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Pharmaceutical modulation of oestrogen during COVID-19 – a nationwide cohort study

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3 **1 Pharmaceutical modulation of oestrogen during COVID-19 – a nationwide cohort study**
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3 24 **Abstract**
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5 25 Objective: Determine if oestrogen augmentation decreases the risk of death following COVID-19.
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9 27 Design: Nationwide registry-based study
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13 29 Participants: Postmenopausal women between 50 and 80 years of age with verified COVID-19 were
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15 30 divided into three groups: 1) Women with previously diagnosed breast cancer and receiving endocrine
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17 31 therapy (decreased systemic oestrogen levels); 2) women receiving hormone replacement therapy
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19 32 (HRT; increased systemic oestrogen levels) and 3) control group not fulfilling requirements for group
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21 33 1 or 2 (postmenopausal oestrogen levels).
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25 35 Main outcome measures: The main outcome was death following COVID-19, and the exposure was
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27 36 pharmaceutical modulation of oestrogen levels. Adjustments were made for potential confounders
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29 37 such as age, annual disposable income (richest group as the reference category), highest level of
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31 38 education (primary, secondary and tertiary (reference)) and the weighted Charlson Comorbidity Index
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33 39 (wCCI).
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37 41 Results: From a nationwide cohort consisting of 49,853 women diagnosed with COVID-19 between
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39 42 the 4th of February to 14th of September 2020 in Sweden, 16,693 were between 50 to 80 years of age.
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41 43 We included 14,685 women in the study with 11,923 (81%) in the control group, 227 (2%) women in
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43 44 group 1 and 2,535 (17%) women in group 2. The unadjusted odds ratio (OR) for death following
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45 45 COVID-19 was 2.35 (95% CI 1.51-3.65) for group 1 and 0.45 (0.34-0.6) for group 2. Only the
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47 46 adjusted OR for death remained significant for group 2 with OR 0.47 (0.34-0.63). The risk of death
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49 47 due to COVID-19 was significantly associated with: Age OR 1.15 (1.14-1.17); annual income
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51 48 (poorest 2.79 (1.96-3.97); poor 2.43 (1.71-3.46) and middle 1.64 (1.11-2.41)); education (primary 1.4
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53 49 (1.07-1.81)) and wCCI 1.13 (1.1-1.16).
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3 51 Conclusions: Oestrogen supplementation in post-menopausal women is associated with a decreased
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5 52 risk of dying from COVID-19 in this nationwide cohort study. These findings are limited by the
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7 53 retrospective and non-randomized design. Further randomized intervention trials are warranted.
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3 54 **Strengths and limitations of the study**
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- 6 55 • This study is based on all diagnosed COVID-19 patients in Sweden
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8 56 • Swedish registry data is well-validated and due to historical registry data and cross-linkage
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10 57 with the registries of Statistics Sweden, the confounding and/or effect modifying effects of
11
12 58 socioeconomic variables and comorbidities could be adjusted for
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14 59 • It investigates the effect of pharmaceutical modulation of oestrogen in post-menopausal
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16 60 women on death due to COVID-19
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19 61 • The findings are limited by the retrospective and non-randomized design.
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62 Introduction

63 The coronavirus disease 2019 (COVID-19) pandemic has swept across the globe causing enormous
64 strain on societies and health care systems. Although women are infected, they appear to be
65 protected from poor outcome when compared to men even after adjustment for confounding risk
66 factors^{1 2}. Similar epidemiological findings have also been described for SARS-CoV and MERS-
67 CoV infections^{3 4 5}. This implies biological differences between the sexes in terms of sensitivity to
68 severe COVID-19, and oestrogen has been identified as a potential therapeutic candidate.

69
70 The majority of breast cancer (BC) patients have oestrogen receptor (ER) positive cancer⁶, and are
71 usually given adjuvant endocrine therapy after surgery in order to reduce the risk of cancer
72 recurrences, leading to *reduced* systemic oestrogen levels. On the other hand, systemic oestrogen
73 levels are *augmented* in women taking hormone replacement therapy (HRT) for relieving menopausal
74 symptoms⁷. We use the opposing effects of endocrine therapy in BC patients and HRT in modulating
75 systemic oestrogen levels in postmenopausal women as a model to test the hypothesis whether
76 increased oestrogen levels are protective towards COVID-19 death in a nationwide cohort.

77 **Materials and methods**

78 *Patients and public involvement statement*

79 All data from the Swedish registries were pseudonymized and therefore patients were not involved
80 in the study.

82 *Participants and sources of data*

83 The personal identification numbers (PINs) from all diagnosed COVID-19 individuals in Sweden
84 (SmiNet) between the 1st of February to 14th of September 2020 were cross-linked with the LISA
85 Register (Longitudinal integrated database for health insurance and labor marker studies)
86 administered by Statistics Sweden; and the following healthcare registers administered by the
87 Swedish National Board of Health and Welfare: Patient; Cancer; Prescribed pharmaceutical and
88 Causes of Death. Post-menopausal women between the ages of 50-80 years of age were stratified into
89 three groups: *Oestrogen decrease (group 1)*: BC as identified by international classification of
90 diseases (ICD) version 10 code C50, and the following treatment: tamoxifen or fulvestrant
91 (anatomical therapeutic chemical (ATC): L02BA01, L02BA03) or an aromatase inhibitor (AI; ATC
92 L02BG03, L02BG04, and L02BG06). *Augmented oestrogen (group 2)*: Drugs classified as HRT
93 (ATC codes: G03CA03, G03CA04, G03CC07, G03CX01, G03FA, G03FB). All ATC codes for
94 group 1 and 2 were identified from the Prescribed Pharmaceutical Register with at least two
95 consecutive withdrawals and at least one time should be during the period 2019-07-01 - to the latest
96 date. *Native oestrogen (control group)*: No BC diagnosis, and no prescription of the above-mentioned
97 pharmaceuticals at any time point during 2019 and 2020. Ethical permit was granted by the Swedish
98 Ethical Review Authority.

100 *Outcome, confounders and effect modifiers*

101 The outcome was death due to COVID-19 as identified by the ICD-10 code U07 as the main or
102 contributing cause of death from the Cause of Death Register. Potential confounders and effect
103 modifiers were included in the model and consisted of the weighted Charlson comorbidity index
104 (wCCI)⁸, age at COVID-19 diagnosis, income (divided into quintiles with the richest group as the

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3 105 reference) and education (primary, secondary and tertiary (=reference)). The wCCI was calculated
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5 106 using the Patient and Cancer Registers, and up to two months prior to the COVID-19 date in order not
6
7 107 to include complications due to COVID-19 as a comorbidity. If there was no information regarding
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9 108 diagnosis codes required for wCCI scoring, the individual was assigned a wCCI of zero. Information
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11 109 regarding income and education was retrieved from the LISA-register.
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15 111 *Statistical methods*

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18 112 The distribution of continuous and categorical variables in the three groups was tested using ANOVA
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20 113 and the χ^2 test, respectively. Each variable was then analyzed with univariate logistic regression
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22 114 models, followed by a multivariable regression model to compare the control group with group 1 and
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24 115 2, respectively, and adjusting for confounders. Descriptive analyses and logistic regression models
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26 116 were performed in R statistical software version 4.0.2 using *finalfit* package 1.0.2.
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3 117 **Results**

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5 118 *Participants*

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7 119 During the study period a total of 49,853 women of all ages were diagnosed with COVID-19 in
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9 120 Sweden, and a total of 14,685 women between the ages of 50-80 years of age were included in our
10
11 121 study (Figure 1). Characteristics of these groups are shown in Table 1. Individuals with decreased
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13 122 oestrogen due to adjuvant endocrine therapy for BC (group 1) were older with a higher comorbidity
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15 123 index. A larger proportion of women in group 2 (increased levels of systemic oestrogen due to HRT)
16
17 124 had high income and tertiary level of education (Table 1).
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22 126 *Oestrogen augmentation protects against death due to COVID-19*

23
24 127 Pharmaceutically decreasing systemic oestrogen levels increased the odds of dying due to COVID-19
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26 128 (group 1; odds ratio (OR) 2.35 95% confidence intervals (CI) 1.51-3.65), but following adjustment for
27
28 129 confounders this association was no longer significant (Figure 2). Interestingly, augmentation of
29
30 130 systemic oestrogen levels decreased the odds of dying due to COVID-19 with OR 0.45 (95% CI:
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32 131 0.34-0.6), which remained significant even after adjustment for confounders (0.47 (95% CI: 0.34-
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34 132 0.63)). As expected, higher age and wCCI increased the odds of dying due to COVID-19. For every
35
36 133 year increase in age the odds of dying was 1.15 (95% CI: 1.14-1.17) and for every increase in wCCI
37
38 134 the odds of dying was 1.13 (95% CI: 1.10-1.16) (Figure 2). Furthermore, low income and having only
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40 135 primary level education were also factors that increased the odds of dying due to COVID-19 (Figure
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137 **Discussion**

138 Principal findings

139 The major finding of this nationwide registry-based study is that pharmaceutically augmenting
140 oestrogen levels is associated with decreased odds of death due to COVID-19.

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142 Comparison with related studies

143 There are several possible biological explanations for the lower risk experienced by women. These
144 include mechanisms directly involved in viral internalization and reproduction, where oestrogen has
145 been shown to decrease expression of vital proteins such as ACE2 and TMPRSS2^{9,10}, inherent sex-
146 linked differences in the immune system and direct oestrogen effects¹¹. Our findings are supported by
147 *in vitro* studies where 17 β -estradiol treatment reduced viral load of SARS-CoV-2⁹. Previous
148 experimental studies in mice on SARS-CoV have moreover shown that female mice were less
149 susceptible to infection, and that this protection was lost upon oophorectomy thus indicating a direct
150 protective role of oestrogen signalling¹². Furthermore, Barh et al. showed using a multiomics
151 approach on SARS-CoV-2 infected host interactome, proteome, transcriptome, and bibliome datasets
152 that oestrogen modulation could be a potential therapeutic option in COVID-19¹³. Our findings are
153 further verified by a smaller study of women taking HRT (n = 439) that showed similar results with
154 oestrogen augmentation being associated lower risk of COVID-19 death, although in that study the
155 risk selection bias was more difficult to discern since the cohort was neither population-based nor
156 adjusted for central confounders¹⁴. In our study the effect of increased systemic oestrogen levels on
157 reducing the risk of COVID-19 death remained significant also after adjusting for education level and
158 income, both factors known to influence COVID-19 outcome¹⁵, which further supports the protective
159 role of oestrogen in women.

160 The hypothetic inverse, worsening, effect of reduced systemic oestrogen levels in women with a
161 previous BC receiving adjuvant endocrine therapy was initially significant but not after adjusting for
162 confounders. This population differs from the control group in that they all have been diagnosed with
163 BC and it has been shown that patients, both men and women, with any cancer form are harder hit by
164 COVID-19¹⁶. However, BC patients were in a previous study shown to be healthier compared to the

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3 165 background population in terms of ischemic cardiac disease and CCI ¹⁷, and the wCCI adjustments
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5 166 may therefore overcompensate for this cancer-related vulnerability. Although not significant, a trend
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7 167 towards worse outcome remained and thus a larger population of BC patients on endocrine therapy is
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9 168 likely needed to verify the finding. Thus, this study cannot exclude an increased risk for death from
10
11 169 COVID-19 if systemic oestrogen levels are pharmaceutically decreased.
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15 171 Strengths and limitations

16 172 Strengths of this study are that this is a nationwide cohort in a country with high COVID-19 incidence
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18 173 using well validated registry data. A weakness is that the level of oestrogen modulation cannot be
19
20 174 exactly measured in each individual, and that the number of BC women on anti-oestrogen medication
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22 175 ended up being too small to show significance although there was a clear trend. The HRT group,
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24 176 however, proved large enough to show the clear protective effect.
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29 178 Implications and conclusion

30 179 The present study shows an association between oestrogen levels and COVID-19 death.
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32 180 Consequently, drugs increasing oestrogen levels may have a role in therapeutic efforts to alleviate
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34 181 COVID-19 severity in post-menopausal women and could be studied in randomized control trials.
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28 **194 Contributors**
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30 **195** MS and KW conceptualized the study. MS and AMFC designed the study. OFR and AMFC prepared
31
32 **196** the study data. OFR performed the statistical analysis. All authors contributed to interpretation of the
33
34 **197** results. MS wrote the first draft of the manuscript. All authors contributed to critical revision of the
35
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37
38 **199** corresponding author attests that all authors meet the criteria for authorship and that all have been
39
40 **200** included.
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43 **201**

44
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46

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53 **206** other relationships or activities that could appear to have influenced the submitted work.
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56 **207**

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58 **208 Ethical approval**
59

60 **209** Ethical approval was obtained by the Swedish Ethical Review Authority (number 2020-02150)

210

211 **Data sharing**

212 The study protocol (R script) is available upon request. The study used secondary registry data which
213 is regulated by the Public Access to Information and Secrecy Act (2009:400) and is protected by strict
214 confidentiality. For the purpose of research though, after formal application to access personal data
215 the responsible authority can grant access to data, though this is contingent on vetting by the Ethical
216 Review Authority of Sweden, according to the Act (2003:460) concerning the Ethical Review of
217 Research Involving Humans. This means that the aggregated registry data cannot be shared.

219

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3 267 **Figure legends**
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5 268 Figure 1: Flow chart of the study
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9 270 Figure 2: Oestrogen augmentation is associated with decreased odds of dying due to COVID-19. Crude
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11 271 and adjusted logistic regression models. Statistical significance: ($p < 0.05$ *, $p < 0.01$ **, $p < 0.001$
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14 272 ***). OR odds ratio; CI confidence intervals; wCCI weighted Charlson Comorbidity Index.
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273 **Table 1.** Characteristics of the study population

| Variable | | Native oestrogen (control group) | Oestrogen decrease (group 1) | Augmented oestrogen (group 2) | p-value |
|--------------------------------|-----------|---|-------------------------------------|--------------------------------------|----------------|
| Total N (%) | | 11,923 (81.2) | 227 (1.5) | 2,535 (17.3) | |
| Age | Mean (SD) | 61.2 (8.3) | 64.4 (8.9) | 60.9 (7.7) | <0.001 |
| wCCI | Mean (SD) | 1.4 (2.4) | 5.0 (3.3) | 1.6 (2.5) | <0.001 |
| Income quintiles, n (%) | Richest | 3,422 (28.7) | 64 (28.2) | 937 (37.0) | <0.001 |
| | Rich | 2,743 (23.0) | 42 (18.5) | 605 (23.9) | |
| | Middle | 2,120 (17.8) | 35 (15.4) | 404 (15.9) | |
| | Poor | 1,703 (14.3) | 47 (20.7) | 334 (13.2) | |
| | Poorest | 1,903 (16.0) | 39 (17.2) | 253 (10.0) | |
| | Missing | 32 (0.3) | 0 (0) | 2 (0.1) | |
| Education, n (%) | Tertiary | 4,186 (35.1) | 82 (36.1) | 1074 (42.4) | <0.001 |
| | Secondary | 5,609 (47.0) | 97 (42.7) | 1150 (45.4) | |
| | Primary | 1,882 (15.8) | 45 (19.8) | 290 (11.4) | |
| | Missing | 246 (2.1) | 3 (1.3) | 21 (0.8) | |

274 wCCI: Weighted Charlson Comorbidity Index. SD standard deviation

Figure 1

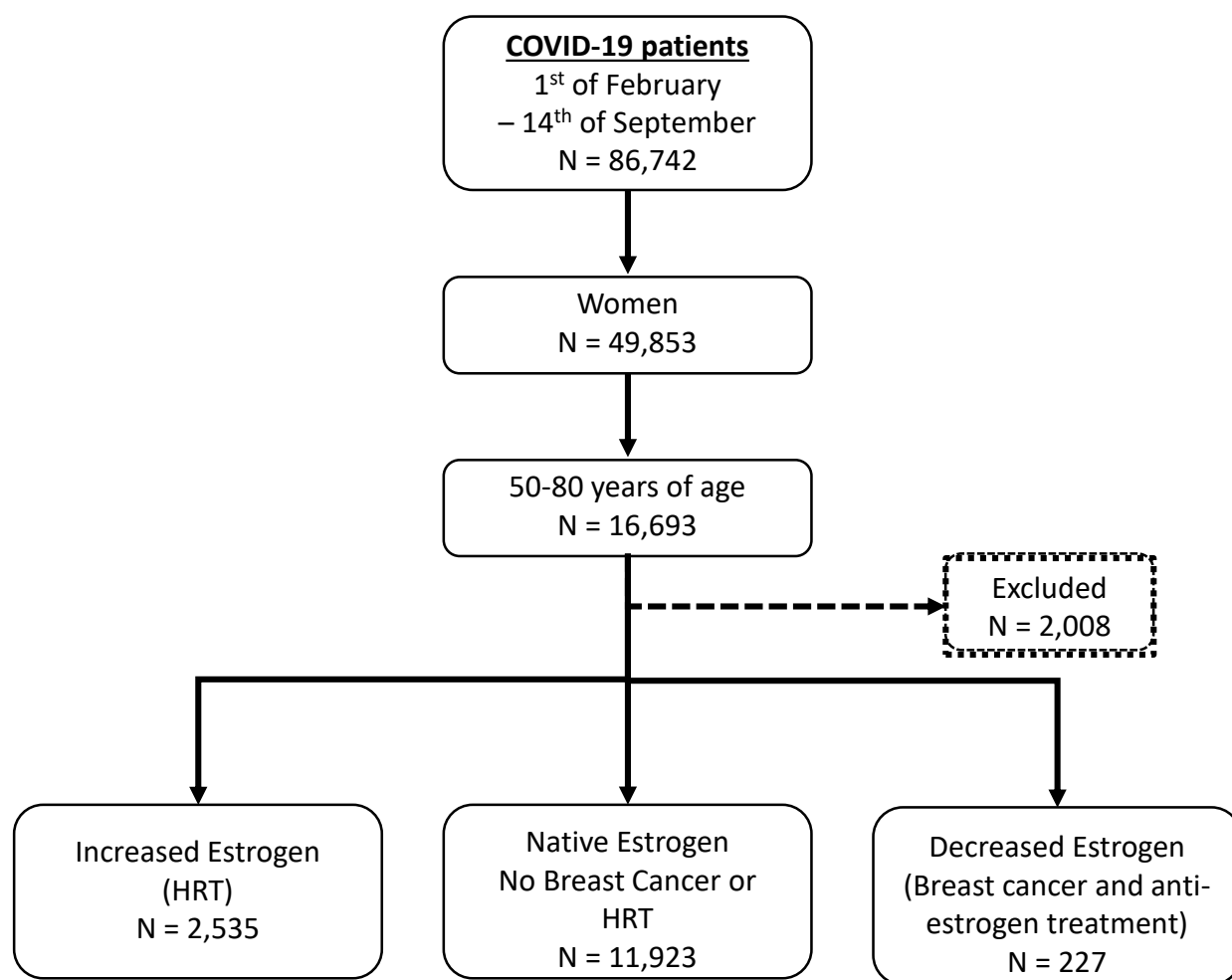
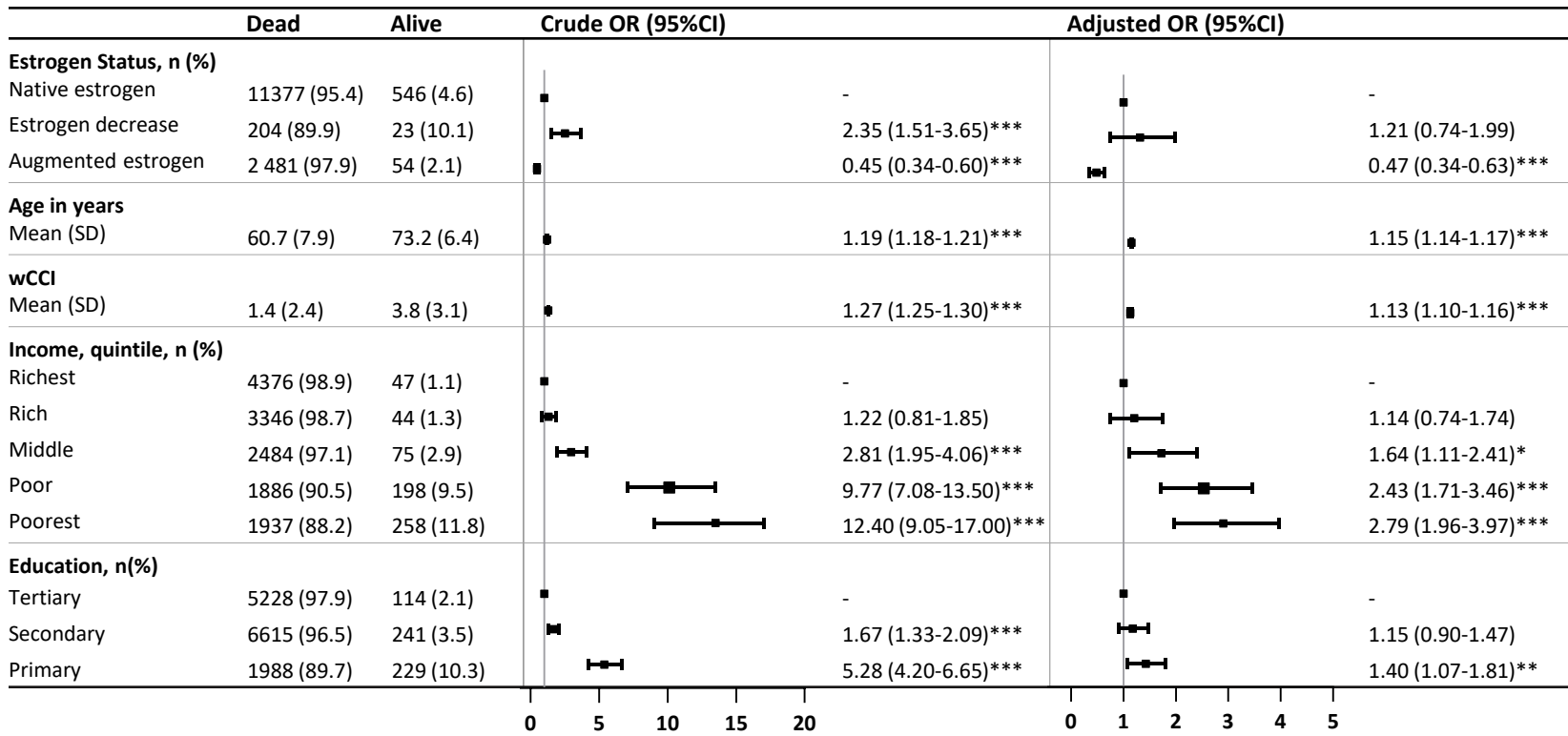


Figure 2



STROBE Statement—checklist of items that should be included in reports of observational studies

| | Item No | Recommendation |
|------------------------------|---------|--|
| Title and abstract | 1 | (a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found |
| Introduction | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses |
| Methods | | |
| Study design | 4 | Present key elements of study design early in the paper |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection |
| Participants | 6 | (a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants (b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group |
| Bias | 9 | Describe any efforts to address potential sources of bias |
| Study size | 10 | Explain how the study size was arrived at |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why |
| Statistical methods | 12 | (a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy (e) Describe any sensitivity analyses |

Continued on next page

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60**Results**

| | | |
|------------------|-----|---|
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount) |
| Outcome data | 15* | <i>Cohort study</i> —Report numbers of outcome events or summary measures over time <i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure <i>Cross-sectional study</i> —Report numbers of outcome events or summary measures |
| Main results | 16 | (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses |

Discussion

| | | |
|------------------|----|--|
| Key results | 18 | Summarise key results with reference to study objectives |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results |

Other information

| | | |
|---------|----|---|
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based |
|---------|----|---|

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Association between pharmaceutical modulation of oestrogen in postmenopausal women in Sweden with death due to COVID-19 – a cohort study

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| | |

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3 **1 Association between pharmaceutical modulation of oestrogen in postmenopausal**
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5 **2 women in Sweden with death due to COVID-19 – a cohort study**
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12 5 PhD⁴⁻⁶, Karin Welen PhD⁶, Anne-Marie Fors Connolly MD PhD^{3*}
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For peer review only

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2
3 **28 Abstract**
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5 29 Objective: Determine whether augmentation of oestrogen in post-menopausal women
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8 30 decreases the risk of death following COVID-19.
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11 31
12 32 Design: Nationwide study in Sweden based on registries from The Swedish Public Health
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14 33 Agency; Statistics Sweden (socioeconomical variables) and the National Board of Health and
15
16 34 Welfare (Causes of death).
17
18

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21 36 Participants: Postmenopausal women between 50 and 80 years of age with verified COVID-
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23 37 19.
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28 39 Interventions: Pharmaceutical modulation of oestrogen as defined by (1) women with breast
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30 40 cancer receiving endocrine therapy (decreased systemic oestrogen levels); (2)
31
32 41 postmenopausal hormone therapy (HT; increased systemic oestrogen levels) and (3) a control
33
34 42 group (postmenopausal oestrogen levels). Adjustments were made for potential confounders
35
36 43 such as age, annual disposable income (richest group as the reference category), highest level
37
38 44 of education (primary, secondary and tertiary (reference)) and the weighted Charlson
39
40 45 Comorbidity Index (wCCI).
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47 47 Primary outcome measure: Death following COVID-19.
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51 49 Results: From a nationwide cohort consisting of 49,853 women diagnosed with COVID-19
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53 50 between February 4 and September 14, 2020 in Sweden, we included 14,685 women in the
54
55 51 study with 11,923 (81%) in the control group, 227 (2%) women in group 1 and 2,535 (17%)
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57 52 women in group 2. The unadjusted odds ratio (OR) for death following COVID-19 was 2.35
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3 53 (95% CI 1.51-3.65) for group 1 and 0.45 (0.34-0.6) for group 2. Only the adjusted OR for
4
5 54 death remained significant for group 2 with OR 0.47 (0.34-0.63). Absolute risk (AR) of death
6
7 55 was 4.6% for the control group vs 10.1% and 2.1%, for the decreased and increased
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9
10 56 oestrogen groups, respectively. The risk of death due to COVID-19 was significantly
11
12 57 associated with: age, annual income, and education.
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17 59 Conclusions: Oestrogen supplementation in post-menopausal women is associated with a
18
19 60 decreased risk of dying from COVID-19 in this nationwide cohort study. These findings are
20
21 61 limited by the retrospective and non-randomized design. Further randomized intervention
22
23 62 trials are warranted.
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27 63

64 **Strengths and limitations of the study**

- 65 • This study is based on all diagnosed COVID-19 patients in Sweden between February
66 1 and September 14, 2020
- 67 • Swedish registry data is well-validated and due to historical registry data and cross-
68 linkage with the registries of Statistics Sweden, the confounding and/or effect-
69 modifying effects of socioeconomic variables and comorbidities could be adjusted for
- 70 • The findings are limited by the retrospective and non-randomized design.
- 71 • Information regarding compliance to pharmaceutical modulation of oestrogen is
72 missing
- 73 • Circulating oestrogen levels are not measured

74 **Introduction**

75 The coronavirus disease 2019 (COVID-19) pandemic has swept across the globe causing
76 enormous strain on societies and health care systems. Although women are infected, they
77 appear to be protected from poor outcomes when compared with men even after adjustment
78 for confounding risk factors^{1 2}. Similar epidemiological findings have also been described
79 for SARS-CoV and MERS-CoV infections^{3 4 5}. This implies biological differences between
80 the sexes in terms of sensitivity to severe COVID-19, and oestrogen has been identified as a
81 potential therapeutic candidate.

82
83 The majority of breast cancer (BC) patients have oestrogen receptor (ER)-positive cancer⁶
84 and are usually given adjuvant endocrine therapy after surgery in order to reduce the risk of
85 cancer recurrences, leading to *reduced* systemic oestrogen levels. On the other hand, systemic
86 oestrogen levels are *augmented* in women taking postmenopausal hormone therapy (HT) to
87 relieve menopausal symptoms⁷. In a nationwide cohort, we used the opposing effects of
88 endocrine therapy in BC patients and women taking postmenopausal HT in modulating
89 systemic oestrogen levels as a model to test the hypothesis that increased oestrogen levels
90 are protective towards COVID-19 death.

91 **Materials and methods**

92 *Patients and public involvement statement*

93 All data from the Swedish registries were pseudonymised and therefore patients were not
94 involved in the study.

96 *Participants and sources of data*

97 The personal identification numbers (PINs) from all diagnosed COVID-19 individuals in
98 Sweden (SmiNet) between the February 1 and September 14, 2020 were cross-linked with the
99 LISA Register (Longitudinal integrated database for health insurance and labour market
100 studies) administered by Statistics Sweden; and the following healthcare registers
101 administered by the Swedish National Board of Health and Welfare: patient, cancer,
102 prescribed pharmaceutical, and causes of death. Post-menopausal women 50-80 years of age
103 were stratified into three groups as follows: Group 1, the *decreased oestrogen group*,
104 included patients with BC as identified by the International Classification of Diseases (ICD)
105 version 10 code C50, and the following treatments: tamoxifen or fulvestrant (anatomical
106 therapeutic chemical (ATC): L02BA01, L02BA03) or an aromatase inhibitor (AI; ATC
107 L02BG03, L02BG04, and L02BG06). Group 2, the *augmented oestrogen group*, included
108 those patients treated with drugs classified as postmenopausal hormone therapy (ATC codes
109 G03CA03, G03CA04, G03CC07, G03CX01, G03FA, and G03FB). All ATC codes for
110 groups 1 and 2 were identified from the Prescribed Pharmaceutical Register with at least two
111 consecutive withdrawals and at least one during the period extending from July 1, 2019 - to
112 the latest date. Group 3, the *native oestrogen (control) group*, included patients with no BC
113 diagnosis and no prescription of the above-mentioned pharmaceuticals at any time during
114 2019 and 2020. Ethical approval was granted by the Swedish Ethical Review Authority.

115

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3 116 *Outcome, confounders and effect modifiers*
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5 117 The outcome was death due to COVID-19, as identified by the ICD-10 code U07, as the main
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7
8 118 or contributing cause of death from the Cause of Death Register. Potential confounders and
9
10 119 effect modifiers were included in the model and consisted of the weighted Charlson
11
12 120 comorbidity index (wCCI)⁸, age at COVID-19 diagnosis, income (divided into quintiles with
13
14 121 the richest group as the reference) and education (primary, secondary and tertiary, which
15
16 122 served as the reference). The wCCI was calculated using the Patient and Cancer Registers, up
17
18
19 123 to 2 months prior to the COVID-19 date in order not to include complications due to COVID-
20
21 124 19 as a comorbidity. If there was no information regarding diagnosis codes required for
22
23 125 wCCI scoring, the individual was assigned a wCCI of zero. Information regarding income
24
25 126 and education was retrieved from the LISA-register.
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31 128 *Statistical methods*
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33 129 The distributions of continuous and categorical variables in the three groups were tested
34
35 130 using ANOVA and the χ^2 test, respectively. Each variable was then analysed with univariate
36
37 131 logistic regression models, followed by a multivariable regression model to compare the
38
39 132 control group with groups 1 and 2, respectively, and adjusting for confounders. Descriptive
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41 133 analyses and logistic regression models were performed using R statistical software version
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43 134 4.0.2, using the *finalfit* package 1.0.2.
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135 **Results**

136 *Participants*

137 During the study period a total of 49,853 women of all ages were diagnosed with COVID-19
138 in Sweden, and a total of 14,685 women aged 50-80 years were included in our study (figure
139 1). Characteristics of these groups are shown in table 1. Individuals with decreased oestrogen
140 due to adjuvant endocrine therapy for BC (group 1) were older with a higher comorbidity
141 index. A larger proportion of women in group 2 (increased levels of systemic oestrogen due
142 to postmenopausal HT) had high income and a tertiary level of education (table 1).

143

144 *Oestrogen augmentation protects against death due to COVID-19*

145 Pharmaceutically decreasing systemic oestrogen levels increased the odds of dying due to
146 COVID-19 (group 1: odds ratio [OR] 2.35, 95% confidence intervals [CI] 1.51 - 3.65), but
147 following adjustment for confounders this association was no longer significant (figure 2).
148 Interestingly, augmentation of systemic oestrogen levels decreased the odds of dying due to
149 COVID-19, with OR 0.45 (95% CI 0.34 - 0.6), and this result remained significant even after
150 adjustment for confounders (OR 0.47, 95% CI 0.34 - 0.63). The absolute risk (AR) of dying
151 was 4.6% for the control group vs. 10.1% and 2.1% for the groups with decreased and
152 increased oestrogen, respectively.

153

154 As expected, higher age and wCCI increased the odds of dying due to COVID-19. For every
155 year increase in age the odds of dying were 1.15 (95% CI 1.14-1.17), and for every increase
156 in wCCI the odds of dying were 1.13 (95% CI 1.10-1.16) (figure 2). Furthermore, low
157 income and having only primary education were also factors that increased the odds of dying
158 due to COVID-19 (figure 2).

159 Discussion

160 Principal findings

161 The major finding of this nationwide registry-based study is that pharmaceutical
162 augmentation of oestrogen levels is associated with decreased odds of death due to COVID-
163 19 in postmenopausal women.

164

165 Comparison with related studies

166 There are several possible biological explanations for the lower risk experienced by women.
167 These include mechanisms directly involved in viral internalization and reproduction, where
168 oestrogen has been shown to decrease expression of vital proteins such as ACE2 and
169 TMPRSS2⁹⁻¹¹, inherent sex-linked differences in the immune system, and direct oestrogen
170 effects¹². As an example, Kalidhindi et al have studied the effect of testosterone and
171 oestrogen on ACE2 expression, a key cell entry for SARS-CoV-2 virus, using *in vitro*
172 experiments on isolated human airway smooth muscle cells of male and female origin¹³. Most
173 interestingly, they show that cells exposed to oestrogen and testosterone behave differently,
174 as testosterone significantly upregulates ACE2 expression in cells from both sexes, whereas
175 oestrogen downregulates ACE2¹³. ACE2 expression and differences in its expression in
176 relation to sex could also be linked to the higher mortality in relation to hypertension, venous
177 thromboembolism and SARS-CoV-2 infection between men and women¹⁴. The observed
178 oestrogen induced reduction of ACE2 expression might however not necessarily translate
179 into a reduction of ACE2 protein at the cell surface *in vivo* in all cell types. Our findings are
180 also supported by *in vitro* studies where 17 β -oestradiol treatment reduced SARS-CoV-2 viral
181 load⁹. Previous experimental studies in mice on SARS-CoV have, moreover, shown that
182 female mice are less susceptible to infection and that this protection was lost upon
183 oophorectomy, thus indicating a direct protective role of oestrogen signalling¹⁵. Furthermore,

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3 184 Barh et al., using a multiomics approach on SARS-CoV-2-infected host interactome,
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5 185 proteome, transcriptome, and bibliome datasets, demonstrated that oestrogen modulation
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7 186 could be a potential therapeutic option in COVID-19 ¹⁶. Our results are in line with those by
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9
10 187 Seeland et al using real world evidence from multiple institutions and the TriNetX platform.
11
12 188 They found by using propensity score matched analysis of data for women aged 50 and above
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14 189 with COVID-19 (n=439), that there was a survival benefit for oestradiol hormone-users
15
16 190 versus non-users (OR 0.33 (95%CI 0.18-0.62)). Although based on a large real-world dataset
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18 191 the risk of selection bias was more difficult to discern since the cohort was neither
19
20 192 population-based nor adjusted for central confounders although likely mitigated by the
21
22 193 propensity score matched analysis ¹⁷. In our study the effect of increased systemic oestrogen
23
24 194 levels on reducing the risk of COVID-19 death remained significant after adjusting for
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26 195 education level and income, both factors known to influence COVID-19 outcome ¹⁸, further
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28 196 supporting the protective role of oestrogen in women.
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31 197 The hypothetical inverse, a worsening effect of reduced systemic oestrogen levels in women
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33 198 with a previous BC receiving adjuvant endocrine therapy, was initially significant but not
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35 199 after adjusting for confounders. This population differs from the control group in that they all
36
37 200 have been diagnosed with BC and it has been shown that patients, both men and women, with
38
39 201 any cancer are harder hit by COVID-19 ¹⁹. However, in a previous study BC patients were
40
41 202 shown to be healthier compared with the background population in terms of ischemic cardiac
42
43 203 disease and CCI ²⁰, and the wCCI adjustments may therefore overcompensate for this cancer-
44
45 204 related vulnerability. Although not significant, a trend towards worse outcome remained and
46
47 205 thus a larger population of BC patients on endocrine therapy is likely needed to verify the
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49 206 finding. Thus, this study cannot exclude an increased risk of death from COVID-19 if
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51 207 systemic oestrogen levels are pharmaceutically decreased.
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3 209 Strengths and limitations
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5 210 The strengths of this study are that this is a nationwide cohort in a country with high COVID-
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7 211 19 incidence using well validated registry data. A weakness is that the level of oestrogen
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9 212 modulation cannot be exactly measured in each individual, and that the number of BC
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11 213 women on anti-oestrogen medication ended up being too small to show significance although
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13 214 there was a clear trend. The postmenopausal HT group, however, proved large enough to
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15 215 show the clear protective effect. A further limitation is that confounding factors such as body
16
17 216 mass index (BMI), nutrition and smoking habits are not available in the nationwide registry
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19 217 data.
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26 219 Implications and conclusion
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28 220 This study shows an association between oestrogen levels and COVID-19 death.
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30 221 Consequently, drugs increasing oestrogen levels may have a role in therapeutic efforts to
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32 222 alleviate COVID-19 severity in post-menopausal women and could be studied in randomized
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34 223 control trials.
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6
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8
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10 227

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21
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23
24

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26
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29
30 236 **Contributors**
31

32 237 MS and KW conceptualized the study. MS and AMFC designed the study, with input from
33
34 238 KW, AJ and OFR. OFR and AMFC prepared the study data. OFR performed the statistical
35
36 239 analysis. MS, KW, AJ, OFR and AMFC contributed to interpretation of the results. MS wrote
37
38 240 the first draft of the manuscript. MS, KW, AJ, OFR and AMFC contributed to critical
39
40 241 revision of the manuscript. MS, KW, AJ, OFR and AMFC approved the final manuscript.
41
42 242 AMFC is the guarantor of this study. The corresponding author attests that all authors meet
43
44 243 the criteria for authorship and that all have been included.
45
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50
51 245 **Competing interests**
52

53 246 All authors have completed the ICMJE uniform disclosure form at
54
55 247 www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the
56
57 248 submitted work, no financial relationships with any organisations that might have an interest
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3 249 in the submitted work in the previous three years, and no other relationships or activities that
4
5 250 could appear to have influenced the submitted work.
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8 251

9
10 252 **Ethical approval**

11
12 253 Ethical approval was obtained by the Swedish Ethical Review Authority (number 2020-
13
14 254 02150)
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19 256 **Data sharing**

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21 257 The study protocol (R script) is available upon request. The study used secondary registry
22
23 258 data that are regulated by the Public Access to Information and Secrecy Act (2009:400) and
24
25 259 are protected by strict confidentiality. For the purpose of research though, after formal
26
27 260 application to access personal data the responsible authority can grant access to data, though
28
29 261 this is contingent on vetting by the Ethical Review Authority of Sweden, according to the Act
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31 262 (2003:460) concerning the Ethical Review of Research Involving Humans. This means that
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33 263 the aggregated registry data cannot be shared.
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3 324 **Figure legends**
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5 325 Figure 1: Flow chart of the study
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10 327 Figure 2: Oestrogen augmentation is associated with decreased odds of dying due to COVID-

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12 328 19. Crude and adjusted logistic regression models. Statistical significance: * $p < 0.05$, ** $p <$

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14 329 0.01 , *** $p < 0.001$). OR, odds ratio; CI, confidence intervals; wCCI, weighted Charlson

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17 330 Comorbidity Index.
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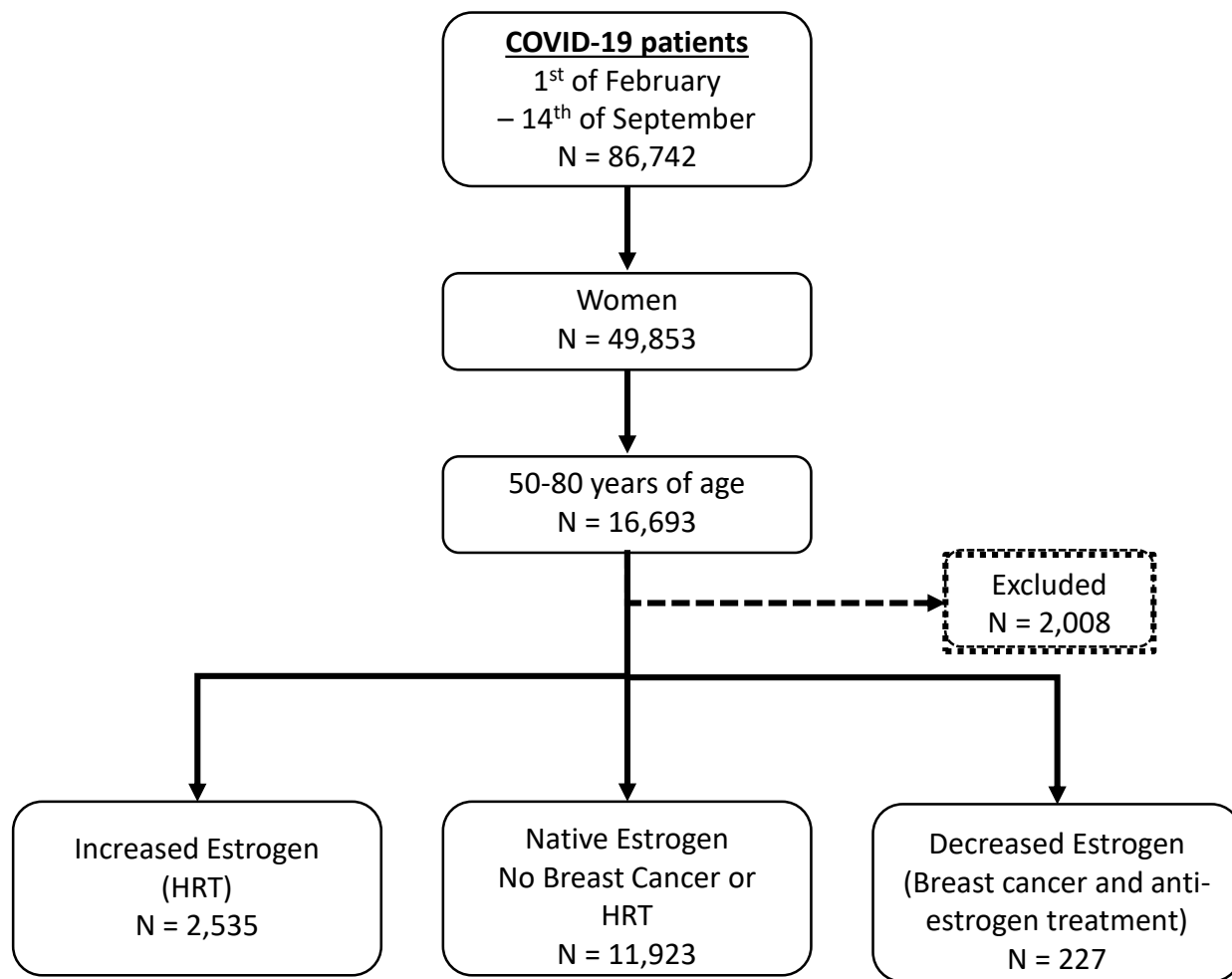
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331 **Table 1.** Characteristics of the study population

| Variable | | Native oestrogen (control group) | Decreased oestrogen (group 1) | Augmented oestrogen (group 2) | p-value |
|--------------------------------|-----------|----------------------------------|-------------------------------|-------------------------------|---------|
| Total N (%) | | 11923 (81.2) | 227 (1.5) | 2535 (17.3) | |
| Deaths | No | 11377 (95.4) | 204 (89.9) | 2481 (97.9) | <0.001 |
| | Yes | 546 (4.6) | 23 (10.1) | 54 (2.1) | |
| Age | Mean (SD) | 61.2 (8.3) | 64.4 (8.9) | 60.9 (7.7) | <0.001 |
| wCCI | Mean (SD) | 1.4 (2.4) | 5.0 (3.3) | 1.6 (2.5) | <0.001 |
| Income quintiles, n (%) | Richest | 3422 (28.7) | 64 (28.2) | 937 (37.0) | <0.001 |
| | Rich | 2743 (23.0) | 42 (18.5) | 605 (23.9) | |
| | Middle | 2120 (17.8) | 35 (15.4) | 404 (15.9) | |
| | Poor | 1703 (14.3) | 47 (20.7) | 334 (13.2) | |
| | Poorest | 1903 (16.0) | 39 (17.2) | 253 (10.0) | |
| | Missing | 32 (0.3) | 0 (0) | 2 (0.1) | |
| Education, n (%) | Tertiary | 4186 (35.1) | 82 (36.1) | 1074 (42.4) | <0.001 |
| | Secondary | 5609 (47.0) | 97 (42.7) | 1150 (45.4) | |
| | Primary | 1882 (15.8) | 45 (19.8) | 290 (11.4) | |
| | Missing | 246 (2.1) | 3 (1.3) | 21 (0.8) | |

332 wCCI: Weighted Charlson Comorbidity Index. SD standard deviation

Figure 1



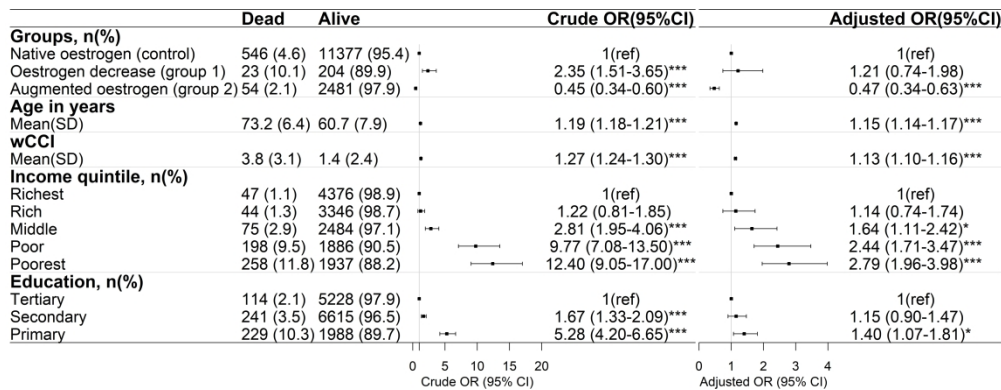


Figure 2

393x152mm (300 x 300 DPI)

STROBE Statement—Checklist of items that should be included in reports of *cohort studies*

| | Item No | Recommendation |
|------------------------------|---------|---|
| Title and abstract | 1 | (a) Indicate the study's design with a commonly used term in the title or the abstract. <i>"Association between pharmaceutical modulation of oestrogen in postmenopausal women in Sweden with death due to COVID-19 – a cohort study". Page 1, line 1-2</i> (b) Provide in the abstract an informative and balanced summary of what was done and what was found. <i>Page 3-4, line 28-62</i> |
| Introduction | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported. <i>Page 5, lines 75-90.</i> |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses. <i>Page 5, lines 87-90</i> |
| Methods | | |
| Study design | 4 | Present key elements of study design early in the paper. <i>Page 6-7, lines 91-134</i> |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection. <i>Page 6, lines 96-114</i> |
| Participants | 6 | (a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up. <i>Page 6, lines 96-114</i> (b) For matched studies, give matching criteria and number of exposed and unexposed. <i>Not applicable.</i> |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable. <i>Page 7, lines 116-126</i> |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. <i>Page 6-7, lines 91-134</i> |
| Bias | 9 | Describe any efforts to address potential sources of bias. <i>Not applicable.</i> |
| Study size | 10 | Explain how the study size was arrived at. <i>Page 6-7, lines 91-134</i> |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why. <i>Page 6-7, lines 91-134</i> |
| Statistical methods | 12 | (a) Describe all statistical methods, including those used to control for confounding. <i>Page 7, lines 128-134</i> (b) Describe any methods used to examine subgroups and interactions. <i>Page 6-7, lines 91-134</i> (c) Explain how missing data were addressed. <i>Case only method.</i> (d) If applicable, explain how loss to follow-up was addressed. <i>Not applicable.</i> (e) Describe any sensitivity analyses. <i>Not applicable.</i> |
| Results | | |
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. <i>Page 8, lines 135-142</i> (b) Give reasons for non-participation at each stage. <i>Not applicable.</i> (c) Consider use of a flow diagram. <i>Figure 1.</i> |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. <i>Page 8, lines 135-142, Table 1</i> |

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(b) Indicate number of participants with missing data for each variable of interest.

Table 1.

(c) Summarise follow-up time (eg, average and total amount). *Not applicable.*

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|--------------------------|-----|---|
| Outcome data | 15* | Report numbers of outcome events or summary measures over time. <i>Page 8, lines 144-158.</i> |
| Main results | 16 | (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included. <i>Page 8, lines 144-158. Figure 2.</i> (b) Report category boundaries when continuous variables were categorized. <i>Not applicable.</i> (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period. <i>Page 8, lines 150-152.</i> |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses. <i>Page 8, lines 154-158.</i> |
| Discussion | | |
| Key results | 18 | Summarise key results with reference to study objectives. <i>Page 9, lines 160-163.</i> |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias. <i>Page 11, lines 209-217.</i> |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence. <i>Page 11, lines 219-223.</i> |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results. <i>Page 9-10, lines 165-207</i> |
| Other information | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based. <i>Page 12, lines 228-234.</i> |

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at <http://www.strobe-statement.org>.

BMJ Open

Association between pharmaceutical modulation of oestrogen in postmenopausal women in Sweden with death due to COVID-19 – a cohort study

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|---------------------------------|---|
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| Article Type: | Original research |
| Date Submitted by the Author: | 18-Nov-2021 |
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| Primary Subject Heading: | Epidemiology |
| Secondary Subject Heading: | Infectious diseases, Oncology |
| Keywords: | COVID-19, Sex steroids & HRT < DIABETES & ENDOCRINOLOGY, EPIDEMIOLOGY, Epidemiology < INFECTIOUS DISEASES |
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3 **1 Association between pharmaceutical modulation of oestrogen in postmenopausal**
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5 **2 women in Sweden with death due to COVID-19 – a cohort study**
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26 Number of words text: 1406

27 Number of words abstract: 298

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3 **28 Abstract**
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5 **29 Objective:** Determine whether augmentation of oestrogen in post-menopausal women
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8 **30** decreases the risk of death following COVID-19.
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10 **31**
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12 **32 Design:** Nationwide registry-based study in Sweden based on registries from The Swedish
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14 **33** Public Health Agency (all individuals that tested positive for SARS-CoV-2); Statistics
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16 **34** Sweden (socioeconomical variables) and the National Board of Health and Welfare (Causes
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18 **35** of death).
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22 **36**
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24 **37 Participants:** Postmenopausal women between 50 and 80 years of age with verified COVID-
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31 **40 Interventions:** Pharmaceutical modulation of oestrogen as defined by (1) women with
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33 **41** previously diagnosed breast cancer and receiving endocrine therapy (decreased systemic
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35 **42** oestrogen levels); (2) women receiving hormone replacement therapy (HRT; increased
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37 **43** systemic oestrogen levels) and (3) a control group not fulfilling requirements for group 1 or 2
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39 **44** (postmenopausal oestrogen levels). Adjustments were made for potential confounders such as
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41 **45** age, annual disposable income (richest group as the reference category), highest level of
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43 **46** education (primary, secondary and tertiary (reference)) and the weighted Charlson
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45 **47** Comorbidity Index (wCCI).
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49 **49 Primary outcome measure:** Death following COVID-19.
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52 **51 Results:** From a nationwide cohort consisting of 49,853 women diagnosed with COVID-19
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56 **52** between February 4 and September 14, 2020 in Sweden, 16,693 were between 50 to 80 years
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3 53 of age. We included 14,685 women in the study with 11,923 (81%) in the control group, 227
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5 54 (2%) women in group 1 and 2,535 (17%) women in group 2. The unadjusted odds ratio (OR)
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8 55 for death following COVID-19 was 2.35 (95% CI 1.51-3.65) for group 1 and 0.45 (0.34-0.6)
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10 56 for group 2. Only the adjusted OR for death remained significant for group 2 with OR 0.47
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12 57 (0.34-0.63). Absolute risk (AR) of death was 4.6% for the control group vs 10.1% and 2.1%,
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14 58 for the decreased and increased oestrogen groups, respectively. The risk of death due to
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17 59 COVID-19 was significantly associated with: age, OR 1.15 (1.14-1.17); annual income,
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19 60 poorest 2.79 [1.96-3.97], poor 2.43 [1.71-3.46] and middle 1.64 [1.11-2.41]; and education
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21 61 (primary 1.4 [1.07-1.81]) and wCCI 1.13 [1.1-1.16].
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26 63 Conclusions: Oestrogen supplementation in post-menopausal women is associated with a
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28 64 decreased risk of dying from COVID-19 in this nationwide cohort study. These findings are
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31 65 limited by the retrospective and non-randomized design. Further randomized intervention
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33 66 trials are warranted.
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38 39 68 **Strengths and limitations of the study**

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42 69 • This study is based on all diagnosed COVID-19 patients in Sweden between February
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44 70 1 and September 14, 2020
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47 71 • Swedish registry data is well-validated and due to historical registry data and cross-
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49 72 linkage with the registries of Statistics Sweden, the confounding and/or effect-
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51 73 modifying effects of socioeconomic variables and comorbidities could be adjusted for
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54 74 • The findings are limited by the retrospective and non-randomized design.
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56 75 • Information regarding compliance to pharmaceutical modulation of oestrogen is
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58 76 missing
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3 77 • Information about the exact duration of the postmenopausal hormone therapy (HT)
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5 78 was not available in the dataset
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8 79 • Circulating oestrogen levels are not measured
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80 **Introduction**

81 The coronavirus disease 2019 (COVID-19) pandemic has swept across the globe causing
82 enormous strain on societies and health care systems. Although women are infected, they
83 appear to be protected from poor outcomes when compared with men even after adjustment
84 for confounding risk factors^{1 2}. Similar epidemiological findings have also been described
85 for SARS-CoV and MERS-CoV infections^{3 4 5}. This implies biological differences between
86 the sexes in terms of sensitivity to severe COVID-19, and oestrogen has been identified as a
87 potential therapeutic candidate.

88
89 The majority of breast cancer (BC) patients have oestrogen receptor (ER)-positive cancer⁶
90 and are usually given adjuvant endocrine therapy after surgery in order to reduce the risk of
91 cancer recurrences, leading to *reduced* systemic oestrogen levels. On the other hand, systemic
92 oestrogen levels are *augmented* in women taking postmenopausal hormone therapy (HT) to
93 relieve menopausal symptoms⁷. In a nationwide cohort, we used the opposing effects of
94 endocrine therapy in BC patients and women taking postmenopausal HT in modulating
95 systemic oestrogen levels as a model to test the hypothesis that increased oestrogen levels
96 are protective towards COVID-19 death.

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3 97 **Materials and methods**
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5 98 *Patients and public involvement statement*
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8 99 All data from the Swedish registries were pseudonymised and therefore patients were not
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10 100 involved in the study.
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15 102 *Participants and sources of data*
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17 103 The personal identification numbers (PINs) from all diagnosed COVID-19 individuals in
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19 104 Sweden (SmiNet) between the February 1 and September 14, 2020 were cross-linked with the
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21 105 LISA Register (Longitudinal integrated database for health insurance and labour market
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23 106 studies) administered by Statistics Sweden; and the following healthcare registers
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25 107 administreed by the Swedish National Board of Health and Welfare: patient, cancer,
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27 108 prescribed pharmaceutical, and causes of death. Post-menopausal women 50-80 years of age
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29 109 were stratified into three groups as follows: Group 1, the *decreased oestrogen group*,
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31 110 included patients with BC as identified by the International Classification of Diseases (ICD)
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33 111 version 10 code C50, and the following treatments: tamoxifen or fulvestrant (anatomical
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35 112 therapeutic chemical (ATC): L02BA01, L02BA03) or an aromatase inhibitor (AI; ATC
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37 113 L02BG03, L02BG04, and L02BG06). Group 2, the *augmented oestrogen group*, included
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39 114 those patients treated with drugs classified as postmenopausal hormone therapy (ATC codes
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41 115 G03CA03, G03CA04, G03CC07, G03CX01, G03FA, and G03FB). All ATC codes for
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43 116 groups 1 and 2 were identified from the Prescribed Pharmaceutical Register with at least two
44
45 117 consecutive withdrawals and at least one during the period extending from July 1, 2019 - to
46
47 118 the latest date. Group 3, the *native oestrogen (control) group*, included patients with no BC
48
49 119 diagnosis and no prescription of the above-mentioned pharmaceuticals at any time during
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51 120 2019 and 2020. Ethical approval was granted by the Swedish Ethical Review Authority.
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3 122 *Outcome, confounders and effect modifiers*
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5 123 The outcome was death due to COVID-19, as identified by the ICD-10 code U07, as the main
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8 124 or contributing cause of death from the Cause of Death Register. Potential confounders and
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10 125 effect modifiers were included in the model and consisted of the weighted Charlson
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12 126 comorbidity index (wCCI)⁸, age at COVID-19 diagnosis, income (divided into quintiles with
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14 127 the richest group as the reference) and education (primary, secondary and tertiary, which
15
16 128 served as the reference). The wCCI was calculated using the Patient and Cancer Registers, up
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19 129 to 2 months prior to the COVID-19 date in order not to include complications due to COVID-
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21 130 19 as a comorbidity. If there was no information regarding diagnosis codes required for
22
23 131 wCCI scoring, the individual was assigned a wCCI of zero. Information regarding income
24
25 132 and education was retrieved from the LISA-register.
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31 134 *Statistical methods*
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33 135 The distributions of continuous and categorical variables in the three groups were tested
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35 136 using ANOVA and the χ^2 test, respectively. Each variable was then analysed with univariate
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37 137 logistic regression models, followed by a multivariable regression model to compare the
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39 138 control group with groups 1 and 2, respectively, and adjusting for confounders. The present
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41 139 study evaluates specific outcomes, and the odds ratio and p-values are adjusted for relevant
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43 140 confounders using the multivariate logistic regression model and there was no need to further
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45 141 adjust using the Bonferroni/Benjamini/FDR approach. Descriptive analyses and logistic
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47 142 regression models were performed using R statistical software version 4.0.2, using the *finalfit*
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50 143 package 1.0.2.
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144 **Results**

145 *Participants*

146 During the study period a total of 49,853 women of all ages were diagnosed with COVID-19
147 in Sweden, and a total of 14,685 women aged 50-80 years were included in our study (figure
148 1). Characteristics of these groups are shown in table 1. Individuals with decreased oestrogen
149 due to adjuvant endocrine therapy for BC (group 1) were older with a higher comorbidity
150 index. A larger proportion of women in group 2 (increased levels of systemic oestrogen due
151 to postmenopausal HT) had high income and a tertiary level of education (table 1).

153 *Oestrogen augmentation protects against death due to COVID-19*

154 Pharmaceutically decreasing systemic oestrogen levels increased the odds of dying due to
155 COVID-19 (group 1: odds ratio [OR] 2.35, 95% confidence intervals [CI] 1.51 - 3.65), but
156 following adjustment for confounders this association was no longer significant (figure 2).
157 Interestingly, augmentation of systemic oestrogen levels decreased the odds of dying due to
158 COVID-19, with OR 0.45 (95% CI 0.34 - 0.6), and this result remained significant even after
159 adjustment for confounders (OR 0.47, 95% CI 0.34 - 0.63). The absolute risk (AR) of dying
160 was 4.6% for the control group vs. 10.1% and 2.1% for the groups with decreased and
161 increased oestrogen, respectively.

162
163 As expected, higher age and wCCI increased the odds of dying due to COVID-19. For every
164 year increase in age the odds of dying were 1.15 (95% CI 1.14-1.17), and for every increase
165 in wCCI the odds of dying were 1.13 (95% CI 1.10-1.16) (figure 2). Furthermore, low
166 income and having only primary education were also factors that increased the odds of dying
167 due to COVID-19 (figure 2).

168 Discussion

169 Principal findings

170 The major finding of this nationwide registry-based study is that pharmaceutical
171 augmentation of oestrogen levels is associated with decreased odds of death due to COVID-
172 19 in postmenopausal women.

173

174 Comparison with related studies

175 There are several possible biological explanations for the lower risk experienced by women.
176 These include mechanisms directly involved in viral internalization and reproduction, where
177 oestrogen has been shown to decrease expression of vital proteins such as ACE2 and
178 TMPRSS2⁹⁻¹¹, inherent sex-linked differences in the immune system, and direct oestrogen
179 effects¹². As an example, Kalidhindi et al have studied the effect of testosterone and
180 oestrogen on ACE2, a key cell entry for SARS-CoV-2 virus, using *in vitro* experiments on
181 isolated human airway smooth muscle cells of male and female origin¹³. Most interestingly,
182 they show that cells exposed to oestrogen and testosterone behave differently, as testosterone
183 significantly upregulates ACE2 expression in cells from both sexes, whereas oestrogen
184 downregulates ACE2¹³. ACE2 expression and differences in its expression in relation to sex
185 could also be linked to the higher mortality in relation to hypertension, venous
186 thromboembolism and SARS-CoV-2 infection between men and women¹⁴. Our findings are
187 also supported by *in vitro* studies where 17 β -oestradiol treatment reduced SARS-CoV-2 viral
188 load⁹. Previous experimental studies in mice on SARS-CoV have, moreover, shown that
189 female mice are less susceptible to infection and that this protection was lost upon
190 oophorectomy, thus indicating a direct protective role of oestrogen signalling¹⁵. Furthermore,
191 Barh et al., using a multiomics approach on SARS-CoV-2-infected host interactome,
192 proteome, transcriptome, and bibliome datasets, demonstrated that oestrogen modulation

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3 193 could be a potential therapeutic option in COVID-19¹⁶. Our results are in line with those by
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5 194 Seeland et al using real world evidence from multiple institutions and the TriNetX platform.
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8 195 They found by using propensity score matched analysis of data for women aged 50 and above
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10 196 with COVID-19 (n=439), that there was a survival benefit for oestradiol hormone-users
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12 197 versus non-users (OR 0.33 (95%CI 0.18-0.62)). Although based on a large real-world dataset
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14 198 the risk of selection bias was more difficult to discern since the cohort was neither
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17 199 population-based nor adjusted for central confounders although likely mitigated by the
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19 200 propensity score matched analysis¹⁷. In our study the effect of increased systemic oestrogen
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21 201 levels on reducing the risk of COVID-19 death remained significant after adjusting for
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23 202 education level and income, both factors known to influence COVID-19 outcome¹⁸, further
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25 203 supporting the protective role of oestrogen in women. Adjusting for income and education is
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27 204 important as we have previously shown the how these affect the risk of dying due to COVID-
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29 205 19 in Sweden¹⁹.
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33 206 The hypothetical inverse, a worsening effect of reduced systemic oestrogen levels in women
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35 207 with a previous BC receiving adjuvant endocrine therapy, was initially significant but not
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37 208 after adjusting for confounders. This population differs from the control group in that they all
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39 209 have been diagnosed with BC and it has been shown that patients, both men and women, with
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41 210 any cancer are harder hit by COVID-19²⁰. However, in a previous study BC patients were
42
43 211 shown to be healthier compared with the background population in terms of ischemic cardiac
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45 212 disease and CCI²¹, and the wCCI adjustments may therefore overcompensate for this cancer-
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47 213 related vulnerability. Although not significant, a trend towards worse outcome remained and
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49 214 thus a larger population of BC patients on endocrine therapy is likely needed to verify the
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51 215 finding. Thus, this study cannot exclude an increased risk of death from COVID-19 if
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53 216 systemic oestrogen levels are pharmaceutically decreased.
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3 218 Strengths and limitations
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5 219 The strengths of this study are that this is a nationwide cohort in a country with high COVID-
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7 220 19 incidence using well validated registry data. A weakness is that the level of oestrogen
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9 221 modulation cannot be exactly measured in each individual, and that the number of BC
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11 222 women on anti-oestrogen medication ended up being too small to show significance although
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13 223 there was a clear trend. Furthermore, we do not have data on the exact duration of
14
15 224 postmenopausal HT for the individuals. The postmenopausal HT group, however, proved
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17 225 large enough to show the clear protective effect. A further limitation is that confounding
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19 226 factors such as body mass index (BMI), nutrition and smoking habits are not available in the
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21 227 nationwide registry data.
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28 229 Implications and conclusion
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30 230 This study shows an association between oestrogen levels and COVID-19 death.
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32 231 Consequently, drugs increasing oestrogen levels may have a role in therapeutic efforts to
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34 232 alleviate COVID-19 severity in post-menopausal women and could be studied in randomized
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36 233 control trials.
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3 234 **Acknowledgments:**
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6
7 236 helping us design figure 2.
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15
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17
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19
20 242 Umeå University; Stroke Research in Northern Sweden; and Molecular Infection Medicine

21
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30 246 **Contributors**
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32 247 MS and KW conceptualized the study. MS and AMFC designed the study. OFR and AMFC

33 248 prepared the study data. OFR performed the statistical analysis. MS, OFR, KW, AJ and

34 249 AMFC all contributed to interpretation of the results. MS wrote the first draft of the

35 250 manuscript. MS, OFR, KW, AJ and AMFC contributed to critical revision of the manuscript.

36 251 MS, OFR, KW, AJ and AMFC approved the final manuscript. AMFC is the guarantor of this

37 252 study. The corresponding author attests that all authors meet the criteria for authorship and

38 253 that all have been included.
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44 255 **Competing interests**
45

46 256 All authors have completed the ICMJE uniform disclosure form at

47 257 www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the

48 258 submitted work, no financial relationships with any organisations that might have an interest
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3 259 in the submitted work in the previous three years, and no other relationships or activities that
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5 260 could appear to have influenced the submitted work.
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8 261

9
10 262 **Ethical approval**

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12 263 Ethical approval was obtained by the Swedish Ethical Review Authority (number 2020-
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14 264 02150)
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19 266 **Data sharing**

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21 267 The study protocol (R script) is available upon request. The study used secondary registry
22
23 268 data that are regulated by the Public Access to Information and Secrecy Act (2009:400) and
24
25 269 are protected by strict confidentiality. For the purpose of research though, after formal
26
27 270 application to access personal data the responsible authority can grant access to data, though
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29 271 this is contingent on vetting by the Ethical Review Authority of Sweden, according to the Act
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31 272 (2003:460) concerning the Ethical Review of Research Involving Humans. This means that
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33 273 the aggregated registry data cannot be shared.
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3 338 **Figure legends**
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5 339 Figure 1: Flow chart of the study
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10 341 Figure 2: Oestrogen augmentation is associated with decreased odds of dying due to COVID-

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12 342 19. Crude and adjusted logistic regression models. Statistical significance: * $p < 0.05$, ** $p <$

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14 343 0.01 , *** $p < 0.001$). OR, odds ratio; CI, confidence intervals; wCCI, weighted Charlson

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17 344 Comorbidity Index.
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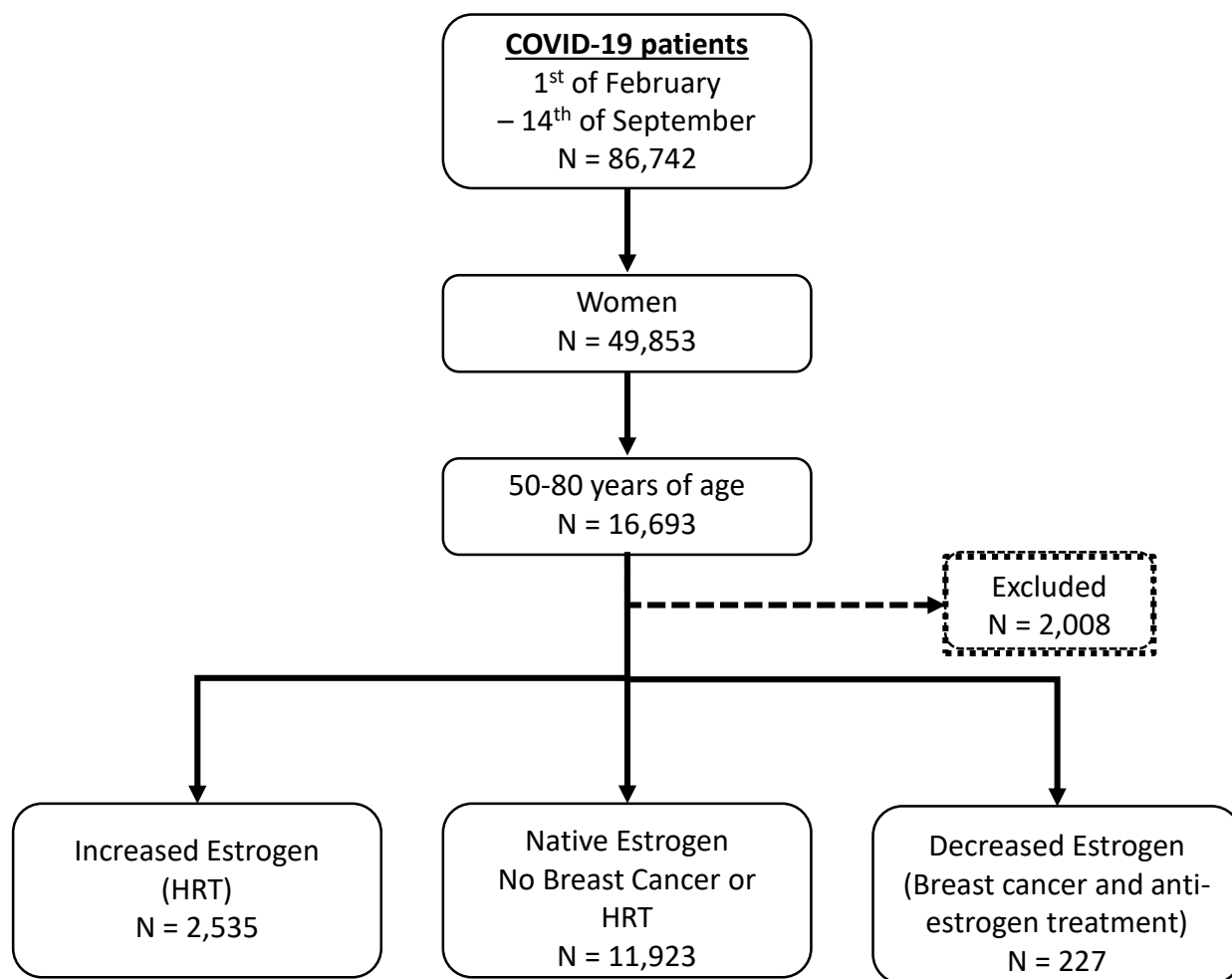
For peer review only

345 **Table 1.** Characteristics of the study population

| Variable | | Native oestrogen (control group) | Decreased oestrogen (group 1) | Augmented oestrogen (group 2) | p-value |
|--------------------------------|-----------|----------------------------------|-------------------------------|-------------------------------|---------|
| Total N (%) | | 11923 (81.2) | 227 (1.5) | 2535 (17.3) | |
| Deaths | No | 11377 (95.4) | 204 (89.9) | 2481 (97.9) | <0.001 |
| | Yes | 546 (4.6) | 23 (10.1) | 54 (2.1) | |
| Age | Mean (SD) | 61.2 (8.3) | 64.4 (8.9) | 60.9 (7.7) | <0.001 |
| wCCI | Mean (SD) | 1.4 (2.4) | 5.0 (3.3) | 1.6 (2.5) | <0.001 |
| Income quintiles, n (%) | Richest | 3422 (28.7) | 64 (28.2) | 937 (37.0) | <0.001 |
| | Rich | 2743 (23.0) | 42 (18.5) | 605 (23.9) | |
| | Middle | 2120 (17.8) | 35 (15.4) | 404 (15.9) | |
| | Poor | 1703 (14.3) | 47 (20.7) | 334 (13.2) | |
| | Poorest | 1903 (16.0) | 39 (17.2) | 253 (10.0) | |
| | Missing | 32 (0.3) | 0 (0) | 2 (0.1) | |
| Education, n (%) | Tertiary | 4186 (35.1) | 82 (36.1) | 1074 (42.4) | <0.001 |
| | Secondary | 5609 (47.0) | 97 (42.7) | 1150 (45.4) | |
| | Primary | 1882 (15.8) | 45 (19.8) | 290 (11.4) | |
| | Missing | 246 (2.1) | 3 (1.3) | 21 (0.8) | |

346 wCCI: Weighted Charlson Comorbidity Index. SD standard deviation

Figure 1



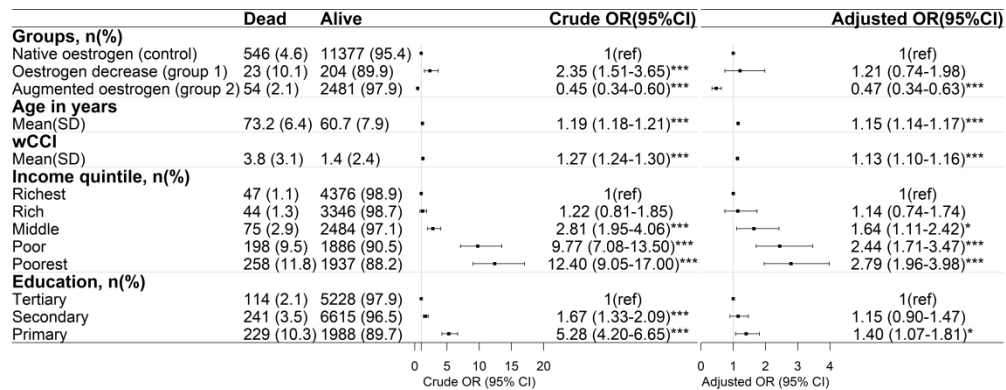


Figure 2

393x152mm (600 x 600 DPI)

STROBE Statement—Checklist of items that should be included in reports of *cohort studies*

| | Item No | Recommendation |
|------------------------------|---------|---|
| Title and abstract | 1 | (a) Indicate the study's design with a commonly used term in the title or the abstract. <i>"Association between pharmaceutical modulation of oestrogen in postmenopausal women in Sweden with death due to COVID-19 – a cohort study". Page 1, line 1-2</i> (b) Provide in the abstract an informative and balanced summary of what was done and what was found. <i>Page 3-4, line 28-62</i> |
| Introduction | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported. <i>Page 5, lines 75-90.</i> |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses. <i>Page 5, lines 87-90</i> |
| Methods | | |
| Study design | 4 | Present key elements of study design early in the paper. <i>Page 6-7, lines 91-134</i> |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection. <i>Page 6, lines 96-114</i> |
| Participants | 6 | (a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up. <i>Page 6, lines 96-114</i> (b) For matched studies, give matching criteria and number of exposed and unexposed. <i>Not applicable.</i> |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable. <i>Page 7, lines 116-126</i> |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. <i>Page 6-7, lines 91-134</i> |
| Bias | 9 | Describe any efforts to address potential sources of bias. <i>Not applicable.</i> |
| Study size | 10 | Explain how the study size was arrived at. <i>Page 6-7, lines 91-134</i> |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why. <i>Page 6-7, lines 91-134</i> |
| Statistical methods | 12 | (a) Describe all statistical methods, including those used to control for confounding. <i>Page 7, lines 128-134</i> (b) Describe any methods used to examine subgroups and interactions. <i>Page 6-7, lines 91-134</i> (c) Explain how missing data were addressed. <i>Case only method.</i> (d) If applicable, explain how loss to follow-up was addressed. <i>Not applicable.</i> (e) Describe any sensitivity analyses. <i>Not applicable.</i> |
| Results | | |
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. <i>Page 8, lines 135-142</i> (b) Give reasons for non-participation at each stage. <i>Not applicable.</i> (c) Consider use of a flow diagram. <i>Figure 1.</i> |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. <i>Page 8, lines 135-142, Table 1</i> |

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(b) Indicate number of participants with missing data for each variable of interest.

Table 1.

(c) Summarise follow-up time (eg, average and total amount). *Not applicable.*

| | | |
|--------------------------|-----|---|
| Outcome data | 15* | Report numbers of outcome events or summary measures over time. <i>Page 8, lines 144-158.</i> |
| Main results | 16 | (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included. <i>Page 8, lines 144-158. Figure 2.</i> (b) Report category boundaries when continuous variables were categorized. <i>Not applicable.</i> (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period. <i>Page 8, lines 150-152.</i> |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses. <i>Page 8, lines 154-158.</i> |
| Discussion | | |
| Key results | 18 | Summarise key results with reference to study objectives. <i>Page 9, lines 160-163.</i> |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias. <i>Page 11, lines 209-217.</i> |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence. <i>Page 11, lines 219-223.</i> |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results. <i>Page 9-10, lines 165-207</i> |
| Other information | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based. <i>Page 12, lines 228-234.</i> |

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at <http://www.strobe-statement.org>.