Received: 14 August 2023 | Revised: 7 December 2023 | Accepted: 12 December 2023

DOI: 10.1111/andr.13581

ANDROLOGY WILEY

ORIGINAL ARTICLE

Sex hormones and the total testosterone:estradiol ratio as predictors of severe acute respiratory syndrome coronavirus 2 infection in hospitalized men

David Ruiz-Ochoa[1] | Armando-Raúl Guerra-Ruiz[2,3,4] | María-Teresa García-Unzueta[2,3,4] | Pedro Muñoz-Cacho[3,5] | Bryan Rodriguez-Montalvan[6] | Carlos Antonio Amado-Diago[3,4,7] | Bernardo-Alio Lavín-Gómez[2,3] | María-Eliecer Cano-García[8] | Daniel Pablo-Marcos[8] | Luis Alberto Vázquez[1,3,4]

[1]Department of Endocrinology and Nutrition, Marqués de Valdecilla University Hospital, Santander, Spain

[2]Department of Clinical Biochemistry, Marqués de Valdecilla University Hospital, Santander, Spain

[3]IDIVAL Health Research Institute, Santander, Spain

[4]University of Cantabria, Santander, Spain

[5]Department of Medicine and Psychiatry, Gerencia de Atención Primaria, Servicio Cántabro de Salud, Santander, Spain

[6]Department of Endocrinology and Nutrition, San Pedro University Hospital, Logroño, Spain

[7]Department of Pneumology, Marqués de Valdecilla University Hospital, Santander, Spain

[8]Department of Microbiology, Marqués de Valdecilla University Hospital, Servicio Cántabro de Salud, Santander, Spain

Correspondence
David Ruiz-Ochoa, Endocrinology and Nutrition, Marqués de Valdecilla University Hospital, Avda. Valdecilla s/n, 39005-Santander, Cantabria, Spain.
Email: david.ruiz@scsalud.es

Trial registration: Not applicable

Funding information
IDIVAL-Instituto de Investigación Marqués de Valdecilla

Abstract

Background: The predictive ability of the early determination of sex steroids and the total testosterone:estradiol ratio for the risk of severe coronavirus disease 2019 or the potential existence of a biological gradient in this relationship has not been evaluated.

Objectives: To assess the relationship of sex steroid levels and the total testosterone:estradiol ratio with the risk of severe acute respiratory syndrome coronavirus 2 infection in men, defined as the need for intensive care unit admission or death, and the predictive ability of each biomarker.

Materials and methods: This was a prospective observational study. We included all consecutive adult men with severe acute respiratory syndrome coronavirus 2 infections in a single center admitted to a general hospital ward or to the intensive care unit. Sex steroids were evaluated at the centralized laboratory of our hospital.

Results: We recruited 98 patients, 54 (55.1%) of whom developed severe coronavirus disease in 2019. Compared to patients with nonsevere coronavirus disease 2019, patients with severe coronavirus disease 2019 had significantly lower serum levels of total testosterone (111 ± 89 vs. 191 ± 143 ng/dL; $p < 0.001$), dehydroepiandrosterone (1.69 ± 1.26 vs. 2.96 ± 2.64 ng/mL; $p < 0.001$), and dehydroepiandrosterone sulfate (91.72 ± 76.20 vs. 134.28 ± 98.261 μg/dL; $p = 0.009$), significantly higher levels of estradiol (64.61 ± 59.35 vs. 33.78 ± 13.78 pg/mL; $p = 0.001$), and significantly lower total testosterone:estradiol ratio (0.28 ± 0.31 vs. 0.70 ± 0.75; $p < 0.001$). The lower the serum level of androgen and the lower the total testosterone:estradiol ratio values, the higher the likelihood of developing severe coronavirus disease 2019, with the linear trend in the adjusted analyses being statistically significant for all parameters except for androstenedione ($p = 0.064$). In the receiver operating characteristic

analysis, better predictive performance was shown by the total testosterone:estradiol ratio, with an area under the curve of 0.77 (95% confidence interval 0.68–0.87; $p < 0.001$).

Discussion and conclusion: Our results suggest that men with severe acute respiratory syndrome coronavirus 2 infection, decreased androgen levels and increased estradiol levels have a higher likelihood of developing an unfavorable outcome. The total testosterone:estradiol ratio showed the best predictive ability.

KEYWORDS
COVID-19, intensive care unit, mortality, predictive ability, testosterone, total testosterone:estradiol ratio

1 | INTRODUCTION

Sex acts as a modifier of the epidemiology, pathophysiology, clinical manifestations, disease progression, and/or response to treatment of several diseases, including coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] The November 2021 report of the COVID-19 Sex-Disaggregated Data Tracker, the world's largest database of sex-disaggregated data on COVID-19, showed that, compared to women, men accounted for a higher proportion of hospitalizations (55% vs. 45%), intensive care unit (ICU) admissions (63% vs. 37%) and deaths (57% vs. 43%).[2] Among other factors, such as differences in lifestyle and behaviors (e.g., greater tobacco and/or alcohol use among men) and the prevalence of comorbidities, which are leading causes of disability and death (e.g., a higher prevalence of diabetes, cardiovascular disease, or chronic obstructive pulmonary disease among men), it has been hypothesized that sex hormones could play a key role in the different burdens of COVID-19 between men and women, with estrogens playing a protective role and androgens playing a somewhat controversial role[3–5]; regarding androgens, increasing evidence indicates that low testosterone levels have a negative impact on COVID-19.[4,6]

Early identification of patients at a higher risk of severe COVID-19 is a major challenge.[7] Overall, low testosterone levels could be a biomarker of poorer health since they have been found in several chronic diseases, such as obesity and diabetes,[8] chronic obstructive pulmonary disease,[9] HIV infection[10] and chronic kidney disease.[11] Moreover, low testosterone levels have been associated with an increased likelihood of mortality among men from the general population[12,13] and among men with chronic kidney disease.[14,15] Among patients with COVID-19, several studies have reported an association between low testosterone levels and severe disease or worse outcomes.[16–20] Using a retrospective design, Salonia et al.[16] found in a multivariate model that the higher the testosterone levels were, the lower the likelihood of ICU admission (odds ratio [OR] 0.53, 95% confidence interval [CI] 0.39–0.74) and death (OR 0.67, 95% CI 0.46–0.99); using an interaction analysis, they showed that for the same severity of the disease, the lower the testosterone level was, the higher the risk of death. Rastrelli et al.,[17] in a cohort of 31 men with SARS-CoV-2 pneumonia admitted to a respiratory intensive care unit, found lower testosterone levels among those who were transferred to the ICU for intubation or who had died than among those who remained stable in the respiratory intensive care unit or were transferred to an internal medicine unit. In a prospective cohort study that included 358 men with COVID-19, Cinislioglu et al.[18] found lower testosterone levels at admission in those subjects with clinically severe disease compared to those with mild-to-moderate disease, in those who required intensive care compared with those who did not, and in those who died vs. survivors. Dhindsa et al.,[19] in a prospective cohort study of 90 men with COVID-19, reported that those with severe disease defined as hypoxia requiring supplemental oxygen, a need for mechanical ventilation, need for ICU treatment, or death due to COVID-19 during hospitalization showed lower testosterone levels during hospitalization than those with milder disease.

Very few studies have assessed the predictive ability of the early determination of sex steroids and the total testosterone:estradiol ratio (T:E2) for the risk of serious COVID-19 or the potential existence of a biological gradient in this relationship. The primary objective of this study was to assess the relationship between sex steroid levels and the T:E2 ratio with the risk of severe SARS-CoV-2 infection in men, defined as the need for ICU admission or death, and the predictive ability of each biomarker.

2 | MATERIALS AND METHODS

2.1 | Study design and subjects

This was a prospective observational study conducted at a single center (Hospital Universitario Marqués de Valdecilla, Santander, Spain). All patients were informed of the purpose of this study and provided their written informed consent. This study was reviewed and approved by the Ethics Committee for Medicines Research (CEIm by its Spanish acronym) of Cantabria (Santander, Spain) and funded by Research Institute IDIVAL (INNVAL20/15). The study was performed according to the Declaration of Helsinki

We included all consecutive men with SARS-CoV-2 infection admitted to the center from March 2020 to April 2021 who met the following selection criteria: aged 18 or older, with a confirmed diagnosis of

SARS-CoV-2 infection by quantitative real-time reverse transcription polymerase chain reaction (qRT–PCR) and/or by compatible symptoms and positive results by serological antibody test, admitted to a general hospital ward or to the ICU, who were not receiving drugs or using substances that could interfere with the evaluation of sex steroids and who provided their written informed consent.

After obtaining blood samples for the assessment of study parameters, all patients received treatment according to the protocols established in our hospital. This regimen consisted of dexamethasone plus hydroxychloroquine and/or lopinavir/ritonavir.

2.2 | Assessments

We recorded information from the clinical charts on age and comorbidities, including obesity, smoking status, hypertension, diabetes, dyslipidemia, cardiovascular disease, and immunosuppression.

Sex steroids were evaluated at the centralized laboratory of our hospital within the first 24 hours after admission; total serum testosterone, estradiol, dehydroepiandrosterone sulfate (DHEAS), and sex hormone-binding globulin (SHBG) were evaluated by a specific and automated chemiluminescent immunoassay (CLIA) with the MAGLUMI 2000 Analyzer (Snibe; Shanghai Int Holding Corp). The sensitivity of these assays was 15 ng/dL for total testosterone, 12 pg/mL for estradiol, 1 pg/dL for DHEAS and 0.8 nmol/L for SHBG. Precision within and between runs was < 6 and < 7% for total testosterone; < 7 and < 6% for estradiol; < 6 and < 8% for DHEAS and < 4 and < 5% for SHBG at any concentration, respectively. Noninterference was found with substances that could potentially interfere, as documented by each assay. DHEA was quantified by specific radioimmunoassay (DRG Instruments): the sensitivity was 0.16 ng/mL; the reproducibility within and between assays was < 10 and 15%, respectively; and nonsignificant reactivities were obtained with several compounds (DHEAS, isoandrosterone and androstenedione). In the same manner, androstenedione was quantified by specific radioimmunoassay (DiaSource Immunoassays; Louvain-la-Neuve): the sensitivity was 0.03 ng/mL; the reproducibility within and between assays was < 5 and 9%, respectively; and nonsignificant reactivities were obtained with several compounds (DHEA, DHEAS, cortisol, estradiol, and testosterone among others) documented by the manufacturer.

Our in-house reference range values obtained from a population of 72 healthy nonobese fertile men aged 25–40 were: 145.8–717.5 ng/dL for testosterone; 20.7-90.3 pg/mL for estradiol; 148.9–540.9 μg/dL for DHEAs; 1.9–19.1 ng/mL for DHEA; 0.9–6.4 ng/mL for androstenedione and 12.8–69.4 ng/mL for SHBG.

We also calculated the total T:E2 as the balance between the two hormones and markers of aromatase activity, the enzyme that converts testosterone into estradiol. The following formula was used for the calculation: testosterone/(10*estradiol).

2.3 | Statistical analysis

Quantitative variables are described using the means and standard deviations or, if not normally distributed, with the medians and interquartile ranges; normality was tested with the Shapiro–Wilk test. Categorical variables are expressed as absolute and relative frequencies.

Patients were categorized as having severe COVID-19 if they were admitted to the ICU or died during hospitalization and nonsevere COVID-19 if this was not the case. The characteristics of the patients and the serum levels of sex hormones between the two groups were compared using Student's t-test or, if not normally distributed, the Mann–Whitney U test for quantitative variables and the chi-square test for categorical variables.

To analyze the presence of a biological gradient in the potential association between sex hormone levels and severe COVID-19, serum levels of sex hormones and the T:E2 ratio were categorized in tertiles. The highest tertile was selected as the reference category except for estradiol, for which we used the lowest tertile, and we tested whether subsequently lower or higher categories were associated with an increased likelihood of the outcome using a crude and adjusted analysis. For the adjusted analysis, we used a multiple logistic regression model that included age, obesity, smoking status (yes/no), diabetes, and chronic kidney disease defined as a glomerular filtration rate below 60 mL/min/1.73 m^2 as covariates.

The predictive ability of each sex hormone parameter was analyzed using receiver operating characteristic (ROC) analysis and calculating the area under the curve (AUC) as an overall summary of prognostic accuracy. Youden's index was used to determine the best cutoff point. For a test with poor diagnostic accuracy, Youden's index is 0, whereas for a perfect test, it is 1.

All analyses were performed using SPSS version 26.0 (IBM SPSS Statistics; IBM Corp.) and Stata 13.0 for Windows (StataCorp LLC). The significance level was set at $p < 0.05$.

3 | RESULTS

3.1 | Baseline characteristics of the patients

We recruited 98 patients, and of them, 54 (55.1%) developed severe COVID-19. Patients had a mean (standard deviation) age of 63.7 (15.5) years, with those exhibiting severe COVID-19 being older, although the differences were not statistically significant (Table 1). With the exception of being a current smoker, which was more frequent among patients with nonsevere COVID-19 (9.5% vs. 5.7%), all comorbidities were more frequent in patients with severe COVID-19, and the largest differences were observed for obesity (47.2% vs. 29.5% for severe and nonsevere COVID-19, respectively); none of the differences in comorbidities were statistically significant (Table 1).

3.2 | Sex hormone concentrations

Compared to patients with nonsevere COVID-19, patients with severe COVID-19 had significantly lower serum levels of total testosterone (111 ± 89 vs. 191 ± 143 ng/dL, $p < 0.001$). The serum levels of DHEA and DHEAS were also significantly lower among patients with

TABLE 1 Baseline characteristics of the patients.

Characteristic	All patients $n = 98$	Nonsevere COVID-19 $n = 44$	Severe[a] COVID-19 $n = 54$	*p*-Value
AGE (YEARS), MEAN ± SD	63.65 ± 15.53	61.66 ± 16.20	65.28 ± 14.91	0.253
OBESITY, *n* (%)	38 (38.8)	13 (29.5)	25 (47.2)	0.096
SMOKING, *n* (%)	7 (7.4)	4 (9.5)	3 (5.7)	0.696
HYPERTENSION, *n* (%)	50 (51.5)	21 (47.7)	29 (54.7)	0.544
DIABETES, *n* (%)	14 (15.6)	6 (14.0)	8 (17.0)	0.776
DYSLIPIDEMIA, *n* (%)	46 (50.5)	19 (46.3)	27 (54.0)	0.530
CARDIOVASCULAR DISEASE, *n* (%)	7 (7.1)	3 (6.8)	4 (7.4)	1.000
IMMUNOSUPPRESSION, *n* (%)	2 (2.5)	2 (5.1)	0 (0)	0.234

[a]Severe COVID-19 was defined as admission to the intensive care unit or death during hospitalization.

TABLE 2 Sex hormone concentrations in patients with nonsevere and severe coronavirus disease 2019 (COVID-19).

Variable	All patients $n = 98$	Nonsevere COVID-19 $n = 44$	Severe[a] COVID-19 $n = 54$	*p*-Value
Testosterone (ng/dL)	147 ± 123 120 (137)	191 ± 143 147 (124)	111 ± 89 82 (94)	< 0.001
DHEAS (microg/dL)	110.80 ± 88.91 92.97 (108.30)	134.28 ± 98.261 110.05 (102.05)	91.72 ± 76.20 68.87 (91.71)	0.009
Estradiol (pg/mL)	50.77 ± 47.40 37.34 (22.60)	33.78 ± 13.78 31.30 (22.74)	64.61 ± 59.35 46.15 (51.77)	0.001
SHBG (nmol/L)	37.23 ± 20.80 34.84 (26.76)	39.03 ± 18.90 35.38 (23.16)	35.72 ± 22.34 30.62 (29.67)	0.192
DHEA (ng/mL)	2.27 ± 2.10 1.65 (1.7)	2.96 ± 2.64 2.30 (1.5)	1.69 ± 1.26 1.26 (1.3)	<0.001
Androstenedione (ng/mL)	1.91 ± 1.36 1.45 (1.8)	2.01 ± 1.14 1.75 (1.5)	1.82 ± 1.52 1.40 (1.8)	0.143
T:E2 ratio	0.47 ± 0.59 0.29 (0.51)	0.70 ± 0.75 0.48 (0.65)	0.28 ± 0.31 0.16 (0.35)	< 0.001

Abbreviations: DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; SHBG, sex hormone-binding globulin; T:E2 ratio, total testosterone:estradiol ratio.
All figures are the means ± standard deviations and medians (interquartile ranges).
[a]Severe COVID-19 was defined as admission to the intensive care unit or death during hospitalization.

severe COVID-19; however, although compared to patients with nonsevere COVID-19, the levels of SHBG and androstenedione were also lower among patients with severe COVID-19, the differences were not statistically significant (Table 2). Serum estradiol levels were significantly higher among patients with severe COVID-19 (64.61 ± 59.35 vs. 33.78 ± 13.78 pg/mL, $p = 0.001$). The T:E2 ratio was significantly lower among patients with severe COVID-19 (0.28 ± 0.31 vs. 0.70 ± 0.75, $p < 0.001$).

Our analysis of the biological gradient in the potential association between sex hormone levels and severe COVID-19 (Table 3) showed a dose–response relationship for most androgens analyzed and the T:E2; thus, the lower the serum level of androgen was, the higher the likelihood of developing severe COVID-19, with the linear trend in the adjusted analyses being statistically significant for all androgens except for androstenedione ($p = 0.064$). The higher the serum levels of estradiol, the higher the likelihood of developing severe COVID-19; however, the linear trend for the adjusted analysis was not statistically significant ($p = 0.065$). A dose–response relationship was also shown for the total T:E2 ratio; thus, compared to patients with the highest ratio values, patients with the lowest ratio values had an OR for severe COVID-19 of 28.82 (95% CI 4.97–166.97), and those with medium ratio values had an OR of 2.80 (95% CI 0.82–9.55); the linear trend for the adjusted analysis was statistically significant.

3.3 | ROC curves

The highest AUC among the eight sex steroids was for the T:E2 at 77.4% ($p < 0.0001$), followed by the AUCs of DHEA and total testosterone at 74.7% ($p < 0.0001$) and 71.6% ($p < 0.001$), respectively (Table S1). The results for SHBG and androstenedione were not significantly different from chance (50%), with AUCs of 58.7% and 59.0%,

TABLE 3 Crude and adjusted odds ratios for the potential association of sex hormones and the total testosterone:estradiol ratio (T:E2) ratio with severe coronavirus disease 2019 (COVID-19).

Variable	Cutoff point	Nonsevere COVID-19 $n = 44$	Severe[a] COVID-19 $n = 54$	Crude OR	(95% CI)	Adjusted OR[b]	(95% CI)
Testosterone (ng/dL) (Tertiles)							
High (reference)	160.3+	21	11	1.00	–	1.00	–
Medium	78.2–160.2	16	17	2.03	0.75–5.51	2.41	0.74–7.78
Low	< = 78.1	7	26	7.09	2.34–21.48	6.62	1.68–26.05
Linear trend				**0.001**		**0.007**	
DHEAS (μg/mL) (Tertiles)							
High (reference)	124.901+	19	13	1.00	–	1.00	–
Medium	56.371–124.90	16	17	1.55	0.58–4.15	1.14	0.31–4.16
Low	< = 56.370	9	24	3.90	1.38–11.04	5.59	1.32–23.71
Linear trend				**0.011**		**0.014**	
Estradiol (pg/mL) (Tertiles)							
Low (reference)	< = 29.860	20	13	1.00	–	1.00	–
Medium	29.861–47.660	16	17	1.64	0.62–4.34	1.42	0.45–4.54
High	47.661	8	24	4.62	1.60–13.35	3.25	0.93–11.34
Linear trend				**0.005**		0.065	
SHBG (nmol/L) (Tertiles)							
High (reference)	42.68+	16	16	1.00	–	1.00	–
Medium	24.74–42.67	19	13	0.68	0.26–1.84	0.68	0.20–2.29
Low	< = 24.73	9	24	2.67	0.95–7.49	3.12	0.75–12.88
Linear trend				0.066		0.113	
DHEA (ng/mL) (Tertiles)							
High (reference)	2.5+	18	10	1.00	–	1.00	–
Medium	1.2–2.4	17	16	1.69	0.60–4.75	1.39	0.41–4.74
Low	< = 1.1	7	24	6.17	1.97–19.35	7.23	1.70–30.80
Linear trend				**0.002**		**0.007**	
Androstenedione (ng/mL) (Tertiles)							
High (reference)	2.5+	14	14	1.00	–	1.00	–
Medium	1.2-2.4	18	14	0.78	0.28–2.15	1.19	0.32–4.40
Low	< = 1.1	10	22	2.20	0.77–6.30	3.37	0.89–12.78
Linear trend				0.133		0.064	
Ratio total testosterone:estradiol (Tertiles)							
High (reference)	0.46+	23	9	1.00	–	1.00	–
Medium	0.17–0.45	17	17	2.56	0.92–7.11	2.80	0.82–9.55
Low	< = 0.16	4	28	17.89	4.87–65.68	28.82	4.97–166.97
Linear trend				**< 0.001**		**< 0.001**	

Abbreviations: CI, confidence interval; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; OR, odds ratio; SHBG, sex hormone-binding globulin.

[a]Severe COVID-19 was defined as admission to the intensive care unit or death during hospitalization.

[b]Adjusted for age, body mass index, smoking status (yes/no), diabetes, and chronic kidney disease defined as a glomerular filtration rate below 60 mL/min/1.73.

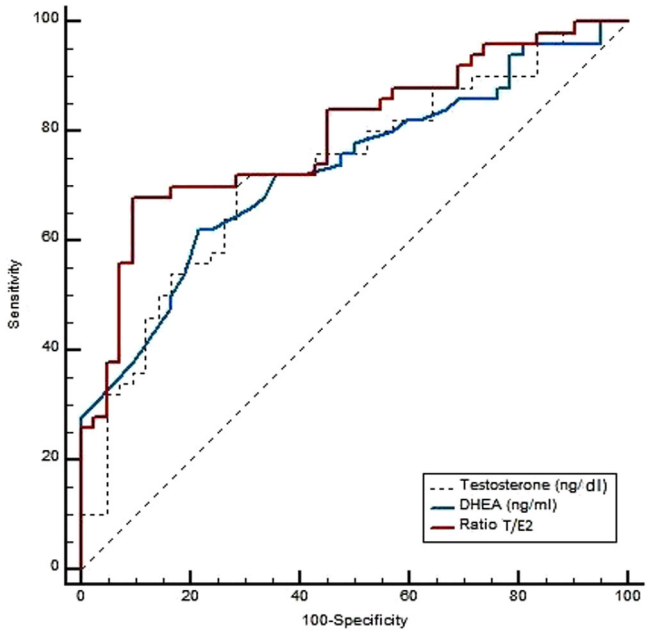

FIGURE 1 Receiver operating characteristic (ROC) analysis of the three biomarkers with better predictive ability for severe coronavirus disease 2019 (COVID-19). Severe COVID-19 was defined as admission to the intensive care unit or death during hospitalization.

respectively. Figure 1 shows the ROC curves of the T:E2, DHEA, and total testosterone. The highest sensitivity and specificity corresponded to a Youden index of 0.55, which in turn corresponded to a T:E2 value of 0.20 and had a sensitivity of 66.7% and a specificity of 88.6% (Table S2). To achieve a sensitivity of 80%, the testosterone/estradiol ratio had to have a value of 0.4, and the corresponding specificity had to be 55.3% (Table S2).

4 | DISCUSSION

Our results show that low serum levels of androgens at admission are associated with a higher likelihood of severe COVID-19 in men during hospitalization, and this relationship shows a biological gradient. When analyzed as a single biomarker, the T:E2 shows a better predictive performance for the occurrence of severe COVID-19 in men during hospitalization.

Except for the study by Rastrelli et al., in which patients with pneumonia admitted to a respiratory intensive care unit had lower testosterone levels among those who were transferred to the ICU for intubation or who had died than among less severe patients,[17] we are not aware of other studies that have used ICU admission or death as a composite outcome measure (i.e., our primary outcome) to evaluate the association between serum androgen levels and severe COVID-19. However, with various designs and analyses, there are several studies that have shown an association between androgens and those component outcomes. Thus, among patients with COVID-19, low serum testosterone levels have been associated with admission to the ICU[16,18,21,22] and in-hospital mortality .[16,18,21–26] Overall, the biological gradient observed in the association between serum levels of sex hormones and the occurrence of severe COVID-19 and the consistency of the results with other studies among patients with COVID-19 reinforces the causality of this association.

The association between the T:E2 ratio and the occurrence or presence of the worst outcomes among patients with COVID-19 has been reported in two previous studies. In a single-center study conducted in Austria in a similar time period, Lanser et al.[21] reported in the univariate analysis that a T:E2 ratio < 3.3 compared to a ratio > 7.3 was associated with a higher likelihood of in-hospital mortality (OR 22.2, 95% CI 2.8–175.3), and the association was maintained in the multivariate analysis. In a second study, Dhindsa et al.[19] reported that the median testosterone:estradiol ratio was higher at admission among men who needed ICU care, men who needed artificial ventilation, and men who died than among men who did not have these outcomes. In addition to these two studies, in a univariate analysis, Infante et al.[23] found that the inverse ratio (i.e., E2:T) was significantly higher among nonsurvivors than among survivors among hospitalized men with COVID-19. This association of the T:E2 with adverse outcomes has been reported in other populations. A low T:E2 has been associated with a higher risk of cardiovascular mortality among men from the general population[12] and a higher risk of major cardiovascular events among men with severe atherosclerosis.[27] Among adult patients with ischemic heart disease, a low sex-specific T:E2 ratio has been associated with higher all-cause mortality.[28] Finally, a high testosterone:estradiol ratio in men was associated with a higher risk of acute ischemic stroke.[29] Overall, these data consistently suggest that the T:E2 ratio could be a useful biomarker of unfavorable outcomes in men.

To the best of our knowledge, no previous studies have evaluated the predictive ability of sex steroids for the risk of developing a severe SARS-CoV-2 infection. In our study, the highest predictive performance for the occurrence of severe COVID-19 was obtained with the T:E2. Interestingly, the predictive ability of this single T:E2 ratio determination could perform similarly to more complex severity scores[30] and better than classically used biomarkers such as C-reactive protein and D-dimer.[31] However, we do not expect that the determination of the T:E2 ratio will be included as a standard evaluation for men with COVID-19 because of its value as a prognostic biomarker, as has been suggested by other authors for serum testosterone levels.[32] Prognostic models are multivariable and are intended for personalizing healthcare resource use. In the most recent publication of a living systematic review on prediction models for the diagnosis and prognosis of COVID-19, the authors found almost 600 models for predicting diverse outcomes in people with COVID-19, including 265 for mortality.[33] To check whether these models included testosterone or the T:E2 ratio among the variables tested in the model is far beyond the goal of this manuscript, but a close look at the predictors included in the final model as described by Wynants et al.[33] in the supplementary material of their article shows that none of them were included in those predictive models. However, in a recent study conducted with UK Biobank data and using machine learning models, Wong et al.[26] reported that testosterone was among the top five factors predictive of mortality among patients with COVID-19 (the other factors were

age, the number of treatments, waist circumference, and red cell distribution width), but contrary to the abovementioned results in our and previous studies, a higher level was associated with an increased risk. We think that the future development of prognostic models for COVID-19 should include the T:E2 ratio as a potential prognostic variable to be tested together with other variables.

Whether improving testosterone levels or the T:E2 ratio would be useful for improving the outcomes of hospitalized patients with COVID-19 is unknown. Interestingly, Toscano-Guerra et al.[34] analyzed the trajectory of serum testosterone levels in patients with COVID-19 and found that among several biochemical and hematological parameters, recovery of serum testosterone levels was the strongest predictor of survival, with an AUC in the ROC analysis of 0.92; the predictive performance for this parameter was better than that for testosterone levels at admission. Whether testosterone replacement therapy could play a role in the management of patients with COVID-19 remains to be elucidated.[4]

Finally, and although it is beyond the scope of this study, we would like to highlight the different trajectories in testosterone and estradiol levels in relation to the severity of SARS-CoV-2 infection. Previous studies have identified an association between the progression of SARS-CoV-2 infection with excessive levels of inflammatory mediators, such as interleukin-6 and tumor necrosis factor-α,[35] and both mediators can stimulate aromatase expression via de PI.4 promoter region.[36,37] Therefore, we believe that patients with severe SARS-CoV-2 infection may have increased aromatase activity. This possibility would be consistent with a previous finding in which 45.7% of SARS-CoV-2 infected male patients had elevated estradiol levels.[38]

Our study has several limitations. This study was conducted at a single center and had a small sample size. The analytical methods used in our study may lack sensitivity in the determination of some of the steroids analyzed, especially at lower levels and mainly related to estradiol measurement[39]; therefore, other studies using more sensitive methods, such as those based on mass spectrometry, should corroborate our results. In addition, this study was largely conducted during a period when COVID-19 vaccines were not available. In addition to the intrinsic need for external validation of the predictive models, it is important to validate our data in the current context of SARS-CoV-2 in terms of variants and vaccination.

In conclusion, our results suggest that men with SARS-CoV-2 infections and decreased androgen levels and increased estradiol levels have a higher likelihood of developing an unfavorable outcome (i.e., death and/or ICU admission). Of all the parameters analyzed in our study, the total T:E2 ratio was the one with the best predictive ability for these outcomes.

AUTHOR CONTRIBUTIONS

Conceptualization and project administration: David Ruiz-Ochoa, María-Teresa García-Unzueta, Armando-Raúl Guerra-Ruiz, and Luis Alberto Vázquez; data curation: David Ruiz-Ochoa, Armando-Raúl Guerra-Ruiz, and Luis Alberto Vázquez; formal analysis and methodology: David Ruiz-Ochoa, Pedro Muñoz-Cacho, and Luis Alberto Vázquez; resources: David Ruiz-Ochoa, María-Teresa García-Unzueta, Bernardo-Alio Lavín-Gómez, and Armando-Raúl Guerra-Ruiz; software: David Ruiz-Ochoa and Armando-Raúl Guerra-Ruiz; supervision: David Ruiz-Ochoa, María-Teresa García-Unzueta, Pedro Muñoz-Cacho, and Luis Alberto Vázquez; writing original draft: David Ruiz-Ochoa; investigation, validation, visualization and writing-review and editing: all authors. All authors approved the final version of this manuscript.

ACKNOWLEDGMENTS

The authors thank Fernando Rico-Villademoros (COCIENTE S.L., Madrid, Spain) for his editorial assistance; this assistance has been funded by Instituto de Investigación Marqués de Valdecilla (IDIVAL; Santander, Spain).

CONFLICT OF INTEREST STATEMENT

Carlos Antonio Amado-Diago has received speaker or consulting fees from Boehringer Ingelheim, Pfizer, AstraZeneca, Novartis, Chiesi, Faes Farma, Esteve, and GSK. Luis Alberto Vázquez is an advisory board member for Eli Lilly, has received a speaker honorarium from Astra Zeneca, Eli Lilly, and Novo Nordisk, has received research grants to institutions from Eli Lilly, and is a minor shareholder of Eli Lilly. The other authors declare no conflict of interest.

FUNDING INFORMATION

This project was funded with aid from the innovation project INN-VAL 20/15 (Instituto de Investigación Marqués de Valdecilla—IDIVAL).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

David Ruiz-Ochoa https://orcid.org/0000-0002-9622-6121
Luis Alberto Vázquez https://orcid.org/0000-0003-4929-4479

REFERENCES

1. Mauvais-Jarvis F, Bairey MN, Barnes PJ, et al. Sex and gender: modifiers of health, disease, and medicine. *Lancet*. 2020;396:565-582.
2. Sex Gender and COVID-19 Project. The COVID-19 sex-disaggregated data tracker November update report. Accessed November, https://globalhealth5050.org/wp-content/uploads/November-2021-data-tracker-update.pdf2021
3. Bechmann N, Barthel A, Schedl A, et al. Sexual dimorphism in COVID-19: potential clinical and public health implications. *Lancet Diabetes Endocrinol*. 2022;10:221-230.
4. Punjani N, Flannigan R. Androgens and COVID-19: exploring the role of testosterone replacement therapy. *Int J Impot Res*. 2022;34:649-651.
5. Twitchell DK, Christensen MB, Hackett G, Morgentaler A, Saad F, Pastuszak AW. Examining male predominance of severe COVID-19 outcomes: a systematic review. *Androg Clin Res Ther*. 2022;3:41-53.
6. Yassin A, Sabsigh R, Al-Zoubi RM, et al. Testosterone and COVID-19: an update. *Rev Med Virol*. 2023;33:e2395.
7. Gallo MB, Aghagoli G, Lavine K, et al. Predictors of COVID-19 severity: a literature review. *Rev Med Virol*. 2021;31:1-10.

8. Dhindsa S, Ghanim H, Batra M, Dandona P. Hypogonadotropic hypogonadism in men with diabesity. *Diabetes Care*. 2018;41:1516-1525.
9. Atlantis E, Fahey P, Cochrane B, Wittert G, Smith S. Endogenous testosterone level and testosterone supplementation therapy in chronic obstructive pulmonary disease (COPD): a systematic review and meta-analysis. *BMJ Open*. 2013;3:e003127.
10. De Vincentis S, Decaroli MC, Fanelli F, et al. Health status is related to testosterone, estrone and body fat: moving to functional hypogonadism in adult men with HIV. *Eur J Endocrinol*. 2021;184:107-122.
11. Romejko K, Rymarz A, Sadownik H, Niemczyk S. Testosterone deficiency as one of the major endocrine disorders in chronic kidney disease. *Nutrients*. 2022;14:3438.
12. Belladelli F, Del Giudice F, Kasman A, Salonia A, Eisenberg ML. The association between testosterone, estradiol and their ratio and mortality among US men. *Andrologia*. 2021;53:e13993.
13. Fantus RJ, Chang C, Platz EA, et al. Serum total testosterone and premature mortality among men in the USA. *Eur Urol Open Sci*. 2021;29:89-92.
14. Li L, Ju H, Jin H, Chen H, Sun M, Zhou Z. Low testosterone level and risk of adverse clinical events among male patients with chronic kidney disease: a systematic review and meta-analysis of cohort studies. *J Healthc Eng*. 2022;2022:3630429.
15. van der Burgh AC, Khan SR, Neggers S, Hoorn EJ, Chaker L. The role of serum testosterone and dehydroepiandrosterone sulfate in kidney function and clinical outcomes in chronic kidney disease: a systematic review and meta-analysis. *Endocr Connect*. 2022;11:e220061.
16. Salonia A, Pontillo M, Capogrosso P, et al. Severely low testosterone in males with COVID-19: a case-control study. *Andrology*. 2021;9:1043-1052.
17. Rastrelli G, Di Stasi V, Inglese F, et al. Low testosterone levels predict clinical adverse outcomes in SARS-CoV-2 pneumonia patients. *Andrology*. 2021;9:88-98.
18. Cinislioglu AE, Cinislioglu N, Demirdogen SO, et al. The relationship of serum testosterone levels with the clinical course and prognosis of COVID-19 disease in male patients: a prospective study. *Andrology*. 2022;10:24-33.
19. Dhindsa S, Zhang N, McPhaul MJ, et al. Association of circulating sex hormones with inflammation and disease severity in patients with COVID-19. *JAMA Netw Open*. 2021;4:e2111398.
20. Giagulli VA, Guastamacchia E, Magrone T, et al. Worse progression of COVID-19 in men: is testosterone a key factor? *Andrology*. 2021;9:53-64.
21. Lanser L, Burkert FR, Thommes L, et al. Testosterone deficiency is a risk factor for severe COVID-19. *Front Endocrinol*. 2021;12:694083.
22. Çayan S, Uğuz M, Saylam B, Akbay E. Effect of serum total testosterone and its relationship with other laboratory parameters on the prognosis of coronavirus disease 2019 (COVID-19) in SARS-CoV-2 infected male patients: a cohort study. *Aging Male*. 2020;23:1493-1503.
23. Infante M, Pieri M, Lupisella S, et al. Low testosterone levels and high estradiol to testosterone ratio are associated with hyperinflammatory state and mortality in hospitalized men with COVID-19. *Eur Rev Med Pharmacol Sci*. 2021;25:5889-5903.
24. Peralta-Amaro AL, Pecero-García EI, Valadez-Calderón JG, Ramírez-Ventura JC, Coria-Moctezuma LA, Hernández-Utrera JE. Low testosterone levels as an independent risk factor for mortality in male patients with COVID-19: report of a single-center cohort study in Mexico. *Rev Int Androl*. 2023;21:100329.
25. Vena W, Pizzocaro A, Maida G, et al. Low testosterone predicts hypoxemic respiratory insufficiency and mortality in patients with COVID-19 disease: another piece in the COVID puzzle. *J Endocrinol Invest*. 2022;45:753-762.
26. Wong KC, Xiang Y, Yin L, So HC. Uncovering clinical risk factors and predicting severe COVID-19 cases using UK Biobank Data: machine learning approach. *JMIR Public Health Surveill*. 2021;7:e29544.
27. van Koeverden ID, de Bakker M, Haitjema S, et al. Testosterone to oestradiol ratio reflects systemic and plaque inflammation and predicts future cardiovascular events in men with severe atherosclerosis. *Cardiovasc Res*. 2019;115:453-462.
28. Raparelli V, Nocella C, Proietti M, et al. Testosterone-to-estradiol ratio and platelet thromboxane release in ischemic heart disease: the EVA project. *J Endocrinol Invest*. 2022;45:1367-1377.
29. Choi JW, Ryoo IW, Hong JY, et al. Clinical impact of estradiol/testosterone ratio in patients with acute ischemic stroke. *BMC Neurol*. 2021;21:91.
30. Fang L, Xie H, Liu L, et al. Early predictors and screening tool developing for severe patients with COVID-19. *BMC Infect Dis*. 2021;21:1040.
31. Timpau AS, Miftode RS, Petris AO, et al. Mortality predictors in severe COVID-19 patients from an East European tertiary center: a never-ending challenge for a no happy ending pandemic. *J Clin Med*. 2021;11:58.
32. Rowland SP, O'Brien BE. Screening for low testosterone is needed for early identification and treatment of men at high risk of mortality from COVID-19. *Crit Care*. 2020;24:367.
33. Wynants L, Van Calster B, Collins GS, et al. Prediction models for diagnosis and prognosis of covid-19: systematic review and critical appraisal. *BMJ*. 2020;369:m1328.
34. Toscano-Guerra E, Martínez-Gallo M, Arrese-Muñoz I, et al. Recovery of serum testosterone levels is an accurate predictor of survival from COVID-19 in male patients. *BMC Med*. 2022;20:129.
35. Ulhaq ZS, Soraya GV. Interleukin-6 as a potential biomarker of COVID-19 progression. *Med Mal Infect*. 2020;50:382-383.
36. Che Q, Liu BY, Liao Y, et al. Activation of a positive feedback loop involving IL-6 and aromatase promotes intratumoral 17β-estradiol biosynthesis in endometrial carcinoma microenvironment. *Int J Cancer*. 2014;135:282-294.
37. Zhao Y, Nichols JE, Valdez R, Mendelson CR, Simpson ER. Tumor necrosis factor-alpha stimulates aromatase gene expression in human adipose stromal cells through use of an activating protein-1 binding site upstream of promoter 1.4. *Mol Endocrinol*. 1996;10:1350-1357.
38. Schroeder M, Schaumburg B, Mueller Z, et al. High estradiol and low testosterone levels are associated with critical illness in male but not in female COVID-19 patients: a retrospective cohort study. *Emerg Microbes Infect*. 2021;10:1807-1818.
39. Rochira V, Carani C. Estrogens, male reproduction and beyond. In: Feingold KR, Anawalt B, Blackman MR, et al., eds. *Endotext*. MDText.com, Inc.; 2023.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Ruiz-Ochoa D, Guerra-Ruiz A-R, García-Unzueta M-T, et al. Sex hormones and the total testosterone:estradiol ratio as predictors of severe acute respiratory syndrome coronavirus 2 infection in hospitalized men. *Andrology*. 2024;1-8. https://doi.org/10.1111/andr.13581