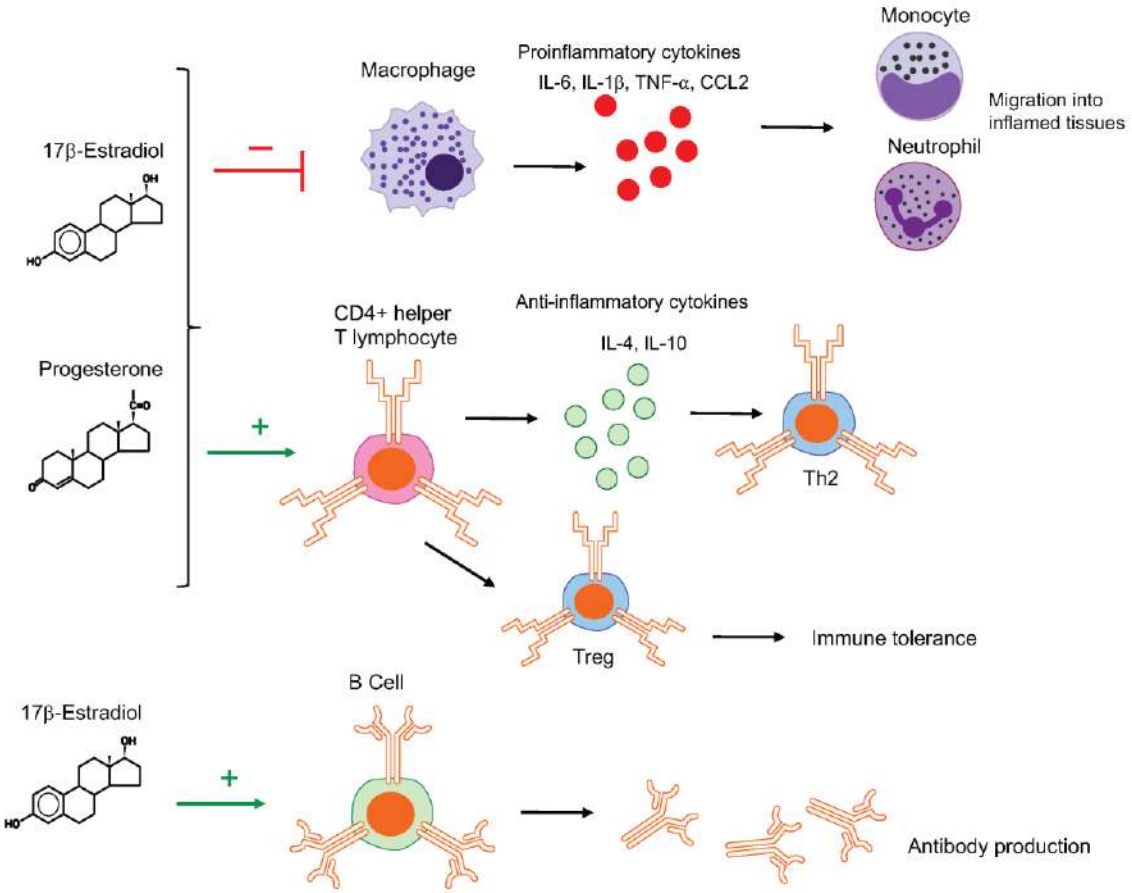
Figure legend

Figure 1. Anti-inflammatory and immunomodulatory actions of estradiol and progesterone. High physiological concentrations of E2 suppress the production of pro-inflammatory cytokines, e.g., IL-6, IL-1β and TNF-α, and chemokine CCL2 by macrophages, thus preventing neutrophils and monocytes migration into inflamed areas. P4 also inhibits pro-inflammatory cytokines IL-1β and IL-12 production by macrophages and dendritic cells. High concentrations of E2 or P4 stimulate CD4+ T helper cells production of anti-inflammatory cytokines, e.g., IL-4, IL-10 and favor Th2-type anti-inflammatory responses. E2 and P4 also enhance the expansion of regulatory T cells (Treg), thus promoting immune tolerance. Finally, E2 stimulates antibody production by B cells.

Figure 1



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Research Article

Unequal Impact of Structural Health Determinants and Comorbidity on COVID-19 Severity and Lethality in Older Mexican Adults: Considerations Beyond Chronological Aging

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Abstract

Background: COVID-19 has had a disproportionate impact on older adults. Mexico's population is younger, yet COVID-19's impact on older adults is comparable to countries with older population structures. Here, we aim to identify health and structural determinants that increase susceptibility to COVID-19 in older Mexican adults beyond chronological aging.

Methods: We analyzed confirmed COVID-19 cases in older adults using data from the General Directorate of Epidemiology of Mexican Ministry of Health. We modeled risk factors for increased COVID-19 severity and mortality, using mixed models to incorporate multilevel data concerning healthcare access and marginalization. We also evaluated structural factors and comorbidity profiles compared to chronological age for COVID-19 mortality risk prediction.

Results: We analyzed 20 804 confirmed SARS-CoV-2 cases in adults aged 60 and older. Male sex, smoking, diabetes, and obesity were associated with pneumonia, hospitalization, and intensive care unit (ICU) admission in older adults, CKD and COPD were associated with hospitalization. High social lag indexes and access to private care were predictors of COVID-19 severity and mortality. Age was not a predictor of COVID-19 severity in individuals without comorbidities and combination of structural factors and comorbidities were better predictors of COVID-19 lethality and severity compared to chronological age alone. COVID-19 baseline lethality hazards were heterogeneously distributed across Mexican municipalities, particularly when comparing urban and rural areas.

Conclusions: Structural factors and comorbidity explain excess risk for COVID-19 severity and mortality over chronological age in older Mexican adults. Clinical decision-making related to COVID-19 should focus away from chronological aging onto more a comprehensive geriatric care approach.

Keywords: COVID-19, Human Aging, Inequality, Mortality, Mexico, SARS-CoV-2

The novel SARS-CoV-2 has disproportionately affected older adults. Notably, the impact of the COVID-19 pandemic has had larger repercussions in countries with older population structures; data from large outbreaks in China, Italy, and most recently, the United States has shown a remarkable impact of SARS-CoV-2 infections on older patients, with increased disease severity, adverse outcomes and increased mortality (1–3). This susceptibility has been suggested to be attributable to deleterious features of aging, particularly in those with cardio-metabolic and respiratory comorbidities. Tissue-specific expression of the *ACE2* gene, which encodes the SARS-CoV-2 receptor, has been linked to specific immune signatures in males and older adults and its expression have been shown to be age-dependent (4,5); moreover, immunosenescence and increased inflammatory responses related to the aging process might lead to increased infection risk and dysregulated immune response to SARS-CoV-2 in older adults (6). Given these mechanisms which may increase susceptibility in older individuals, the role of comorbidities and social inequities, which condition an increased burden on functionality and dependence, has often been overlooked over chronological aging as significant predictors of COVID-19 severity and lethality. In Mexico, COVID-19 has shown similar patterns on its impact on older adults, despite an apparent demographic dividend by having, on average, a younger population (7). Mexico's younger population structure compared to European countries is nonetheless affected by increased rates of cardio-metabolic diseases, particularly Type 2 diabetes, obesity and the metabolic syndrome, with most individuals reaching older age with a significant number of comorbidities (8,9). Recent shifts on aging structures within Mexico have resulted in an increased number of older adults who continue working into old age, most of whom live in poverty, do not have adequate healthcare access, and are dependent on the working-age population (7). Poverty and low socioeconomic status are the result of structural inequality. According to the WHO report on social determinants of health, the circumstances of daily life are determined by structural drivers such as the nature and degree of stratification in society; biases, norms, and values within society; global and national economic and social policy; as well as processes of governance at the global, national, and local level. The combination of unequal structural factors, an aging population with increased prevalence of chronic diseases, and the co-existence of infectious diseases position Mexican older adults as a particularly susceptible population during the COVID-19 pandemic (10). Given that most studies have linked an increased risk of COVID-19 with chronological age, many resource allocation decisions have been made purely on this basis, which represents an issue for geriatric care in the patient population facing the highest disease burden (11). To address these gaps, we investigated health and structural determinants which could contribute to increased COVID-19 severity and lethality beyond chronological age in older Mexican adults to better characterize the impact of COVID-19 in older populations.

Methods

Data Sources

We analyzed data collected by the General Directorate of Epidemiology of the Mexican Ministry of Health, which is an open-source data set comprising daily updated suspected COVID-19 cases that have been tested using real-time RT-PCR to confirm SARS-CoV-2 according to the Berlin Protocol (12), and were certified by the National Institute for Diagnosis and Epidemiological Referral (13). Data to estimate population rates at different ages and population density was extracted from 2020 population projections obtained by the Mexican National Population Council (CONAPO). The 2015 social lag index (SLI), which is a composite of several factors which are measured to estimate social disadvantage and structural inequality at the municipal level based on population census data, was obtained from the Mexican National Evaluation Council (CONEVAL); SLI is a principal component score which comprises percentages of literacy, access to basic education, healthcare services, living conditions including drainage, dirt floor, access to water, electricity and electrical appliances for each Mexican municipality (14). Data on available hospital beds at the end of 2019 were extracted from the Mexican Ministry of Health and were standardized to projected 2020 population figures from CONAPO (15). For this work we considered all SARS-CoV-2 PCR-positive cases up to June 3, 2020 in individuals aged 60 and older.

Definitions of COVID-19 Cases, Predictors, and Outcomes

Suspected COVID-19 cases were defined as an individual of any age whom in the last 7 d has presented two or more of the following: cough, fever, or headache, accompanied by either dyspnea, arthralgias, myalgias, sore throat, rhinorrhea, conjunctivitis, or chest pain. Among suspected cases, the Ministry of Health establishes two protocols for case confirmation: (i) SARS-CoV-2 testing is done widespread for suspected COVID-19 cases with severe acute respiratory infection and signs of breathing difficulty or deaths with suspected COVID-19, (ii) for all other cases, a sentinel surveillance model is being utilized, whereby 475 health facilities which comprise a nationally representative sample evaluate ~10% of mild outpatient cases to provide estimates of confirmed mild cases (16). Demographic and health data are collected and uploaded to the epidemiologic surveillance database by personnel from each corresponding healthcare facility. Available variables include age, sex, nationality, state, and municipality where the case was detected, immigration status as well as identification of individuals who speak indigenous languages from Mexico. Health information includes the status of diabetes, obesity, chronic obstructive pulmonary disease (COPD), immunosuppression, pregnancy, arterial hypertension, cardiovascular disease, chronic kidney disease (CKD), and asthma. Date of symptom onset, hospital admission, and death are available for all cases as are outpatient or hospitalized status, information regarding the diagnosis of pneumonia, ICU admission, and whether the patient required invasive mechanical ventilation.

Statistical Analysis

Population-based statistics

We estimated age-specific incidence and mortality rates by standardizing cases per 1-y increment to the corresponding projected population sizes and normalized it to reflect a rate per 100 000 inhabitants. Incidence and mortality rates were plotted against age and smoothed splines were fitted to model trends according to age profiles. Categorical variables were compared using the chi-squared statistic and stratified according to age.

COVID-19 outcome models

Given the large structural inequalities in healthcare access across Mexico, we fitted mixed-effects models which considered municipality of case occurrence as a random effect to control for potential inequalities in healthcare access, aging structures, and unequal

regional evolution of the epidemic. These models would also allow to include multilevel data, to increase the precision of outcome estimates considering structural factors for each municipality. Models were fitted using mixed-effects logistic regression using the municipality of case occurrence as a random effect; *p* values and confidence intervals were estimated using the Laplace approximation within the *lme4* and *lmerTest* R packages.

Risk of COVID-19 lethality in older adults

To model COVID-19 mortality risk in older adults we fitted Cox proportional hazard regression models stratified by sex, including frailty penalties to accommodate multilevel data and random effects for the municipality of case occurrence, approximating iterations using the Newton–Raphson algorithm. Random effect estimates were exponentiated to calculate baseline mortality hazards across municipalities to represent geographical heterogeneity. Models were selected based on the Bayesian Information Criterion (BIC) and performance was assessed using Harrel's c-statistic. To visualize increases in lethality risk for continuous variables and quantify uncertainty for estimations, we performed postestimation simulations using bootstrapping with the *simPH* package. Proportional hazard assumptions were verified using Schoenfeld residuals.

Impact of age, structural determinants, and comorbidities on mortality risk

To investigate the role of age in predicting mortality compared to structural factors and comorbidities, we fitted sequential Cox proportional hazard models compared to only COVID-19 pneumonia as a predictor and introduced either age, structural factors (ie, SLI, private care), or increasing number of comorbidities as blocks. We compared models using changes in the BIC; the better models were those which either minimized or had large changes in BIC. We also separately analyzed outcomes and mortality for older adults without comorbidities to examine the role of structural factors compared to age in relation to disease severity and COVID-19 lethality. A p value of $< .05$ was considered as the statistical significance threshold. All analyses were performed using R version 4.0.0.

Results

COVID-19 in Mexican Adults Aged 60 and Older

As of June 3, 2020, we observed 20 804 confirmed SARS-CoV-2 cases in adults aged 60 and older, representing 20.5% of all confirmed COVID-19 cases (n = 101 238, Table 1). Overall, the rate of positivity in older adults has been 43.8% which represents a ratio of 1.39:1 in positivity among all evaluated cases. Despite the higher positivity rate, mortality rates are disproportionally higher in this population segment. Among all 11 728 confirmed lethal cases, 6136 have been recorded in older adults, indicating significantly higher lethality (6.95% vs. 29.49%). When standardizing incidence rates to population estimates per each 1-y increase, the highest incidence rates occur between 55 and 65 y with a decrease in those aged older than 65 y; regardless, mortality remains steadily higher for adults aged 60 and older (Figure 1A and B). Similarly, higher disease severity as assessed by hospitalization and ICU admission rates, as well as requirements for invasive ventilation, were more frequent in older adults. Furthermore, most comorbidities were clustered in older individuals and the rates of individuals having 2 or more comorbidities were markedly higher for older adults (24.0% vs. 11.6%, $p < .001$, Figure 1C and D).

COVID-19 Outcomes and Disease Severity in Older Adults

We identified that increasing age, male sex, smoking, diabetes, and obesity were associated with increased pneumonia risk; similarly, for hospitalization we identified an increased risk for older adults with obesity, CKD, COPD, diabetes, increasing age, and male sex. Notably, both pneumonia and hospitalization risk were higher among older adults living in municipalities with high SLI, indicating an effect of structural inequalities besides the aforementioned comorbidities in further increasing COVID-19 severity (Figure 2). When assessing ICU admission risk factors, we identified increased likelihood with obesity, male sex and higher SLI; similarly, the risk for invasive ventilation was higher for older adults with obesity and municipalities with high SLI. Notably, in older adults without comorbidities (n = 5746), age alone was not a significant predictor of requirement for invasive ventilation or ICU admission. We only observed a significant role for age in predicting pneumonia and risk of hospital admission; moreover,

Table 1. Descriptive Statistics Positive SARS-CoV-2 Cases in Mexico on June 3, 2020

Comorbidities	Age<60 n = 80 434	Age≥60 n = 20 804	p value
Diabetes (%)	9956 (12.4)	7533 (36.2)	<.001
COPD (%)	692 (0.9)	1298 (6.2)	<.001
Asthma (%)	2510 (3.1)	420 (2)	<.001
Immunosuppression (%)	1036 (1.3)	519 (2.5)	<.001
Hypertension (%)	11 362 (14.1)	9593 (46.1)	<.001
Other (%)	2273 (2.8)	920 (4.4)	<.001
CVD (%)	1211 (1.5)	1383 (6.6)	<.001
Obesity	16 463 (20.5)	4136 (19.9)	.0622
CKD (%)	1316 (1.6)	1023 (4.9)	<.001
Smoking (%)	6518 (8.1)	1815 (8.7)	.0039
Men(%)	44 742 (55.6)	12 257 (58.9)	<.001
Pneumonia(%)	16 735 (20.8)	10 190 (49)	<.001
Hospitalization(%)	21 503 (26.7)	13 374 (64.3)	<.001
ICU admission (%)	1903 (2.4)	1556 (7.5)	<.001
Mortality (%)	5592 (vii)	6136 (29.5)	<.001

Note: COPD = chronic obstructive pulmonary disease; CKD = chronic kidney disease; CVD = cardiovascular disease; ICU = intense care unit.

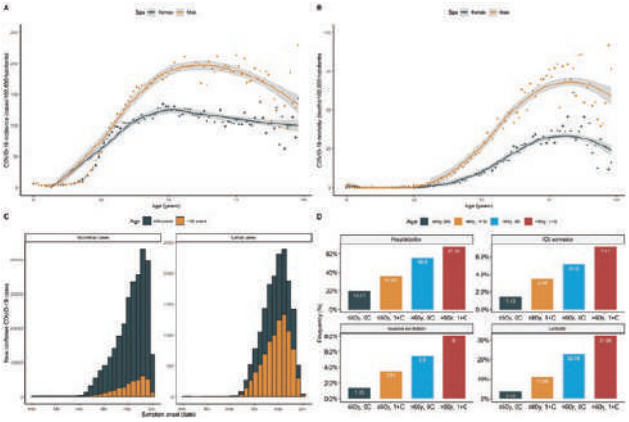

Figure 1. COVID-19 cases (**A**) and mortality rates (**B**) standardized according to age-specific population projections for 2020 for each 1-y increment. The figure also displays the distribution of lethal and nonlethal cases stratified by age group, comparing younger and older adults according to date of symptom onset to demonstrate the disproportionate number of lethal cases in older adults (**C**) and distribution of COVID-19 outcomes comparing rates between those ≤60 and >60 y, with (1+C) and without comorbidity (0C), to demonstrate the impact of comorbidity in modifying outcomes independent of age (**D**).

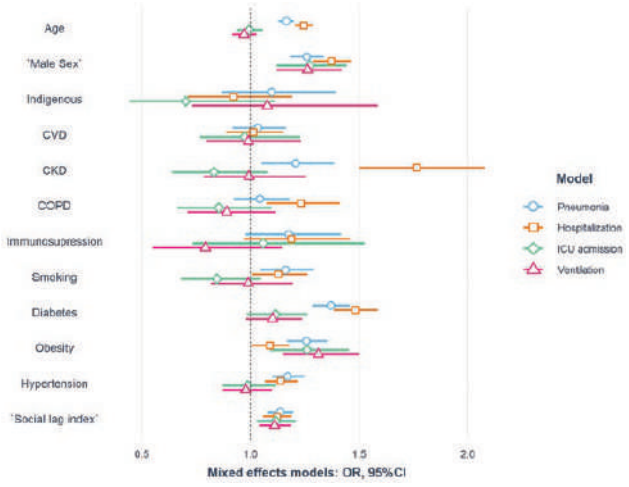

Figure 2. Risk factors for COVID-19-related pneumonia, hospitalization, and ICU admission in older Mexican adults, modeled using logistic with mixed effects, including municipality of case occurrence as a random effect to control for regional inequalities.

the SLI alone was associated with increased risk of pneumonia (OR 1.45, 95% CI 1.21–1.74), hospitalization (OR 1.52, 95% CI 1.24–1.87), and requirements for invasive ventilation (OR 1.38, 95% CI 1.07–1.78) in older adults without comorbidity.

Health and Structural Determinants of COVID-19 Mortality in Older Adults

Besides comorbidities, we identified that the SLI was a predictor of COVID-19 lethality adjusted for age and sex (HR 1.133, 95% CI 1.054–1.217); the number of hospital beds per 1000 individuals was not a protective factor for mortality (HR 0.996, 95% CI 0.990–1.002) but receiving treatment within a private healthcare facility was (HR 0.444, 95% CI 0.368–0.535). In multivariable analyses,

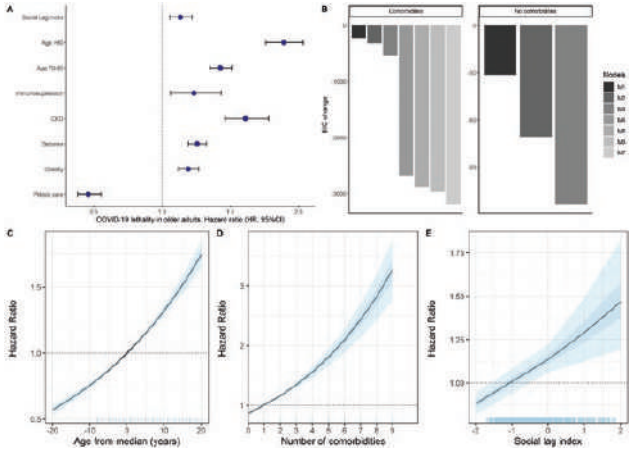

Figure 3. Risk factors for COVID-19 lethality in older Mexican adults, using Cox proportional Hazard regression models with frailty penalty to accommodate multilevel data (**A**). (**B**) shows the changes in the Bayesian Information Criteria to estimate the role of age, structural factors, and comorbidities in improving COVID-19 lethality risk predictions M0 is the reference model, which includes sex and COVID-19 pneumonia, M1 = M0 + Social lag index, M2 = M0 + Age, M3 = M0 + Age + Social lag index, M4 = M0 + Comorbidities, M5 = M0 + Comorbidities + Social lag index, M6 = M0 + Comorbidities + Age, M7 = M0 + Age + Social lag index + Comorbidities comparing subjects with and without comorbidities. We also include postestimation simulations to quantify the effect of covariates in increasing COVID-19 lethality risk for age compared to the median in older adults (72 y, **C**), an increasing number of comorbidities (**D**) and increases in the SLI (**E**).

we identified a higher mortality risk associated with age, comorbid diabetes, obesity, immunosuppression, and CKD. Being treated in a public care facility and living in municipalities with increased values of the SLI were also predictors of COVID-19 lethality (Figure 3A).

Role of Structural Determinants, Comorbidities, and Age on COVID-19 Severity and Lethality

To assess improvements in model performance, we examined the effect of age, structural factors, and comorbidities in improving prediction of COVID-19 lethality. Overall, comorbidities offered the larger changes in predictive improvement for mortality, followed by its combination with structural factors; models that included age had smaller changes in BIC. Similarly, the combination of comorbidities and structural factors showed lower BIC values compared to the combination of either with age (Figure 3B). The better model, which included age, structural factors, and comorbidities only showed marginal improvements in predictive ability compared to the model which excluded age (c-statistics 0.661 vs. 0.642, respectively). To isolate the effect of age, we analyzed older adults without comorbidities; in these individuals, the SLI was a risk factor (HR 1.21, 95% CI 1.06–1.38) and treatment in private care facilities a protective factor (HR 0.39, 95% CI 0.26–0.58) for mortality (c-statistic = 0.676). Addition of age offered additional decreases in BIC, and increased the overall predictive capacity of the model (c-statistic = 0.687). Overall, in individuals without comorbidities, additional consideration of structural factors improved prediction of mortality compared to age alone. Similarly, obesity remained as a significant predictor of mortality even in individuals without other comorbidities (HR 1.17, 95% CI 1.01–1.34) and was also a predictor of increased risk of pneumonia (OR 1.76, 95% CI 1.51–2.06), hospitalization risk (OR 1.45, 95% CI 1.23–1.71), ICU admission

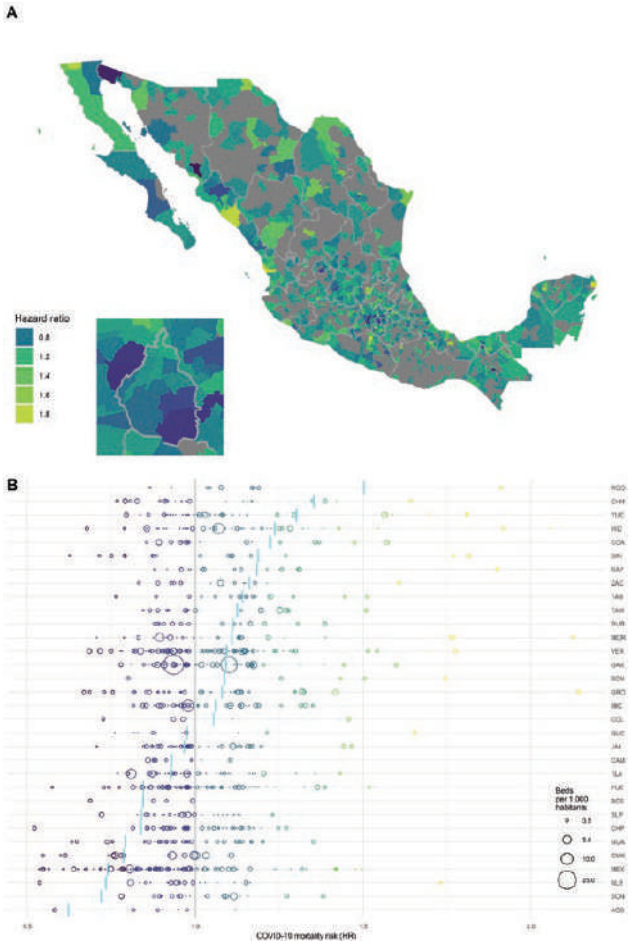

Figure 4. Geographical distribution of baseline COVID-19 lethality hazards across Mexican municipalities as modeled by mixed-effects Cox proportional risk regression models with frailty penalties (**A**). We also show hazard of individual municipalities and the number of beds per 1000 inhabitants in each municipality considering also a state-wide mortality hazard (blue line, **B**).

(OR 1.53, 95% CI 1.14–2.05) and requirement of invasive ventilation (OR 1.50, 95% CI 1.13–2.05), over chronological age. These latter findings suggest that the obesity paradox observed in other acute respiratory infections is not present in older individuals (17).

Postestimation Simulation of Mortality Risk Prediction for Continuous Variables

When running postestimation simulations in the model with all three categories, we identified that compared to median age among older adults (72 y), those younger than the median had lower mortality risk compared to those above. However, despite age being a predictor of mortality, we did not observe significantly higher risk in individuals aged older than 80 compared to individuals aged 70–80 y. Similarly, an increasing number of comorbidities and living in municipalities with increasing SLI were also related to monotonic increases in mortality risk (Figure 3C–E). We hypothesized that COVID-19 outcomes would be geographically heterogeneous; therefore, we fitted mixed-effect models considering the municipality of case occurrence as a random effect. When considering baseline mortality hazard for older adults with COVID-19 across Mexican municipalities, we identified significant disparities; notably, higher risk areas were related to municipalities with higher SLI which were mostly nearby metropolitan areas (Figure 4A). Furthermore, when plotting baseline hazard ratios using the number of hospital beds available per 1000 inhabitants, we observed that higher risk occurred in municipalities with five or fewer beds per 1000 inhabitants. Since the distribution of cases has not been homogeneous and most high-risk areas were near metropolitan areas, we performed a subanalysis restricted to those locations (n = 15 965, 4592 deaths). Interestingly, the SLI remained as a significant predictor of mortality in metropolitan areas (HR 1.37, 95% CI 1.13–1.65), as was being treated in a private care facility (HR 0.39, 95% CI 0.30–0.49), adjusted for age and sex; however this was not observed for nonmetropolitan areas where the SLI was not predictive of mortality (HR 1.01, 95% CI 0.91–1.13), suggesting a rural-urban dissociation on the factors which impact lethality.

Discussion

Here, we demonstrate the role of health and structural socioeconomic determinants—as proxied by the SLI and private versus public healthcare delivery—in increasing COVID-19 lethality and disease severity independent of age in older Mexican adults. Notably, we observed that while age is a significant independent predictor of COVID-19 lethality, comorbidities, and structural health determinants likely play a larger role in increasing disease severity and conditioning risk of COVID-19 lethality in older patients. In older adults without comorbidities, age was not a predictor of disease severity with structural factors playing a larger role; in addition, mortality prediction in individuals without comorbidities improved only marginally when considering chronological age added to structural factors. We therefore confirmed a worrisome association of increased COVID-19 lethality with increasing values of the SLI, which considers deficiencies or unequal access to education, healthcare, living conditions, and basic services; furthermore, being treated in private facilities also decreases the risk of COVID-19 lethality, suggesting a significant gap between care received in public compared to private facilities for older adults or better accessibility for private care. These results suggest that the consequences of large socioeconomic inequalities within Mexico represent added burdens to multimorbidity in older adults, and both of which are better predictors of mortality compared to chronological age alone. These conditions must be addressed as the pandemic transitions to more rural areas where limited access to health services and older population structures with adverse multimorbidity profiles increase mortality risk.

Data from countries affected by the COVID-19 pandemic have reported higher mortality rates in older adults, particularly in those with an increasing number of comorbidities (18). These observations have led to distinct problems concerning healthcare decisions in the face of COVID-19. Given the overwhelming effect of COVID-19 on healthcare burden for countries with older population structures, many systems have opted for health resources to be allocated based mainly on chronological age (19,20). While evidence of an age-dependent increase in the expression of the *ACE2* gene has been reported and an increasing number of comorbidities are readily observed in older adults with COVID-19, data which demonstrates biological effects of chronological aging over multimorbidity and health disparities on outcomes has not been reported (5,21). Instead, additional effort should be carried out to reduce disparities in healthcare access, reduce socioeconomic inequalities, and improve care in older adults with multimorbidity to reduce its effect

on COVID-19 outcomes, given their concomitant effects along with chronological age in increasing COVID-19 severity and lethality (22,23). A previous analysis by our group which considered COVID-19 cases in Mexico highlighted how the cardio-metabolic burden in younger patients approximated mortality risk to those observed in older COVID-19 patients without comorbidity (24). Our results in this present study show that chronological age only offers modest improvements in mortality risk prediction and does not predict disease severity in older adults without comorbidities. This evidence advocates for bioethical decisions on resource allocation in overwhelmed healthcare systems to not be based on chronological age as an exclusive criterion, but rather to integrally consider comorbidity profiles, functional status and other markers of biological aging along with establishing a framework which works towards ameliorating the impact of socioeconomic inequalities on COVID-19 outcomes for older adults as the pandemic progresses.

The response of Mexican authorities to promote social distancing among older adults has thus far been consistent. A nation-wide stipend was established the Mexican government to support older adults throughout Mexico and, recently, advanced payments were released to reduce the financial burden of COVID-19 (25). Despite these policies, our data shows that older adults who live in highly marginalized municipalities remain at higher risk of COVID-19 severity and mortality, which might likely indicate unaddressed gaps that must be considered to reduce the impact of COVID-19 on older adults, particularly from highly marginalized communities and ethnic groups within Mexico (26). Inequalities which might arise once social distancing is lifted must also be addressed to facilitate reincorporation of older adults into daily life after the pandemic and geriatric care must be secured to reduce the impact of social distancing on the general health and function of older adults, particularly those living in isolation and with multimorbidity (27).

Obstacles for healthcare access in older adults have been consistently associated with worsened outcomes and increased all-cause mortality, particularly for those facing social inequalities (28). Previous research has shown that negative self-perceived aging influences healthcare-seeking behavior, particularly in settings of limited healthcare access (29,30). Our results suggest that reduced healthcare access, as measured by increases in the SLI, is associated with increased COVID-19 severity and lethality in older adults, likely reflecting the effect of structural inequalities across Mexican municipalities. A recent geroscience approach to the COVID-19 pandemic in the United States, Italy, and China demonstrated an exponential increase in COVID-19 lethality with age and similarly observed male-biased mortality as in the Mexican data, potentially implicating aging mechanisms into COVID-19 lethality in older adults (31). However, as shown by our results, in older adults without comorbidities the effect of age was not a predictor of disease severity; furthermore, structural factors and comorbidities were better predictors of mortality compared to chronological age alone. These findings on the adverse impacts of structural factors in individual clearly relate the individual with its environment are in line with the multidimensional concept encapsulated by intrinsic capacity, which also includes a composite of physical and mental capacities which define and individual's functional ability. These deficits might be responsible for the increased COVID-19 risk observed in older adults. Intrinsic capacity should be evaluated as a risk factor for COVID-19 and other infectious diseases in older adults in future studies to better understand it as a predictor of mortality over chronological aging (32,33). Similarly, biological markers and clinical measures could be used to estimate biological aging and its potential impact on COVID-19 outcomes, and which are likely to better predict fatality risk in older adults; these metrics should be evaluated to better reflect risk attributable to aging in COVID-19 (34,35).

Ageism and self-perceived aging might impact healthcare-seeking decisions in older adults, particularly under a widespread perception of increased healthcare burden during the COVID-19 pandemic (11,36). An additional factor to consider in this setting is the fact that awareness of COVID-19 symptoms is directed towards typical disease presentations and might not account for atypical symptoms and presentations which are more likely to occur in older patients (37,38). Overall, given the significant impact of COVID-19 on older adults, our findings call for more integral approaches to spread awareness of COVID-19 in older adults in Mexico and other countries, addressing information gaps which might reduce healthcare-seeking behavior and reducing ageism as a criterion for bioethical decision-making. Moreover, evidence regarding atypical COVID-19 presentations and identifying factors that decrease mortality in older adults to increase awareness and promote healthcare-seeking behavior in high-risk remain as a relevant unaddressed gap which must be undertaken by future studies

Our approach is robust given that it considers national data on all confirmed COVID-19 cases in older adults with significant representation of most regions within Mexico. Inclusion of multilevel data allowed us to identify structural factors related to COVID-19 lethality and outcomes and to contrast the role of these factors and comorbidities as predictors of COVID-19 severity and mortality, highlighting areas of opportunity to address these and future affections which will likely also impact older adults. A potential limitation of our study is the lack of symptom-specific data which could be helpful to characterize atypical presentations of COVID-19 in older adults and identify early predictors of disease severity and potential recovery as has been seen in some series, and which remains to be addressed in future studies to better characterize the course of COVID-19 in older adults (39). A relevant limitation of our work could be related to underreporting of mild cases in marginalized areas due to a lack of widespread testing, which might inflate mortality estimates for those regions, but which also would indicate a significant challenge for handling the COVID-19 epidemic in marginalized communities moving forward. Similarly, care should be taken when interpreting data on COVID-19 outcomes in private and public facilities, as it is impossible to discern to what extent improved outcomes result from differences in the severity of admitted and reported cases not captured in the gross data, with increased admission and reporting of mild cases in private hospitals, differences in socioeconomic status and other un-identified predictors of COVID-19 outcomes in patient populations, or differences in quality of care. Finally, consideration of additional measures not included in our study for assessment of disability, functionality and dependence are required to adequately analyze subjects with and without comorbidities and quantify the impact of such conditions on COVID-19, remaining as an area of opportunity for future research. Our findings could inform public policy related to ethical decision making for older adults by prompting consideration of factors beyond chronological age and promote further studies in older populations, which remains imperative to address aging in populations undergoing an epidemiological transition with increased morbidity burden, such as Mexico.

In conclusion, we characterized predictors of COVID-19 outcomes in older Mexican adults, including potential predictors related to structural inequalities which increase COVID-19 severity and lethality risk. We demonstrated that comorbidity profiles and

structural factors are better predictors of COVID-19 severity and mortality compared to chronological age. Overall, baseline COVID-19 mortality hazards are unequally distributed for older adults in Mexico. Special attention should be given to underprivileged older populations to ameliorate the impact of these factors which increase lethality in older adults with multimorbidity. These findings could aid in shifting the current clinical and ethical decision-making focus away from chronological aging and onto a more integral and comprehensive geriatric care approaches.

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Conflict of Interest

Nothing to disclose.

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Author Contributions

Research idea and study design O.Y.B.C., A.G.D., L.M.G.R.; data acquisition: O.Y.B.C., A.G.D.; data analysis/interpretation: O.Y.B.C., J.P.B.L., N.E.A.V., A.V.V., A.G.D., C.G.P., C.A.A.S., L.M.G.R.; statistical analysis: O.Y.B.C., A.G.D.; manuscript drafting: O.Y.B.C., A.G.D., N.E.A.V., J.P.B.L., A.M.S., C.A.F.M.; supervision or mentorship: O.Y.B.C., L.M.G.R. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions about the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

Data Availability

All data sources and R code are available for reproducibility of results at https://github.com/oyaxbell/covid_aging_mx.

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The association between obesity, type 2 diabetes, and hypertension with severe COVID-19 on admission among Mexicans

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Authors' contribution: SB, HL-G, TB-G, JAR and ED-G came up with the idea and analysis plan for the present study; RC-A, JA-Z, CZ-J, DD-L, TV-R, RG-V, KR-G, CE-M, and HL-G had full access to all data and take responsibility for the integrity of the data; ED-G and SB conducted the data analysis. EDG and SB contributed to writing the first version of the manuscript. ED-G, RC-A, JA-Z, CZ-J, DD-L, HL-G, TB-G, JAR and SB critically revised the manuscript. All authors reviewed and approved the final version.

Word count: 2149 words

What is already known about this subject?

Previous studies have highlighted the relationship between obesity and higher risk of infectious diseases. Some of these studies have reported the association between obesity and the risk of a severe form of COVID-19.

What are the new findings in your manuscript?

We analyzed data from 3,844 patients who tested positive for COVID-19. After adjusting for age, sex, smoking status, history of chronic diseases (hypertension, diabetes, cardiovascular disease, chronic kidney disease, immunosuppression) and drug treatment, people with obesity showed a 1.43-fold higher odds of developing severe COVID-19 on admission compared to patients without obesity. Additionally, this is the first study that evaluates the association between obesity, diabetes, and hypertension with severe COVID-19 on admission among the Mexican population.

How might your results change the direction of research or the focus of clinical practice?

If causality exists between obesity, diabetes, hypertension, and COVID-19, it will help the health sector better target vulnerable populations and assess the risk of deterioration.

Abstract

Objective: To explore the association between obesity, type 2 diabetes, hypertension, and severe COVID-19 on admission.

Methods: In the present study, a total of 23,593 patient samples were evaluated by a laboratory from the Mexican Institute of Epidemiological Diagnosis and Reference (InDRE, for its acronym in Spanish). Of these: 18,443 were negative for COVID-19, 3,844 were positive for COVID-19, and 1,306 were positive for other respiratory viruses. Severe types of respiratory disease were defined by

the presence of pneumonia and other organ failure that requires intensive care. Multivariable logistic regression models were used to explore factors associated with severe COVID-19 on admission.
Results: Patients who tested positive for COVID-19 had a higher proportion of obesity (17.4%), diabetes (14.5%), and hypertension (18.9%), compared to those without a confirmed diagnosis. Compared to non-obese patients, those with obesity showed a 1.43-fold higher odds of developing severe COVID-19 on admission, while subjects with diabetes and hypertension showed a 1.87-fold and 1.77-fold higher odds of developing severe COVID-19 on admission, respectively.
Conclusion: Obesity, diabetes, and hypertension were significantly associated with severe COVID-19 on admission and the association of obesity was stronger in patients < 50 y.

Introduction

The new coronavirus 2019 epidemic (COVID-19), which is caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has spread worldwide and poses a critical threat to public health around the world. Rapidly communicating information related to the virus is a current priority for disease prevention and control (1-3).

Obesity has been linked with a greater risk of inflammation and other chronic conditions (4,5). In addition, previous studies have highlighted the relationship between obesity, diabetes, and a higher risk of infectious diseases (6,7). For example, obesity has long been recognized as a risk factor for increased morbidity and mortality associated with the influenza A (H1N1) infection (8). This is a major challenge in countries like Mexico where obesity prevalence (40.1% in women and 26.6% in men) ranks as one of the highest in the world and has continued to increase during the last three decades, particularly in low- and middle-income groups (9).

Previous reports (1,3,10,11) suggest that people over age 60 and those with obesity have a higher risk of severe COVID-19 complications. For that reason, understanding and quantifying this risk is key to enabling patients, care givers, and healthcare professionals to make informed decisions about ways to manage risk in patients with obesity, type 2 diabetes or hypertension during the COVID-19 pandemic. Thus, the present study hypothesized that patients with obesity, diabetes, and hypertension would experience greater odds of developing severe COVID-19 on admission. If so, this might provide insights for Mexican health authorities and other middle-income or developing countries with a high prevalence of obesity.

Methods

Design and study participants

In Mexico, COVID-19 reporting follows two general procedures. Hospital surveillance keeps track of all deaths and hospitalizations, providing a census of confirmed COVID-19 cases. In addition, sentinel surveillance is carried out through a system of selected health units that monitor respiratory diseases (USMER, by its Spanish acronym) (Figure 1). USMERs include medical units from the first, second, or third level of care that have enough material and human resources to follow strict clinical evaluation protocols to identify respiratory disease, as well as the infrastructure to produce timely and complete epidemiological reports (12). A total of 475 USMER units are distributed across the country and every Mexican state has at least one reporting unit. Although sentinel units were not selected randomly, sentinel surveillance is the most effective way to collect good quality and timely data on respiratory diseases in Mexico. The spectrum of severity of COVID-19 infections varies greatly. In this sense, the sentinel surveillance system focuses on mild manifestations of mostly community cases, while severe patients that require hospital treatment are documented by hospital-based surveillance.

In the present study, the information collected from National Epidemiological Surveillance System (SINAVE, SISVER, for its acronym in Spanish) started on February 27, 2020, when the first case of COVID-19 in Mexico was confirmed, and ended-on April 10, 2020. A total of 33,893 subjects were included in the Epidemiological Surveillance System for viral respiratory disease (SISVER, for its acronym in Spanish) platform (**Figure 1**). Of these, 23,593 patient samples were tested for SARS-

CoV-2 by the laboratory from the Mexican Institute of Epidemiological Diagnosis and Reference (InDRE, for its acronym in Spanish) or by any other public or private laboratory in Mexico. Of the 23,593 samples for which results were available, 18,443 were negative for COVID-19, 3,844 were positive for COVID-19, and 1,306 were positive for other respiratory viruses such as H1N1.
For the initial analysis, we only included those who were negative or positive for COVID-19 and who had complete information. In the final analysis, only those who were positive for COVID-19 were included.

Data collection

Confirmation of COVID-19

This study utilized the information collected by every state through first, second, and third level medical care units, which evaluate suspected cases of viral respiratory disease, based on an operational definition (person of any age who in the past 7 days has had at least two of the following signs and symptoms: cough, fever, or headache, accompanied by at least one of the following signs or symptoms: dyspnea, arthralgia, myalgia, odynophagia, rhinorrhea, conjunctivitis or chest pain.)
Among these suspected cases, two protocols were followed: a) SARS-CoV-2 testing for suspected COVID-19 cases with severe acute respiratory infection with signs of breathing difficulty, and b) for all other suspected cases, sentinel surveillance was utilized (13). This definition was approved by the National Epidemiological Surveillance Committee (CONAVE, by its Spanish acronym) (14). Once collected, this information was uploaded to the SISVER online platform.
The presence of SARS-CoV-2 was diagnosed by the real-Time Reverse Transcription Polymerase Chain Reaction method, based on the Berlin protocol (15,16). Only laboratory-confirmed cases were included in the final analysis.
A consent disclaimer was obtained for the purposes of this study. This cross-sectional study was performed in line with the Strengthening the Reporting of Observational Studies in Epidemiology statement.

Outcome assessment

For the present study, the primary outcome was a severe form of COVID-19. All patients were classified into severe and mild respiratory disease based on results from a clinical examination and their symptoms. Patients who only had symptoms like cough, expectoration, and other upper respiratory tract symptoms were classified as non-severe. Severe types of respiratory disease on admission were defined by the presence of pneumonia and other organ failure that requires monitoring and treatment in the intensive care unit (ICU).

Additionally, information on the date of onset of symptoms and hospital admission are available for all cases, as well as the status of treatment (outpatient or hospitalized), information on the diagnosis of pneumonia, and admission to the ICU (13).

Assessment of covariates

In most cases (68% approximately), information on obesity, type 2 diabetes, and hypertension was obtained by the attending physician by self-report from the patient. For the rest (32% approximately), information was obtained by self-report and was corroborated with medical records.

The attending physician collected epidemiological, other clinical information (presence of comorbidities), laboratory, and treatment, as well as demographic characteristics (e.g., age, sex), and tobacco consumption, using a standardized questionnaire. This information was subsequently recorded on the SISVER online platform.

Statistical Analyses

Descriptive analyses of the main characteristics of interest were performed. Categorical variables were described as percentages and continuous variables as mean and standard deviation. Means for continuous variables were compared using independent group t-tests. Comparisons for categorical variables were done using the chi square test or the Fisher exact test. Multivariable logistic regression models (one model for each condition) were used to explore obesity, diabetes and hypertension with a severe COVID-19 on admission; odds ratios (OR) and 95% confidence intervals (95% CIs) were estimated.

To assess the possible effect of modification, we explored stratified analyses by age (two categories: <= 50 years vs ≥ 51 years). We tested the significance of the interaction with a likelihood ratio test by

comparing a model with the main effect variable and the interaction terms with a reduced model with only the main effects.

All *P* values presented are two sided; $P < 0.05$ was considered statistically significant. The statistical analyses were performed using the STATA statistical software package, version 13.0 (Stata Corp. LP: College Station, TX).

Results

When comparing the patients who tested positive for COVID-19 to those who tested negative, we observed that the positive patients had a mean age of 45.4 years and a higher proportion of them were over age 60 (19.4%). Additionally, patients who were positive for COVID-19 had a higher proportion of obesity (17.4%), diabetes (14.5%), and hypertension (18.9%), compared to those without a confirmed diagnosis (**Table 1**).

The characteristics of patients who tested positive for COVID-19 were stratified by obesity condition (**Table 2**). Of them, 17.4% had obesity and 82.6% did not have obesity. We observed a higher number of older patients with obesity than in the patients without obesity (23.0% vs 18.7%, P<0.001). Patients with obesity also had a higher prevalence of other chronic diseases, such as diabetes (29.3%), hypertension (36.1%), cardiovascular disease (5.9%), and chronic kidney disease (2.3%). Patients with obesity were more likely to have symptoms such as fever (P<0.001), cough (P = 0.005) and dyspnea (P<0.001), compared to patients without obesity. Finally, a higher proportion of patients with obesity required ICU support and invasive medical ventilation.

Table 3 shows that after adjusting for age, sex, smoking status, history of chronic diseases (cardiovascular disease, chronic kidney disease, immunosuppression), place of care, USMER, and drug treatment, patients with obesity showed a 1.43-fold higher odds of developing severe COVID-19 on admission compared to patients without obesity. Also, patients with diabetes had a 1.87-fold higher odds of severe COVID-19 on admission compared to those without diabetes. Finally, patients with hypertension had a 1.77-fold higher odds of severe COVID-19 on admission compared to those without hypertension.

We examined the statistical interaction between age (< 50y vs >= 50 y) and the presence of obesity on their odds for severe COVID-19 on admission. The reference group did not have obesity and were < 50 y of age. Relative to the reference group, the odds ratio of the group with obesity (also < 50y) was 1.88 (95% CI: 1.26, 2.55). The odds ratio for the group without obesity > 50y was 1.51 (95% CI: 1.03, 2.20) versus 1.67 for the group with obesity >50y (95% CI: 1.01, 2.63) (Figure 2) (P for interaction 0.03). Additionally, we evaluated the possible interaction between hypertension and diabetes with age. However, these interactions were not statistically significant.

Finally, we performed a sensitivity analysis according to the origin of the patients (USMER vs non-USMER). Although the patients from USMER had higher odds of severe COVID-19 on admission, they were not statistically different from those who came from non-USMER institutions (data not shown).

Discussion

To our knowledge, this is the first study that evaluates the association between obesity, diabetes, and hypertension with severe COVID-19 on admission among the Mexican population. Our data suggests that these conditions are associated with severe COVID-19 on admission.

We observed that among patients with COVID-19, 17.4% had obesity, 14.5% had diabetes, 18.9% had hypertension, and 2.8% had cardiovascular disease. Similar to our data, recent studies from China claim that hypertension was prevalent in approximately 17% of the patients with COVID-19, while diabetes, cardiovascular diseases, and chronic kidney disease were present in 8%, 5%, and 2% of the cases, respectively (17-21).

Our analysis found that compared to patients without obesity, patients with obesity had 1.42 times the odds of developing severe COVID-19 on admission. A recent study by Xu et al. (3) found a similar association between higher body mass index and greater odds of developing severe disease. Compared to patients with normal weight, patients with obesity showed 2.42-fold higher odds (95% CI: 1.42, 8.27; $P = 0.004$) of developing a severe form of COVID-19 (3). Additionally, recent studies conducted by Kalligeros et al. (11), and Simmonet et al. (10) found that obesity is a risk factor for severe COVID-19.

People living with diabetes have been considered at higher risk of infections (17), which is consistent with our finding of 1.87-fold higher odds of developing severe COVID-19 on admission in patients with diabetes. However, a recent systematic review and meta-analysis found that the odds of developing a severe form of COVID-19 in people living with diabetes was not statistically significant (OR = 2.07; 95% CI: 0.89, 4.82) (18).

Specific comorbidities associated with an increased risk of infection and severity of COVID have been reported. In this sense, one of the most commonly reported comorbidities was hypertension (22,23). However, there is still no evidence that hypertension is related to the COVID-19 results. Our data suggest that patients with hypertension had 77% greater odds of developing severe COVID-19 on admission, compared to patients without hypertension.

To our knowledge, there are no previous studies in Mexico evaluating the joint effect of age and the presence of obesity. Our findings suggest that patients with obesity aged <= 50 years were 1.88 times more likely to develop severe COVID-19 on admission, while patients with obesity aged >= 50 years were 1.67 times more likely to develop severe COVID-19 on admission, compared to patients without obesity aged <= 50 years. A study found that patients with obesity under age 60 were more likely to be admitted to acute and critical care (24).

Despite the fact that the exact mechanisms by which obesity increases the severity of COVID-19 have not been clearly described, multiple mechanisms may play a role. Patients with obesity have an affected respiratory physiology, involving decreased functional residual capacity and expiratory reserve volume, as well as hypoxemia and ventilation/perfusion abnormalities (25). Obesity and other chronic conditions, like diabetes, might be significant in the pathogenesis of COVID-19 infection. The immune system, which plays an important role in the pathogenesis of COVID-19, is also a crucial element in obesity-induced adipose tissue inflammation. This inflammation of adipose tissue has been linked to metabolic dysfunction, which in turn has been associated with dyslipidemia, type 2 diabetes, hypertension, and cardiovascular disease (26,27). In addition, a number of possible mechanisms have been proposed for the increased risk of severe clinical outcomes in COVID-19 for people living with diabetes, including elevated plasmin levels, imbalance of angiotensin converting enzyme 2 and cytokines, reduced viral clearance, insulin resistance, and increased inflammatory markers (26-29). Also, obesity and diabetes have been linked with impaired immune responses to viral and bacterial

infections (6,7,19) like influenza A; therefore, by analogy, obesity may play an important role in COVID-19 transmission and the severity of disease. For example, in the case of influenza A, obesity increases the duration of virus shedding; symptomatic patients with obesity shed the virus 42% longer than adults without obesity (26,30). Moreover, diabetes was proven to be an important risk factor for mortality in patients infected with influenza A (19).

The present study has some important limitations. First, this is an administrative dataset which was developed to monitor the epidemic and not specifically designed to follow-up patients; thus, information is only available at the moment in which people were registered in the system. Therefore, no information is available regarding the severity of the patients later on. Second, despite the fact that the data is from patients from all over Mexico, patients who were asymptomatic or treated at home are not part of the data, so our study represents only the more severe cases of COVID-19, and the results cannot be extrapolated to non-severe COVID-19 cases. Furthermore, since sentinel units were not randomly selected, our findings are not likely to be representative of the entire population. Third, in most cases, obesity was defined by self-report. In this sense, a classification error cannot be ruled out, and this would bias the observed associations towards the null value. In addition, self-report of obesity may be the reason behind the low obesity prevalence in the present study, compared to the prevalence observed at the national level. An additional potential source of bias is the origin of the patients' information (USMER vs non-USMER); however, the separate sensitivity analyses of patients according to their origin found consistent results between groups. Notwithstanding the limitations, this study has some strengths. First, comparative studies between obesity and chronic conditions associated with severe disease caused by SARS-CoV-2 are scarce; therefore, our results may help identify these associations. Second, the sample size permitted a multivariable analysis, consequently reducing the possibility of confounding factors. Third, although it is probable that our findings are not representative of the entire population, our study contains nationwide data.

In conclusion, obesity, diabetes and hypertension—important public health problems in Mexico—were significantly associated with severe COVID-19 on admission. In addition, the association of obesity was stronger in patients < 50 y. As previously suggested (26), this pandemic has shown us that more must be done to combat and prevent obesity in our societies in order to reduce the burden of chronic diseases and adverse outcomes to viral pandemics. Finally, our data suggest the need for

studies that evaluate the mechanisms associated with increased severity of COVID-19 in patients with obesity, as well as the need for prevention strategies for these patients.

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Aknowledments

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Data sharing

The data that support the findings of this study are available from the corresponding author on reasonable request. A proposal with a detailed description of study objectives and a statistical analysis plan will be needed to evaluate requests for our data. The corresponding author will decide based on these materials.

Table 1. Characteristics of patients analyzed by the laboratory of the Mexican Institute of Epidemiological Diagnosis and Reference.

Characteristics	COVID-19 n = 3844	Negative to COVID-19 n = 18443	P-value
Age, years[1]	45.4 ± 15.8	38.8 ± 17.5	<0.001
Age, %			
< 19 years	2.6	8.9	
20 to 59 years	78.0	78.5	<0.001
> 60 years	19.4	12.6	
Sex, %			
Women	42.0	54.5	<0.001
Men	58.0	45.5	
Smoking status, %			
Yes	9.5	10.5	0.06
Chronic conditions			
Obesity, %	17.4	12.8	<0.001
Diabetes, %	14.5	9.6	<0.001
Hypertension, %	18.9	14.4	<0.001
Cardiovascular disease, %	2.8	3.3	0.09
Chronic kidney disease, %	1.7	2.1	0.07
Immunosuppression	1.0	1.0	0.55
Initial symptoms			
Fever	79.5	66.3	<0.001
Cough	86.2	83.5	<0.001
Sore throat	45.0	49.8	<0.001
Nasal congestion	41.0	46.2	<0.001
Dyspnea	37.2	27.6	<0.001
Headache	78.9	76.9	0.02
Muscle pain	64.7	52.9	<0.001
Arthralgia	58.3	48.0	<0.001
Diarrhea	20.7	16.0	<0.001
Vomiting	7.7	7.6	0.17
Abdominal pain	15.4	14.2	0.07

Conjunctivitis	14.9	13.9	0.09
Treatment, %			
Antiviral treatment, %			
Yes	19.2	15.7	<0.001
Need ICU care, %			
Yes	12.7	6.9	<0.001
Invasive mechanical ventilation, %			
Yes	12.1	5.7	<0.01

[1] Mean ± standard deviation.

[2] Instituto Mexicano del Seguro Social (IMSS, by its Spanish acronym).

[3] Instituto de Seguridad y Servicios Sociales de los Trabajadores del Estado (ISSSTE, by its Spanish acronym).

[4] Secretaria de Salud (SSA, by its Spanish acronym).

Table 2. Characteristics of patients with COVID-19 according to the obesity condition.

Characteristics	Total n = 3844	Non-obese n = 3176	Obese n = 668	P-value
Age, years[1]	45.4 ± 15.8	44.9 ± 16.1	48.2 ± 14.0	<0.001
Age, %				
< 19 years	2.6	2.9	0.9	
20 to 59 years	78.0	78.4	76.1	0.001
> 60 years	19.4	18.7	23.0	
Sex, %				
Women	42.0	42.2	41.8	0.85
Men	58.0	57.8	58.2	
Smoking status, %				

Yes	9.5	8.5	13.9	<0.001
Chronic conditions				
Diabetes, %	17.4	11.3	29.3	<0.001
Hypertension, %	14.5	15.3	36.1	<0.001
Cardiovascular disease, %	18.9	2.1	5.9	<0.001
Chronic kidney disease, %	2.8	1.6	2.3	0.27
Immunosuppression	1.0	0.85	1.1	0.55
Initial symptoms				
Fever	79.5	78.0	86.2	<0.001
Cough	86.2	85.5	89.7	0.005
Sore throat	45.0	44.4	47.5	0.03
Nasal congestion	41.0	40.6	42.8	0.03
Dyspnea	37.2	34.2	51.2	<0.001
Headache	78.9	78.9	78.7	0.76
Muscle pain	64.7	63.8	68.7	0.01
Arthralgia	58.3	57.1	64.1	<0.001
Diarrhea	20.7	18.9	29.1	<0.001
Vomiting	7.7	7.2	11.2	<0.001
Abdominal pain	15.4	14.5	21.1	<0.001
Conjunctivitis	14.9	14.4	17.4	0.02
Treatment, %				
Antiviral treatment, %				
Yes	19.2	18.7	21.7	<0.001
Need ICU care, %				
Yes	12.7	3.5	6.7	<0.001
Invasive mechanical ventilation, %				
Yes	12.1	3.2	6.3	<0.01
Disease progression, %				
Home monitoring	15.5	16.1	12.3	
In treatment	56.6	58.4	47.2	
Medical release	2.7	2.8	2.6	<0.001
Severe case	16.4	15.1	22.9	
Death	8.8	7.5	15.0	

[1] Mean ± standard deviation.

[2] Instituto Mexicano del Seguro Social (IMSS, by its Spanish acronym).

[3] Instituto de Seguridad y Servicios Sociales de los Trabajadores del Estado (ISSSTE, by its Spanish acronym).

[4] Secretaria de Salud (SSA, by its Spanish acronym).

Table 3. Association between obesity, diabetes and hypertension with severe COVID-19 on admission.

Variable	Age adjusted model		Multivariate model	
	OR	95% CI	OR	95% CI
		Obesity[1]		
Total				
Non-obese	1.0	--	1.0	--
Obese	1.76	1.39, 2.23	1.43	1.11, 1.83
Men				
Non-obese	1.0	--	1.0	--
Obese	2.25	1.55, 3.25	1.75	1.15, 2.57
Women				
Non-obese	1.0	--	1.0	--
Obese	1.52	1.12, 2.08	1.30	1.03, 1.81
		Diabetes[2]		
Total				
Non-diabetes	1.0	--	1.0	--
Diabetes	3.53	2.78, 4.48	1.87	1.41, 4.26
Men				
Non-diabetes	1.0	--	1.0	--
Diabetes	2.61	1.73, 3.81	1.87	1.19, 2.94
Women				
Non-diabetes	1.0	--	1.0	--
Diabetes	2.10	1.52, 2.89	1.86	1.30, 2.64
		Hypertension[3]		
Total				
Non-hypertension	1.0	--	1.0	--
Hypertension	2.12	1.68, 2.68	1.77	1.37, 2.29

Men				
Non-hypertension	1.0	--	1.0	--
Hypertension	2.85	1.96, 4.15	2.33	1.56, 3.49
Women				
Non-hypertension	1.0	--	1.0	--
Hypertension	1.73	1.28, 2.34	1.50	1.07, 2.08

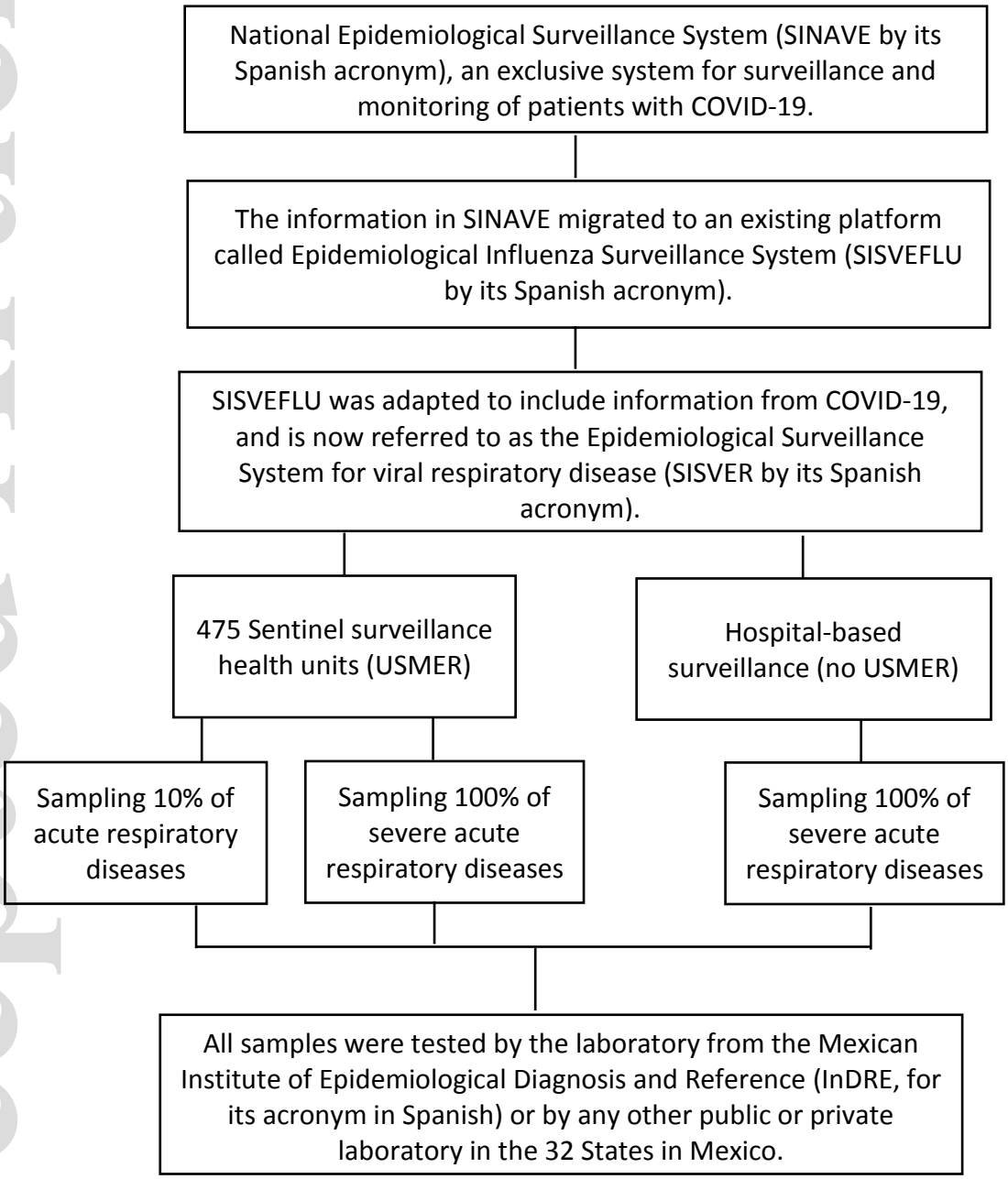
[1] Adjusted for age, sex, smoking status, history of chronic diseases (diabetes, hypertension, cardiovascular disease, chronic kidney disease, immunosuppression), place of care, USMER[2], and drug treatment.

[2] Adjusted for age, sex, smoking status, obesity, history of chronic diseases (hypertension, cardiovascular disease, chronic kidney disease, immunosuppression), place of care, USMER[4], and drug treatment.

[3] Adjusted for age, sex, smoking status, obesity, history of chronic diseases (diabetes, cardiovascular disease, chronic kidney disease, immunosuppression), place of care, USMER[2], and drug treatment.

[4] USMER: Health units of the sentinel surveillance system

Figure 1. Flow chart of the Mexican National Epidemiological Surveillance System for viral respiratory diseases.



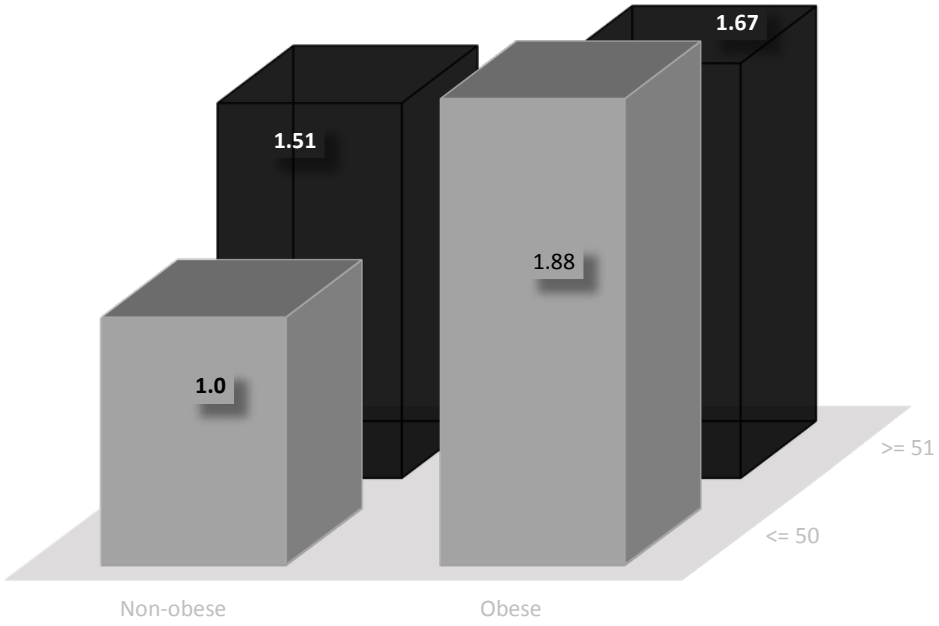


Figure 2. Joint association of Obesity and age (<= 50 years and >= 51 years) with severe COVID-19 on admission in the Mexican population.

Reference group for comparisons were subjects without obesity and <= 50 years. Odds ratio were adjusted for: age (years), sex, smoking status, history of chronic diseases (hypertension, diabetes, cardiovascular disease, chronic kidney disease, immunosuppression), place of care, USMER[1], date of symptoms onset and drug treatment

[1] USMER: Health units of the sentinel surveillance system

Predicting mortality due to SARS-CoV-2: A mechanistic score relating obesity and diabetes to COVID-19 outcomes in Mexico

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ABSTRACT

BACKGROUND: The SARS-CoV-2 outbreak poses challenge to healthcare systems due to high complication rates in patients with cardiometabolic diseases. Here, we identify risk factors and propose a clinical score to predict COVID-19 lethality, including specific factors for diabetes and obesity and its role in improving risk prediction.

METHODS: We obtained data of confirmed and negative COVID-19 cases and their demographic and health characteristics from the General Directorate of Epidemiology of Mexican Ministry of Health. We investigated specific risk factors associated to COVID-19 positivity and mortality and explored the impact of diabetes and obesity on modifying COVID-19 related lethality. Finally, we built a clinical score to predict COVID-19 lethality.

RESULTS: Among 177,133 subjects at May 18th, 2020, we observed 51,633 subjects with SARS-CoV-2 and 5,332 deaths. Risk factors for lethality in COVID-19 include early-onset diabetes, obesity, COPD, advanced age, hypertension, immunosuppression, and CKD; we observed that obesity mediates 49.5% of the effect of diabetes on COVID-19 lethality. Early-onset diabetes conferred an increased risk of hospitalization and obesity conferred an increased risk for ICU admission and intubation. Our predictive score for COVID-19 lethality included age ≥65 years, diabetes, early-onset diabetes, obesity, age <40 years, CKD, hypertension, and immunosuppression and significantly discriminates lethal from non-lethal COVID-19 cases (c-statistic=0.823).

RESULTS: Here, we propose a mechanistic approach to evaluate risk for complications and lethality attributable to COVID-19 considering the effect of obesity and diabetes in Mexico. Our score offers a clinical tool for quick determination of high-risk susceptibility patients in a first contact scenario.

Keywords: COVID-19; SARS-CoV-2; Diabetes; Obesity, Lethality; Mexico

INTRODUCTION

The first cases of SARS-CoV-2 infection in Mexico were reported at the end of February [1]; since then, the number of COVID-19 cases has been steadily increasing, with most fatal cases being associated with the presence of comorbidity and, particularly, cardiometabolic comorbidities. A high prevalence of cardiometabolic diseases worldwide represents a challenge during the COVID-19 epidemic; an elevated number of patients with SARS-CoV-2 infection have a preexisting disease such as obesity, hypertension, cardiovascular disease, diabetes, chronic respiratory disease or cancer [2,3]. Diabetes mellitus and obesity represent a large share of the cardiometabolic morbidity burden of the region [4]; moreover, most cases of diabetes remain either undiagnosed or lack adequate glycemic control, posing them at risk of increased COVID-19 severity. Despite several reports evaluating the burden of comorbidities including obesity, diabetes and hypertension on the clinical course of COVID-19, the joint role of obesity and diabetes in modifying COVID-19 outcomes has not been fully explored [5].

Several studies have demonstrated a higher susceptibility to acute respiratory infectious diseases in people with diabetes [6]. Moreover, diabetes and obesity have been described as independent risk factors for severe pulmonary infection [7,8]. Obesity influences the clinical outcomes during acute severe respiratory distress syndrome (ASRDS); obesity has been proposed as a protective factor for mortality following lung injury due to reverse causality or as a cause of mortality and adverse clinical outcomes for severe influenza cases due to mechanical and immunologic factors. In the cases of the COVID-19 outbreak, obesity has been consistently associated with adverse outcomes [9,10]. Furthermore, a large proportion of obesity cases in Mexico live in geographical areas of increased social vulnerability, which poses a structural inequality that might also increase mortality for COVID-19 associated to both diabetes and obesity [11]. Chronic inflammation in obesity might worsen the acute inflammatory response triggered by SARS-CoV-2 infection, which

might be associated to a cytokine release syndrome [5,12]. Here, we investigate the role of both diabetes and obesity in determining propensity for SARS-CoV-2 infection and its associated clinical outcomes including disease severity and COVID-19 lethality; using these associations, we further construct a clinically useful predictive model for COVID-19 mortality using national epidemiological surveillance data from Mexico.

METHODS

Data sources

We extracted data from the General Directorate of Epidemiology of the Mexican Ministry of Heath, which is an open-source dataset comprising daily updated information of suspected COVID-19 cases which have been confirmed with a positive test for SARS-CoV-2 certified by the National Institute for Diagnosis and Epidemiological Referral [13].

Definitions of suspected and confirmed COVID-19 cases

The Ministry of Health defines a suspected COVID-19 case as an individual of any age whom in the last 7 days has presented cough, fever or headache (at least two), accompanied by either dyspnea, arthralgias, myalgias, sore throat, rhinorrhea, conjunctivitis or chest pain. Amongst these suspected cases, the Ministry of Health establishes two protocols for case confirmation: 1) SARS-CoV-2 testing is done widespread for suspected COVID-19 cases with severe acute respiratory infection with signs of breathing difficulty or deaths in suspected COVID-19 cases, 2) for all other suspected cases, a sentinel surveillance model is being utilized, whereby 475 nationally representative health facilities sample ~10% of mild outpatient cases and all suspected severe acute respiratory infection [14]. Demographic and health data are collected and uploaded to the epidemiologic surveillance database by personnel from the corresponding individual facility.

Variables and definitions

Available information for all confirmed, negative and suspected COVID-19 cases includes age, sex, nationality, state and municipality where the case was detected, immigration status as well as identification of individuals who speak indigenous languages. Health information includes status of diabetes, obesity, chronic obstructive pulmonary disease (COPD),

immunosuppression, pregnancy, arterial hypertension, cardiovascular disease, chronic kidney disease (CKD) and asthma. Date of symptom onset, hospital admission and death are available for all cases as well as treatment status (outpatient or hospitalized), information regarding diagnosis of pneumonia, ICU admission and whether the patient required invasive mechanical ventilation. Early-onset diabetes was defined as a medically diagnosed case of diabetes mellitus in subjects younger than age 40 years. The majority of early-onset diabetes cases are patients with type 2 diabetes. This phenotype is common in Mexico; it is characterized for having a more aggressive form of the disease usually associated with obesity, rapidly declining β-cell function, higher risk of microvascular complications compared to late-onset type 2 diabetes [15]. We considered this form of diabetes given its high prevalence in Mexican and other populations as well as its higher propensity for complications [16,17].

Statistical analysis

Comorbidities associated to SARS-CoV-2 positivity

We investigated the association of demographic and health data associated with SARS-CoV-2 positivity using logistic regression analyses, excluding individuals who were only suspected but unconfirmed cases of COVID-19. Next, we stratified these analyses for individuals with only diabetes or only obesity to identify specific risk factors within these populations, especially focusing on individuals who were <40 years and likely acquired the disease early.

COVID-19 mortality risk

In order to investigate risk factors predictive of COVID-19 related 30-day lethality, we fitted Cox Proportional risk regression models estimating time from symptom onset up to death or censoring, whichever occurred first in cases with confirmed positivity for SARS-CoV-2. To identify diabetes and obesity-specific risk factors, we carried out stratified analyses. Given the availability of SARS-CoV2 negative cases within the dataset, we fitted Cox models for mortality which included SARS-CoV-2 positivity as an interaction term with different comorbidities, hypothesizing that some factors increase mortality risk specifically for COVID-

19. Finally, we fitted a logistic regression model only for mortality cases to evaluate associations with lethality rates in COVID-19 related and non-related deaths.

Influence of obesity and diabetes in COVID-19 related outcomes

Finally, we estimated factors associated to admission to hospital facilities, intensive care units (ICUs) and requirements for mechanical ventilation in all confirmed COVID-19 cases using logistic regression. To identify specific factors for all COVID-19 and non-COVID-19 patients related to outcomes, we included interaction effects with comorbidities factors; we also performed Kaplan-Meier analyses to identify the role of comorbidities in modifying lethality risk in individuals with diabetes and obesity and compared across categories using Breslow-Cox tests. Finally, we performed causal-mediation analyses with causally-ordered mediators using a previously validated approach to investigate whether obesity mediates the decreases in COVID-19 survival attributable to diabetes, particularly in early-onset cases <40 years [18].

Mechanistic mortality risk score for COVID-19

Finally, we constructed a clinically useful model to predict lethality in COVID-19 cases which might be useful to apply in first contact settings, including variables and interactions which were identified in mortality analyses. The model was trained in 80% of the dataset split using random sampling stratified by mortality status with the *caret* R package and was later validated in the remaining 20%. Points were assigned by standardizing all β coefficients with the minimum absolute β coefficient obtained from Cox regression. Points were stratified according to categories of Low risk (≤0), Mild risk (1-3), Moderate risk (4-6), High risk (7-9) and very high risk (≥10). Risk across categories was verified using Kaplan-Meier analyses. C-statistics and D_{xy} values were corrected for over-optimism using k-fold cross-validation (k=10) using the *rms* R package. A p-value <0.05 was considered as statistical significance threshold. All analyses were performed using R software version 3.6.2.

RESULTS

COVID-19 cases in Mexico

At the time of writing this report (May 18th, 2020), a total of 177,133 subjects had been treated initially as suspected COVID-19 cases. Amongst them, 51,633 had been confirmed as positive and 98,567 tested negative for SARS-CoV-2 infection; additionally, 26,933 cases were still being studied as suspected cases pending testing reports. Amongst confirmed cases, 5,332 deaths were reported (10.33%) whilst 2,009 deaths were SARS-CoV-2 negative cases (2.04%) and 656 deaths of suspected but unconfirmed cases (2.44%) had been reported. Compared to SARS-CoV-2 negative cases, confirmed cases were older, predominantly male (1.37:1 ratio), had higher rates of hospitalization and showed a higher prevalence of diabetes, hypertension and obesity. SARS-CoV-2 cases were also more likely have higher rates of ICU admission and requirements for invasive ventilation compared to negative cases (**Table 1**).

Factors associated with COVID-19 positivity

We investigated cases related to COVID-19 positivity within Mexico. We found that odds of SARS-CoV-2 positivity was higher with diabetes, hypertension, obesity, age >65 and male sex. When assessing age, we observed reduced odds of SARS-CoV-2 positivity in patients <40 years and, in contrast, when exploring its interaction with diabetes, we observed an increased probability of SARS-CoV-2 infection. In stratified models, we observed that for patients with diabetes, SARS-CoV-2 positivity was associated with obesity and male sex; for patients with obesity, diabetes and male sex were also significant as was the interaction between diabetes and age <40 years [19].

Predictors for COVID-19 related 30-day mortality

We identified that COVID-19 cases were associated with a near four-fold increase in mortality due to acute respiratory infection (HR 3.967, 95%CI 3.739-4.210) compared to non-COVID-19 cases. Of interest, the only comorbidity which conferred increased lethality risk exclusively for COVID-19 compared to non-COVID-19 was obesity (HR 1.261, 95%CI 1.109-1.433, **Figure 1A**). Factors associated to increased lethality in COVID-19 cases were age

>65 years, diabetes mellitus, obesity, CKD, COPD, immunosuppression and hypertension, whilst asthma showed a protective effect (**Figure 1B**). We searched for an interaction between diabetes mellitus and age <40 years to account for early-onset diabetes mellitus, adjusted for sex and obesity; a higher mortality risk was found for early-onset diabetes cases (HR 2.754, 95%CI 2.259-3.359). Adjusting the model for pneumonia to account for SARS-CoV-2 severity as a predictor of mortality (HR 5.264, 95%CI 4.933-5.618), we observed that asthma was no longer associated to decreased mortality, whilst all other predictors remained constant.

COVID-19 in patients with diabetes mellitus

Confirmed COVID-19 cases with diabetes had a mean age of 57.16 (±12.83) years and were predominantly male. This population had particularly higher mortality rate (21.8% vs. 7.7%), hospitalization, ICU admission, requirement for invasive ventilation and confirmed pneumonia compared with those without diabetes. When stratifying mortality,those with early onset diabetes (<40 years) had higher mortality rates compared to individuals <40 years without diabetes (11.3% vs. 1.3%); similarly, those aged >40 years without diabetes had lower rates compared to those >40 years with diabetes (12.0% vs. 22.7%). As expected, obesity, hypertension, COPD, CKD, CVD, and immunosuppression were also more prevalent in this population. [19]. In patients with diabetes mellitus, COVID-19 related mortality was higher in those with concomitant immunosuppression, COPD, CKD, hypertension and those aged >65 years (**Figure 1C)**. When assessing the role of comorbidities, COVID-19 patients with diabetes, coexistent obesity, those with early onset diabetes (<40 years) or an increase in the number of comorbidities had increased risk of COVID-19 lethality (Breslow test p<0.001, **Figure 2**). When comparing cases with and without COVID-19 amongst patients with diabetes mellitus we observed a threefold higher risk of mortality associated with COVID-19 (HR 3.375, 95%CI 3.103-3.672) after adjustment for age, sex and comorbidities [19].

COVID-19 in patients with obesity

Similar to patients with diabetes, confirmed COVID-19 cases with obesity had particularly higher rates of mortality (13.5% vs. 9.4%), hospitalization and confirmed pneumonia. Furthermore, patients with obesity also had higher rates of ICU admission (5.0% vs. 3.3%) and were more likely to be intubated (5.2% vs. 3.3%). As expected, diabetes, hypertension, COPD, smoking, CVD and asthma were also more prevalent in patients with obesity [19]. As previously mentioned, obesity was identified as a risk factor which displayed differential risk for COVID-19 infection, specific risk factors for lethality in obese patients with COVID-19 infection included COPD, CKD, hypertension, male sex, age >65, diabetes and, particularly, early-onset type 2 diabetes (**Figure 1D**); overall, COVID-19 increased risk of mortality in obesity nearly five-fold (HR 4.989, 95%CI 4.444-5.600). The addition of obesity to any number of comorbidities significantly increased the risk for COVID-19 lethality (Breslow test $p<0.001$, **Figure 2-C**). Using causally ordered mediation analysis we investigated whether the effect of diabetes (E) on COVID-19 related lethality (Y) was partially mediated by obesity (M); the direct effect of diabetes on COVID-19 lethality was significant ($\Delta_{E1\rightarrow Y}$=1.45, 95%CI 1.36-1.54) as was the indirect effect of obesity ($\Delta_{M1\rightarrow Y}$ =1.42, 95%CI: 1.36-1.49), representing 49.5% of the total effect of diabetes.

COVID-19 outcomes and comorbidities

Given the increased risk of diabetes and obesity in modifying COVID-19 related lethality, a secondary objective of our study was to investigate its associations with inpatient outcomes, including hospitalization rate, ICU admission and requirement for mechanical ventilation. In general, early-onset diabetes patients and those with obesity had higher risk of hospitalization, whilst patients with obesity also had increased risk for ICU admission and required intubation. Patients with diabetes mellitus overall had higher risk of hospitalization , risk of ICU admission and intubation (**Figure 3**).

Mechanistic score for mortality in COVID-19

Using the identified predictors for mortality and the observed interaction for early-onset diabetes, we designed a predictive score for COVID-19 mortality using Cox regression with a random split of 80% of the dataset stratified by mortality (n=41,307, deaths=4,276). We identified as significant predictors age>65 years, diabetes mellitus, obesity, CKD, COVID-19 related pneumonia, COPD, and immunosuppression (**Table 2**); age<40 was a protective factor which was modified by its interaction with T2D (R^2=0.154, c-statistic= 0.817, D_{xy}=0.647); assigning the point system did not significantly reduce the model's performance (R^2=0.154, C-statistic=0.822, D_{xy}=0.645). Finally, category stratification reduced only moderately performance statistics (R^2=0.152, C-statistic=0.810, D_{xy}=0.620), and were not significantly modified after cross-validation correction (R^2=0.167, D_{xy}=0.645). The score was then validated using the remaining 20% of the population (n=10,326, deaths=1,056); we observed that the score retained its predictive and discriminative ability (R^2=0.167, C-statistic=0.830, D_{xy}=0.660) as did the categories (R^2=0.170, C-statistic=0.821, D_{xy}=0.642). Distribution of the score significantly discriminates between lethal and non-lethal COVID-19 cases (**Figure 4**).

DISCUSSION

Our results demonstrate that diabetes, particularly early-onset diabetes, obesity and comorbidity burden modify risk profiles in patients with COVID-19 in Mexico and significantly improve mortality prediction related to COVID-19 lethality. These findings position the notion that early-onset type 2 diabetes might carry a higher risk of mortality in younger patients and the risk is similar to older patients with other comorbidities and only higher in older patients with diabetes. Inclusion of the interaction term (Diabetes*Age <40 years) within the risk score effectively offsets the protective effect of younger age, indicating the higher risk attributable to early-onset diabetes and the utility of including this term within the risk score to improve prediction of mortality. Furthermore, our results suggest that obesity is a COVID-19 specific risk factor for mortality, risk of ICU admission, tracheal intubation and hospitalization and even increases risk in patients with comorbid diabetes and COVID-19

infection. Overall, this positions the co-existence of obesity and diabetes, particularly early-onset diabetes, as a considerable risk factor for COVID-19 mortality in Mexicans, whom have reported an alarmingly high burden of both conditions in recent health surveys.

The relationship between increased risk of mortality attributable to acute severe respiratory infections in patients with diabetes mellitus has been extensively reported, particularly for the acute respiratory syndrome caused by SARS-CoV-1 [20–22]. Evidence relating SARS-CoV-2 infections in China demonstrated increased rates of diabetes mellitus in hospitalized patients and in those with increased disease severity as assessed by ICU admission and requirement for invasive ventilation. Additionally, hospitalized patients have shown increased rates of both obesity and diabetes for COVID-19 compared to non-hospitalized cases in the US, China and Italy [5,23,24]. Increased susceptibility for COVID-19 in patients with diabetes may be explained for several potential mechanisms including an increased lung ACE2 expression and elevated circulating levels of furin, a protease involved in viral entry to cells, and a decreased clearance of SARS-CoV-2 viral particles in subjects with diabetes and/or hypertension associated with ACE2 expression [25–28]. Impairments in immunity observed in patients with diabetes are characterized by initial delay in activation of Th1 cell-mediated immunity and late hyper-inflammatory response and are consistent with the increased risk associated with additional immunosuppression observed with our data [29]. Additional factors which have been proposed to modify COVID-19 mortality risk and worsen glycemic control in diabetes include corticosteroid therapy, inadequate glucose monitoring, the effect of social distancing on diabetes care and the use of antihypertensive medication; however these factors remain to be confirmed by clinical evidence [30]. Given the large proportion of undiagnosed diabetes cases in Mexican and poor glycemic control reported by recent estimates, the burden of COVID-19 might be higher than expected in Mexico and poses a challenge for the Mexican healthcare system to give particular attention to this sector as the epidemic moves forward [31–33].

Diabetes mellitus is one of the main causes of morbidity and it accounts for a large proportion of mortality risk in Mexican population [4]. Of relevance, Mexicans have increased

risk of diabetes and diabetes-related obesity attributable to genetic variants associated to its Amerindian ancestry, and an earlier age of onset independent of body-mass index [34,35]. Data on the incidence of early-onset type 2 diabetes in Mexican population position obesity and insulin resistance as significant risk factors, which are also highly prevalent in younger patients and increase metabolic risk [16,36]. These associations partly explain the increased risk of COVID-19 lethality in younger patients within our cohort despite the younger average age of Mexican population and poses early-onset diabetes mellitus as a significant risk factor for COVID-19 mortality and increased severity of infection in younger patients [5,37].

In our work, we demonstrate that compared to non-COVID-19 infections, obesity significantly modifies the risk of mortality attributable to COVID-19 infection. Obesity and, in particular, abdominal obesity, is one of Mexico's main public health problems; in recent years the socio-economic burden of obesity as well as its impact on mortality have increased drastically, with the Ministry of Health declaring a state of epidemiological emergency [38]. Evidence from different regions has supported the notion that obesity increases mortality risk and severity of COVID-19 infections, which holds particularly true for younger patients [2,39]. Obesity is characterized by low-grade inflammation , whereby mononuclear cells increase transcription of pro-inflammatory cytokines; obesity also interacts with insulin resistant states and metabolic syndrome traits often comorbid in subjects with obesity to further promote inflammatory and a pro-thrombotic states which might lead to deleterious responses to infectious pathogens [40,41]. Furthermore, obesity has been shown to lead to decrease immune response to infectious pathogens which in turn also may affect the lung parenchyma, increasing the risk for inflammatory lung diseases of infectious causes, like influenza and SARS-CoV-2 [42–44]. Similar inflammatory responses have been attributed to the low number of asthmatics with confirmed SARS-CoV-2 infection. Immune Th2 response observed in asthma may counter inflammation related to SARS-CoV-2 infection, as was previously reported for cases in Wuhan; however, increased pro-inflammatory processes in severe forms of COVID-19 likely outweigh the effect of asthma. Additionally, abdominal obesity reduces the compliance of lung, chest wall and the entire respiratory system,

resulting in impaired ventilation of the base of the lungs and reduced oxygen saturation of blood [45]. Recently, Simonnet et al. explored the high prevalence of obesity in patients with COVID-19 reporting that obesity is a risk factor for SARS-CoV-2 infection severity independent of age, diabetes and hypertension. Notably, ACE2 expression in adipose tissue is higher than in the lung and its expression profile is not different in obese and non-obese subjects; however, obese subjects have more adipocytes; thus, they have a greater number of ACE2-expressing cells and thus higher likelihood of SARS-CoV-2 entry [2,46]. Our data shows that obesity is a specific risk factor for COVID-19 related outcomes and that it partly mediates the risk associated with diabetes mellitus. Public health efforts by the Mexican government in epidemiological surveillance have largely focused in identifying patient's at highest risk of complications; these findings could inform public health decisions and increase awareness on the role of obesity in modifying risk of COVID-19 outcomes.

Our study had some strengths and limitations. First, we analyzed a large dataset which included information on both confirmed positive and negative SARS-CoV-2 cases, which provides a unique opportunity to investigate COVID-19 specific risk factors and develop a predictive model for COVID-19 mortality. Additionally, with the database being nationally-representative it allows for reasonable estimates on the impact of both diabetes and obesity despite the possibility of important regional differences in cardiometabolic risk which might influence risk estimates. A potential limitation of this study is the use of data collected from a sentinel surveillance system model, which is skewed towards investigating high risk cases or only those with specific risk factors which on one hand increases power to detect the effect of comorbidities and on the other hand might not be representative of milder cases of the disease; this is demonstrated in the risk of COVID-19 positivity, which is higher for high risk cases. The updating daily estimates of COVID-19 cases are unlikely to change the direction of the identified associations though it might modify numeric estimates. The role of a risk-gradient related to BMI and increasing degrees of obesity could not be explored with available data and remains as an area to be explored in further studies. Implementation of

our proposed model might be useful to allocate prompt responses to high risk cases and improve stratification of disease severity.

In conclusion, we show that both diabetes and obesity increase the risk of SARS-CoV-2 infection in Mexico. In particular, diabetes increases the risk of COVID-19 related mortality and, specifically, increases mortality risk in early-onset cases. Obesity is a COVID-19 specific risk factor for mortality and for increased disease severity; obesity also is a partial mediator on the effect of diabetes in decreasing survival associated with COVID-19 infection. This mechanistic interpretation on the risk of comorbidities allowed the development of a model with good performance to predict mortality in COVID-19 cases. Given the burden of obesity and diabetes in Mexico, COVID-19 lethality might be higher in younger cases. Special attention should be given to susceptible individuals and screening should be conducted for all symptomatic cases with either obesity and/or diabetes to decrease the burden associated with COVID-19 in Mexico.

Conflict of interest: Nothing to disclose.

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DATA AVAILABILITY

All data sources and R code are available for reproducibility of results at https://github.com/oyaxbell/covid_diabetesmx.

AUTHOR CONTRIBUTIONS

Research idea and study design OYBC, JPBL, CAAS; data acquisition: OYBC, AGD; data analysis/interpretation: OYBC, JPBL, NEAV, AVV, AGD, JJN, CAAS; statistical analysis: OYBC, NEAV; manuscript drafting: OYBC, NEAV, AVV, JPBL, AMS, CAFM, JJN; supervision or mentorship: OYBC, CAAS. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

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Table 1. Descriptive statistics comparing negative, positive and suspected cases for SARS-CoV-2 in Mexico at 04/27/2020.

Abbreviations: ICU= intense care unit; COPD= chronic obstuctive pulmonary disease; CKD= chronic kidney disease; CVD= cardiovascular disease**.**

Parameter	Positive for SARS-CoV-2 n=51633	Negative for SARS-CoV-2 n=98567	Suspected for SARS-CoV-2 n=26933
Age (mean ± sd)	46.65 ± 15.83	40.25 ± 17.33	43.3 ± 16.55
Male sex (%)	29803(57.7)	47177(47.9)	13602(50.5)
Mortality (%)	5332(10.3)	2009(2.0)	656(2.4)
Hospitalization (%)	19831(38.4)	18586(18.9)	6674(24.8)
Pneumonia (%)	14919(28.9)	11928(12.1)	4723(17.5)
ICU admission (%)	1893(3.7)	1456(1.5)	464(1.7)
Invasive ventilation (%)	1959(3.8)	1162(1.2)	406(1.5)
Diabetes (%)	9460(18.3)	10553(10.7)	3442(12.8)
COPD (%)	1131(2.2)	2238(2.3)	444(1.6)
Asthma (%)	1602(3.1)	4530(4.6)	725(2.7)
Immunosuppression (%)	849(1.6)	2472(2.5)	393(1.5)
Hypertension (%)	11151(21.6)	14858(15.1)	4453(16.5)

Parameter	Positive for SARS-CoV-2 n=51633	Negative for SARS-CoV-2 n=98567	Suspected for SARS-CoV-2 n=26933
Obesity (%)	10708(20.7)	14011(14.2)	4364(16.2)
CKD (%)	1265(2.4)	2270(2.3)	525(1.9)
CVD (%)	1381(2.7)	2891(2.9)	595(2.2)
Smoking (%)	4366(8.5)	9624(9.8)	2451(9.1)

Table 2. Cox proportional risk models for lethality using the mechanistic COVID-19 lethality score in confirmed cases of COVID-19 using individual components, single score point and risk stratification categories. Abbreviations: COPD= chronic obstructive pulmonary disease; CKD= chronic kidney disease; CVD= cardiovascular disease.

Model	Parameter	B	Score	SE	Wald	HR	95% CI	P-Value
Individual variables *C-statistic= 0.817*	Age ≥65 years	0.705	3	0.034	20.868	2.02	1.89-2.16	<0.001
	Diabetes	0.294	1	0.034	8.668	1.34	1.26-1.43	<0.001
	Diabetes*Age <40 years	1.052	5	0.138	7.618	2.86	2.19-3.76	<0.001
	Age <40 years	-1.344	-6	0.070	-19.32	0.26	0.23-0.29	<0.001
	Obesity	0.225	1	0.035	6.376	1.25	1.17-1.34	<0.001
	Pneumonia	1.650	7	0.037	44.812	5.21	4.84-5.60	<0.001
	CKD	0.686	3	0.066	5.145	1.99	1.77-2.23	<0.001
	COPD	0.337	1	0.066	5.145	1.40	1.23-1.59	<0.001
	Immunosuppression	0.239	1	0.088	2.271	1.27	1.07-1.51	0.007
Score point training	1-Point increment	0.238		0.003	68.49	1.27	1.26-1.28	<0.001

C-statistic= 0.823							
Risk Categories training *C-statistic= 0.810*	Low-Risk (≤0 pts)			Reference			
	Mild-Risk (1-3 pts)	1.794	0.071	25.40	6.01	5.24-6.91	<0.001
	Moderate-Risk (4-6 pts)	2.762	0.067	41.22	15.83	13.88-18.05	<0.001
	High-Risk (7-9 pts)	3.243	0.064	50.34	25.61	22.57-29.06	<0.001
	Very High-Risk (≥10 pts)	3.678	0.069	53.43	39.58	34.58-45.30	<0.001

FIGURE LEGENDS

Figure 1. Cox proportional risk regression analysis to evaluate lethality of SARS-CoV-2 in Mexico, compared to SARS-CoV2 negative cases for all suspected cases with SARS-CoV2 status available (A) and stratified by diabetes mellitus (B) and obesity (C). *Abbreviations*: ICU= intense care unit; COPD= chronic obstructive pulmonary disease; CKD= chronic kidney disease; CVD= cardiovascular disease, HR= Hazard ratio.

Figure 2. Kaplan-Meier survival curves to evaluate lethality of SARS-CoV-2 positivity in patients with diabetes and comorbidities (A), diabetes and obesity (B), obesity and comorbidities (C) and diabetes with age 40 years (D). Abbreviations: DM= Diabetes mellitus; OB= Obesity; Comorb= Comorbidities.

Figure 3. Logistic regression analyses to evaluate COVID-19 related outcomes in all patients with SARS-CoV2 positivity for admission to ICU (A), mechanical ventilation (B) and hospital admission risk (C).

Figure 4. Symptom onset among lethal and non-lethal cases in new-confirmed COVID 19 cases, stratified by diabetes and obesity status (A), density histogram of scores of the mechanistic COVID-19 score (B). Points and score intervals considered for clinical score scale, where Diabetes & Age<40 represent the score of the interaction term (C) and Kaplan-Meir Survival analysis curves to evaluate lethality using risk categories in the training (D) and validation cohorts (E). Abbreviations: OB= Obesity; DM= Diabetes mellitus; CKD= chronic kidney disease; COPD= Chronic obstructive pulmonary disease.

Figure 1

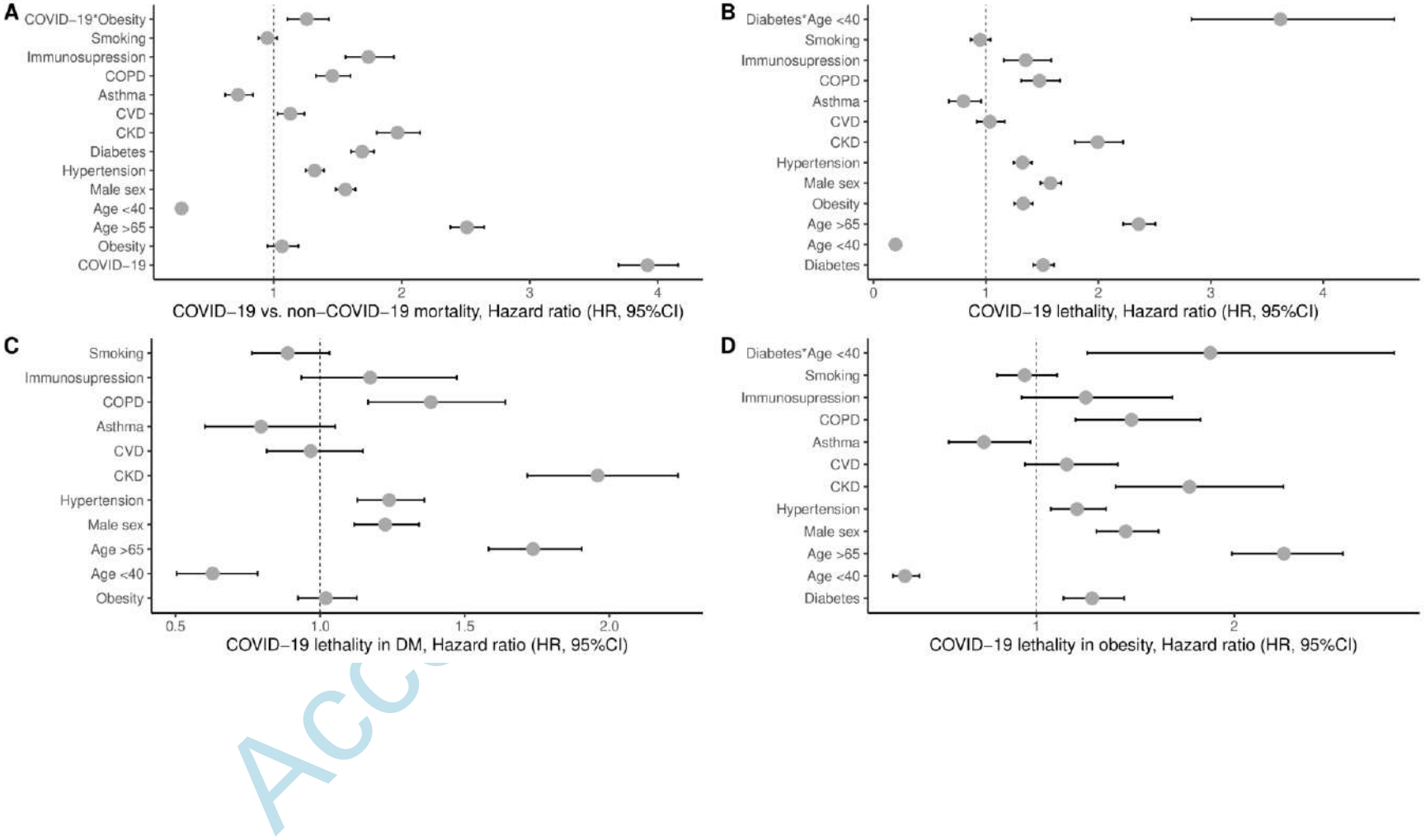


Figure 2

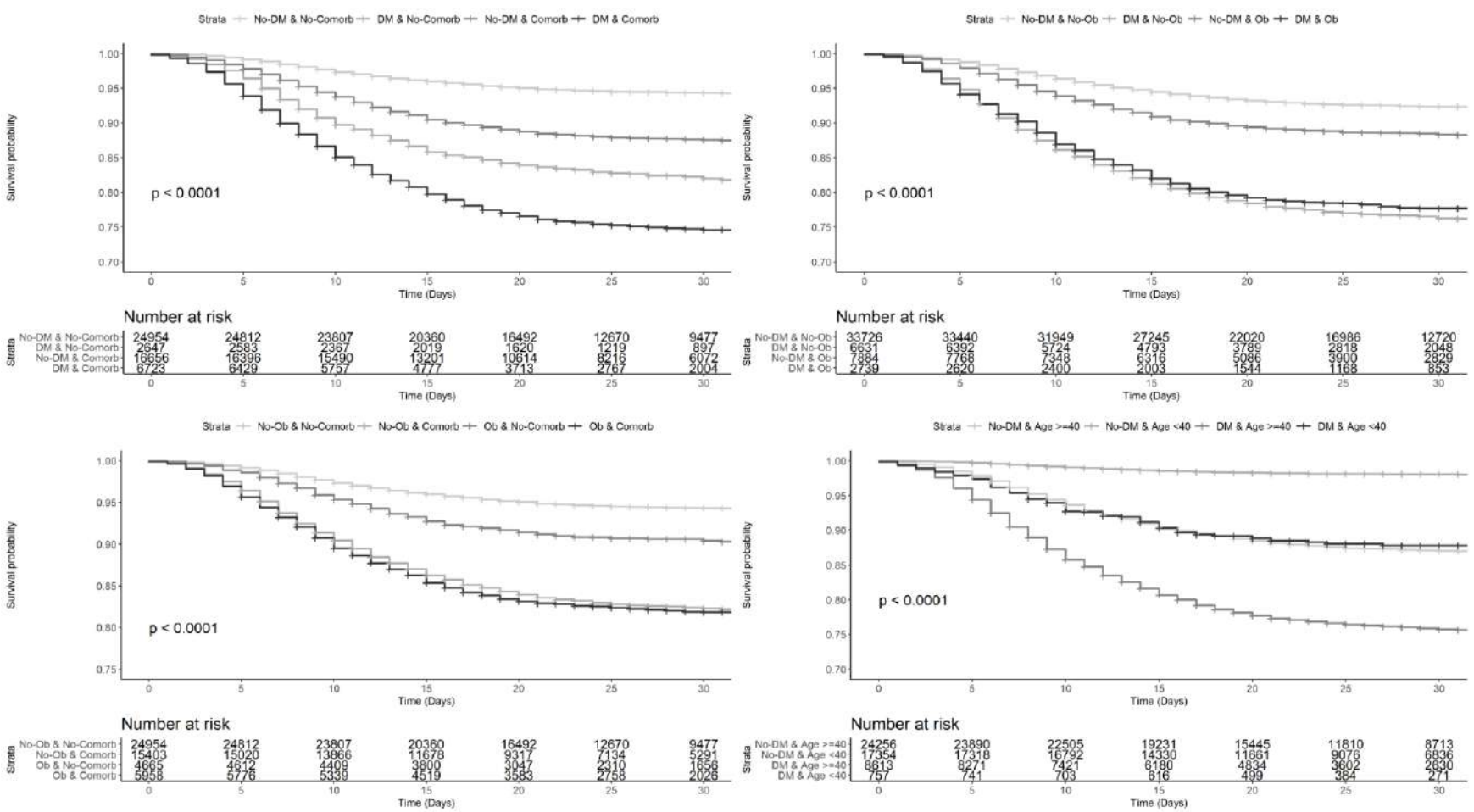

Strata No-DM & No-Comorb DM & No-Comorb No-DM & Comorb DM & Comorb
Survival probability
p < 0.0001
Time (Days)
Number at risk
No-DM & No-Comorb 24954 24812 23807 20360 16492 12670 9477
DM & No-Comorb 2647 2583 2367 2019 1620 1219 897
No-DM & Comorb 16656 16396 15490 13201 10614 8216 6072
DM & Comorb 6723 6429 5757 4777 3713 2767 2004
Strata No-DM & No-Ob DM & No-Ob No-DM & Ob DM & Ob
Survival probability
p < 0.0001
Time (Days)
Number at risk
No-DM & No-Ob 33726 33440 31949 27245 22020 16986 12720
DM & No-Ob 6631 6392 5724 4793 3789 2818 2048
No-DM & Ob 7884 7766 7348 6316 5086 3900 2829
DM & Ob 2739 2620 2400 2003 1544 1168 853
Strata No-Ob & No-Comorb No-Ob & Comorb Ob & No-Comorb Ob & Comorb
Survival probability
p < 0.0001
Time (Days)
Number at risk
No-Ob & No-Comorb 24954 24812 23807 20360 16492 12670 9477
No-Ob & Comorb 15403 15020 13866 11678 9317 7134 5291
Ob & No-Comorb 4665 4612 4409 3800 3047 2310 1656
Ob & Comorb 5958 5776 5339 4519 3583 2758 2026
Strata No-DM & Age >=40 No-DM & Age <40 DM & Age >=40 DM & Age <40
Survival probability
p < 0.0001
Time (Days)
Number at risk
No-DM & Age >=40 24256 23890 22505 19231 15445 11810 8713
No-DM & Age <40 17354 17318 16792 14330 11661 9076 6836
DM & Age >=40 8613 8271 7421 6180 4834 3602 2630
DM & Age <40 757 741 703 616 499 384 271

Figure 3

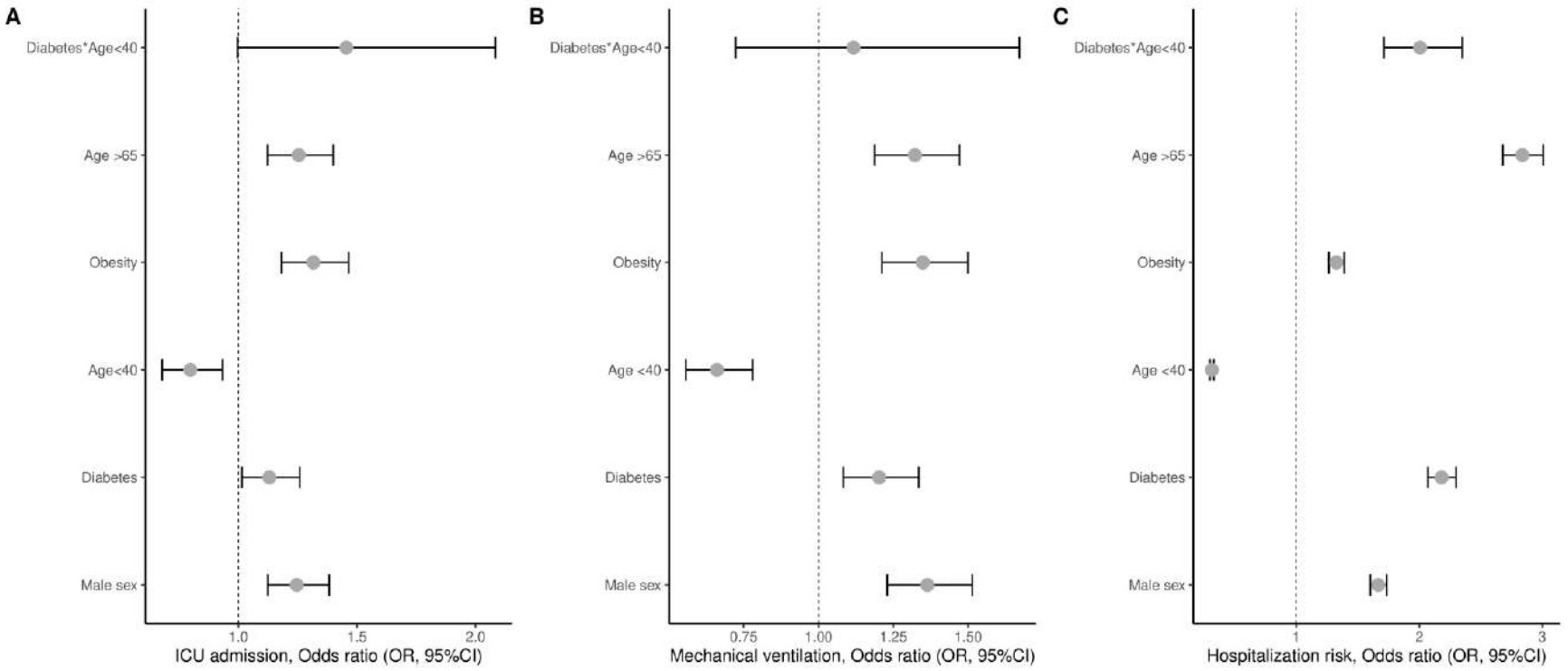
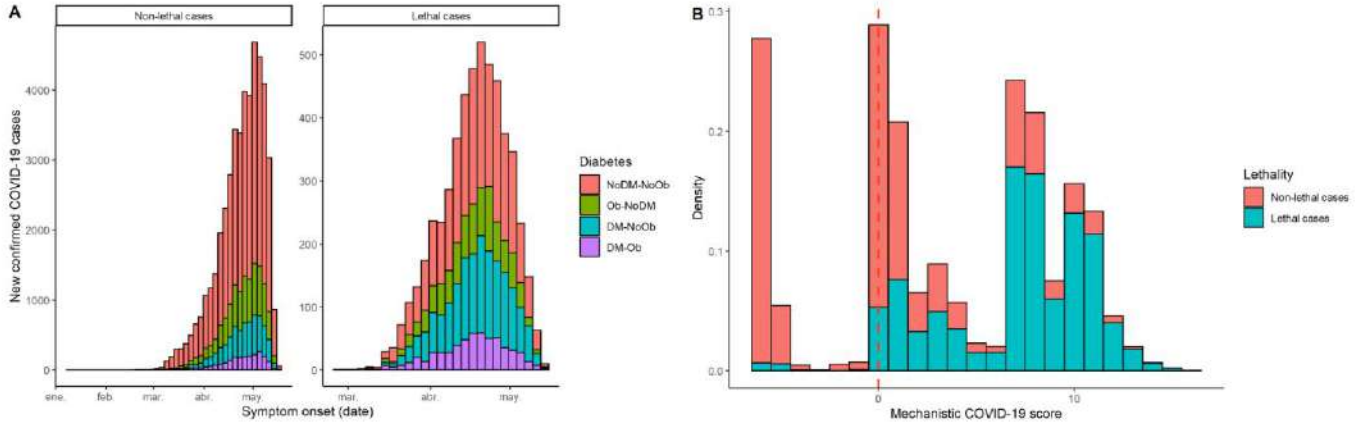
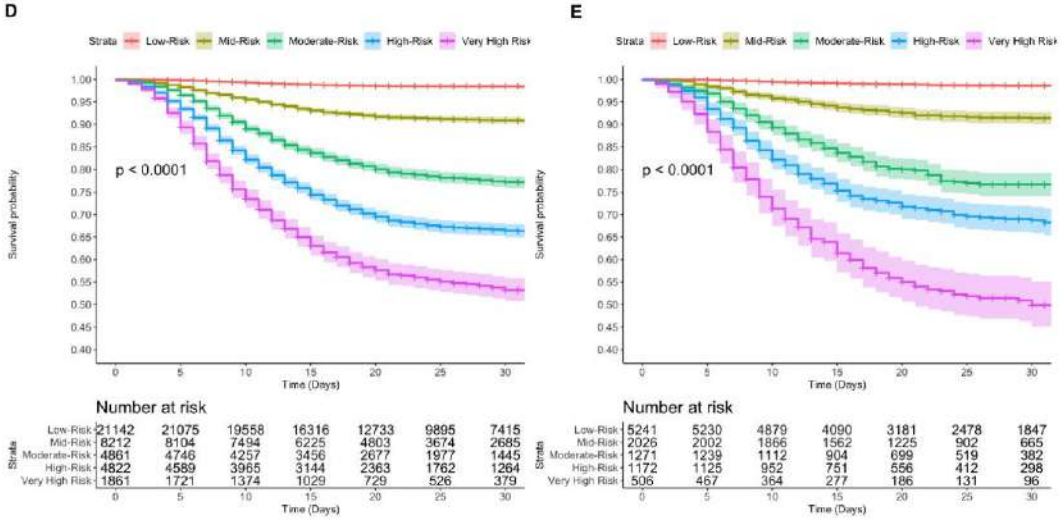


Figure 4



C

Condition	Score
Pneumonia	7
Diabetes & Age <40 years	5
Age ≥65 years	3
CKD	3
Immunosuppression	1
COPD	1
Obesity	1
Diabetes mellitus	1
Age <40 years	-6
Risk Categories	*Score Intervals*
Very High-Risk	≥12
High-Risk	8-11
Moderate-Risk	4-7
Mild-Risk	1-3
Low-Risk	≤0



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Rev Invest Clin. 2020;72(3):151-8 BRIEF COMMUNICATIONS

Impact of Comorbidities in Mexican SARS-CoV-2-Positive Patients: A Retrospective Analysis in a National Cohort

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ABSTRACT

Background: The coronavirus disease 2019 outbreak is a significant challenge for health-care systems around the world. **Objective:** The objective of the study was to assess the impact of comorbidities on the case fatality rate (CFR) and the development of adverse events in patients positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the Mexican population. **Materials and methods:** We analyzed the data from 13,842 laboratory-confirmed SARS-CoV-2 patients in Mexico between January 1, 2020, and April 25, 2020. We investigated the risk of death and the development of adverse events (hospitalization, pneumonia, orotracheal intubation, and intensive care unit [ICU] admission), comparing the number of comorbidities of each patient. **Results**: The patient mean age was 46.6 ± 15.6 years, 42.3% (n = 5853) of the cases were women, 38.8% of patients were hospitalized, 4.4% were intubated, 29.6% developed pneumonia, and 4.4% had critical illness. The CFR was 9.4%. The risk of hospitalization (odds ratio [OR] = 3.1, 95% confidence interval [CI]: 2.7-3.7), pneumonia (OR = 3.02, 95% CI: 2.6-3.5), ICU admission (OR = 2, 95% CI: 1.5-2.7), and CFR (hazard ratio = 3.5, 95% CI: 2.9-4.2) was higher in patients with three or more comorbidities than in patients with 1, 2, or with no comorbidities. **Conclusions:** The number of comorbidities may be a determining factor in the clinical course and its outcomes in SARS-CoV-2-positive patients. (REV INVEST CLIN. 2020;72(3):151-8)

Key words: Coronavirus disease 2019. Comorbidities. Mortality. Adverse events. Severe acute respiratory syndrome coronavirus 2. Demographic characteristic.

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INTRODUCTION

In early December 2019, a group of cases of "pneumonia of unknown origin" was reported in Wuhan, capital of the Chinese province of Hubei[1]. Over the next 2 months, the outbreak spreads rapidly throughout China, and the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified shortly after as the responsible pathogen[2]. What was initially an epidemic quickly spread to the rest of the world, declared as a global pandemic and named coronavirus disease 2019 (COVID-19)[3].

The presence of any comorbidity had previously been shown to condition an increased risk of developing acute respiratory distress syndrome in patients with influenza[4]. In the first reports of SARS-CoV-2 disease, 32% of confirmed patients had concomitant comorbidities and among them, patients admitted to the intensive care unit (ICU) had significantly more[5,6]. Many elderly patients who became seriously ill had evidence of underlying disease, of cardiovascular, hepatic, and/or kidney origin or malignant tumors, and they often died as a result of their original comorbidities[5]. Other studies have reported that overweight and obese patients are at increased risk of admission to the ICU and of a fatal outcome[2].

COVID-19 is an ongoing global pandemic, without a vaccine or effective treatment in the near horizon, so only public health measures may potentially decrease its impact; therefore, all the original comorbidities of individuals infected with SARS-CoV-2 must be accurately assessed[7].

Since comorbidities could be a risk factor for adverse outcomes, the objective of this study is to assess their impact on the case fatality rate (CFR) and on the development of adverse events in patients positive for SARS-CoV-2 in the Mexican population.

MATERIALS AND METHODS

This retrospective, observational study was conducted with a multicenter national cohort of 13,842 patients positive for SARS-CoV-2 in Mexico, between January 1, 2020, and April 25, 2020. The data were obtained from the General Directorate of Epidemiology of the Mexican Ministry of Health that updates daily an open-source data set with information on patients with a suspicious, negative, and definitive diagnosis of COVID-19[8].

This study included patients with a confirmed diagnosis of COVID-19 based on a positive result of the SARS-CoV-2 test by real-time reverse transcription polymerase chain reaction, certified by the National Institute of Epidemiological Diagnosis and Reference. Data were obtained from different medical units in the 32 Mexican states that belong to 14 different institutions integrating the Mexican health sector. The collection of patient data from each medical center caring for patients with COVID-19 is forwarded to the Ministry of Health of Mexico and, once validated, it is uploaded to the epidemiological surveillance platform, and real-time patient data are updated in the cohort daily.

All demographic data (age, origin, sex, nationality, pregnancy, smoking status, date of symptom onset, date of medical attention, contact with another confirmed case, and comorbidities) and clinical data (onset of symptoms, presence of pneumonia, requirement for orotracheal intubation, and the need for intensive care) were collected on arrival at the medical center for hospital care. Depending on the clinical criteria, patients were admitted to a hospital area to continue their treatment and observation or were discharged with outpatient treatment. The death date was updated daily.

Comorbidities were determined by self-report at the time of medical care and classified as present or absent. The defined comorbidity groups were diabetes, chronic obstructive pulmonary disease, asthma, immunosuppression, hypertension, cardiovascular disease (CVD), obesity, and chronic kidney disease (CKD). Comorbidities were classified according to their number in every individual into the following categories: without comorbidities, 1 comorbidity, 2 comorbidities, and ≥ 3 comorbidities.

The primary endpoint was all-cause of death during follow-up, and secondary endpoints were the presence of adverse events defined as hospitalization, the development of pneumonia, intubation, and ICU admission.

Statistical analysis

Data are presented as frequencies and percentages in the case of categorical variables and as means ± standard deviation for continuous or discrete variables. Comparisons were made with the Chi-square test, Student's t-test, and one-way ANOVA with Tukey test *post hoc* analysis. The impact of the number of comorbidities on overall survival was analyzed with the Kaplan-Meier methods with pairwise comparisons and between categories with the log-rank test. Multivariate logistic regression analysis was applied to determine the risk of adverse events for pre-existing comorbidities. Multivariate Cox proportional hazards regression models determined the prediction of the CFR in patients with COVID-19. Variables were entered into the multivariate models at an initial significance level of $p < 0.1$ in the bivariate analysis, using the Enter method to establish the independent contribution of each covariate on adverse events or CFR. Multivariate models were adjusted by sex, age, smoking status, and time from onset of symptoms to initial care. The multivariate-adjusted Cox regression models were rerun in three subgroup analyses: in hospitalized patients, intubated patients, and patients admitted to the ICU. $p < 0.05$ was considered statistically significant. All analyses were performed in the SPSS statistical program version 21 and GraphPad Prism version 6.

RESULTS

Most of the positive cases for SAR-CoV-2 were men (57.7%) with a mean age of 46.6 ± 15.6 years, and 98.7% of cases were Mexican. The prevalence of COVID-19 increases with age. More than a quarter of current cases were in contact with a confirmed case of COVID-19 (Table S1).

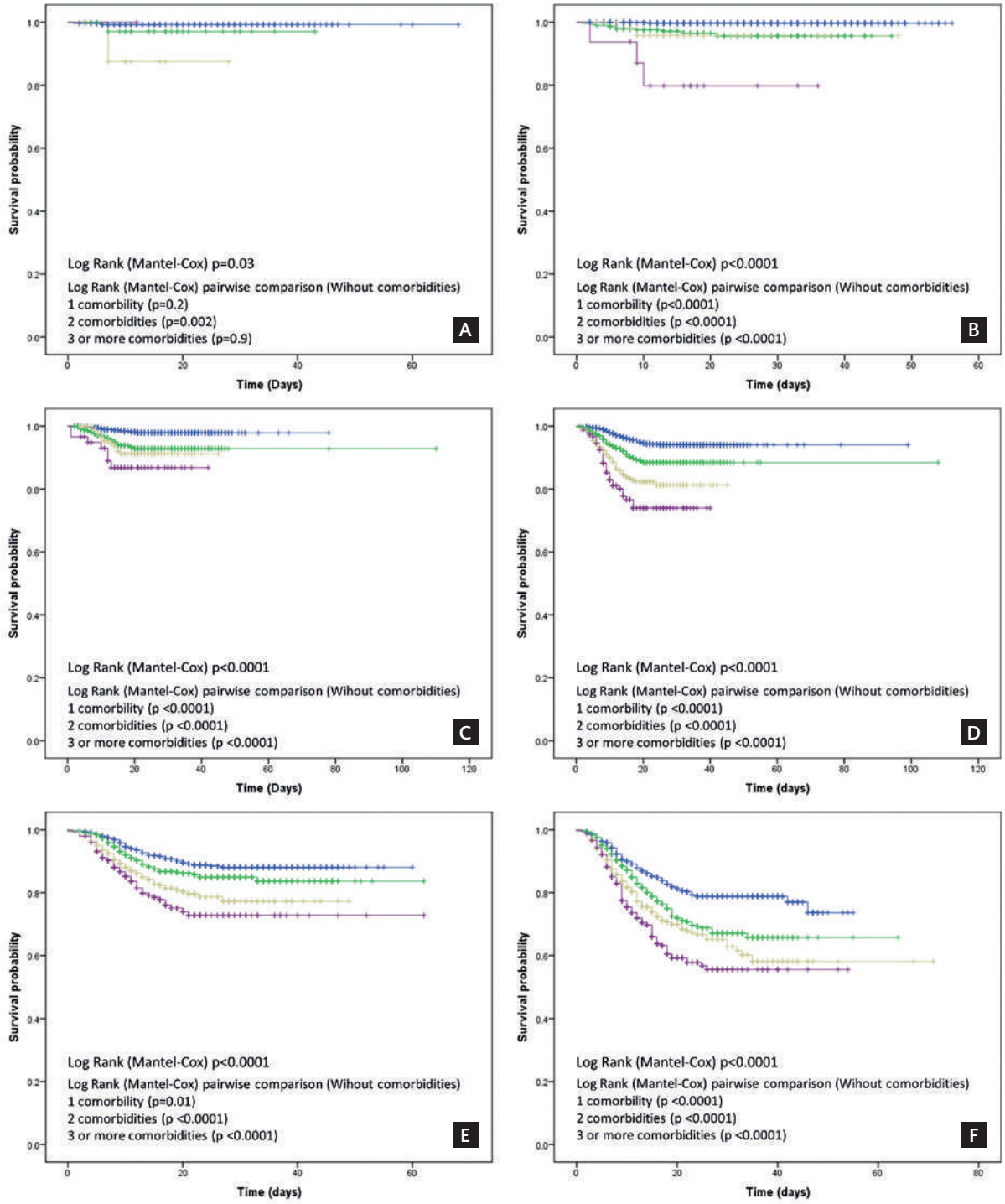
Of 13,842 confirmed cases, 5373 (38.8%) required hospitalization, and 67.7% (n = 3635) of these patients had pneumonia on admission. Among the hospitalized patients, 11.4% (n = 611) required orotracheal intubation and 55.5% (n = 339) of these were admitted to ICU. The total CFR was 9.4% (men: 11.1%, women: 7.1%, $p < 0.0001$), but the CFR in COVID-19 hospitalized patients was 21.9%, and the rates in patients who required orotracheal intubation and in those who required intensive care were 50.2% and 41.5%, respectively.

Among the SARS-CoV-2-positive cases, 45.3% had at least one comorbidity. About 26% of the patients had 1 comorbidity, 12.9% had 2 comorbidities, and 6.4% had ≥ 3 comorbidities. The patients' age increased according to the number of comorbidities (without comorbidities: 41.6 ± 14.3, 95% confidence interval (CI): 41.3-41.9; 1 comorbidity: 49.6 ± 14.9, 95% CI: 49.1-50.1; 2 comorbidities 52.3 ± 14.1, 95 CI %: 54.6-55.9; and ≥ 3 comorbidities: 59.1 ± 13.5, 95% CI: 58.2-60.01; $p < 0.0001$). The proportion of patients who developed adverse events increased with the number of comorbidities and was higher in the groups with two and three or more comorbidities (Table S2).

Survival analysis showed that 95.6% of patients without comorbidities survived, while in patients with 1 comorbidity (88.5%), 2 comorbidities (81.8%), and ≥ 3 comorbidities (73.7%), survival was statistically decreased (log-rank Mantel-Cox, $p < 0.0001$). The greater the patients' age, the lower the survival (≤ 20 years: 98.8%; 21-30 years: 99%, 31-40 years: 97.1%; 41-50 years: 92.6%; 51-60 years: 88%, and > 60 years: 76.3%; log-rank Mantel-Cox, $p < 0.0001$). When survival analysis was performed according to the number of comorbidities and per age group, we observed that comorbidity determines survival regardless of age since it decreases even in younger cases (Fig. 1). Similarly, in hospitalized patients, in those who required orotracheal intubation, and in those who required intensive care, survival was inversely proportional to the number of comorbidities: hospitalized patients: without comorbidities: 85.5%, 1 comorbidity: 77.7%; 2 comorbidities: 71.2%; and ≥ 3 comorbidities: 65.5%; (log-rank Mantel-Cox, $p < 0.0001$); intubated patients: without comorbidities: 58.1%; 1 comorbidity: 49.5%; 2 comorbidities: 40.3%; and ≥ 3 comorbidities: 46.7%; (log-rank Mantel-Cox, $p = 0.002$) and; ICU patients: without comorbidities: 66.4%; 1 comorbidity: 59.4%; 2 comorbidities: 49.1%; and ≥ 3 comorbidities: 48.1%; (log-rank Mantel-Cox, $p < 0.0001$).

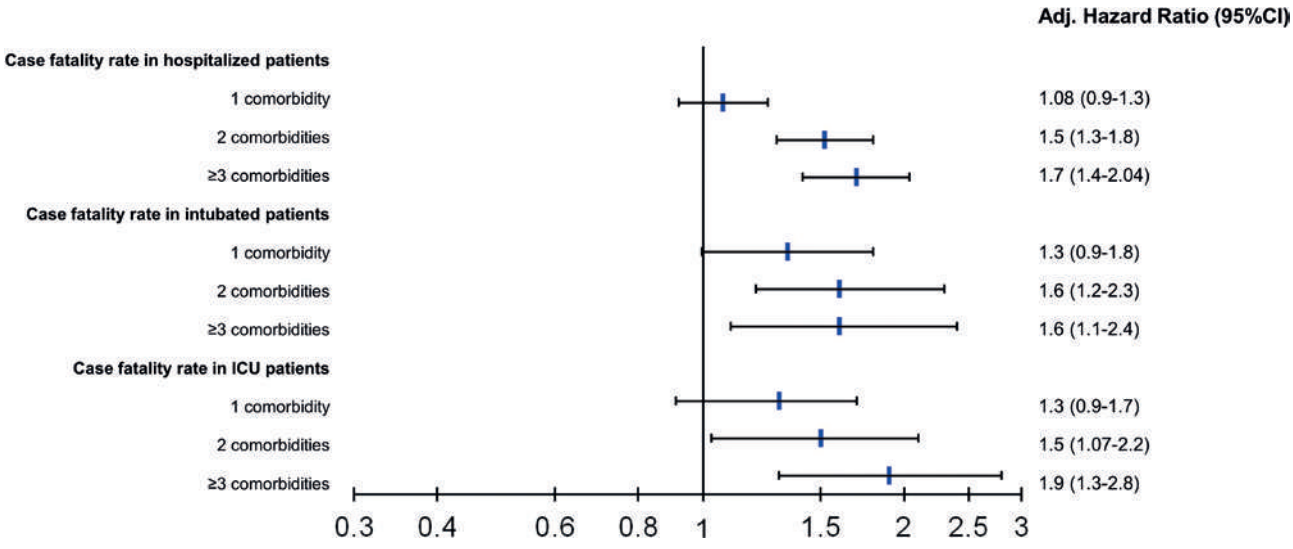
Regression analysis established that the presence of comorbidities increases the risk of hospitalization, development of pneumonia, the requirement for orotracheal intubation, ICU admission, and the CFR.

Figure 1. Survival curves according to age ranges and number of comorbidities. **A**: patients < 20 years, **B**: patients between 21 and 30 years, **C**: patients between 31 and 40 years, **D**: patients between 41 and 50 years, **E**: patients between 51 and 60 years, **F**: patients > 60 years.



Survival probabilities												
	≤20 years		21-30 years		31-40 years		41-50 years		51-60 years		>60 years	
Number of comorbidities	n	%	n	%	n	%	n	%	n	%	n	%
Without comorbidities	282	99.3	1456	99.7	2013	98.5	1793	95.9	1080	91.4	615	83.9
1 comorbility	36	97.3	285	96.6	679	94.7	813	91.3	738	88.4	638	77
2 comorbidities	7	87.5	52	96.3	190	93.6	341	85.3	409	83.6	456	73.1
3 or more comorbidities	1	100	13	81.3	52	88.1	119	78.8	195	78.6	274	66.5

Figure 2. Multivariate analyses of different groups of patients according to the number of comorbidities. Model adjusted for age, sex, and time from onset of symptoms to initial care, the hazard ratio and 95% confidence interval are compared with those obtained in patients with no associated comorbidity.



Diabetes, hypertension, and obesity were the comorbidities established as risk factors for all outcomes. Patients with three or more comorbidities have a higher risk of developing adverse events in comparison with cases with two or one comorbidities; further, compared with those without any comorbidity (Table S3), the combination of diabetes and obesity was the most significant in all outcomes, and diabetes and CKD led to an increased risk of hospitalization, pneumonia, and CFR (Table S4). Different subanalyses were performed in patient subgroups to predict the CFR in hospitalized, intubated, and ICU patients; we observed that the number of comorbidities remains a risk factor for the CFR in SARS-CoV-2-positive patients (Fig. 2).

DISCUSSION

This study analyzes the demographic characteristics of the infected Mexican population and the impact of the number of comorbidities on the development of adverse events and the CFR in SARS-CoV-2-positive patients in Mexico. The most frequent comorbidities in this population were hypertension, obesity, and diabetes, similar findings to those observed in China at the onset of the pandemic[5,9,10,11].

We found that 45.3% of the cases had at least one comorbidity. Italy reported that the vast majority of patients who died from COVID-19 until April 2, 2020, had chronic comorbidities (2.7 diseases on average): 97.2% of patients had at least one comorbidity, 51.3% had three comorbidities, 23.9% two comorbidities, and 22.1% one comorbidity. Hypertension was the most common comorbidity in 72.1% of patients followed by diabetes mellitus in 31.5% and ischemic heart disease in 27.4% of cases[12,13].

The effect of comorbidities on fatality rates is well-known; however, in Mexico, this takes on a new and alarming dimension since the country ranks second in the world in obesity prevalence[14]; according to the ENSANUT 2018 survey[15], 75.2% of the Mexican population over the age of 20 is overweight or obese. Furthermore, the prevalence of diabetes in Mexicans above the age of 20 is 10.3% (8.6 million individuals) and the prevalence of hypertension is 18.4% in patients over 20 years of age and particularly, in those above the age of 70, with a prevalence of 26.7%. This prevalence of metabolic diseases characterizes Mexico as an extremely vulnerable country to the development of complications caused by COVID-19.

Similar to our findings, multiple studies have been conducted to determine the risk factors associated with the development of critical illness requiring mechanical ventilation and leading to death in patients with COVID-19. Thus, the following are consistently

identified as the main risk factors for severe disease: age over 65 years, chronic lung disease, systemic arterial hypertension, CVD, diabetes mellitus, obesity (body mass index [BMI] ≥ 30), immunosuppression, end-stage CKD, and liver disease[1,5,10,16-18].

Diabetes, hypertension, and obesity were the only comorbidities that were statistically significant in all models analyzing adverse events in this cohort of Mexican patients, suggesting that metabolic diseases are a determining factor in the severity of COVID-19. Of these three, only obesity was unaffected by the presence of other risk factors after multivariate adjustment, which would suggest an association between abdominal adiposity and disease severity.

In the context of SARS-CoV-2 infection, BMI has been reported to be significantly higher in critically ill patients compared with other COVID-19 patients (27 ± 2.5 vs. 22 ± 1.3; $p < 0.001$)[19]. Another study established that the BMI of the group of patients with COVID-19 and critical illness was higher than that of patients without critical illness (25.5, interquartile range [IQR]: 23-27.5 vs. 22.0, IQR: 20-24, $p = 0.003$) and that 88.2% of the patients who died from COVID-19 had a BMI above 25[20].

A study in France showed that the risk of requiring invasive mechanical ventilation (IMV) in patients with COVID-19 and with a BMI > 35 is 7 times higher than in patients with a BMI < 25[21]. In New York, patients with a BMI of 30-34 (odds ratio [OR] = 1.8, 95% CI: 1.2-2.7) and patients with a BMI > 35 (OR = 3.6, 95% CI: 2.5-5.3) had a greater risk of requiring admission to the ICU than patients with a BMI < 30[22]. When survival analyses were performed according to the number of comorbidities in each age group, we observed that comorbidity determines survival regardless of age since it decreases it, even in the youngest cases. In the age group between 21 and 30 years in which survival is 99%, in patients with three or more comorbidities, it decreases almost 20% and since most of the Mexican population harbors the three most frequent comorbidities in COVID-19 cases (arterial hypertension, obesity, and diabetes mellitus), it appears that there is a high disposition to the development of adverse events in the Mexican population. This fact could perhaps be related to the chronic pro-inflammatory state associated with obesity and the metabolic syndrome, which favors a prothrombotic and pro-inflammatory environment with higher tissue expression of angiotensin-converting enzyme 2, a protein associated to the binding of SARS-CoV-2 in the alveolar epithelium[23]. Furthermore, adipose tissue has proven to be a viral reservoir for other pathogens such as Ad-36 adenovirus, influenza A virus, HIV, cytomegalovirus, *Trypanosoma gondii,* and *Mycobacterium tuberculosis;* hence, SARS-CoV-2 could remain viable in the adipose tissue of these patients[24].

The population of critically ill patients with COVID-19 represented 4.4% of all cases (n = 611) and 11.4% of these cases were hospitalized, a similar proportion to that reported in other populations with this disease, such as New York[25], Lombardy,[26] and China[17]. Moreover, in a study of 1043 patients admitted to the ICU, 68% had at least one comorbidity, with hypertension being the most frequent (49%), followed by CVD (21%), hypercholesterolemia (18%), and diabetes mellitus (17%). It should be noted that in this study, age as the only risk factor was not found to be a significant variable in terms of requiring admission to the ICU[26].

Results in other cohorts[13,20,26] have reported that their general CFR is lower than that of our cohort in Mexico (9.4% vs. 2.4-7.2% in China and Italy, respectively). However, the CFR in patients who were hospitalized and critically ill was proportionately similar to that reported in other international cohorts, underscoring the fact that in the group of critically ill patients, it is approximately 50%, according to various published series[17,27,28]. There is, however, wide variation in the CFR reported in the subgroup of patients with critical illness and requiring *in vitro* maturation, ranging from 26% to 97%[16]. These variations may be related to the geographical location of hospital centers, their capacity, the availability of ICU, the implementation of specific care protocols in this group of patients, and the specific characteristics of each population. Besides, several of these studies have reported results on patients who were still hospitalized at the time of publication[13,25,26]. Compared with data obtained in the Mexican population during the influenza A H1N1 virus pandemic in 2009[29], the case fatality found in our study in critically ill patients is higher (50.2% in COVID-19 vs. 41.4 %% in H1N1 influenza), which underscores the impact that this disease has

had on the Mexican population and the major challenge it represents for the country's health system.

Another result that should be emphasized in this cohort is that only 55.5% of critically ill patients on IMV were admitted to ICUs; this may be a consequence of the limited availability of beds in critical care units in the national health system (approximately 5200 beds in the country, for a population of just over 126 million inhabitants). This point is relevant, since in a retrospective cohort of patients who died from COVID-19 in China (that has 3.6 intensive care beds per 100,000 inhabitants), the management of this type of patients by a medical team that is not led and coordinated by intensive care physicians and the delay in the implementation of IMV is probably associated with unfavorable outcomes[30].

The main limitation of this study is the validation of the database since we did not directly collect the data included in the database and therefore could not corroborate each of the analyzed variables; all datasets were directly reviewed and validated only by the Mexican Ministry of Health. Other limitations were failure to report the initiation dates of each adverse event, the fact that self-reporting comorbidities could lead to underreporting of cases, particularly since many are subclinical and lead to underdiagnosis, as well as the lack of information on hospital discharges and the underreporting of COVID-19 cases in the Mexican population.

In conclusion, patients with comorbidities are at greater risk of developing adverse events, and their CFR is also increased when compared with previously healthy patients. The number of comorbidities could be a determining factor in the patients' clinical course and outcomes in cases that are positive for SARS-CoV-2. These findings allow us to identify areas of opportunity on which to focus research, improve the quality of information, as well as the clinical outcomes in Mexican patients with COVID-19.

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SUPPLEMENTARY DATA

Supplementary data are available at Revista de Investigación Clínica online (www.clinicalandtranslationalinvestigation.com). These data are provided by the corresponding author and published online for the benefit of the reader. The contents of supplementary data are the sole responsibility of the authors.

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COVID-19 and androgen targeted therapy for prostate cancer patients

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Abstract

The current pandemic (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a global health challenge with active development of antiviral drugs and vaccines seeking to reduce its significant disease burden. Early reports have confirmed that transmembrane serine protease 2 (TMPRSS2) and angiotensin converting enzyme 2 (ACE2) are critical targets of SARS-CoV-2 that facilitate viral entry into host cells. TMPRSS2 and ACE2 are expressed in multiple human tissues beyond the lung including the testes where predisposition to SARS-CoV-2 infection may exist. *TMPRSS2* is an androgen responsive gene and its fusion represents one of the most frequent alterations in prostate cancer. Androgen suppression by androgen deprivation therapy and androgen receptor signaling inhibitors form the foundation of prostate cancer treatment. In this review, we highlight the growing evidence in support of androgen regulation of TMPRSS2 and ACE2 and the potential clinical implications of using androgen suppression to downregulate TMPRSS2 to target SARS-CoV-2. We also discuss the future directions and controversies that need to be addressed in order to establish the viability of targeting TMPRSS2 and/or ACE2 through androgen signaling regulation for COVID-19 treatment, particularly its relevance in the context of prostate cancer management.

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Introduction

The current pandemic of Coronavirus Disease 2019 (COVID-19) is a disease caused by a new coronavirus termed SARS-CoV-2 by the World Health Organization (WHO) and has rapidly spread to at least 114 countries (Park, 2020), with nearly 3 million confirmed cases resulting in over 200,000 confirmed deaths as of April 28, 2020 (https://www.who.int/emergencies/diseases/novel-coronavirus-2019). The clinical spectrum of this novel disease ranges from asymptomatic or mildly symptomatic to severe and critical illness. COVID-19 symptoms include fever, respiratory symptoms (cough and dyspnea), gastrointestinal symptoms (diarrhea and anorexia) (Han et al., 2020), and olfactory and taste disorders (Xydakis et al., 2020). Headache and myalgias are also prominent. It is clear that cancer patients on the whole are at greater risk of contracting COVID-19 and having a poorer prognosis due to a compromised immune system. Patients with severe COVID-19 mount a proinflammatory immune response characterized by excess cytokine production and a paradoxically immune suppressed state with persistently depressed peripheral T cell counts (Zhou et al., 2020). Progressive disease leads to acute respiratory distress syndrome (ARDS), multi-organ failure with cardiac complications, thrombosis, and death, prompting multifaceted approaches to mitigation including immunomodulation in addition to antiviral therapy. Increasing epidemiologic data indicate that although the incidence of COVID-19 is similar between genders, the severity and progression of COVID-19 is significantly greater in men than women. One hypothesis to account for this infection discrepancy is that viral entry is potentially enhanced in the lungs and other tissues of men.

The SARS-CoV-2 virus particle is 60-100 nm in diameter with a single sense strand of nearly 30 kb of RNA. The cellular entry mechanism involves interaction of a viral spike glycoprotein with transmembrane angiotensin-converting enzyme 2 (ACE2) (Jin et al., 2020b). The subsequent cleavage of the spike protein by transmembrane protease serine 2 (TMPRSS2) is necessary for viral infection. Both of these proteins are androgen regulated. While other proteases are involved, TMPRSS2 is of particular interest. It is an exquisitely androgen regulated gene associated with prostate cancer (Bertram et al., 2013). The androgen receptor (AR), and associated signaling, is prominent in prostate tissues as an essential determinant of its development and

progression of both benign and adenocarcinoma tissue. Additionally, the inhibition of the androgen signaling axis impacts organs throughout the body inclusive of the lungs. Androgen targeted therapy may impact susceptibility or mortality risk in prostate cancer patients. This review discusses the current considerations for prostate cancer patients and their treatment in the context of the COVID-19 pandemic, as our understanding of the ramifications of this new prostate cancer comorbidity emerges.

Hypothalamic-pituitary-gonadal axis

Androgens, in particular testosterone, are critical in the pathogenesis and evolution of prostate cancer as underscored by seminal work by Huggins in the 1940s that formed the theoretical basis for androgen deprivation therapy (Huggins, 1942). In a healthy adult male, approximately 90% of circulating testosterone levels are secreted by Leydig cells in the testes with about 5-10% being produced by the adrenal glands. The biosynthesis of testosterone is regulated by the hypothalamic-pituitary-gonadal (HPG) axis whereby pulsatile release of gonadotropin-releasing hormone (GnRH) from the hypothalamus regulates synthesis and secretion of gonadotropins (luteinizing hormone or LH and follicle-stimulating hormone or FSH) from the pituitary gland (Figure 1). LH, in turn, stimulates synthesis of testosterone in the gonads (testicles). GnRH and LH signaling is inhibited by increasing systemic concentrations of testosterone (Kluth et al., 2014). Testosterone is the primary circulating male hormone with only 1-2% existing in a free state, the remaining bound to sex hormone-binding globulin or albumin (Imamoto et al., 2010). DHT is the primary androgen in peripheral tissues resulting from testosterone conversion by the enzyme 5α-reductase in the prostate, testes, skin, hair follicles, and adrenals (Imamoto et al., 2008).

Although pulsatile stimulation of the pituitary by GnRH is required for generation of gonadotropins, continuous stimulation of pituitary GnRH receptors results in desensitization and downregulation of these receptors with eventual decrease in circulating sex steroids inclusive of androgens (Kluth et al., 2014). This negative-feedback loop serves as the mechanism of action of luteinizing-hormone-releasing hormone (LHRH)

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agonists, while antagonizing the LHRH receptor represents another method of suppressing testosterone secretion through inhibition of downstream LH signaling (Figure 1).

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)

SARS-CoV-2 was originally isolated from the bronchoalveolar lavage fluid of 3 infected patients from Wuhan in December 30, 2019. It is an enveloped, single-stranded sense RNA virus with a genome size of 29.9 kb with a nucleoprotein (N) wrapping the RNA genome, forming a coiled tubular structure called the nucleocapsid (Jin et al., 2020b). The viral envelope houses the nucleocapsid and is where several associated structural proteins are located including the spike glycoprotein (S), membrane (M) protein, and envelope (E) protein. The SARS-CoV-2 genome has 5' and 3' terminal sequences containing the 5 essential genes encoding for the 4 structural proteins with a gene order 5′-replicase open reading frame (ORF) 1ab-S-envelope(E)-membrane(M)-N-3′, highly conserved amongst the β-coronaviruses (Jin et al., 2020b; Park, 2020).

The inflammatory response to infection can potentiate a cytokine storm involving the upregulation of tumor necrosis factor-α (TNFα), interleukin 1β (IL-1β), IL-6, monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory proteins 1-α (MIP1α). Although the source of the cytokine storm is not yet clear contributing to the multi-organ dysfunction, uncontrolled viral replication and deleterious inflammatory responses may both contribute to tissue damage evident in the lungs. In later stages of disease, evidence of abnormal coagulation with prothrombotic tendency is a poor prognostic factor. The elevated expression of ACE2 on vascular endothelia is thought to be a potential mechanism for the coagulation and associated hypotension (Lukassen et al., 2020). However, the predominant site of ACE2 expression is on type II alveolar (AT2) cells of the lung, resulting in the greatest damage to these cells during SARS-CoV-2 infection. Long term outcomes of lung damage caused by SARS-CoV-2 are not yet well-described as most patients are still in the early stages of infection or recovery, but based on our knowledge of ARDS in other infections including influenza, recovery can be protracted, with lasting fibrosis. Other organ systems including the heart, digestive,

and urinary tracts have been shown to express ACE2 and may serve as additional at-risk targets for SARS-CoV-2 (Zou et al., 2020). Thus, infection prevention is the holy grail.

SARS-CoV-2 transmission, host entry, and antigenicity

All human coronavirus infections are caused by 2 of 4 subfamilies of coronaviruses, the α- and β-coronaviruses; these viruses cause respiratory, hepatic, neurologic, and enteric diseases (Jin et al., 2020b). Respiratory secretions or droplets and direct contact have been recognized as the main modes of SARS-CoV-2 transmission, while reports exist of fecal-oral transmission (Jin et al., 2020b; Park, 2020). The transmissibility of SARS-CoV-2 through aerosols and vertical transmission remains poorly described (Jin et al., 2020b; Park, 2020). The mean incubation period has been shown to be 5.2 days (95% confidence interval or CI 4.1-7.0) with a basic reproductive number estimated to be 2.2 (95% CI 1.4-3.9) (Li et al., 2020b).

Early investigations identified that ACE2 was expressed in human airway epithelia with more abundant expression in the apical surface and well-differentiated cells and served as the functional receptor for viral entry into host cells for the original SARS-CoV (Jia et al., 2005). It has been shown that human ACE2 also mediates SARS-CoV-2 entry into host cells through tight binding of the viral S protein domain B (S^B) to human ACE2 (Walls et al., 2020). In elegant sequencing studies of SARS-CoV-2, the presence of a furin cleavage site at the S_1/S_2 boundary that is processed during viral biosynthesis sets SARS-CoV-2 apart from SARS-CoV and may enhance the tissue tropism and/or transmissibility of SARS-CoV-2 compared to SARS-CoV given the near-ubiquitous distribution of furin-like proteases in the human body (Walls et al., 2020). The cellular serine protease, TMPRSS2, mediate S protein cleavage at the S_1/S_2 site, seemingly a required priming event for subsequent SARS-CoV-2 interaction with ACE2 and cell entry (Hoffmann et al., 2020). Co-expression of ACE2 and TMPRSS2 has been identified in type II pneumocytes, absorptive enterocytes of the small intestine, and nasal goblet secretory cells, all typical sites of COVID-19 symptoms (Ziegler et al., 2020).

Because of its crucial role in cell entry and viral propagation, the coronavirus S glycoprotein is suspected to be the primary target of neutralizing antibodies following infection and is the focus of current therapeutic and vaccine designs. Mapping of the S protein demonstrated a more conserved S_2 subunit while

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the S_1 subunit showed the highest divergence given that it is more exposed at the viral surface and potentially subjected to more selection pressure from the host immune system. Given that the SARS-CoV-2 and SARS-CoV S^B domains share 75% amino acid sequence identity and most SARS-CoV neutralizing antibodies target the S^B receptor binding domain, cross-reactivity across coronaviruses may not necessarily be achieved (Yuan et al., 2020).

COVID-19 clinical presentation

In the initial cohort of 41 patients diagnosed with laboratory-confirmed SARS-CoV-2, majority of the infected patients were aged 25-49 (49%) with a median age of 49.0 years and male (73%) (Huang et al., 2020). The median time from onset of illness to shortness of breath or dyspnea was 8.0 days (interquartile range or IQR 5.0-13.0), 7.0 days (IQR 4.0-8.0) for hospital admission, and 9.0 days for ARDS (IQR 8.0-14.0). Laboratory parameters showed leukopenia (white blood cell or WBC count less than 4 X 10^9/L) in 25%, lymphopenia (lymphocyte count < 1.0 X 10^9/L) in 63%, elevated lactate dehydrogenase (LDH, > 245 U/L) in 73%, elevated aspartate aminotransferase (> 40 U/L) in 37%, and a normal procalcitonin level (< 0.1 ng/mL) in 69%. On admission, abnormalities in chest computed tomography (CT) were detected in all patients with bilateral involvement of the lungs in 98% of patients. The most common complications included ARDS (29%), acute cardiac injury (12%), and secondary infection (10%) with mechanical ventilation required in 10% of patients. At the time of their report, 68% of patients had been discharged, while 15% had died (Huang et al., 2020)

Higher levels of prothrombin time and D-dimer on admission were also identified in patients requiring intensive care unit (ICU) care compared to non-ICU patients (Huang et al., 2020). Indeed, coagulopathy has emerged as a key aspect impacting COVID-19 mortality (Tang et al., 2020) although it is unclear yet whether this is different in male versus female patients. Initial plasma concentrations of IL-2, IL-7, IL-10, GCSF, CXCL10, CCL2, CCL3, and TNFα were also higher in ICU patients than non-ICU patients (Huang et al., 2020) raising the potential that innate immune factors contribute to severity of the COVID-19 course. Notably, there are known influences of androgens and estrogens on immune biology relating specifically to viral infection

which may be important (Klein, 2012). Influences of sex hormones also act differently in men and women, accounting in part for the persistent differences in male patients and post-menopausal women. In both the Italian and Chinese datasets there was higher incidence of severe disease in male patients; of 355 fatal COVID-19 cases in Italy, 30% were women (Onder et al., 2020). In all the aforementioned series, fatal cases occurred more often in patients with diabetes (20-35% compared to <10% in non-fatal cases), coronary artery disease (10-30% compared to about 10%), and in the Italian series 20% had active cancer (Onder et al., 2020). Future studies may reveal the cumulative impact of the inflammatory and metabolic insults on prostate cancer progression.

Mechanisms of SARS-CoV-2 and gonadal dysfunction

There is growing evidence to suggest that the testes may be predisposed for damage by SARS-CoV-2. ACE2 expression in tissues has previously been shown to be programmable by stresses including hypertension, whereby the lungs were the only site of increased ACE2 activity in preclinical models (Riviere et al., 2005). A separate group using adult human testis single-cell RNA sequencing datasets identified that ACE2 was primarily enriched in spermatogonia and Leydig and Sertoli cells (the latter group of Leydig and Sertoli cells could not be separated into individual clusters on analysis), whereas early spermatocytes, late spermatocytes, spermatids and other somatic cells had very low expression levels of ACE2 (Wang and Xu, 2020). Interestingly, ACE2 expression in Leydig and Sertoli cells was 3-fold higher compared to ACE2-expressing AT2 cells (4.25% vs. 1.40%). Further analysis showed that ACE2-positive spermatogonia express a higher number of genes associated with viral reproduction and transmission with a lower number of genes related to spermatogenesis compared to ACE2-negative spermatogonia, while ACE2-positive Leydig and Sertoli cells express a higher number of genes involved in cell-cell junction and immunity with a lower number of genes associated with mitochondria and reproduction (Wang and Xu, 2020). A second study has illustrated that high ACE2 expression was found in seminiferous ducts and Leydig cells of the testes as well (Fan et al., 2020).

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Preclinical work demonstrated that overexpression of TMPRSS2 to approximately 10-fold higher than normal human lung tissues conferred high susceptibility to SARS-CoV-2 infection compared to control (Fan et al., 2020). Moreover, one group using adult human testis single-cell RNA sequencing demonstrated that TMPRSS2 expression was concentrated in spermatogonia and spermatids with relatively low levels in other cell types of the testis (Wang and Xu, 2020). The constellation of these findings suggest that the testis may represent a high-risk organ vulnerable to SARS-CoV-2 infection that could result in spermatogenic failure. As described earlier, this process is likely mediated by SARS-CoV-2 S protein binding to ACE2 and S protein priming by TMPRSS2, (Hoffmann et al., 2020) of which these 2 critical targets for viral entry are specifically expressed in male gonads.

SARS-CoV-2, androgen regulation, and prostate cancer

The impact of SARS-CoV-2 infection on male gonadal function was first described in a retrospective series by Ma et al. (Ma et al., 2020). In this single-institution study, 81 male patients with laboratory-confirmed SARS-CoV-2 infection underwent evaluation of serum sex hormone levels that were compared to 100 age-matched healthy men. The median age of the SARS-CoV-2 group was 38 years with 14.81%, 44.44%, 33.33%, and 51.85% having received corticosterone, arbidol, oseltamivir, and intravenous (IV) antibiotics respectively. Thirty-one patients (38.27%) in this group had elevated serum alanine transaminase and/or serum aspartate transaminase. Compared to age-matched healthy male controls, those with SARS-CoV-2 infections had significantly higher serum LH ($p < 0.0001$) and serum prolactin ($p < 0.0001$). Whereas there was no statistical difference between serum testosterone or FSH levels between groups, there was a significantly decreased testosterone:LH ratio ($p < 0.0001$) and FSH:LH ratio ($p < 0.0001$) seen in the SARS-CoV-2-positive group. On multivariable analysis, C reactive protein levels were significantly associated with serum testosterone:LH ratio in SARS-CoV-2 patients ($p = 0.0128$). It is worthwhile to note that this study was limited by its retrospective nature, relatively small sample size, lack of confirmation of SARS-CoV-2 in semen or testes, proportion of steroid use and potential other factors influencing the HPG axis (*e.g.*, stress), and uncertainty of timepoint of collection of samples. As more recently the presence of SARS-CoV-2 was identified

in semen of infected men (Li et al., 2020a), the compelling analysis implicate SARS-CoV-2-infected males impaired testosterone production may stimulate LH release to maintain testosterone levels during early stages of hypogonadism based on negative feedback regulation (Ma et al., 2020). This phenomenon may be one explanation for the dramatically decreased testosterone:LH ratios observed compared to age-matched controls. Furthermore, the authors argue that the observed elevated prolactin levels in infected males could contribute to pituitary suppression and decreased gonadotropins, but prolactin secretion can be affected by a multitude of factors, in general. FSH is mainly suppressed by inhibin B from Sertoli cells and given that there was no statistically significant difference in FSH levels between infected and healthy men, Leydig cells rather Sertoli cells appear more susceptible to injury from SARS-CoV-2.

The original form of androgen deprivation therapy as described by Huggins, bilateral orchiectomy, remains in use worldwide but has largely been replaced by pharmaceutical castration via manipulation of the HPG axis as the cornerstone of prostate cancer therapy where such drugs are available. The recent treatment landscape for prostate cancer has evolved with the advent of agents that target the androgen signaling pathway by direct antagonism of the receptor or specific inhibition of CYP17. This broader class of agents that inactivate the androgen signaling pathway is better described as androgen-targeted therapy (Crawford et al., 2019), but will be briefly summarized below.

First-generation oral nonsteroidal antiandrogens such as bicalutamide, flutamide, and nilutamide compete with DHT for binding AR are assocaited with a moderate rise in testosterone. Hence, these agents are most commonly used in combination with LHRH agonists to reduce the impact of a testosterone surge (Boccardo, 2000; Crawford et al., 2019). Leuprolide acetate (LUPRON®) is among the most common LHRH agonists used in the achieving testosterone levels below the U.S. Food and Drug Administration (FDA) defined castration level of 50 ng/dL in 93-100% of patients receiving the drug, usually by day 21 (Crawford et al., 2019). As with leuprolide, goserelin acetate (ZOLADEX®) is another LHRH analogue that is associated with a testosterone rise during the first week but fall within castrate range (< 3 nmol/L) by 21 days (Cockshott, 2000). A LHRH antagonist, degarelix (FIRMAGON®) is approved for the treatment of advanced prostate cancer and is available as a once monthly subcutaneous dose and competitively binds to the LHRH receptor that inhibits

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downstream LH signaling and suppresses testosterone without an initial testosterone surge seen with LHRH agonists (Klotz et al., 2008). Degarelix has been shown to achieve castrate levels of testosterone (≤ 0.5 ng/mL) within 1-3 days of administration in 99-100% of patients (Klotz et al., 2008). Abiraterone acetate (ZYTIGA®) is an oral, androgen biosynthesis inhibitor that blocks testosterone production through inhibition of the enzyme CYP17 (O'Donnell et al., 2004; Attard et al., 2008). It is most commonly combined with prednisone and LHRH agonists to reduce androgen production from all sources including the testes, adrenal glands, and prostate cancer cells. In castrate-naïve prostate cancer patients (testosterone level of > 9 nmol/L), treatment with abiraterone results in castrate levels of testosterone (≤ 2.0 nmol/L) rapidly but often is not sustained beyond 3 days (O'Donnell et al., 2004). Notably, the combination of abiraterone and LHRH agonists (leuprolide) profoundly suppresses testosterone levels to those generally seen with LHRH agonists alone (Taplin et al., 2014). Next-generation orally available nonsteroidal androgen receptor inhibitors enzalutamide (XTANDI®), apalutamide (ERLEADA™) and darolutamide (NUBEQA™) competitively bind the ligand binding domain of the AR to limit nuclear translocation and downstream signaling. The principal gonadal suppressing effects are likely from the LHRH agonism given that monotherapy studies of enzalutamide in castrate-naïve (testosterone levels ≥ 230 ng/dL) prostate cancer patients show that testosterone levels rise sharply between 1 and 5 weeks, after which testosterone leveled off between 13 and 49 weeks with enzalutamide (Tombal et al., 2015). Apalutamide and darolutamide have limited penetration in the central nervous system compared to enzalutamide, suggesting a lower tendency to affect the HPG axis (Clegg et al., 2012; Moilanen et al., 2015). Infectious complications were not noted to be higher in phase III randomized trials, suggesting no clinically relevant immune compromise associated with androgen receptor targeting. The impact of these agents on overall immune function has not been well described.

Clinical relevance of SARS-CoV-2 and androgen suppression in prostate cancer

Although men and women have a similar susceptibility to SARS-CoV-2, men appear to be more prone to greater disease severity and mortality independent of age (Jin et al., 2020a). *TMPRSS2* gene fusions have historically been shown to contribute to prostate tumorigenesis with approximately 50% of prostate cancer

cases being positive for TMPRSS2-ERG fusions (Tomlins et al., 2008). Being that *TMPRSS2* is an androgen responsive gene, one group analyzed publicly available genomic datasets and determined that *TMPRSS2* mRNA expression levels in human normal lung samples in men were higher than those in women ($p = 0.029$) (Asselta et al., 2020). In searching for genetic variants of *TMPRSS2* that could account for variations in disease severity in the European population (particularly in Italy where SARS-CoV-2 cases and mortality are among the highest in the world), a frequent European haplotype linked to a known androgen-responsive enhancer for *TMPRSS2* and thus expected to upregulate *TMPRSS2* in an androgen-dependent manner was virtually absent in the Asian population. A second haplotype was also significantly increased in Italians compared to East Asians and was putatively associated with higher *TMPRSS2* expression. Of 4 single nucleotide polymorphisms (SNPs) found to significantly differ between the Italian population and East Asians, one was found to be of lower frequency in Italians compared to East Asians and other Europeans. Interestingly, this one variant involved a missense substitution of an exon variant, p.Val160Met, which affects a residue far from the serine protease catalytic triad and has been associated with genomic rearrangements in *TMPRSS2* and high-risk prostate cancer (Asselta et al., 2020). In an Italian population where the male cancer patients had a higher risk of having COVID-19 ($n = 4532$; OR 1.79; CI 1.62-1.98), Montololi et al. recently reported that ARSIs may provide partial protection from SARS-CoV-2 infection (Montopoli et al., 2020). Of note, although one group identified a higher homozygous mutation rate of *ACE2* in males than females, ACE2 mRNA expression levels in the lung from a large dataset were not substantially different between males and females (Asselta et al., 2020; Cao et al., 2020).

This collection of data of a more aggressive course of COVID-19 in men that is possibly associated with TMPRSS2 expression and androgen regulation can suggest an intriguing possibility where androgen signaling influences disease severity and mortality with SARS-CoV-2 infection. It has been well documented that TMPRSS2 is expressed in the lung and human epithelial airway cells where its activity is important to facilitate viral entry into host cells for human coronaviruses including SARS-CoV-2 (Bertram et al., 2013; Hoffmann et al., 2020). However, lung tissues in mice and humans did not support *TMPRSS2* as a determinant of sex differences in SARS-CoV-2 infection as there were similar levels of gene expression in males and females

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(Baratchian et al., 2020; Stopsack et al., 2020). Other studies have shown that camostat mesylate, a clinically proven serine protease inhibitor that is active against TMPRSS2 inhibited SARS-CoV-2 S protein-driven viral entry into TMPRSS2-expressing human lung cell lines, but not in TMPRSS2-null cell lines (Hoffmann et al., 2020). The decreased infection with SARS-CoV-2 supported TMPRSS2 as a viable target in the treatment of SARS-CoV-2.

Androgen receptors have been shown to be present in both normal human lungs and lung cancer specimens where administration of androgens in cultured human lung cancer cells resulted in a significant upregulation of *TMPRSS2* with androgen-dependent loading of the AR protein onto the *TMPRSS2* enhancer (Mikkonen et al., 2010). Conversely, mining of public genomic datasets has shown that treatment with estradiol (prolonged) or AR antagonists (*i.e*., enzalutamide) significantly downregulated *TMPRSS2* (Wang et al., 2020). As expected, *TMPRSS2* expression increased between 1.4- and 20-fold following treatment with androgens, synthetic androgens, or short-doses of estradiol. In addition to estrogen and androgen-related compounds, other treatments that decreased *TMPRSS2* expression included dual TGF-ß and EGF treatment (Wang et al., 2020).

Chromosomal rearrangements fusing the androgen-regulated gene *TMPRSS2* to the oncogenic ETS transcription factor *ERG* occurs in about 50% of prostate cancers and attenuates androgen signaling through direct inhibition of AR expression and downregulating AR signaling at gene-specific loci (Yu et al., 2010). In preclinical prostate cancer models, ERG overexpression represses AR target genes and significantly suppresses *TMPRSS2* when comparing ERG+ tumors to ETS- prostate tumors (Yu et al., 2010). Accordingly, knockdown of *ERG* resulted in *AR* upregulation. This is of potential significance given that one SNP that has been associated with genomic rearrangements in *TMPRSS2* and high-risk prostate cancer was found in significantly lower frequency in an Italian cohort, a group with notably higher COVID-19 incidence and mortality (Asselta et al., 2020). Altogether, these results possibly indicate that expression of TMPRSS2, a target of SARS-CoV-2, in human lungs is modulated by estrogens and androgens and can be repressed by treatment with estrogens or androgen receptor antagonists as a therapeutic strategy against COVID-19. Men receiving

ARSI therapy because of their diagnosed *TMPRSS2*:*ERG* fusion expressing prostate cancer may represent a subgroup of patients with reduced infectivity by SARS-CoV-2 and a lesser disease severity with COVID-19.

ACE2 is recognized as both the entry receptor and serving a lung protective function from injury to human coronaviruses including SARS-CoV-2. Viral S protein binding and downregulation of ACE2 may promote excess angiotensin II production and enhanced lung vascular permeability, acute lung injury, and tissue damage (Imai et al., 2005). Using VeroE6 cells, treatment with clinical-grade human recombinant soluble ACE2 (hrsACE2), which has already been tested in phase 1 and phase 2 clinical trials, significantly inhibited SARS-CoV-2 infection in a dose-dependent manner as compared to controls. Treatment with mouse recombinant soluble ACE2 did not inhibit the infection. Inhibition of SARS-CoV-2 infection was also demonstrated with hrsACE2 treatment in human capillary and kidney organoids that modeled the ability of the virus to infect blood vessels prior to local organ infections (Monteil et al., 2020).

Androgen axis regulation also appears to affect ACE2 expression. In early animal studies, testosterone and DHT downregulated ACE2 mRNA and protein expression in the vasculature in an ERK1/2 MAP kinase-signaling dependent manner while testosterone withdrawal by castration significantly elevated ACE2 mRNA and protein levels.(Mishra et al., 2016) A separate study has shown that chronic blockade of the androgen receptor with the antiandrogen flutamide in the absence of a change in estradiol significantly increased renal ACE2 mRNA expression in rats compared to control (Dasinger et al., 2016). In an analysis of RNA-sequencing/microarray datasets, transgender males who underwent androgen targeted therapy for 1 year showed significantly higher ACE2 expression and more ACE2-expressing cells among Sertoli cells in the testis (Chen et al., 2020). However, cell line screens for transcriptional inhibitors of ACE2 have shown that the synthetic androgen R1881 was among the compounds that upregulated ACE2 although ACE2 expression was poorly expressed in cell lines profiled by the drug transcriptome screen (Wang et al., 2020). Therefore, although SARS-CoV-2 may be targeted through TMPRSS2 and androgen signaling suppression, developing a similar approach through androgen suppression and ACE2 is less clear. Evidence to date suggests that ACE2 expression may be enhanced with androgen suppression. It is unknown whether this would result in a net increase in risk for severe infection, or a potential benefit from preserving the protective effects of ACE2 protein

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expression, while hindering a key step in conformational change and viral entry to nonimmune cells. Further understanding of these factors is essential to optimizing use of androgen suppression in the setting of SARS-CoV-2.

Current management of prostate cancer during the COVID-19 pandemic and controversies

Androgen signaling inhibition is a hallmark treatment strategy for prostate cancer across multiple stages of disease. Despite the previously described evidence in support of a potential and serendipitous therapeutic strategy against SARS-CoV-2 by targeting TMPRSS2 in human lung tissue using a standard therapy in prostate cancer patients, where do we stand in its clinical application during the current COVID-19 pandemic? Further investigation is certainly needed prior to clinical application. Firstly, it would be beneficial through observational or retrospective analyses to describe the impact of TMPRSS2 expression in non-tumor lung tissues from patients with prostate cancer treated with AR signaling inhibitors and those from individuals with normal androgen levels. If the data is supportive, clinical trials could be rapidly implemented to test the strategy of downregulating TMPRSS2 by androgen suppression as a treatment for COVID-19 given the widespread use, familiarity with the safety profiles, and knowledge of the onset of androgen suppression of classical androgen deprivation drugs (*e.g.*, leuprolide) and oral AR signaling inhibitors (*e.g.*, enzalutamide, apalutamide, or darolutamide) that are currently used in prostate cancer treatment.

However, the impact of androgen signaling suppression on ACE2, another critical target of SARS-CoV-2 infectivity, would also need to be further evaluated across larger populations. Preclinical work suggests that chronic androgen blockade could in fact upregulate ACE2 expression and this potentially negative consequence would need to be counter-balanced in considering its effect on the severity and mortality from COVID-19. Leuprolide is also a frequently used drug in fertility preservation for women. The role of estrogen in mediating infection by SARS-CoV-2 needs to be evaluated, particularly through its impact on viral entry receptors in the lung. Estrogen therapy significantly increases ACE2 expression in the mouse thymus and human testes, while estrogen has been shown to exert a tissue-specific effect by regulating ACE2 expression in the kidneys, but not in lungs (Mishra et al., 2016; Chen et al., 2020). Additional study on a broader level of

how androgens and estrogens impact immune biology and how sex hormones between men and women differentially influence viral infection is beyond the scope of this review but is certainly warranted amidst the current pandemic. Within this topic, one could also look into whether hormone levels in an older population affect the expression of ACE2 and TMPRSS2 and/or risk of SARS-CoV-2 infection when compared to a younger population. Moreover, the effects of SARS-CoV-2 on gonadal function in humans is limited to one retrospective study, with its effects on gonadal function in human subjects with prostate cancer treatment remain unknown. The long-term impact on gonadal function in humans infected with SARS-CoV-2 needs to be established. Beyond the acute phase of COVID-19, the theoretical implications of long-term, chronic effects of SARS-CoV-2-related gonadal suppression for therapeutic efficacy in prostate cancer patients would also be an area of particular interest.

Current prostate cancer management recommendations

Cancer patients and cancer survivors are an at-risk population for COVID-19 with a pooled prevalence of 2.0% (Desai et al., 2020). The infection rate of SARS-CoV-2 in patients with cancer from a single-institution series is higher (0.79%) than the cumulative incidence of all diagnosed COVID-19 cases in the city of Wuhan over the same period (0.37%) (Yu et al., 2020). In general, patients with metastatic cancers infected with COVID-19 have had poorer outcomes including death, admission to the intensive care unit, requiring mechanical ventilation, and severe symptoms; patients with COVID-19 and non-metastatic (stage I-III) cancer had similar outcomes in patients without cancer (Dai et al., 2020; Liang et al., 2020).

Until the areas of controversy with respect to androgen suppression and TMPRSS2 and ACE2 are addressed, expert consensus or national oncology practice guidelines for use of androgen targeted therapy in prostate cancer during the COVID-19 pandemic are a resource and can be followed (Hanna et al., 2020; Kutikov et al., 2020; National Comprehensive Cancer Network, 2020) whereby: 1) androgen deprivation therapy should be avoided in those with very low, low, and intermediate risk prostate cancer; 2) avoid initiating androgen deprivation therapy for patients with a prostate-specific antigen (PSA) doubling time of >9 months in non-metastatic disease; 3) consider neoadjuvant androgen deprivation therapy for up to 4-6 months as

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necessary in asymptomatic, unfavorable intermediate risk and high-risk prostate cancer patients planned for definitive radiation therapy (RT); 4) prioritize androgen deprivation therapy and other non-myelosuppressive regimens when alternatives exist to minimize risk of immunosuppression and infectious complications for symptomatic or advanced prostate cancer patients; and 5) 3-, 4-, or 6-month formulations of androgen deprivation therapy should be preferred over 1-month injections in all cases.

Conclusions and future directions

As development of antiviral drugs and vaccines against SARS-CoV-2 are underway during the COVID-19 pandemic, androgen regulation of TMPRSS2 and ACE2 as a means to inhibit SARS-CoV-2 viral entry and thus infection may represent a novel strategy in treating COVID-19. This concept would be serendipitous for men with prostate cancer being treated by this foundational therapeutic strategy used for decades. A growing concern is the explosion of early reports on the prognosis of cancer patients, efficacy of therapeutic interventions for COVID-19, and safety of anticancer therapies during the COVID-19 pandemic that have produced conflicting results and are, for the most part, non-peer-reviewed. It would be important for compiling of all these fragmented studies into systemic registries for comprehensive retrospective analyses and to inform future studies. In the current era with social media and internet technology, the importance of crowdsourcing cannot be underestimated as a tool to assist in disaster risk reduction and emergency management through information dissemination, data monitoring, and the direct engagement of diverse sets of actors to spur risk reduction efforts (Alexander, 2014; Wukich, 2015). Given this lack of knowledge and concern for extreme vulnerability in cancer patients, the COVID-19 and Cancer Consortium (CCC19) was formed. This national collaborative effort began organically, primarily through social media channels but has grown to more than 65 physicians and nurses representing over 30 institutions and organizations in the United States. Most institutional members are large NCI-designated Comprehensive Cancer Centers. The driving goal of the consortium is to collect prospective, granular, uniformly organized information on cancer patients infected with COVID-19 as rapidly as possible (https://ccc19.org/). While this review is being written during the first wave of COVID-19 infection in the U.S., there will inevitably be many more to follow where prostate cancer patients,

and cancer patients as a whole, may not be spared. Efforts to guide the management of these at-risk patients and the safe delivery of systemic anticancer therapies will require a concerted effort by a multidisciplinary task force.

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Declaration of interest

The authors declare no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Figure 1. The hypothalamic-pituitary-gonadal axis in prostate cancer and androgen regulation of TMPRSS2 and ACE2.

Left, Pulsatile release of gonadotropin-releasing hormone (GnRH) or luteinizing hormone releasing hormone (LHRH) from the hypothalamus regulates production and release of gonadotropins (luteinizing hormone or LH and follicle-stimulating hormone) from the anterior pituitary gland. LH stimulates testosterone synthesis in the testes while increasing systemic concentrations of testosterone inhibits GnRH and LH signaling. Androgens, in particular, testosterone are fundamental to the pathogenesis and evolution of prostate cancer. Dehydroepiandrosterone (DHEA) is converted to testosterone and dihydrotestosterone (DHT) in the skin and hair follicles and is produced in the adrenal gland under the control of adrenocorticotropic hormone (ACTH) and in the gonads under the control of GnRH. ***Right,*** Continuous stimulation of pituitary GnRH receptors results in desensitization and downregulation of these receptors with eventual decrease in circulating sex steroids inclusive of androgens. This negative-feedback loop serves the basis of androgen deprivation therapy via LHRH agonists, while antagonizing the LHRH receptor represents another method of suppressing testosterone secretion through inhibition of downstream LH signaling. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) viral entry into host cells is dependent on transmembrane serine protease 2 (TMPRSS2) and angiotensin converting enzyme 2 (ACE2), which have been found to be expressed in human lungs and other tissues. *TMPRSS2* and *ACE2* appear to be androgen regulated genes where androgen receptors are expressed in human lungs. Androgen receptor (AR) signaling has been shown to increase TMPRSS2 expression while decreasing ACE2 expression. Thus, while SARS-CoV-2 could be putatively targeted through TMPRSS2 and androgen signaling suppression (a standard treatment paradigm in prostate cancer), ACE2 expression may be enhanced with androgen suppression. It is unknown whether this would result in a net increase in risk for severe infection, or a potential benefit from preserving the protective effects of ACE2 protein expression, while hindering a key step in conformational change and viral entry to nonimmune cells.

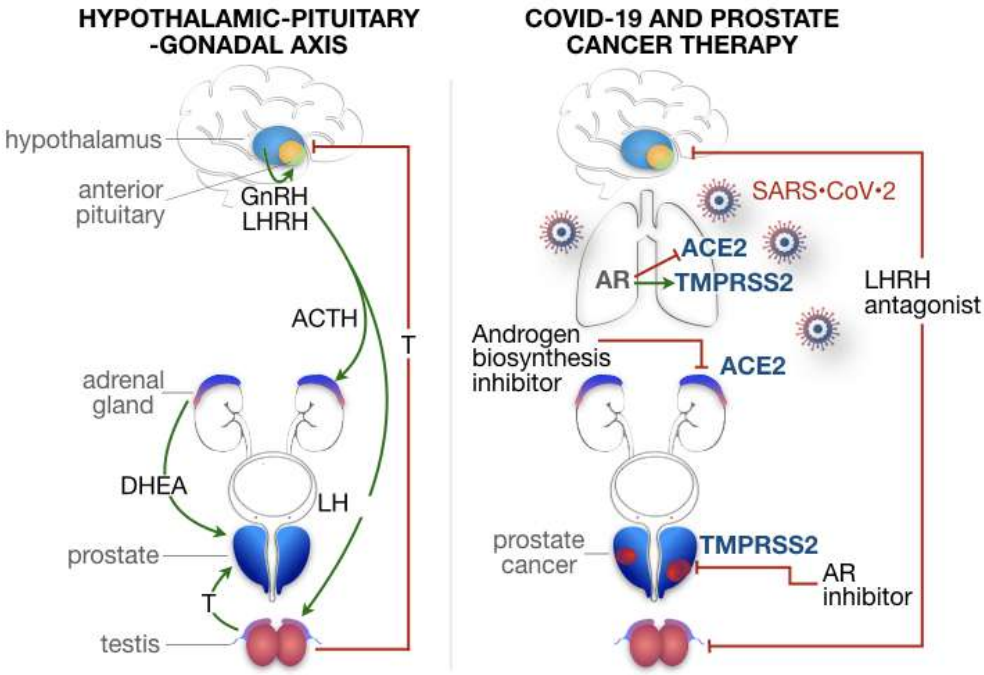

Figure 1. The hypothalamic-pituitary-gonadal axis in prostate cancer and androgen regulation of TMPRSS2 and ACE2.

254x173mm (72 x 72 DPI)

Metabolic Syndrome and COVID-19 Mortality Among Adult Black Patients in New Orleans

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OBJECTIVE

Coronavirus disease 2019 (COVID-19) mortality is high in patients with hypertension, obesity, and diabetes. We examined the association between hypertension, obesity, and diabetes, individually and clustered as metabolic syndrome (MetS), and COVID-19 outcomes in patients hospitalized in New Orleans during the peak of the outbreak.

RESEARCH DESIGN AND METHODS

Data were collected from 287 consecutive patients with COVID-19 hospitalized at two hospitals in New Orleans, LA from 30 March to 5 April 2020. MetS was identified per World Health Organization criteria.

RESULTS

Among 287 patients (mean age 61.5 years; female, 56.8%; non-Hispanic black, 85.4%), MetS was present in 188 (66%). MetS was significantly associated with mortality (adjusted odds ratio [aOR] 3.42 [95% CI 1.52–7.69]), intensive care unit (ICU) (aOR 4.59 [CI 2.53–8.32]), invasive mechanical ventilation (IMV) (aOR 4.71 [CI 2.50–8.87]), and acute respiratory distress syndrome (ARDS) (aOR 4.70 [CI 2.25–9.82]) compared with non-MetS. Multivariable analyses of hypertension, obesity, and diabetes individually showed no association with mortality. Obesity was associated with ICU (aOR 2.18 [CI, 1.25–3.81]), ARDS (aOR 2.44 [CI 1.28–4.65]), and IMV (aOR 2.36 [CI 1.33–4.21]). Diabetes was associated with ICU (aOR 2.22 [CI 1.24–3.98]) and IMV (aOR 2.12 [CI 1.16–3.89]). Hypertension was not significantly associated with any outcome. Inflammatory biomarkers associated with MetS, CRP, and lactate dehydrogenase (LDH) were associated with mortality (CRP [aOR 3.66] [CI 1.22–10.97] and LDH [aOR 3.49] [CI 1.78–6.83]).

CONCLUSIONS

In predominantly black patients hospitalized for COVID-19, the clustering of hypertension, obesity, and diabetes as MetS increased the odds of mortality compared with these comorbidities individually.

Coronavirus disease 2019 (COVID-19), first described in Wuhan, China in December 2019, is caused by the severe acute respiratory syndrome coronavirus 2 (1). It has spread rapidly worldwide, infecting >7 million people as of 18 June 2020, with the U.S. leading the world both in number of cases (~2 million) and fatalities (>100,000) (2). New Orleans, LA was an early epicenter, with the highest death rate per capita in the U.S. (37.93 per 100,000 people) noted in early April (3). One-third of individuals hospitalized for COVID-19 have severe pneumonia requiring admission to an intensive

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See accompanying article, p. XXX.

care unit (ICU) (4), often resulting in acute respiratory distress syndrome (ARDS) (5). A subset of critically ill patients with COVID-19 develop a "cytokine storm" (6,7), similar to that described in prior β-coronavirus outbreaks (8). It is proposed that high levels of proinflammatory chemokines and cytokines are seen in the cytokine storm, and the subsequent recruitment of effector inflammatory cells into the lungs, rather than the virus itself, drives COVID-19–related ARDS (8).

Case subjects with severe and fatal COVID-19 are associated with comorbid conditions including hypertension, obesity, diabetes, and cardiovascular disease (1,4,9–12). Among case subjects with fatal COVID-19, obesity, hypertension, and diabetes are almost always present in higher proportion than chronic heart or pulmonary disease. However, a pathophysiological mechanism explaining these associations has not been established. Metabolic syndrome (MetS) is defined by the coexistence of metabolic comorbidities that contribute to an increased risk of cardiovascular disease and is diagnosed if three or more of the following five metabolic comorbidities exist: obesity, prediabetes/diabetes, hypertension, hypertriglyceridemia, and reduced HDL levels (13). MetS is a chronic low-grade inflammatory state, with elevated circulating concentrations of CRP, interleukin 6 (IL-6), and IL-1β (14,15). We studied the relationship between hypertension, obesity, and diabetes—individually and clustered as MetS—and COVID-19 severe and fatal outcomes in the urban population of New Orleans, LA, a population with high risk of metabolic disease. Louisiana is among the states with the highest prevalence of diabetes, obesity, and hypertension, with some of the worst health outcomes in the U.S. (16).

RESEARCH DESIGN AND METHODS

We performed a retrospective, observational study of hospitalized patients with COVID-19 (confirmed by severe acute respiratory syndrome coronavirus 2 PCR) at two tertiary academic hospitals in New Orleans, LA from 30 March to 5 April 2020. This study was reviewed and approved with waivers of consent by the Tulane University Biomedical Institutional Review Board and the University Medical Center New Orleans Research Review Council.

Data Collection, Study Cohorts, and Outcome Measures

Demographic and clinical data were collected via chart review by four study investigators with duplicated data collection efforts to ensure fidelity of data, with any discrepancies resolved by chart review from the primary investigator. The data set, including information from hospital admission to discharge or death, was complete as of 27 May 2020, with no patients remaining in the hospital. Data included age, sex, race, ethnicity, admission/discharge dates, ICU admission, invasive mechanical ventilation (IMV), hospital mortality, comorbid conditions to calculate the Charlson Comorbidity Index (17), last recorded hemoglobin A_{1c}, BMI on admission calculated from height and weight, history of hypertension or antihypertensive medication use, last recorded triglyceride (TG) level, last recorded HDL level along with history of hyperlipidemia and concurrent statin use, and other laboratory values on admission (specifically ferritin, CRP, and lactate dehydrogenase [LDH]). Both TG and HDL levels used were obtained from the patient's record prior to admission when possible as a more accurate measure, recognizing that these levels may fluctuate with decreased diet and acute inflammation (18). TG levels were not included in the MetS calculation or in subgroup analyses if the patient had received or was currently receiving propofol infusion, as propofol infusions may lead to hypertriglyceridemia (19). In these cases, TG levels were recorded from either before admission or prior to propofol initiation, if available.

Patients were divided into two cohorts, MetS and non-MetS, according to modified World Health Organization criteria (13). MetS was defined as having at least three of the following five factors: *1*) prediabetes (hemoglobin $A_{1c} \geq 5.7\%$) or documented history of diabetes or diabetic medication use, *2*) obesity (BMI ≥ 30 kg/m^2), *3*) history of hypertension or antihypertensive medication use, *4*) TG ≥ 150 mg/dL, and *5*) HDL <50 mg/dL for women and <40 mg/dL for men or use of a cholesterol-lowering medication with documented history of hypercholesterolemia. These modified criteria allow for improved reproducibility of these study results using similar large data sets (20). The primary outcome for all analyses was hospital mortality. Secondary outcomes included need for ICU, IMV, a diagnosis of ARDS using the ratio of PAo_2 to fractional inspired oxygen as defined by Berlin criteria (21), hospital length of stay (LOS), and hospital readmission after initial discharge. To examine the association between clinically significant elevations in inflammatory markers and patient outcomes in MetS, appropriate cutoff values were chosen from published data that have previously been described to affect mortality in patients with COVID-19. These cutoff values include: CRP >41.2 mg/L (22), LDH >365 units/L (22), and ferritin >300 ng/mL (1). These inflammatory markers were also analyzed as continuous variables in a separate analysis. Prespecified subgroup analyses were also completed to determine the association of the individual MetS-associated factors defined above with primary and secondary outcomes. Given the high percentage of black non-Hispanic patients, post hoc analyses examining these subjects were performed in a similar manner as described for the overall population.

Statistical Analysis

Student *t* test was used for statistical comparison of numerical variables in different groups, with a two-tailed *P* value ≤ 0.05 set as statistically significant. Pearson χ^2 test was used to compare categorical variables. For the comparison of outcomes among MetS and non-MetS, a multivariable logistic regression model (or multivariable linear regression model when appropriate) was constructed including age, sex, race, individual hospital site, and Charlson Comorbidity Index as covariates. Separate but similar models were constructed for each subgroup analysis to evaluate the risk of each MetS diagnostic criteria individually (i.e., prediabetes/diabetes, hypertension, obesity, elevated TG, and low HDL) without inclusion of MetS itself in these models. Statistical analyses were performed using SAS Enterprise Guide, version 6.1, and SAS, version 9.4 (both from SAS Institute).

RESULTS

Study Population

We collected data from 287 hospitalized patients with confirmed COVID-19. Mean age was 61.5 years, 56.8% were females, and the majority (245; 85.4%) self-identified

as non-Hispanic black. Baseline characteristics of our patient population are presented in Table 1. The most common comorbid conditions were hypertension (80%), obesity (65%), diabetes (54%), and low HDL (39%). These were present in higher proportion than congestive heart failure (14%) chronic obstructive pulmonary disease (10%), and asthma (10%). The median LOS was 10 days (interquartile range [IQR] 10). In total, 130 patients (45%) required admission to the ICU, of whom 108 (83%) required IMV, 81 (62%) developed ARDS, and 58 (20%) died during the study period. Characteristics of disease severity are presented for the patients who required ICU admission at any point during the hospitalization compared with patients who never required ICU admission (Table 1).

Metabolic Syndrome Is Associated With Hospital Mortality

A total of 188 patients (66%) met the criteria for MetS, and the remaining 99 (34%) were included in the non-MetS control group (Table 2). The two cohorts did not differ in age or sex, but non-Hispanic black patients were present in higher proportion in the MetS group than in the non-MetS group (91% vs. 75%, respectively; $P = 0.0009$). Among the entire cohort, CRP, LDH, and ferritin levels were available in 270, 273, and 275 patients, respectively. CRP and LDH serum concentrations were more elevated in the MetS group than in the non-MetS group (Table 2). Similar observations were made in the non-Hispanic black population when analyzed by race (Supplementary Table 1).

In adjusted outcome analyses, MetS was associated with 3.42 increased odds of hospital mortality (CI 1.52–7.69), 4.59 increased odds of ICU requirement (CI 2.53–8.32), 4.71 increased odds of IMV (CI 2.50–8.87), and 4.70 increased odds of ARDS (CI 2.25–9.82) when compared with non-MetS (Table 3). Similar observations were noted in the non-Hispanic black population when analyzed by race (Supplementary Table 2).

In contrast to comorbidities clustered as MetS, in separate subgroup multivariable analyses, none of the individual MetS-associated comorbidities were significantly associated with hospital mortality (Fig. 1). However, obesity was associated with increased odds of ICU requirement (adjusted odds ratio [aOR] 2.18 [95% CI 1.25–3.81]), ARDS (aOR 2.44 [95% CI 1.28–4.65]), and IMV (aOR 2.36 [95% CI 1.33–4.21]). Similarly, prediabetes/diabetes was associated with increased odds of ICU requirement (aOR 2.22 [95% CI 1.24–3.98]) and IMV (aOR 2.12 [95% CI 1.16–3.89]). Low HDL was associated with increased odds of ICU requirement (aOR 2.16 [95% CI 1.29–3.60]), ARDS (aOR 2.29 [95% CI 1.28–4.09]), and IMV (aOR 1.72 [95% CI 1.02–2.89]). TG and hypertension were not associated with any primary or secondary outcomes (Fig. 1). In non-Hispanic black patients, MetS was similarly associated with hospital mortality (aOR 3.30 [95% CI 1.31–8.32]), but none of the individual MetS-associated comorbidities were in separate subgroup multivariable analyses (Supplementary Fig. 1). In non-Hispanic black patients, obesity was associated with increased odds of ICU requirement (aOR 2.76 [95% CI 1.49–5.11]), ARDS (aOR 2.88 [95% CI 1.43–5.79]), and IMV (aOR 3.14 [95% CI 1.64–6.03]). Diabetes and hypertension were not associated with primary or secondary outcomes (Supplementary Fig. 1).

Inflammatory Markers Are Associated With Hospital Mortality

Using previously described cutoff values shown to correlate with increased disease severity or mortality in patients with COVID-19, the inflammatory markers CRP and LDH were significantly associated with MetS (Supplementary Table 3). Ferritin was the only biomarker that did not show a significant association with MetS. In non-Hispanic black patients, MetS was associated only with increased CRP (Supplementary Table 4). When inflammatory markers were compared with patient outcomes (Supplementary Fig. 2), CRP, ferritin, and LDH were all significantly associated with the need for ICU, IMV, and a diagnosis of ARDS, but only CRP and LDH were significantly associated with hospital mortality: CRP (aOR 3.66 [CI 1.22–10.97]) and LDH (aOR 3.49 [CI 1.78–6.83]). Similar observations were made in non-Hispanic black patients, except CRP was also not associated with hospital mortality (Supplementary Fig. 3).

CONCLUSIONS

The main finding of this multicenter, observational study of hospitalized

Table 1—Patient characteristics and severity of disease

Characteristics	Total ($n = 287$)	ICU ($n = 130$)	Ward ($n = 157$)
Age, mean ± SD, years	61.5 ± 15.2	63.2 ± 14.3	60.0 ± 15.8
Female, *n* (%)	163 (56.8)	72 (55.4)	91 (58.0)
Race/ethnicity, *n* (%)			
Non-Hispanic black	245 (85.4)	118 (90.8)	127 (80.9)
Non-Hispanic white	25 (8.7)	10 (7.7)	15 (9.6)
Other[a]	17 (5.9)	2 (1.5)	15 (9.6)
BMI[b], mean ± SD, kg/m^2	33.8 ± 8.5	35.3 ± 8.0	32.6 ± 8.8
Charlson Comorbidity Score, mean ± SD	3.5 ± 2.3	3.7 ± 2.3	3.3 ± 2.4
Selected comorbidities, *n* (%)			
Hypertension	230 (80.1)	112 (86.2)	118 (75.2)
Obesity (BMI ≥30 kg/m^2)	187 (65.2)	94 (72.3)	93 (59.2)
Severe obesity (BMI ≥40 kg/m^2)	63 (22.0)	35 (26.9)	28 (17.8)
Diabetes (type 1 or 2)	154 (53.6)	72 (55.4)	82 (52.2)
HLD	112 (39.0)	65 (50.0)	47 (29.9)
MetS	188 (65.5)	106 (81.5)	82 (52.2)
Congestive heart failure	41 (14.3)	22 (16.9)	19 (12.1)
COPD	29 (10.1)	18 (13.9)	11 (7.0)
Asthma	30 (10.5)	24 (18.5)	6 (3.8)
Obstructive sleep apnea	29 (10.1)	17 (13.1)	12 (7.6)
Disease severity characteristics			
ARDS, *n* (%)	80 (27.9)	76 (58.5)	4 (2.6)
IMV, *n* (%)	108 (37.6)	108 (83.1)	0 (0.0)
LOS, mean ± SD, days	12.9 ± 10.5	17.5 ± 10.7	9.2 ± 8.8
LOS, median (IQR), days	10 (6–16)	15 (10–24)	7 (4–11)
Death, *n* (%)	58 (20.21)	57 (43.9)	1 (0.6)
Hospital readmission, *n* (%)	22/229 (9.6)	9/73 (12.3)	13/156 (8.3)

Hospital readmission data were available only for case subjects who survived to discharge. COPD, chronic obstructive pulmonary disease; HLD, hyperlipidemia. [a]Includes Hispanic, Asian, and unknown. [b]BMI is weight in kilograms divided by the square of height in meters.

Table 2—MetS characteristics

Characteristics	MetS (n = 188)	Non-MetS (n = 99)	P value
Age, mean ± SD, years	61.6 ± 13.9	61.2 ± 17.4	0.8181
Female, n (%)	111 (59.0)	52 (52.5)	0.2894
Race, n (%)			0.0009
Non-Hispanic black	171 (90.9)	74 (74.8)	
Non-Hispanic white	11 (5.9)	14 (14.1)	
Other[a]	6 (3.2)	11 (11.1)	
BMI[b], mean ± SD, kg/m^2	35.4 ± 8.5	30.8 ± 7.8	<0.0001
Charlson Index Score, mean ± SD	3.9 ± 2.3	2.8 ± 2.3	0.0002
MetS comorbidities, n (%)			
Prediabetes/diabetes[c]	169 (89.9)	31 (31.3)	<0.0001
Obesity[d]	136 (72.3)	51 (54.5)	0.0004
Hypertension[e]	176 (93.6)	54 (54.5)	<0.0001
TG >150 mg/dL	40/136 (29.4)	1/24 (4.1)	0.0090
Low HDL[f]	139 (73.9)	8 (8.1)	<0.0001
Laboratory values, mean ± SD (n measured)			
Ferritin, ng/mL	922 ± 1,503 (180)	771 ± 886 (95)	0.2958
CRP, mg/L	126 ± 88 (176)	96 ± 87 (94)	0.0079
LDH, units/L	403 ± 162 (177)	357 ± 173 (96)	0.0306

TG levels were available for a limited number of patients, and some were excluded if propofol had been administered prior. [a]Includes Hispanic, Asian, and unknown. [b]BMI is weight in kilograms divided by the square of height in meters. [c]Prediabetes defined by World Health Organization criteria: hemoglobin A_{1c} ≥5.7%. [d]BMI >30 kg/m^2. [e]Defined as history of hypertension or antihypertensive medication. [f]HDL <50 mg/dL for women and <40 mg/dL for men or those on a statin with documented history of hyperlipidemia.

patients with COVID-19 in New Orleans during the peak of the outbreak is that patients with MetS exhibited a roughly four times greater odds of severe and especially fatal COVID-19 outcomes compared with those without MetS, following multivariable analyses that accounted for age, sex, race, hospital site, and the Charlson Comorbidity Index. In separate subgroup multivariable analyses, however, hypertension, obesity, prediabetes/diabetes, and low HDL, although associated individually with disease severity, were not associated individually with mortality. This suggests that MetS should be considered a composite predictor of COVID-19 lethal outcome, increasing the odds of mortality by the combined effects of its individual components. Previous studies have reported that obese patients are at increased risk for the severe manifestations of COVID-19 (10,23,24). In this study, hospital mortality was not increased by obesity alone. Diabetes has also been reported to increase the risk of ICU requirement by two- to threefold in patients with COVID-19, as well as mortality rates, compared with the overall population (25). However, hospital mortality was not significantly increased by diabetes alone in our population. Hypertension has been reported as the most frequently associated comorbidity in fatal COVID-19 outcomes (1,4,9–12). In our population, hypertension was not associated with any primary or secondary outcomes. Together, this suggests that a combined effect of these comorbidities may be driving the association of MetS with COVID-19 fatal outcomes.

The relationship between hypertension, obesity, and diabetes and critical COVID-19 outcomes has been observed in many large cohort studies (1,4,9–12). Among case subjects with fatal COVID-19, hypertension, obesity, and diabetes were always present in higher proportion than pulmonary or heart disease, suggesting that metabolic diseases predict worse outcomes in COVID-19 than diseases in which lung and heart functions are compromised. One possible explanation, as our study suggests, is that these metabolic comorbidities (combined as MetS) are characterized by a low-grade systemic inflammation (14). Previous reports show that compared with case subjects with moderate COVID-19, case subjects with severe COVID-19 exhibited higher serum levels of inflammatory markers such as CRP, ferritin, D-dimer, and LDH, as well as markedly higher levels of proinflammatory cytokines such as IL-6 and tumor necrosis factor-α (26,27). Similarly, our MetS cohort exhibited increased inflammatory biomarkers (CRP and LDH) when using cutoffs shown to predict COVID-19 mortality. Notably, CRP, ferritin, and LDH were all associated with two- to threefold increased odds of severe outcomes, and CRP and LDH were associated with 3.5-fold increased odds of hospital death. Therefore, the chronic low-grade systemic inflammation that characterizes individuals with MetS may provide a permissive inflammatory environment that intensifies the evolution toward ARDS and death (12). Further research into the underlying mechanisms by which MetS increases COVID-19 mortality is needed.

Our cohort is unique. Our hospitals care for a predominantly non-Hispanic black population (85% of our cohort) with a high prevalence of comorbidities

Table 3—Multivariable analyses, MetS vs. non-MetS

Outcomes	MetS (n = 188)	Non-MetS (n = 99)	Risk difference, % (95% CI)	Adjusted OR[a] (95% CI)	P value
Hospital mortality, n (%)	48 (25.5)	10 (10.1)	15.4 (6.8–24.0)	3.42 (1.52–7.69)	0.0030
ICU requirement, n (%)	106 (56.4)	24 (24.2)	32.1 (21.1, 43.2)	4.59 (2.53–8.32)	<0.0001
ARDS[b], n (%)	69 (36.7)	11 (11.1)	25.6 (16.3–34.9)	4.70 (2.25–9.82)	<0.0001
IMV, n (%)	90 (47.9)	18 (18.2)	29.7 (19.3–40.1)	4.71 (2.50–8.87)	<0.0001
LOS, mean ± SD, days	14.1 ± 10.6	10.7 ± 10.1	n/a	n/a	0.0097
LOS, median (IQR), days	11 (7.0–20.5)	7 (4.0–14.0)	n/a	n/a	0.0062
Hospital readmission, n (%)	16/140 (11.4)	6/89 (6.7)	4.7 (−1.7 to 12.1)	1.19 (0.40–3.61)	0.7533

Hospital readmission data were available only for case subjects who survived to discharge. n/a, not available. [a]Multivariable logistic regression model adjusted for age, sex, race, hospital site, and Charlson Comorbidity Index. [b]ARDS defined according to Berlin criteria.

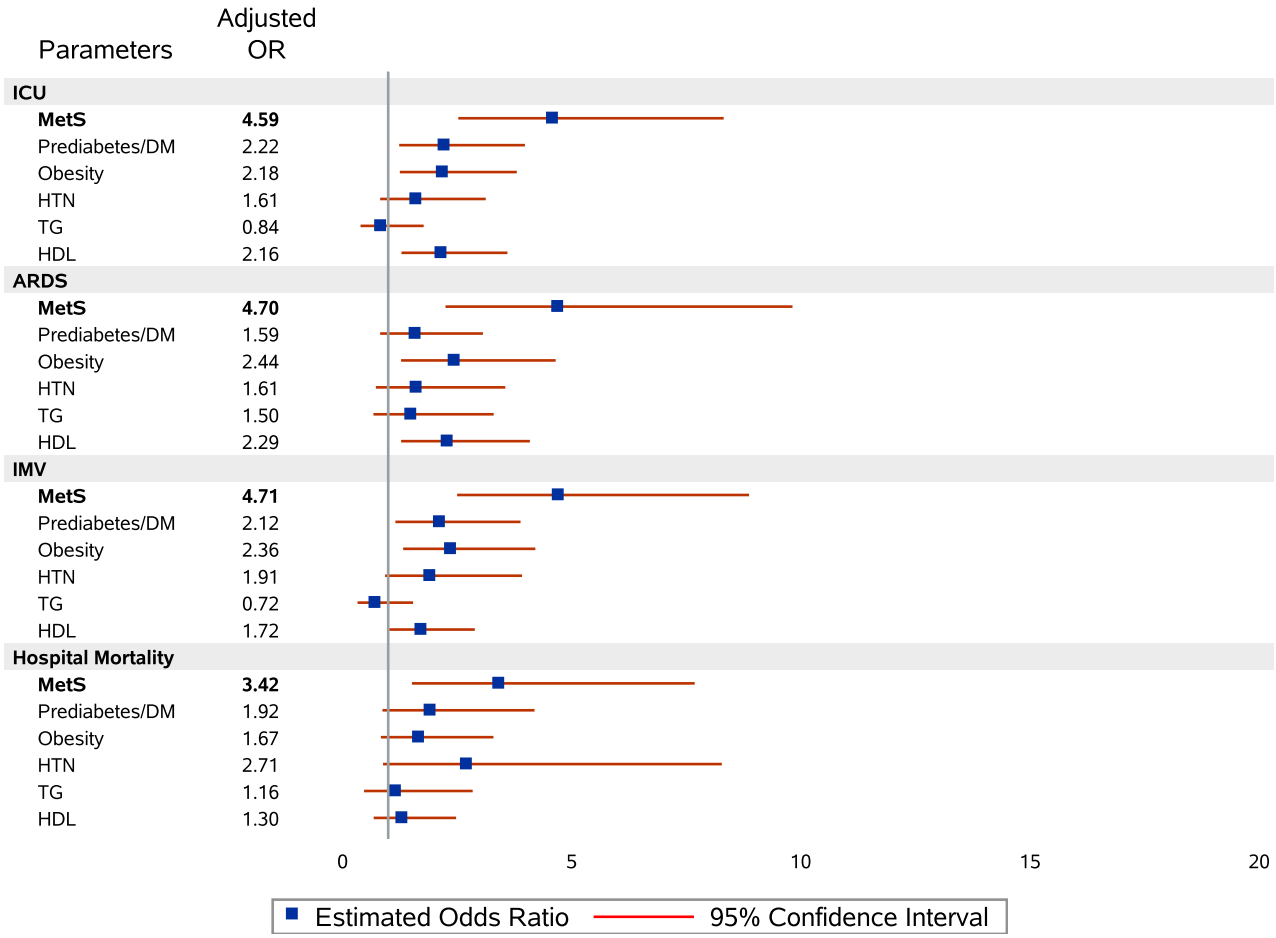

Figure 1—Forrest plot of MetS and individual MetS components on primary and secondary outcomes. Multivariable regression analysis for MetS itself and separate analyses for MetS components were performed. All analyses were adjusted for age, sex, race/ethnicity, hospital site, and the Charlson Comorbidity Index. DM, diabetes mellitus; HTN, hypertension.

who have been greatly affected by the COVID-19 pandemic (3,28). A recent study including >1,000 hospitalized non-Hispanic black patients from a hospital system in Louisiana caring for a healthier population (only 9% were uninsured) (29) reported a mean Charlson Comorbidity Index of 1.3 (29). By contrast, our cohort of 245 hospitalized black non-Hispanic patients in downtown New Orleans (74% from a public hospital and mostly uninsured) exhibited a mean Charlson Comorbidity Index of 3.5. This is further highlighted by our control group, whom, despite not meeting criteria for MetS, showed elevated rates of hypertension (54%), obesity (53%), prediabetes/diabetes (31%), and mean Charlson Comorbidity Score (2.8).

Another important consideration of our study is the effect of sex on COVID-19 outcomes. Our hospitalized population exhibited a predominance of women (56%), which was also observed in another study in non-Hispanic black patients from southeastern Louisiana (29). This predominance of women contrasts with most large studies in predominantly non-Hispanic white, European, or Asian patients in whom COVID-19 hospitalization showed a strong predominance of men, averaging 70% (11,30–34). Thus, in our population, COVID-19 could affect women and men differently.

This study has several limitations. As a retrospective observational study, our conclusions can only be based on associations and do not imply causation. Although we used multivariable regression models to adjust for relevant covariates and clinically relevant data evaluations (e.g., taking into account the consideration of propofol infusion with regard to TG levels), these data cannot fully account for all unknown potential confounders.

Conclusion

In a predominantly non-Hispanic black population hospitalized for COVID-19, the clustering of hypertension, obesity, and diabetes as MetS and inflammatory markers increased the odds of mortality compared with these comorbidities individually. These findings suggest that MetS is a composite predictor of COVID-19 lethal outcome, in which the combined effect of its related comorbidities was significantly associated with mortality, possibly via inflammation.

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ORIGINAL ARTICLE

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Low testosterone levels predict clinical adverse outcomes in SARS-CoV-2 pneumonia patients

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Abstract

Background: The pandemic of new severe acute respiratory syndrome (SARS) due to coronavirus (CoV) 2 (SARS-CoV-2) has stressed the importance of effective diagnostic and prognostic biomarkers of clinical worsening and mortality. Epidemiological data showing a differential impact of SARS-CoV-2 infection on women and men have suggested a potential role for testosterone (T) in determining gender disparity in the SARS-CoV-2 clinical outcomes.

Objectives: To estimate the association between T level and SARS-CoV-2 clinical outcomes (defined as conditions requiring transfer to higher or lower intensity of care or death) in a cohort of patients admitted in the respiratory intensive care unit (RICU).

Materials and methods: A consecutive series of 31 male patients affected by SARS-CoV-2 pneumonia and recovered in the respiratory intensive care unit (RICU) of the "Carlo Poma" Hospital in Mantua were analyzed. Several biochemical risk factors (ie, blood count and leukocyte formula, C-reactive protein (CRP), procalcitonin (PCT), lactate dehydrogenase (LDH), ferritin, D-dimer, fibrinogen, interleukin 6 (IL-6)) as well as total testosterone (TT), calculated free T (cFT), sex hormone–binding globulin (SHBG), and luteinizing hormone (LH) were determined.

Results: Lower TT and cFT were found in the transferred to ICU/deceased in RICU group vs groups of patients transferred to IM or maintained in the RICU in stable condition. Both TT and cFT showed a negative significant correlation with biochemical risk factors (ie, the neutrophil count, LDH, and PCT) but a positive association with the lymphocyte count. Likewise, TT was also negatively associated with CRP and ferritin levels. A steep increase in both ICU transfer and mortality risk was observed in men with TT < 5 nmol/L or cFT < 100 pmol/L.

Giulia Rastrelli and Vincenza Di Stasi equally contributed to this study.

Discussion and conclusion: Our study demonstrates for the first time that lower baseline levels of TT and cFT levels predict poor prognosis and mortality in SARS-CoV-2-infected men admitted to RICU.

KEYWORDS
COVID-19, inflammatory markers, mortality, prognosis, sex hormones

1 | INTRODUCTION

The new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has spread rapidly around the world in the last few months, thus resulting in more than 3.8 million persons globally infected and 265,961 deaths as of May 9, 2020 (https://covid19.who.int/). The SARS-CoV-2 infection presents with a broad range of clinical manifestations, encompassing asymptomatic infection, mild upper respiratory tract illness, and dyspnea, to a life-threatening SARS, with a great number of patients being hospitalized with pneumonia (https://www.who.int/docs/default-source/coronaviruse/situation-reports). The sharp increase in SARS-CoV-2 infection and the dramatic strain on healthcare systems worldwide have pressured international researchers to urgently investigate SARS-CoV-2 disease pathogenesis and to explore factors, which could have an impact toward its prognosis. Earlier prediction models suggest that elderly patients or patients with comorbidities (eg, hypertension, diabetes mellitus, chronic obstructive pulmonary disease, cardiovascular disease) run a greater risk for disease progression and even fatal outcomes.[1-3] However, delineating efficient prognostic factors for both worse clinical course and mortality is claimed as a major unmet need in order to tackle clinical management of the severe cases.[4]

SARS-CoV-2 infection has been shown to have a differential impact on women and men. Sex disaggregated data indeed showed greater mortality rates in men as compared with women. In particular, men are up at threefold higher odds of lethality than women,[5] thus indicating a firm gender inequality in SARS-CoV-2-associated sequelae. Several social factors, genetic, immunological, and hormonal differences, as well as lifestyle habits (ie, smoking and alcohol consumption), have been considered to play a role in this gender disparity.[6] Among sex hormones, a potential role for testosterone (T) in defining sex-related differences in terms of SARS-CoV-2 infection clinical outcomes has been hypothesized.[7,8] Obviously, due to the known higher level of T in men than in women, the prevailing theory is that T might be a promoter of SARS-CoV-2 infection and progression.[7,8] This theory stems from the positive regulatory effect of androgen receptor (AR) and T on the transcription of a transmembrane protease, serine 2 (TMPRSS2), a critical factor enabling cellular infection by coronaviruses, including SARS-CoV-2. Conversely, others have hypothesized that low T levels could theoretically be detrimental, increasing the risk of severe disease among patients with SARS-CoV-2 infection,[8] as for other comorbidities.[9,10] Indeed, T has been also described to induce the angiotensin-converting enzyme 2 (ACE2) expression,[11] an important lung protective enzyme.[12] This view would agree with the evidence reporting that in some countries (at least Italy and Spain), SARS-CoV-2 infection among female health workers is twice that of their male counterpart (https://data.unwomen.org/resources/Covid-19-emerging-gender-data-and-why-it-matters). Of relevance, it is well established that T level declines according to aging and in the presence of comorbidities like obesity, diabetes mellitus, and cardiovascular diseases, which have been found to be highly prevalent in SARS-CoV-2 patients.[13] Owing to the high global prevalence of hypogonadism (estimated to be 15%-20% among middle-aged/elderly men),[14,15] the relation between hypogonadism and SARS-CoV-2 infection outcomes deserves deep a thorough investigation. More importantly, the prevalence of hypogonadism in elderly male patients admitted to the hospital for acute illness raises up to 53%.[16,17]

Thereof, we sought to estimate the association between the T levels and the SARS-CoV-2 infection clinical outcomes (defined as conditions requiring to transfer to a higher or lower intensity of care units or even death) as well as the biochemical prognostic predictors of severe and fatal SARS-CoV-2 infection in a cohort of patients admitted in the respiratory intensive care unit (RICU) of a single Hospital in Mantua, one of the epicenter of the global SARS-CoV-2 pandemic in Italy. This study reports findings from 31 patients with SARS-CoV-2 infection and follows 25 of them through to discharge or death. The follow-up until discharge or death is a point of difference from other case series to date.

2 | METHODS

Data from a consecutive series of 31 male patients with SARS-CoV-2 pneumonia and recovered in the respiratory intensive care unit (RICU) of the "Carlo Poma" Hospital in Mantua, Italy, were analyzed. A laboratory (pharyngeal-nose swab positivity) of SARS-CoV-2 infection was confirmed by chest X-ray. Comorbidity burden was assessed by the Charlson comorbidity index (CCI).

Acute respiratory distress syndrome (ARDS) was defined by "Berlin definition," and patients were segregated into mild ARDS (PaO2/FiO2 ≤ 300 mm Hg and > 200 mm Hg), moderate ARDS (PaO2/FIO2 ≤ 200 mm Hg and > 100 mm Hg), and severe ARDS (PaO2/FiO2 ≤ 100 mm Hg)[18]; therefore, for the specific purpose of this analysis, patients have been further subdivided into either men with severe ARDS or with mild-moderate ARDS at the RICU admission.

According to the protocol (approved by Local Ethics Committee Val Padana, Mantua, Italy), each patient underwent a standardized diagnostic workup. Specifically, blood samples were drawn the first morning after the admission at the RICU within 8.00 AM, after an overnight fast, for determination of blood count and leukocyte formula, creatinine, uric acid, electrolytes, transaminases, creatine phosphokinase (CPK), C-reactive protein (CRP), procalcitonin (PCT), lactate dehydrogenase (LDH), ferritin, D-dimer, fibrinogen, interleukin 6 (IL-6), total testosterone (TT), sex hormone–binding globulin (SHBG), and luteinizing hormone (LH). Blood sample analyses were performed in the central laboratory of the "Carlo Poma" Hospital (Mantua, Italy) with commercially available kits normally used for clinical practice of the hospital. TT was measured once by immunoassay (Electrochemiluminescence Immunoassay, ECLIA), and free T was calculated by the Vermeulen formula (http://www.issam.ch/freetesto.htm).[19]

Hence, patients were divided according to the outcome throughout the hospitalization in RICU. For the first analyses, patients have been segregated in four groups: (a) The first group composed by patients with improved clinical conditions overtime who have been transferred to the internal medicine (IM) units; clinically, they no longer needed non-invasive ventilation (NIV) but only low-flow oxygen therapy; (b) the second group composed by patients still in RICU and under NIV at the time of the current analysis (data on the definitive outcomes are not available yet); (c) the third composed by patients transferred to intensive care unit (ICU) for intubation; and (d) the fourth group composed by patients who eventually died.

2.1 | Statistical methods

Differences among groups with different prognoses were evaluated eventually merging the two groups with the worst outcomes (the aforementioned third and fourth groups). Thereafter, a sensitivity analysis was performed to compare the groups of which post-RICU outcome was known at the time of the analysis (thus excluding the aforementioned second group). Either Mann-Whitney U test or one-way ANOVA on ranks (Kruskal-Wallis test) was used to test the differences concerning continuous variables between two or more than two groups, respectively. Differences between continuous categorical variables were assessed by the likelihood-ratio test. Data were expressed as medians [interquartile ranges] or percentage for continuous and categorical variables, respectively. Univariate relationships were firstly assessed by Spearman's rank correlation and afterward checked for non-linearity by the locally weighted scatterplot smoothing (LOWESS) analysis. When linearity could not be assumed, threshold levels for TT or calculated free T (cFT) were identified by the LOWESS analysis and further confirmed by linear regression models with linear spline functions for TT and cFT levels. This analysis allowed identifying threshold levels at which a significant change in the slope of the association between T and other blood markers occurred. The relationship between the clinical outcomes (transferred to IM, in charge to RICU, transferred to ICU or deceased) and TT or cFT was assessed by ordinal logistic regressions. The probability of each outcome based on T according to this regression model was calculated and fitted in a LOWESS curve as a function of TT or cFT.

All the analyses were performed by Statistical Package for the Social Sciences Statistics 26 (IBM Corporation). Spline linear functions were carried out by Stata MP 13 (StataCorp).

3 | RESULTS

Of 31 patients, 21 (67.7%) were transferred from RICU to IM after overall improvement of the respiratory conditions, six (19.4%) were stable at time of the present analysis and maintained in RICU, and four (12.9%) worsened their conditions and were either transferred to ICU (n = 2) or eventually died (n = 2).

Table 1 details descriptive statistics of the whole cohort of patients as segregated according to the different outcomes. Overall, significant difference among groups was found in terms of previous history of psychiatric diseases and of prevalence of severe ARDS at RICU admission. Moreover, lymphocyte count was the lowest in both RICU and ICU/deceased patients, whereas PCT and LDH had the highest values in the ICU/deceased group. Of note, no differences in D-dimer serum levels were observed. LH, TT, and cFT were significantly different among groups, with higher LH and lower TT and cFT in the ICU/deceased group. These differences were confirmed when comparing the groups transferred from RICU because of either improved respiratory conditions or adverse outcomes, respectively (Table 1). In addition, the comparison of these two groups revealed that men in the adverse outcome group (ie, transferred to ICU or deceased) more frequently had a history of arrhythmia and were less frequently obese. Besides the aforementioned biochemical and hormonal parameters, men in the ICU/deceased group had significantly higher neutrophil count, potassium, and CRP levels, as compared with men transferred to IM.

In order to further explore the trends of hormonal levels according to the severity of the outcome, hormones were analyzed in each category after splitting the adverse outcome group into men transferred to ICU and men eventually deceased. Figure 1 confirms the aforementioned differences and shows a stepwise decrease in both TT and cFT according to the severity of the outcome at the time of the final assessment. Despite not achieving the statistical significant, LH had higher values in men with stable or adverse outcome categories. SHBG had comparable values among the four groups. Besides the outcome classes, LH, TT, and cFT were significantly different in patients with or without severe ARDS (LH = 12.0 U/L [8.5-21.7] vs 6.9 U/L [5.0-10.4]; TT = 2.19 nmol/L [1.25-4.58] vs 7.0 nmol/L [4.07-13.8] and cFT = 50.5 pmol/L [18.6-84.0] vs 138.0 pmol/L [102.0-221.0], respectively; all $P < .05$). No differences in SHBG were found (data not shown). In this cohort, TT and cFT were not significantly associated with age (B= −0.93 [−0.287; 0.101], $P = .337$ and B= −2.84 [−5.96; 0.274], $P = .072$, respectively); similarly, TT and cFT did not differ in obese and non-obese men (TT = 6.75 nmol/L [3.19-14.86]

TABLE 1 Descriptive statistics of the whole cohort as stratified according to clinical outcomes

	Reference range	Transferred to IM (n = 21)	In charge in RICU (n = 6)	Transferred to ICU/deceased (n = 4)	p for trend	p[1]
Demographics and previous medical history						
Age (years)	---	63.0 [55.0-66.5]	72.0 [33.0-83.5]	74.5 [59.5-85.0]	0.162	*0.068*
		Smoking habits (%)				
Former smoker	---	42.9	0.0	50.0	0.128	0.823
Current smoker	---	4.8	0.0	0.0		
Obesity (%)	---	42.9	16.7	0.0	0.860	**0.046**
Hypertension (%)	---	57.1	33.3	50.0	0.584	0.793
Dyslipidemia (%)	---	23.8	33.3	25.0	0.899	0.959
Diabetes (%)	---	28.6	33.3	0.0	0.267	0.119
Hypothyroidism (%)	---	14.3	0.0	25.0	0.347	0.610
Chronic renal failure (%)	---	4.8	0.0	0.0	0.672	0.550
Arrhythmia (%)	---	0.0	0.0	25.0	0.114	**0.048**
Psychiatric diseases (%)	---	0.0	33.3	25.0	**0.023**	**0.048**
Hematologic diseases (%)	---	4.8	16.7	0.0	0.501	0.550
CVD (%)	---	4.8	0.0	0.0	0.672	0.550
Liver diseases (%)	---	4.8	0.0	0.0	0.672	0.550
Parameters during hospitalization in RICU						
Time in RICU (days)	---	7.0 [4.0-9.0]	10.0 [7.0-14.3]	5.0 [4.0-12.0]	0.338	0.456
PaO2/FiO2 (mm Hg)	<300 for ARDS	130.4 [104.8-165.4]	119.5 [99.5-155.0]	87.3 [80.8-157.9]	0.226	0.132
Severe ARDS (%)	PaO2/FiO2 ≤100 mm Hg	14.3	16.7	75.0	**0.050**	**0.016**
WBC (10^3/μL)	4.4-11	7.3 [4.3-9.0]	7.2 [5.3-8.6]	11.6 [7.7-17.3]	0.115	0.056
Neutrophils (10^3/μL)	2.0-7.5	4.0 [2.6-6.7]	6.3 [4.2-7.2]	10.6 [6.4-16.4]	*0.085*	**0.027**
Lymphocytes (10^3/μL)	1.3-4.8	1.2 [0.9-1.6]	0.7 [0.5-0.9]	0.7 [0.5-0.9]	**0.017**	**0.035**
Hemoglobin (g/dL)	13.5-17-5	11.8 [9.4-14.0]	12.4 [10.3-14.0]	11.6 [9.4-13.0]	0.849	0.794
Hematocrit (%)	40-50	34.8 [28.4-42.1]	37.4 [33.1-41.8]	36.2 [30.2-42.1]	0.771	0.911
Platelets (10^3/μL)	150-400	278.5 [173.5-387.3]	299.5 [229.0-346.8]	284.0 [197.3-391.0]	0.993	0.911
Creatinine (mg/dL)	0.6-1.3	0.9 [0.8-1.1]	1.1 [0.8-1.2]	0.8 [0.7-2.3]	0.592	0.592
Uric acid (mg/dL)	2.5-7.2	4.0 [2.9-5.1]	3.4 [2.7-5.9]	4.2 [2.7-8.4]	0.973	0.803
Sodium (mEq/L)	135-145	137.0 [132.0-138.5]	137.5 [131.5-139.5]	137.5 [133.0-154.8]	0.726	0.452
Potassium (mEq/L)	3.4-4.7	3.8 [3.6-3.9]	3.7 [3.4-4.0]	4.4 [3.8-4.5]	*0.088*	**0.047**
AST (U/L)	10-33	35.0 [30.5-63.0]	31.0 [27.5-45.0]	46.5 [21.8-90.8]	0.530	0.748

(Continues)

TABLE 1 (Continued)

	Reference range	Transferred to IM (n = 21)	In charge in RICU (n = 6)	Transferred to ICU/deceased (n = 4)	p for trend	p[1]
ALT (U/L)	5-37	47.0 [32.5-65]	28.5 [21.5-51.8]	79.0 [28.0-114.3]	0.108	0.231
CPK (U/L)	25-200	56.0 [28.0-95.3]	39.5 [22.3-143.8]	94 [53.5-582.3]	0.284	0.183
CRP (mg/L)	0-5	15.7 [3.4-64.2]	24.9 [10.5-72.5]	143.1 [46.1-257.2]	*0.060*	**0.023**
Procalcitonin (ng/mL)	0-0.09	0.08 [0.05-0.15]	0.10 [0.05-0.33]	1.33 [0.46-2.88]	**0.006**	**<0.001**
LDH (U/L)	150-450	414.5 [347.0-515.0]	621.0 [563.3-954.8]	935.5 [623.8-1070.3]	**0.002**	**0.003**
Fibrinogen (mg/dL)	150-450	426.0 [303.0-535.0]	455.5 [386.0-617.5]	471.0 [152.3-756.0]	0.811	0.969
D-dimer (ng/mL)	<500	1836.0 [515.0-3697.0]	1343.5 [631.0-3197.3]	2108.5 [858.0-8171.0]	0.867	0.667
Ferritin (ng/mL)	30-400	993.0 [656.0-1365.0]	1679.5 [511.0-3791.0]	1809.0 [876.0-2199.8]	0.167	0.081
IL-6 (pg/mL)	<7	50.3 [13.1-86.0]	56.1 [30.1-77.6]	137.2 [50.3-205.8]	0.291	0.148
Total T (nmol/L)	8.6-29	8.8 [4.1-16.2]	5.0 [1.8-7.6]	1.0 [0.2-1.9]	**0.005**	**0.001**
Calculated free T (pmol/L)	<225	146.5 [93.8-287.0]	118.0 [40.8-133.5]	17.5 [5.8-37.0]	**0.006**	**0.001**
SHBG (nmol/L)	18.3-54.1	35.6 [22.0-59.0]	24.0 [19.6-37.4]	21.3 [12.2-39.6]	0.159	0.157
LH (U/L)	1.7-8.6	6.6 [4.6-9.6]	16.3 [7.9-20.3]	11.2 [9.0-19.3]	**0.043**	**0.037**

Note: Data are reported as median and interquartile range for continuous variables and as percentage for categorical variables. Differences in continuous variables were assessed by one-way ANOVA on ranks (Kruskal-Wallis test) for comparison among the three groups or by Mann-Whitney *U* test for comparison between the groups with better (transferred to IM) or adverse (transferred to ICU/deceased) outcomes. Differences in categorical variables were evaluated by the likelihood-ratio test. p for trend refers to the comparisons between all the groups. p[1] refers to comparison between men transferred to IM inpatient clinics and men transferred to ICU/deceased.

Abbreviations: ARDS, acute respiratory distress syndrome; AST, alanine transaminase; AST, aspartate transaminase; CPK, creatine phosphokinase; CRP, C-reactive protein; CVD, cardiovascular disease; ICU, intensive care unit; IL-6, interleukin 6; IM, internal medicine; LDH, lactate dehydrogenase; LH, luteinizing hormone.; RICU, respiratory intensive care unit; SHBG, sex hormone–binding globulin; T, testosterone; WBC, white blood cell.

Bold values denote statistically significant *P*-values.

Italics values denote *P*-values close to the statistically significance.

vs 4.61 nmol/L [2.12-11.65], *P* = .633 and cFT = 127.0 pmol/L [58.1-167.0] vs 120.0 pmol/L [46.8-204.5], respectively; *P* = .929).

As both TT and cFT showed a significant and progressive decline according to worsening outcomes, we evaluated the relationship between T and markers of disease severity, as emerged by Table 1. Table 2 shows the correlation for TT and cFT with markers of disease severity. Both TT and cFT showed a negative significant correlation with the neutrophil count, LDH, and PCT levels and a positive one with the lymphocyte count. TT was also negatively correlated with CRP and ferritin; cFT showed a similar but not statistically significant trend. Neither TT nor cFT was correlated with potassium. In order to further explore these relationships, we conducted LOWESS analyses to check for their non-linearity. Figures 2 and 3 show the results for TT and cFT, respectively. Several non-linear relationships were observed with TT. The visual inspection of LOWESS curves allowed hypothesizing a possible threshold for TT levels around 5 nmol/L (Figure 2). For cFT, the linearity could be assumed for all of the variables but LDH, which showed a steeper increase for cFT levels below 100 pmol/L (Figure 3). In order to check formally these putative thresholds, linear regressions with linear spline functions were performed. Table 3 reports the results of these analyses: PCT, LDH, and ferritin levels depicted different trends above and below TT 5 nmol/L. In particular, for TT < 5 nmol/L, PCT, LDH, and ferritin increased on average of 0.18 ng/mL, 72.72 U/L, and 232.17 ng/mL, for each nmol/L decrease in TT, respectively; these values were higher than those predicted assuming linearity. This was not the case for the neutrophil and lymphocyte counts for which a linear association

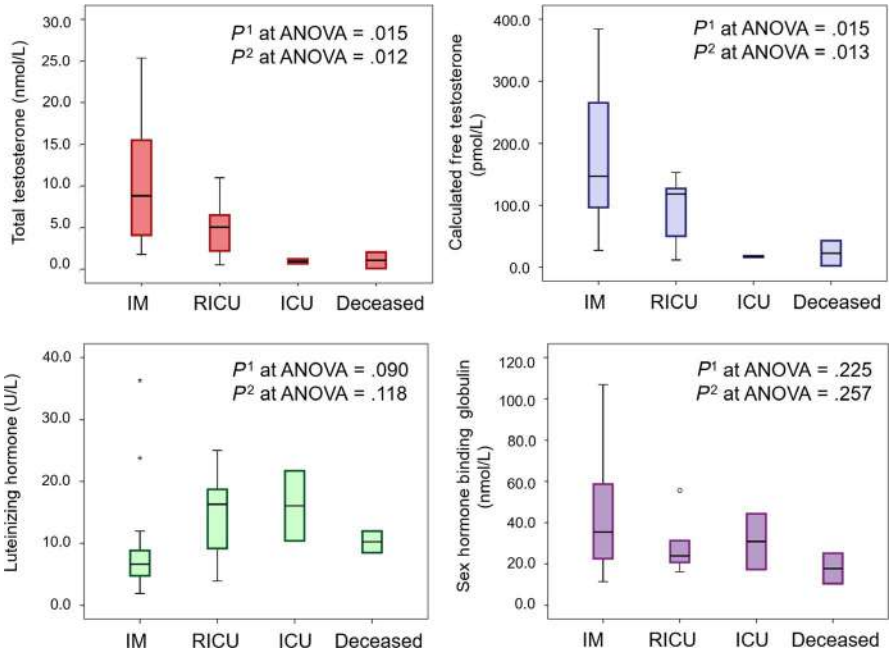


FIGURE 1 Hormone and sex hormone–binding globulin levels according to different clinical outcomes. Data are expressed as box plot, with median and interquartile range; whiskers represent minimum and maximum values; circles and asterisks represent the outliers and extreme outliers. p^1 is the significance level for one-way ANOVA on ranks (Kruskal-Wallis test) for testing the differences among the four groups; p^2 is the significance level for one-way ANOVA on ranks (Kruskal-Wallis test) after excluding the RICU group. Abbreviations: IM = internal medicine inpatient clinics; RICU = respiratory intensive care unit; ICU = intensive care unit

with TT may be assumed. The association between TT and CRP was not confirmed in this analysis. After adjustment for age and comorbidities, all the above-mentioned linear relationships were confirmed (neutrophils: B= −0.19 [−0.36;-0.03], *P* = .026; lymphocyte: B = 0.03 [0.00;0.06]; *P* = .043; PCT: B= −0.03 [−0.07; 0.01]; *P* = .137; LDH: B= −16.45 [−28.22;-4.69], *P* = .008; CRP: B= −2.81 [−6.57;0.94], *P* = .135; ferritin: B= −21.76 [−80.36;36.83]; *P* = .451).

A threshold effect for cFT of 100 pmol/L was confirmed for PCT and LDH that decreased on average of 0.09 ng/mL and 41.46 U/L, for each 10 pmol/L increase in cFT, respectively; again, these values were higher than those predicted by assuming linearity. Conversely, linearity could be assumed for the relationship between cFT and neutrophil and lymphocyte counts. After adjustment for age and comorbidities, all the above-mentioned linear relationships were confirmed (neutrophils: B= −0.10 [−0.21;0.00], *P* = .064; lymphocyte: B = 0.02 [0.00;0.03]; *P* = .045; PCT: B= −0.02 [−0.04; 0.00]; *P* = .135; LDH: B= −10.93 [−18.58;-3.29], *P* = .007).

After confirming the association between TT and cFT with several markers of disease severity, the relationship of these parameters with the clinical outcomes was evaluated. Ordinal linear regressions showed that for each nmol/L decrease in TT and for each 10 pmol/L decrease in cFT, the probability of having worse outcomes significantly increased (OR = 1.42 [1.06;1.89]; *P* = .017 and OR = 1.25 [1.06;1.48]; *P* = .007 for TT and cFT, respectively) being unaffected by the adjustment for age and CCI (OR = 1.35 [1.03;1.76]; *P* = .029 and OR = 1.23 [1.04;1.46]; *P* = .015 for TT and cFT, respectively). The estimated probability of being transferred to IM and ICU, or to die based on T levels is reported in Figure 4. A non-linear change in the probability of different outcomes is well recognizable. When applying the aforementioned thresholds, the probability of being

TABLE 2 Correlation between total and free testosterone with parameters associated with different outcomes in SARS-CoV-2 pneumonia patients

	Total testosterone (nmol/L)		Calculated free testosterone (pmol/L)	
	Spearman's rho	*P*	Spearman's rho	*P*
Neutrophils (10^3/μL)	−0.462	**.012**	−0.450	**.014**
Lymphocytes (10^3/μL)	0.493	**.007**	0.461	**.012**
CRP (mg/L)	−0.385	**.035**	−0.357	.053
Procalcitonin (ng/mL)	−0.448	**.015**	−0.454	**.013**
LDH (U/L)	−0.490	**.006**	−0.465	**.010**
Ferritin (ng/mL)	−0.401	**.031**	−0.320	.091
Potassium (mEq/L)	−0.134	.471	−0.202	.285

Note: Data derived from Spearman's rank correlation test.

Abbreviations: CRP, C-reactive protein; LDH, lactate dehydrogenase; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Bold values denote statistically significant *P*-values.

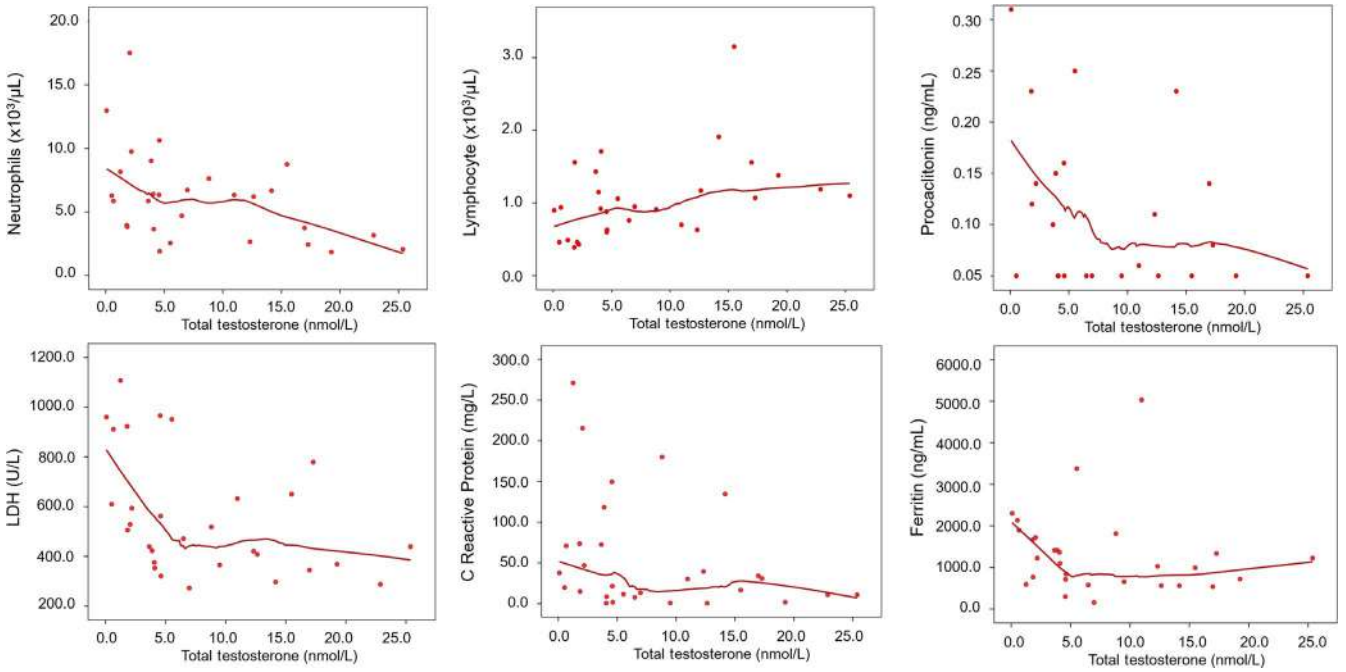
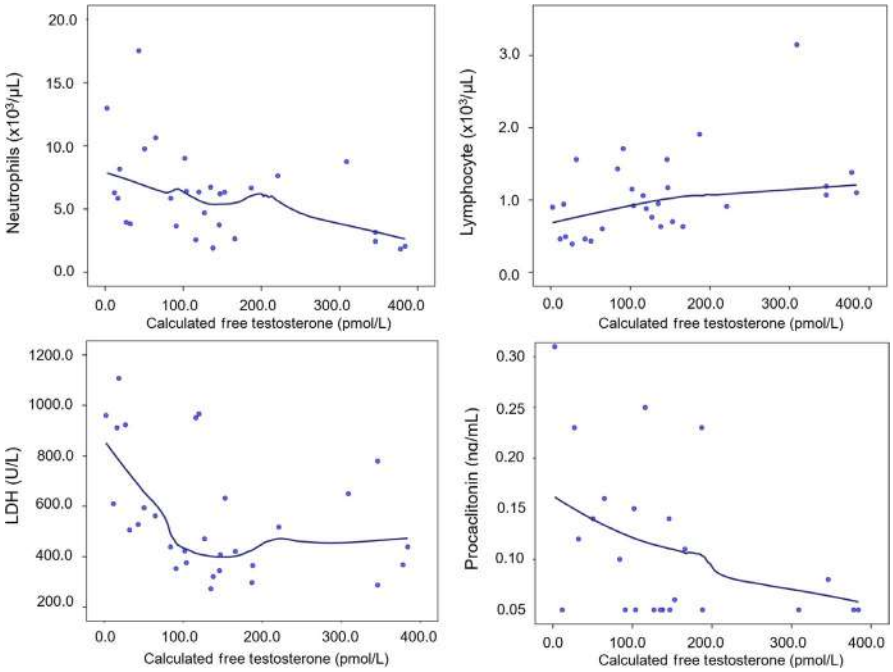


FIGURE 2 Relationship between total testosterone and blood inflammatory markers of severity of SARS-CoV-2 pneumonia. The smooth curves were carried out by locally weighted scatterplot smoothing (LOWESS) analysis. Abbreviations: LDH = lactate dehydrogenase

FIGURE 3 Relationship between calculated free testosterone and blood inflammatory markers of severity of SARS-CoV-2 pneumonia. The smooth curves were carried out by locally weighted scatterplot smoothing (LOWESS) analysis. Abbreviations: LDH = lactate dehydrogenase



transferred to IM was 36.51% [29.28-52.86] and 95.44% [78.05-99.09] below and above TT 5 nmol/L, respectively ($P < .0001$). The probability of being transferred to the ICU or dying below and above TT 5 nmol/L was 14.18% [8.89-17.03] or 0.60% [0.12-3.32], $P < .0001$ or 12.40% [6.77-16.43] and 0.39% [0.07-2.26], $P < .0001$, respectively.

As for actual cases, eight out of 21 (38.1%) transferred to IM, two out of two (100%) transferred to ICU, and two out of two (100%) deceased had TT < 5 nmol/L ($P = .035$). As for cFT, the probability of being transferred to IM, ICU, or dying in men with cFT below and above 100 pmol/L was 25.58% [19.35-41.76] vs 84.08% [74.58-99.45], 18.43% [11.80-21.43] vs 2.10% [0.06-3.65], and 16.39% [8.59-21.93] vs 1.26% [0.04-2.25], respectively; all $P < .0001$. As for actual cases, five out of 21 (23.8%) transferred to IM inpatient clinics, two out of two (100%) transferred to ICU, and two out of two (100%) deceased had cFT < 100 pmol/L ($P = .010$).

TABLE 3 Confirmation of thresholds for total and free testosterone toward blood inflammatory markers using linear regressions with linear spline functions

	Total testosterone			Calculated free testosterone		
	Linear	<5 nmol/L	≥5 nmol/L	Linear	<100 pmol/L	≥100 pmol/L
Neutrophils (10^3/μL)	**B = −0.23 [−0.40;-0.06] *P* = .012**	B = −0.63 [−1.53;0.27] *P* = .161	B = −0.15 [−0.40;0.09] *P* = .200	**B = −0.14 [−0.25;-0.03] *P* = .012**	B = −0.32 [−0.73;0.09] *P* = .121	B = −0.10 [−0.25;0.05] *P* = .191
Lymphocytes (10^3/μL)	**B = 0.03 [0.01;0.06] *P* = .023**	B = 0.07 [−0.08;0.22] *P* = .355	B = 0.03 [−0.01;0.07] *P* = .175	**B = 0.02 [0.00;0.04] *P* = .017**	B = 0.04 [−0.03;0.11] *P* = .234	B = 0.02 [−0.01;0.042] *P* = .160
Procalcitonin (ng/mL)	B = −0.03 [−0.07;0.00] 0.084	**B = −0.18 [−0.35;-0.01] *P* = .042**	B = −0.00 [−0.05;0.05] 0.920	B = −0.02 [−0.04;0.00] *P* = .069	**B = −0.09 [−0.16;-0.01] *P* = .026**	B = −0.00 [−0.03;0.03] *P* = .884
LDH (U/L)	**B = −14.49 [−26.49;-2.49] *P* = .020**	**B = −72.72 [−130.02;-15.43] *P* = .015**	B = −3.39 [−18.98;12.20] 0.659	**B = −8.06 [−15.78;-0.35] *P* = .041**	**B = −41.46 [−67.18;-15.73] *P* = .003**	B = 0.71 [−8.81;10.22] *P* = .880
CRP (mg/L)	B = −3.00 [−6.67;0.68] *P* = .106	B = −10.02 [−28.78;8.75] *P* = .283	B = −1.66 [−6.76;3.45] 0.511	---	---	---
Ferritin (ng/mL)	B = −21.68 [−80.77;37.40] *P* = .458	**B = −232.17 [−397.37;-66.97] *P* = .010**	B = −49.76 [−196,47;96,94] *P* = .474	---	---	---

Note: Data are reported as B coefficients and 95% confidence interval. Data derived from linear regressions ("Linear" columns) with total and free testosterone used as continuous variables using the whole range of values. Columns reporting thresholds (< and ≥ 5 nmol/L; < and ≥ 100 pmol/L) report data derived from linear regressions linear spline functions. Significant associations from linear regressions with the whole range of testosterone values without significant associations with spline functions denote that linearity could be assumed. Significant and non-significant associations from linear regressions with the whole range of testosterone values with significant associations with spline functions denote that linearity could not be assumed and confirm the indicated threshold value. Non-significant associations from linear regressions with the whole range of testosterone values with non-significant associations with spline functions denote a lack of association.

Abbreviations: CRP, C-reactive protein; LDH, lactate dehydrogenase.

Bold values denote statistically significant *P*-values.

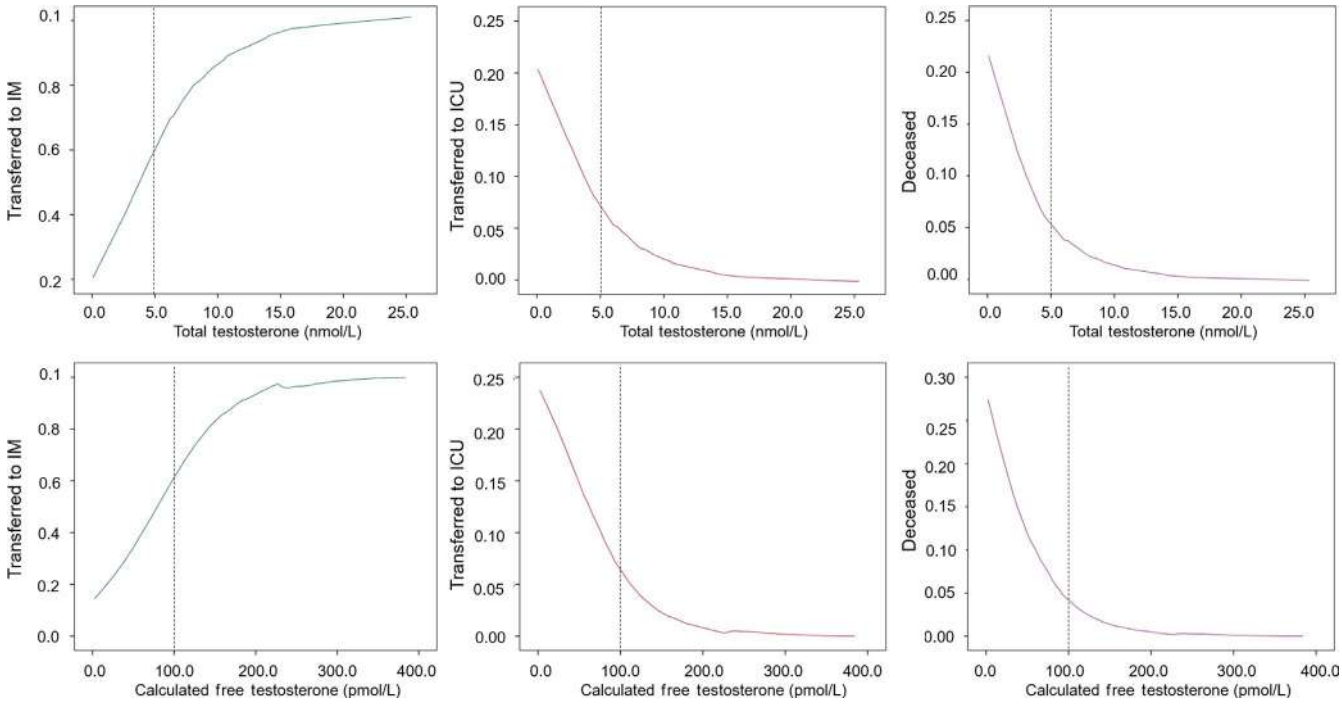


FIGURE 4 Relationship between total or calculated free testosterone and estimated probability of different clinical outcomes in SARS-CoV-2 pneumonia patients based on T levels. The smooth curves were carried out by locally weighted scatterplot smoothing (LOWESS) analysis. Abbreviations: IM = internal medicine inpatient clinics; ICU = intensive care unit

4 | DISCUSSION

Our study demonstrates for the first time that lower levels of TT and cFT (assessed the first day after the admission in RICU) are novel predictors of poor prognosis in SARS-CoV-2 men admitted in RICU for pneumonia. Noteworthy, we found a longitudinal relationship between lower TT and cFT levels and a higher risk of clinical deterioration, thus leading to ICU transfer or to death. Accordingly, we found that lower TT and cFT are significantly associated with higher serum LDH, ferritin, PCT, as well as to an increased level of neutrophils and decrease in lymphocyte count. This latter finding confirms the relevant clinical importance of our observations as all the above-mentioned inflammatory biomarkers had emerged as poor prognostic factors for SARS-CoV-2 infection.[20] Of note, no differences in D-dimer, an important parameter for the diagnosis of disseminated intravascular coagulation, were observed. Interestingly, for several of these markers (ie, PCT, LDH, and ferritin) a non-linear association with T was found with an apparent threshold effect (namely, TT at 5 nmol/L or cFT at 100 pmol/L). For TT < 5 nmol/L, the increase in PCT, LDH, or ferritin associated with one nmol/L decline in TT emerged to be sixfold, fivefold, and 10-fold higher than the linear prediction. Similar results were obtained for cFT below 100 pmol/L for PCT and LDH. Overall, this means that below these threshold values, a further decrease in TT or cFT of 0.5-1.3 nmol/L or 10-23 pmol/L, respectively, could be sufficient to cause an increase in these prognostic markers from the lower limit of normal range above the levels that were found associated with SARS-CoV-2-associated in-hospital death.[2]

Also, in this case, statistical modeling for estimating probability demonstrated a non-linear relationship with mortality or ICU transfer risk. Below TT 5 nmol/L or cFT 100 pmol/L, a steep increase in mortality or ICU transfer risk was observed, with 20- to 30-fold or 10- to 15-fold higher estimated average risk of adverse outcomes as compared to TT or cFT above thresholds, respectively. In contrast, a gradual improvement of clinical outcomes (transfer to non-intensive care IM) with increasing TT or cFT levels was observed.

We also showed that low baseline TT and cFT levels are related to a more severe ARDS at RICU admission. Noteworthy, these data highlight a novel potential mechanism of frailty and mortality by identifying low T as a risk factor for the severe respiratory failure and inflammatory storm in SARS-CoV-2 infections. Our observations are further substantiated by several epidemiological evidence demonstrating that male hypogonadism represents a risk factor for a higher morbidity and mortality.[10,21] In particular, it has been well demonstrated that the so-called functional hypogonadism is associated with conditions like obesity and inflammation in males.[22] Therefore, it could be also speculated that obesity and low T could even foster the cytokine storm aggravating further the clinical condition.

Consistently, over the last decades it has become clear that T is involved in a multitude of biological processes in males, other than reproduction and sexuality. In particular, a novel aspect of the physiology of T is its anti-inflammatory role.[23,24] Several preclinical and clinical evidence showed that low T boosted pro-inflammatory cytokines and that T treatment blunted their levels.[23,25,26] There is evidence for an immunomodulatory and protective effect of T by regulating differentiation of T lymphocytes.[23,27,28] Interestingly, we observed that lymphocyte count increased, while neutrophil levels decreased, as a function of increasing TT and cFT.

Accumulating evidence suggests that SARS-CoV-2 infection severity is influenced by a dysregulation of the immune response. A drastic lymphopenia with reduction in numbers of CD4 + T cells, CD8 + T cells, B cells, and natural killer (NK) cells is a common feature in patients with severe SARS-CoV-2 infection, but not in patients with mild disease,[29] with neutrophil-to-lymphocyte ratio reflecting an enhanced inflammatory process and a poor prognosis.[30] AR is expressed on CD4 + T lymphocytes, CD8 + T lymphocytes, and macrophages supporting the possibility of direct action of T on these cells.[31]

A worsening of clinical status was coupled not only with reduced T level, but also with increased LH levels, even though the latter association did not maintain significance when the four groups were compared, thus supporting the presence of a primary hypogonadism. Accordingly, an orchitis-like syndrome has been hypothesized in SARS-CoV-2 men.[32] Several pathogenic mechanisms occurring during the SARS-CoV-2 infection might be responsible for the impairment of testicular function. First, ACE2 is highly expressed within the human testis, being a constitutive product of adult-type Leydig cells.[33,34] Because angiotensin II reduced both basal and LH-stimulated testosterone synthesis by Leydig cells, ACE2 has been hypothesized to modulate the steroidogenic activity of these cells and to shield testis by limiting angiotensin II detrimental effects.[35,36] ACE2 on Leydig cells could also alter local microvascular flow and permeability[33] and favoring inflammation.[37] Moreover, increased LH levels could contribute further to testicular alterations by modulating testicular blood flow, endothelial cell permeability, and fluid accumulation within the testis.[38] Finally, in our study, SARS-CoV-2 men are affected by a severe form of hypogonadism associated with a primary testicular impairment. A number of well-designed longitudinal studies have shown that late-onset hypogonadism (LOH) represents a common clinical entity among aging males. In most men, there is a slow decline in serum TT levels with aging, even in the absence of disease.[39] However, in our cohort of men T level was sharply reduced without showing any significant age-dependent modulation, further supporting the view of a potential direct effect of SARS-CoV-2 on testicular function.

A study on 6 men (mean age 39 years; ranged from 20 to 58 years old) has previously reported LH and T levels in SARS men.[35] The authors compared SARS men with age-matched healthy men, showing a rise of LH similar to our study, but associated with normal T level.[37] Our study is the first to report novel evidence of a clear-cut reduction in T level along with an LH increase in SARS-CoV-2 men; however, while TT and cFT were associated with a higher risk of clinical deterioration, increased LH levels tended to have a similar relationship, without reaching a statistical significance. We would like to recognize that low serum T levels might also be a consequence

and not a reason for the patients' condition. However, the causal effect relationship could only be tested by randomized trials with testosterone treatment vs placebo in hypogonadal men upon submission to the ICU. Also, the presence of either a psychiatric disorder or cardiac arrhythmias was associated with a higher risk of a poor prognosis, whereas obese patients were paradoxically less prone to worse outcomes.

Some limitations should be recognized. Firstly, the sample size is relatively small with a limited number of adverse outcomes. However, a small sample size represents a concern for lack of significant associations, more than for significant ones. Secondly, six patients were still in charge of the RICU at the time of the present analysis; hence, we do not know their definitive outcome. However, the days passed in RICU are comparable among the groups. Unfortunately, information on the onset of infection before RICU was not collected. Moreover, this study lacks of a control group of patients not affected by SARS-CoV-2 but hospitalized and evaluated in the same way. This comparison could have strengthen our study as the prevalence of hypogonadism in elderly male patients admitted to the hospital for acute illness is reported to be close to 50%.[16,17] Finally, T was assessed only one time and not by the gold standard method (such as mass spectrometry, which was not available in this clinical setting) but it was measured by a commercially available immunoassay used in a high-volume hospital and undergoing quality control programs. Free T was calculated rather than measured.

5 | CONCLUSIONS

Our study demonstrated that, besides a clear pattern of inflammatory, hematologic, biochemical, and immune biomarker abnormalities, lower TT and cFT levels enable significant discrimination between SARS-CoV-2 patients with or without poor clinical outcomes. Therefore, both TT and cFT level assessment may potentially aid in terms of risk stratification modeling to predict severe and even fatal SARS-CoV-2 infection. To the best of our knowledge, this is the first study unraveling the prognostic role of T levels toward either severity or mortality associated with SARS-CoV-2 pneumonia. Whether testosterone therapy could theoretically be beneficial, mitigating the steep increase in clinical deterioration among severely hypogonadal SARS-CoV-2 men may be worth further studies.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest in connection with this article.

AUTHORS' CONTRIBUTIONS

Giulia Rastrelli, Vincenza Di Stasi, Linda Vignozzi, Mario Maggi, and Giuseppe De Donno conceived and designed the study. Vincenza Di Stasi, Francesco Inglese, Massimiliano Beccaria, Martina Garuti, Domenica Di Costanzo, Fabio Spreafico, Graziana Francesca Greco, Giulia Cervi, Antonietta Pecoriello, and Giuseppe De Donno acquired the data. Giulia Rastrelli, Vincenza Di Stasi, Angela Magini, Mario Maggi, Giuseppe De Donno, and Linda Vignozzi analyzed and interpreted the data. Giulia Rastrelli, Vincenza Di Stasi, and Linda Vignozzi drafted the article. Giulia Rastrelli, Andrea Salonia, Andrea Lenzi, Mario Maggi, and Linda Vignozzi revised the article for intellectual contents. Giulia Rastrelli, Vincenza Di Stasi, Francesco Inglese, Massimiliano Beccaria, Martina Garuti, Domenica Di Costanzo, Fabio Spreafico, Graziana Francesca Greco, Giulia Cervi, Antonietta Pecoriello, Angela Magini, Tommaso Todisco, Sarah Cipriani, Elisa Maseroli, Giovanni Corona, Andrea Salonia, Andrea Lenzi, Mario Maggi, Giuseppe De Donno, and Linda Vignozzi provided the final approval of the completed article.

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Alerta Epidemiológica
COVID-19 durante el embarazo
13 de agosto de 2020

Estudios y resultados de la vigilancia de COVID-19 publicados recientemente indicaron un mayor riesgo de la mujer embarazada de presentar formas graves de COVID-19 y por ende de ser hospitalizadas y admitidas a Unidades de Cuidados Intensivos. La Organización Panamericana de la Salud / Organización Mundial de la Salud (OPS/OMS) solicita a los Estados Miembros a redoblar esfuerzos para asegurar el acceso a los servicios de atención prenatal, así como también a implementar medidas preventivas para reducir la morbilidad y mortalidad asociada a la COVID-19 en todos los niveles del sistema de salud, manteniendo los logros y el compromiso de reducir la mortalidad materna y perinatal.

Introducción

Además de los desafíos propios de la respuesta a la pandemia de COVID-19 los países y territorios en la Región han tenido que enfrentar el reto de mantener los logros alcanzados en el ámbito de la salud pública y al mismo tiempo de seguir ofreciendo, sin interrupciones, los servicios de atención que requieren las mujeres en edad fértil y en particular las embarazadas.

La implementación de medidas restrictivas en la movilidad de las personas, o el cierre de algunos centros de atención de salud, han dificultado que las embarazadas reciban el número de controles prenatales adecuados a su edad gestacional. Esto podría implicar la detección tardía de problemas en el embarazo (como por ejemplo diabetes gestacional o estados hipertensivos) o en el feto (como por ejemplo las restricciones en el crecimiento intrauterino) y por lo tanto poner en riesgo la vida de ambos.

A ello, habrá que sumar la escasa información científica disponible sobre el efecto del virus SARS-CoV-2 en el embarazo y en el feto, lo cual dificulta la adopción oportuna de medidas correctivas.

Aunque el perfil de las embarazadas en la Región de las Américas podría ser diferente al perfil de las embarazadas en Europa, se sugiere tener en cuenta algunos estudios realizados en mujeres embarazadas en Europa a la hora de detectar factores de riesgo, de manera tal de mitigar el potencial impacto de la COVID-19 en el embarazo y el feto.

Un estudio realizado en una cohorte de 427 mujeres embarazadas ingresadas en el hospital con infección confirmada por SARS-CoV-2 entre el 1 de marzo de 2020 y el 14 de abril de 2020 en el Reino Unido, encontró que la mayoría de las ingresadas en el hospital se encontraban a finales del segundo o tercer trimestre de embarazo; 233 eran de raza negra o de otros grupos étnicos minoritarios, 281 tenían sobrepeso u obesidad, 175 tenían 35 años o más y 145 tenían comorbilidades preexistentes. Cuarenta y una mujeres ingresadas en el hospital necesitaron asistencia respiratoria y cinco fallecieron.

Cita sugerida: Organización Panamericana de la Salud / Organización Mundial de la Salud. Alerta Epidemiológica: COVID-19 en el embarazo, 13 de agosto de 2020, Washington, D.C. OPS/OMS. 2020

Con relación al término del embarazo, el estudio indicó que 266 mujeres tuvieron un parto o perdieron el embarazo; 196 tuvieron un parto a término. Doce de 265 recién nacidos dieron positivo al RNA del SARS-CoV-2, seis de ellos dentro de las primeras 12 horas después del nacimiento[1].

En otro estudio realizado en España, se realizaron pruebas de anticuerpos para el SARS-CoV-2 en 874 mujeres embarazadas que asistieron consecutivamente a la detección del embarazo en el primer trimestre (entre las 10-16 semanas de gestación, 372 mujeres) o al parto (502 mujeres) entre el 14 de abril y el 5 de mayo de 2020, en tres hospitales universitarios[2] en Barcelona. La seroprevalencia fue similar entre las mujeres en el primer trimestre del embarazo y las mujeres en el tercer trimestre, lo que sugiere un riesgo similar de infección, pero la proporción de mujeres con síntomas y la proporción de mujeres que requirieron hospitalización fue mayor en el grupo del tercer trimestre que en el grupo del primer trimestre[3].

A continuación, se presenta un resumen de la situación de mujeres embarazadas y mortalidad materna en el contexto de COVID-19, en los países de la Región de las Américas, para los cuales se dispone de información.

Lista de paises que han notificado casos y defunciones de embarazadas con COVID-19 .

Tabla 1. Casos y defunciones de embarazadas con COVID-19, según país. Región de las Américas. 1 de enero de 2020 al 11 de agosto de 2020.

Embarazadas con COVID-19		
País	Vivas	Fallecidas
Argentina	155	1
Bolivia	50	5
Brasil	2.256	135
Colombia	571	2
Ecuador	849	20
Estados Unidos de América	15.735	37
Haití	39	2
México*	3.916	106
Perú	4.782	36
República Dominicana	304	12

*México notifica embarazadas y puérperas.

Fuentes: Centro Latinoamericano de Perinatología, Salud de la Mujer, y Reproductiva (CLAP/SMR)
Información publicada en los sitios web de los Ministerios de Salud, Agencias de Salud o similares y reproducidos por la OPS/OMS.

[1] Marian Knight 1, Kathryn Bunch 2, Nicola Vousden et al. Characteristics and outcomes of pregnant women admitted to hospital with confirmed SARS-CoV-2 infection in UK: national population-based cohort study. BMJ 2020 Jun 8;369:m2107. doi: 10.1136/bmj.m2107.
[2] Hospital Sant Joan de Déu, Hospital Clínic y Hospital Sant Pau
[3] Francesca Crovetto, Fàtima Crispi, Elisa Llurba et al. Seroprevalence and presentation of SARS-CoV-2 in pregnancy. The Lancet. DOI: https://doi.org/10.1016/S0140-6736(20)31714-1

En **Brasil**, entre 1 de enero y el 1 de agosto de 2020 fueron hospitalizadas 5.174 embarazadas, lo que corresponde a 0.9% del total de hospitalizaciones por Infección Respiratoria Aguda Grave (IRAG). Del total de embarazadas hospitalizadas por IRAG, 2.256 (44%) fueron confirmadas para COVID-19 y de ellas, 135[4] fallecieron.

La distribución geográfica de las embarazadas hospitalizadas por IRAG confirmadas para COVID-19, en orden decreciente, según región de residencia es la siguiente: Sudeste (885 casos), Nordeste (744 casos), Norte (312 casos), Centro-Oeste (163 casos) y Sur (152 casos). Mientras que las defunciones fueron notificadas en las regiones Nordeste (52 defunciones), Sudeste (49 defunciones), Norte (23 defunciones), Centro-Oeste (10 defunciones) y Sur (1 defunción).

Con relación a las características de las embarazadas fallecidas, el grupo etario con más casos fue el de 30 a 39 años, seguido por el grupo de 20 a 29 años; 56,3% de las embarazadas con COVID-19 fallecieron en el tercer trimestre, 33,3% en el segundo trimestre y 4,4% en el primer trimestre (**Tabla 2**).

Las comorbilidades más frecuentes entre las fallecidas fueron: diabetes (16,3%), cardiopatía (13,3%), obesidad (11,9%), e hipertensión (5,9%).

Tabla 2. Distribución de embarazadas con COVID-19, según grupo etario y edad gestacional. Brasil, 1 de enero al 1 de agosto de 2020.

Embarazadas con COVID-19		
Caracteristicas	Vivas	Fallecidas
Grupo etario	# Casos	# Casos
10-19	203	6
20-29	941	51
30-39	956	64
40-49	156	14
Total	2.256	135
Edad Gestacional	# Casos	# Casos
1° Trimestre	177	6
2° Trimestre	523	45
3° Trimestre	1.450	76
E.G. desconocida	106	8
Total	2.256	135

Fuente: Datos publicados por el Ministerio de Salud de Brasil y reproducidos por la OPS/OMS.

En los **Estados Unidos de América**, un estudio publicado por los Centros para el Control y Prevención de enfermedades (CDC, por sus siglas en inglés)[5] informó que entre el 22 de enero

[4] Datos preliminares, sujetos a revisión.

[5] Sascha Ellington; Penelope Strid; Van T. Tong; et al. Characteristics of Women of Reproductive Age with Laboratory-Confirmed SARS-CoV-2 Infection by Pregnancy Status — United States, January 22–June 7, 2020. MMWR Morb Mortal Wkly Rep 2020;69: [769-775].

y el 7 de junio de 2020, como parte de la vigilancia de COVID-19, fueron notificados 326.335 casos positivos de infección por SARS-CoV-2 en mujeres en edad reproductiva[6]. De éstas, 9% (8.207 de un total de 91.412[7]) estaban embarazadas.

Si bien el estudio encontró que la frecuencia de enfermedad pulmonar crónica, diabetes mellitus y enfermedad cardiovascular fue mayor entre las mujeres embarazadas en comparación con las mujeres no embarazadas, la proporción de hospitalizadas fue mayor entre las embarazadas que las mujeres no embarazadas (31,5% vs 5,8%).

Después de ajustar los datos por edad, presencia de condiciones subyacentes y raza / etnia, se observó que las mujeres embarazadas presentaron 5.4 veces más probabilidades de ser hospitalizadas (IC 95% = 5,1–5,6), 1,5 veces más de probabilidades de ser admitidas en la UCI (IC 95% = 1,2–1,8) y 1,7 veces más probabilidades de recibir ventilación mecánica (IC 95% = 1,2–2,4) que las no embarazadas.

Adicionalmente se observó que la admisión a la UCI fue notificada con mayor frecuencia entre mujeres embarazadas asiáticas no hispanas (3,5%) que entre todas las mujeres embarazadas (1,5%).

Con relación a las defunciones, se notificaron 16 relacionadas con COVID-19 (0,2%) entre mujeres embarazadas y 208 (0,2%) entre mujeres no embarazadas (aRR = 0,9, IC 95% = 0,5–1,5).

Estos hallazgos sugieren que entre las mujeres en edad reproductiva con COVID-19, las mujeres embarazadas tienen más probabilidades de ser hospitalizadas y tienen un mayor riesgo de ingreso en la UCI y de recibir ventilación mecánica.

Si bien es cierto, este estudio tiene limitaciones que deben ser consideradas, lo que destaca es la necesidad de que las embarazadas estén conscientes de su potencial riesgo de desarrollar enfermedad grave por COVID-19.

De acuerdo con la información publicada por los CDC, entre el 22 de enero y el 4 de agosto de 2020, en los Estados Unidos de América, fueron notificados 15.735 casos de mujeres embarazadas con COVID-19, incluidas 37 defunciones. Del total de mujeres embarazadas, 4.086 fueron hospitalizadas (26%)[8], 183 fueron admitidas a la UCI y 66 requirieron ventilación mecánica[9].

En **México**, desde la confirmación de los primeros casos de COVID-19 en el país[10] y hasta el 9 de agosto de 2020, se notificaron 3.916 mujeres embarazadas y en puerperio en seguimiento incluidas 106 defunciones (2,7%) en las que se confirmó la infección por SAR-CoV-2.

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[6] 15 a 44 años
[7] Para las cuales el dato sobre el estado del embarazo estaba disponible.
[8] No hubo datos disponibles para distinguir la hospitalización por indicaciones relacionadas con COVID-19 (como por ejemplo el empeoramiento del estado respiratorio), de la hospitalización por indicaciones relacionadas con el embarazo, como el parto.
[9] Se recopilaron datos de 15.735 mujeres, pero los datos de ingreso a la UCI solo estaban disponibles para 4.319 (27,4%) mujeres y los datos de ventilación mecánica sólo estaban disponibles para 3.533 (22,5%) mujeres.
[10] 27 de febrero de 2020

Las entidades federativas, con más casos de embarazadas y las puérperas con COVID-19 son: la Ciudad de México (480 casos, 13 defunciones) y los Estados de Tabasco (307 casos, 11 defunciones), México (293 casos, 13 defunciones), Sonora (202 casos, 5 defunciones), Nuevo León (199 casos, 3 defunciones), Veracruz (189 casos, 6 defunciones) y Guanajuato (182 casos, 3 defunciones).

Con relación a las características de las embarazadas y puérperas fallecidas, la mediana de edad fue 30 años (rango 19 a 42), el 43,3% fallecieron en el tercer trimestre de embarazo, el 34% en el puerperio, 16% en el segundo trimestre y 5,6% en el primer trimestre (**Tabla 3**). En este grupo, 33 estuvieron intubadas (31%) y 35 en la Unidad de Cuidados Intensivos (33%).
Las comorbilidades más frecuentes entre las fallecidas fueron: obesidad (17,9%), diabetes (10,4%), hipertensión (7,6%) y asma (4,7%).

Tabla 3. Distribución de embarazadas y puérperas fallecidas con COVID-19, según grupo etario y edad gestacional. México 27 de febrero al 9 de agosto de 2020.

Embarazadas y puérperas fallecidas con COVID-19	
Grupo etario	# Casos
15-19	2
20-24	21
25-29	24
30-34	31
35-39	20
40-44	8
Total	106
Edad Gestacional	# Casos
1° Trimestre	6
2° Trimestre	17
3° Trimestre	47
Puerperio	36
Total	106

Fuente: Datos publicados por la Secretaría de Salud de México y reproducidos por la OPS/OMS.

La razón de mortalidad materna (RMM) para COVID-19 a la semana epidemiológica (SE) 31 de 2020[11] en México es 8,1 muertes maternas por 100.000 recién nacidos vivos.

Hasta el 9 de agosto de 2020, fueron notificados en plataforma de SISVER[12] 4.066 recién nacidos, de los cuales 832 (20,5 %) fueron positivos a SARS-CoV-2. Respecto de la condición de sus madres,130 son hijos de madres positivas a SARS- CoV-2, 4 hijos de madres sospechosas a COVID-19, 66 hijos de madres negativas y para los 632 restantes no hubo registro de sus madres en SISVER.[13].

[11] SE 31 de 2020 finalizó el 1 de agosto.
[12] Sistema de Vigilancia Epidemiológica de Enfermedades Respiratorias de México
[13] Secretaria de Salud de México. Informe epidemiológico semanal de embarazadas y puérperas estudiadas, ante sospecha de COVID-19. Semana epidemiológica 33 de 2020. Disponible en: https://bit.ly/33z4x4Q accedido el 12 de agosto de 2020.

Orientaciones para las autoridades nacionales

Los datos disponibles hasta el momento sugieren que las mujeres embarazadas están en mayor riesgo de desarrollar una forma grave de COVID-19 y, en algunos casos, podrían evolucionar a la muerte. En este contexto, la Organización Panamericana de la Salud / Organización Mundial de la Salud (OPS/OMS) recomienda a los Estados Miembros abordar los riesgos y vulnerabilidades específicas que enfrenta este grupo de la población, asegurar la continuidad de los servicios de atención prenatales y la oportuna atención a los signos y síntomas de gravedad por COVID-19 en las embarazadas. Asimismo, se recomienda procurar mantener la comunicación con las embarazadas, para que sepan dónde consultar en caso de emergencia y coordinar los controles virtuales o presenciales o incluso domiciliarios si lo amerita.

A continuación, se presenta una serie recomendaciones en relación con vigilancia epidemiológica, laboratorio y manejo clínico de la embarazada en el contexto de la pandemia de COVID-19.

Vigilancia

Como se ha enfatizado en Alertas y Actualizaciones Epidemiológicas anteriores[14], para interrumpir la transmisión de COVID-19 deben realizarse las actividades listadas abajo, las cuales también se aplican a mujeres embarazadas:

- Detección temprana de casos sospechosos
- Confirmación por laboratorio
- Aislamiento
- Rastreo y cuarentena de contactos

Para la detección temprana de casos sospechosos se propone utilizar la definición de caso actualizada de la OPS/OMS disponible en: https://bit.ly/3kOk9YC

Laboratorio

La confirmación de la circulación de COVID-19 en una población requiere de pruebas de laboratorio. La OPS/OMS recomienda que todos los casos sospechosos se analicen para detectar COVID-19 mediante ensayos virológicos, de acuerdo con las definiciones de casos.

Es importante asegurar el acceso a pruebas para la confirmación del diagnóstico; sin embargo, en áreas con alta incidencia y/o de limitada capacidad o acceso a pruebas de laboratorio, es importante establecer un criterio de priorización para la realización de las pruebas con el fin de implementar medidas que puedan reducir la propagación. En esta

[14] Disponibles en: https://www.paho.org/es/alertas-actualizaciones-epidemiologicas

situación, la priorización de ensayos para casos sospechosos en embarazadas debe ser considerada, teniendo en cuenta que:

- Son personas a riesgo de desarrollar formas graves de la enfermedad.

- En algún momento durante el tiempo de embarazo, requerirán hospitalización.

Toda embarazada sospechosa de COVID-19, a la cual no fuera posible confirmar por pruebas de laboratorio por cualquier razón, debe ser consideradas como caso de COVID-19.

Manejo clínico

Toda embarazada y puérpera debe ser manejada de acuerdo con las directrices y normativas establecidas y vigentes en cada país y territorio de las Américas.

Debido a que la mujer embarazada en la cual se sospeche o se haya confirmado la infección por SARS-CoV- 2, requerirá medidas adicionales para la atención específica debido a la COVID-19, se recomienda considerar los siguientes documentos a la hora de la toma de decisiones:

- Guía para el cuidado de pacientes adultos críticos con COVID-19 en las Américas. Versión 2, 29 de julio del 2020. Disponible en: https://bit.ly/3it5AHK

- Algoritmo de manejo de pacientes con sospecha de infección por COVID-19 en el primer nivel de atención y en zonas remotas de la región de las Américas, julio del 2020 Disponible en: https://bit.ly/33SzCk2

- Atención inicial de personas con infección respiratoria aguda (IRA) en el contexto de la infección por coronavirus (COVID-19) en establecimientos de salud: evaluar el riesgo, aislar, referir. Recomendaciones provisionales, versión 1 (12 de abril de 2020. Disponible en: https://bit.ly/2DHEWwb

- Manejo clínico de la COVID. Recomendaciones provisionales, 27 de mayo de 2020. Disponible en: https://bit.ly/3gRScMR

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