Mathematical analysis of Córdoba calcifediol trial suggests strong role for Vitamin D in reducing ICU admissions of hospitalized COVID-19 patients

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Abstract
A randomized controlled trial of calcifediol (25-hydroxyvitamin D\textsubscript{3}) as a treatment for hospitalized COVID-19 patients in Córdoba, Spain, found that the treatment was associated with reduced ICU admissions with very large effect size and high statistical significance, but the study has had limited impact because it had only 76 patients and imperfect blinding, and did not measure vitamin D levels pre- and post-treatment or adjust for several comorbidities. Here we reanalyze the results of the study using rigorous and well established statistical techniques, and find that the randomization, large effect size, and high statistical significance address many of these concerns. In particular, we show that decreased ICU admissions were not due to uneven distribution of comorbidities or other prognostic indicators, to imperfect blinding, or to chance, but were instead associated with the calcifediol intervention. We conclude that the Córdoba study provides sufficient evidence to warrant immediate, well-designed pivotal clinical trials of calcifediol in a broader cohort of inpatients and outpatients with COVID-19, and to consider broad adoption of calcifediol treatment for vitamin-D-deficient hospitalized COVID-19 patients.

Introduction
As of this writing, the 2019-2020 COVID-19 pandemic has resulted in more than one million deaths worldwide, and at times overloaded hospital and intensive care unit (ICU) capacities in the worst-affected areas. Effective treatments to decrease the severity of the disease are urgently needed.

Several lines of evidence have suggested a relationship between the vitamin D endocrine system and both the incidence and severity of COVID-19 [1]. Here we focus on the latter. Most of the risk factors known to be associated with poor COVID-19 outcome [2,3] are also risk factors for vitamin D (25-hydroxyvitamin D (25OHD)) deficiency [4,5], including advanced age, obesity, darker skin, hypertension, cardiovascular disease, kidney disease, and diabetes. European countries with higher 25OHD deficiency tended to have higher COVID-19 mortality rates [6,7], and an analysis of 117 countries found that those with high northern latitude, which presumably correlates with lower UVB light and lower 25OHD serum levels, have higher mortality rates when adjusted for age [8].

Serum 25OHD levels were predictive of COVID-19 severity and mortality in several cohort and retrospective studies. A retrospective study of 80 patients with confirmed COVID-19 in Madrid, Spain, found that vitamin D deficiency measured within the three preceding months predicted more severe disease [OR 3.2, (95% CI: 0.9-11.4), p = 0.07] [9]. A cohort study found that 17 hospitalized patients ≥50 years of age with COVID-19 admitted to a Singapore hospital after a treatment regimen with vitamin D, magnesium, and vitamin B\textsubscript{12} was initiated had significantly less need for oxygen or ICU admission than the 26 patients admitted before the regimen was initiated (17.6% versus 61.5%, p = 0.006), and the difference remained substantial and significant when adjusted for age or hypertension [10]. A study in Heidelberg, Germany, of 185 COVID-19 patients found 25OHD levels below 12 ng/mL were significantly associated with higher risk of the need for mechanical ventilation (HR 6.12, 95% CI 2.79–13.42, p < 0.001) or death (HR 14.73, 95% CI 4.16–52.19, p < 0.001, respectively) when adjusted for age, gender, and comorbidities [11]. A retrospective observational study of 149 COVID-19 patients in Istanbul, Turkey found that 25OHD serum levels were lower in patients with severe illness, and found that vitamin D levels independently predicted mortality after adjusting for age and several comorbidities [12]. A retrospective study in a Mexican hospital found that among 172 hospitalized COVID-19 patients, those with 25OHD serum levels below 8 ng/mL had 3.68 higher risk of dying from COVID-19 than those with higher levels, though the study did not control for possible confounders [13].

Several mechanisms have been proposed for how activation of the vitamin D receptor (VDR) could decrease acute lung injury and Acute Respiratory Distress Syndrome (ARDS), which are the major factors determining poor prognosis of hospitalized COVID-19 patients [14–16], namely decreasing cytokine storm, modulating
neutrophil activity, maintaining the pulmonary epithelial barrier, stimulating epithelial repair, and decreasing hypercoagulability and thrombosis [17,18]. An analysis of gene expression differences between COVID-19 patients and controls found that dysregulation of the Renin Angiotensin System (RAS) in COVID-19 patients leads to increased levels of bradykinin (a “bradykinin storm”), which could account for many symptoms of ARDS, suggesting this could be corrected by vitamin D supplementation to decrease levels of renin [19].

High dose D$_3$ supplementation has been found to be safe in critically ill patients with low 25OHD serum levels. The consensus recommendation of the European Society for Clinical Nutrition and Metabolism is that a high dose of vitamin D$_3$ (500,000 IU or, equivalently, 12,500 mcg) can be administered to critically ill patients with low plasma vitamin D levels, based on seven randomized trials of 716 critically ill adult patients that found no side effects in six months of follow up after supplementation with 200,000-540,000 IU (5,000-13,500 mcg) [20].

A small randomized controlled trial in Córdoba, Spain, of calcifediol (25-hydroxyvitamin D$_3$, or 25(OH)D$_3$) for hospitalized COVID-19 patients (henceforth, “the Córdoba study”) found dramatic reduction in the need for ICU admission [21]. This study has been viewed as a small preliminary study, suggesting at most that further study might be warranted. It has gotten relatively little attention, though its strengths and weaknesses were discussed in this article [22], and a Bayesian cost/benefit analysis found the expected benefits, in terms of lives saved and severe illness avoided, of immediately adopting the treatment protocol were considerably higher than the expected costs [23].

Here we apply rigorous and well established statistical techniques to reanalyze the results of the Córdoba study. We show that the randomization and large effect size address many of the concerns that have been raised about the study, including the small study size, the possibility that the results are due to uneven distribution of comorbidities between treated and control groups, and imperfect blinding, indicating with high confidence that the reduction in ICU admission for the treated group was associated at least in part with the calcifediol treatment. Specifically, we show that the probability of obtaining such a large effect size by chance if the treatment had no effect is less than one in a million, that the probability that the effect was due to differences in comorbidities between the treatment and control groups is less than one in 60,000, and for the results to be due to imperfect blinding would have required placebo and related effects to cause an implausibly large risk reduction. We investigate the question of whether these results will generalize to different cohorts, and consider next steps.

**Results and Discussion**

**The Córdoba calcifediol study**

The Córdoba study was a randomized, controlled, partly-masked clinical trial at Reina Sofia University Hospital in Córdoba, Spain, to test whether treatment with calcifediol could decrease the need for ICU admission among hospitalized COVID-19 patients, which would also likely decrease the risk of death. The study was preregistered (NCT04366908), with ICU admission and death as the primary outcome measures. The study report was ambiguous with regard to who was masked; we discuss this in the section on blinding.

Electronic randomization by hospital statisticians assigned 76 consecutive hospitalized confirmed COVID-19 patients to treatment or control groups in a two-to-one ratio. All received the hospital standard of care at the time, which was hydroxychloroquine and azithromycin. Treated patients also received oral calcifediol consisting of one 532 mcg soft capsule on the day of admission and 266 mcg on days 3 and 7, then weekly until discharge. Calcifediol was used rather than alternative vitamin D formulations because of its reliable intestinal absorption and rapid restoration of serum concentration. The dosage is well within the safe dosage guidelines for critically ill patients with low 25OH Damien plasma levels (12,500 mcg as a single dose) from the European Society for Clinical Nutrition and Metabolism [20], even when adjusted for the higher absorption of calcifediol compared to vitamin D$_3$. ICU admission was determined by a blinded multidisciplinary selection committee based on previously specified criteria.

A dramatic decrease in the need for ICU admission was observed in the treated group (1 out of 50, 2%) as compared to the control group (13 out of 26, 50%). In order to determine if this difference was due to different characteristics of the patients in the two groups, the authors reported statistics for 15 prognostic risk factors (Table 1), and used a multivariate logistic regression to compute the adjusted odds ratio correcting for the two risk factors, hypertension and type 2 diabetes mellitus, that were significantly higher in the control group. After correcting for these imbalances, ICU admissions were still dramatically lower among the treated patients (odds
ratio 0.03, 95% CI: 0.003-0.25).

The mortality rate among treated patients was also lower (0 out of 50 treated patients, 0%, versus 2 out of 26 control patients, 8%). The number of deaths was too small to achieve statistical significance against a null hypothesis of no effect (one-sided hypergeometric \( p = 0.11 \)) but the result is consistent with the plausible hypothesis that the decrease in mortality would be similar to the decrease in ICU admissions.

**Statistical significance is extremely high**

Although the study included a small number of patients in absolute terms, the very large effect size observed allows us to make confident inferences.

The primary concern with a small trial is that differences in the outcomes of the treated and control groups could be due not to the treatment but instead due to chance, under the null hypothesis that assignment to the treatment group has no effect. However, the number of patients needed to rule out this null hypothesis depends on the size of the effect, with large studies being necessary for subtle effects but a smaller number of patients being sufficient to detect a more robust effect. The probability that an effect as large as the one observed could arise due to chance under the null hypothesis, the p-value, tells us whether the number of patients in the study provides enough statistical power for the observed effect size.

The authors of the study calculated the statistical significance of the lower rate of ICU admission in the treated group as compared to the control group using the \( \chi^2 \) approximation, and reported a p-value \( p<0.001 \), but we find that the exact p-value is much smaller, indicating much greater statistical significance. Since 14 of the 76 patients in the study required ICU admission, and under the null hypothesis the probability of a patient requiring ICU admission does not depend on whether the patient is in the treated or control groups, the one-sided p-value is, by definition, the probability that if we randomly choose 14 out of the 76 patients, one or fewer of them would be in the treated group. That probability is given by the hypergeometric distribution, the one-sided version of Fisher’s exact test, which is defined as the probability that among \( n \) elements randomly drawn from a finite population without replacement, \( k \) of them will have a specified feature. In our case, the random draws are the 14 out of 76 patients requiring ICU admission, and the specified feature is being in the treated group. Using the hypergeometric distribution, we find that the p-value is \( p = 0.00000077 = 7.7 \times 10^{-7} \). A one-sided p-value is appropriate, because the hypothesis being tested is that calcifediol decreases disease severity, rather than that it has some effect in either direction.

There is a qualitative difference between results having a p-value of 0.001 and a p-value less than \( 10^{-6} \). As of April 30, 2020 when the Córdoba study was registered with clinicaltrials.gov, there were approximately 500 randomized intervention studies of COVID-19 treatments registered there. Even if none of those treatments were effective, one would expect approximately one of them to obtain a p-value as low as \( \frac{1}{500} = 0.002 \) simply due to chance, and it is plausible that one of them would obtain a p-value less than 0.001. P-hacking and HARKing (hypothesizing after results are known) effectively allow several hypotheses to be tested at once, with only the most significant result being reported [24]. However, these effects are severely limited by preregistration, since the hypothesis being tested is specified before the trial is conducted. It is not plausible that in a preregistered clinical trial with a single intervention, a combination of these effects could explain a p-value less than \( 10^{-6} \), which is less than one thousandth of the smallest p-value that would be expected by chance among 500 studies if the intervention had no effect. In short, we can be confident that if assignment to the treatment group had no effect, we would not have observed these results simply due to chance.

**Randomization protects against comorbidities and other prognostic risk factors**

A related concern is that the control group could have been enriched for comorbidities or other prognostic risk factors that make a severe outcome more likely. As noted above, the authors found that the effect size and statistical significance remained high (odds ratio 0.03, 95% CI: 0.003-0.25) after correcting for the two prognostic risk factors that were significantly enriched in the control group, namely hypertension and type 2 diabetes mellitus, out of the fifteen risk factors that were measured (Table 1), but other prognostic risk factors such as obesity and overall disease severity at hospital admission were not reported. It is generally recommended that all known confounders be checked and their effects modeled, but the concerns that such explicit modeling are meant to address are alleviated for the Córdoba study by the large effect size and statistical significance.

In a retrospective study, it is essential to ensure that the treated and control groups are well matched for known
prognostic risk factors, and confounding by unknown risk factors can invalidate the results. However, in a
randomized study with large effect size and statistical significance, the randomization protects against that. On
the one hand, a very large imbalance in prognostic risk factors would be needed to explain the factor of 25
difference in relative risk of ICU admission observed in the Córdoba study. A difference of, say, three-fold in
the rates of some risk factor such as obesity between the treatment and control groups cannot explain more
than a three-fold difference in outcomes. That is because if a risk factor were 100% causal and there were no
other factors affecting the outcome then a three-fold difference in the rates of that risk factor would explain
exactly a three-fold difference in the outcomes, and the effect of that risk factor on outcomes will be diluted if it
is less than 100% causal and there are other factors affecting the outcome. On the other hand, randomization
limits how large an imbalance is likely to occur: for example, if we flip a coin 50 times we will get 5 or fewer
heads only about twice in a billion attempts ($p = 2.1 \times 10^{-9}$).

There can be any number of prognostic risk factors, but if we knew what all of them were, and their effect
sizes, and the interactions among them, we could combine their effects into a single number for each patient,
which is the probability, based on all known and yet-to-be discovered risk factors at the time of hospital
admission, that the patient will require ICU care if not given the calcifediol treatment. Call this (unknown)
probability $P_{\text{prognostic}}(\text{Patient})$. Even though we do not know the actual values of these probabilities, we will show
that we can make some inferences about them. While knowing the particular comorbidities that contribute can
be valuable for understanding the mechanism of the disease, only the combined probability is needed for
determining whether they explain the difference in outcomes between the treated and control groups. It might
seem counterintuitive that we can use a single number to replace a large number of prognostic risk factors,
some of which could have large imbalances. For example, some individual risk factor such as obesity could be
substantially overrepresented in the control group. However, looking at one risk factor at a time does not give
the complete picture. A large overrepresentation of obesity in the control group might be counterbalanced by a
large overrepresentation of elderly patients in the treated group, or by the combined effect of several smaller
overrepresentations in the treated group. It is only by combining the effects of all risk factors that we can
accurately judge whether one group of patients or the other has a greater predisposition to severe disease.

We can infer something about how the values of $P_{\text{prognostic}}(\text{Patient})$ are distributed between the treatment and
control groups from the fact that patients were assigned to these groups randomly. If we knew this distribution,
it would be possible to calculate how likely it would be that we would observe a difference in ICU admissions
as large as the actual observed difference, under the null hypothesis that assignment to the treatment group
has no effect. If this likelihood were larger than our significance threshold, say 0.05, then that would mean that
the result would no longer be statistically significant after adjusting for all prognostic risk factors. The following
mathematical theorem allows us to estimate how likely it is that the randomization would distribute the
prognostic risk factors so unevenly between the treatment and control groups that after adjusting for them the
result would no longer be statistically significant.

**Theorem:** In a randomized study, let $p$ be the p-value of the study results, and let $q$ be the probability that the
randomization assigns patients to the control group in such a way that the values of $P_{\text{prognostic}}(\text{Patient})$ are
sufficiently unevenly distributed between the treatment and control groups that the result of the study would no
longer be statistically significant at the 95% level after controlling for the prognostic risk factors. Then $q < \frac{p}{0.05}$.

We supply the proof in Methods. The basic idea is that if the difference in outcomes results from a chance
imbalance of prognostic risk factors, that is a special case of the difference in outcomes resulting from chance.
The only assumption is that the randomization is truly random, i.e., that all patients have the same probability
of being assigned to the control group, and that these assignments are independent for different patients. It
does not rely on any assumption about the number or effect sizes of the prognostic risk factors.

Applying this theorem, we find that $q < \frac{0.00000077}{0.05} = 0.0000154 < \frac{1}{60,000}$.

So, there is less than a one in 60,000 chance that an uneven distribution of comorbidities or other prognostic
risk factors was so extreme as to make the results no longer significant at the 95% level. Similarly, there is less
than a one in 12,000 chance of that at the 99% significance level. Consequently, we can very confidently rule
out imbalances in comorbidities or other prognostic risk factors as the explanation for the large difference in
ICU admissions between the treated and control groups that was observed in the study.

**Large effect size protects against imperfect blinding**

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We have defined the null hypothesis to be that assignment to the treatment group had no effect, but the hypothesis we really want to rule out is that the treatment itself had no effect. These are different because assignment to the treatment group could have effects other than the treatment itself, such as placebo effect. The purpose of blinding and placebo control is to eliminate any such effects. The Córdoba study was performed during the peak of COVID-19 infections in Spain which overwhelmed many health care facilities, so a full double blinded placebo-controlled trial with extensive blood sampling was not feasible. However, steps were taken to minimize non-treatment effects of being in the treatment group. We describe these below, based on our conversations with Dr José Manuel Quesada Gómez, senior author of the Córdoba study.

Most importantly, the decision of whether a patient should be admitted to the ICU was made by a committee of specialists who were blinded, and that decision was based on the prespecified hospital protocol. The data collectors and statisticians were also blinded, eliminating those potential sources of bias. Although no placebo was used, patients were not told which group they were in, and because they were receiving several other drugs as part of the hospital standard of care, and were hospitalized with severe illness, it is unlikely that many, if any, of the patients became aware of whether they were receiving the calcifediol treatment or not. The calcifediol was administered by nurses, and the treating physicians were not specifically told which patients received the treatment. The information was available to the treating physicians if they looked up the daily drug treatment information, but in practice it is unlikely that many of them became aware of which patients received the calcifediol.

Despite these precautions, there could have been some residual effects of imperfect blinding, such as placebo effect, experimenter bias, etc., among any patients or their treating physicians who became aware of which group they were in. However, we calculated that these residual effects would need to have been implausibly large to invalidated the conclusions of the study. We define “unblinded effect” as the combined effect of all consequences of imperfect blinding to decrease the need for ICU care for a patient who would otherwise need it. We calculated that unless unblinded effect cut the risk of requiring ICU care by more than a factor of 4.8, the decreased ICU admissions observed in the treated group would still be statistically significant at the 95% level after adjusting for the unblinded effect (see Methods). For a given strength of the unblinded effect, we can also calculate how likely it is that prognostic risk factors would be so unevenly distributed that the results would no longer be statistically significant at the 95% level after adjusting for this uneven distribution and unblinded effect. We find that unless unblinded effect cut the risk of requiring ICU care by more than a factor of 2.6, this likelihood would be less than the usual threshold of 0.05. We conclude that it is not plausible that the decreased ICU admissions in the treated group were due to imperfect blinding, uneven distribution of prognostic risk factors, or a combination of the two.

No evidence of incorrect randomization
A key assumption in our analysis has been that the randomization was truly random. It has been suggested that the significant enrichment of hypertension in the control group is evidence that it was not random [22]. The Córdoba study reported that 15 of the 26 patients in the control group (56.69%) had hypertension as compared to 11 of the 50 patients in the treatment group (24.19%) with a nominal p-value of 0.0023, meaning that random assignment of patients to the control group would only lead to an enrichment of hypertension in the control group this large 0.23% of the time. Taken by itself, this would appear to be strong evidence that the patient assignment was not random. However, there were 15 prognostic risk factors reported, and the p-value of 0.0023 was a one-sided p-value, so there were actually 30 hypotheses tested, namely that one of the 15 prognostic risk factors was higher in the control group or higher in the treated group. When testing many hypotheses, it is expected that some of them will have low nominal p-values simply due to chance, so to correct for these a Bonferroni factor of 30 needs to be applied, giving a corrected p-value of 0.069 for the excess hypertension in the control group. This is not significant evidence that the assignment was not random at the usual 95% confidence level. As noted earlier, the effect of the treatment was still very large and very significant after adjusting for the excess hypertension in the control group.

Will the results generalize to other cohorts?
Showing that the decreased ICU admissions among the treated patients in the Córdoba study were associated with the calcifediol treatment is not sufficient to show that the effect will be similar in other cohorts, because the Córdoba patients might not be representative. One source of possibly relevant differences is known prognostic risk factors for the disease. To investigate this, we compared characteristics of the Córdoba cohort to those of
the patients in the SOLIDARITY trial [25], a huge trial of repurposed drugs on 11,266 hospitalized COVID-19 patients in 405 hospitals in 30 countries (Table 1). Among the many patient characteristics reported in each study, the ones in common between the two were age, sex, lung disease, diabetes, and heart disease. Counts for the age ranges reported in the SOLIDARITY trial (<50, 50-69, and 70+) were not reported in the Córdoba study, so we estimated counts using normal distributions matching the means and standard deviations reported for the treatment and control groups. Among the characteristics reported for both studies, the Córdoba cohort had significantly fewer patients with heart disease, diabetes, and over age 70, and significantly more aged 50-69. If calcifediol treatment is less likely to help patients with heart disease or diabetes, or over age 70, or more likely to help those aged 50-69, then there could be a smaller effect in the broad SOLIDARITY cohort than in the Córdoba cohort. Furthermore, since few drugs have as large an effect size as the observed association with calcifediol in the Córdoba cohort, from a Bayesian point of view we would expect the effect size in other cohorts to be somewhat lower.

Another possible difference between the Córdoba cohort and other hospitalized COVID-19 patients is the treatment background. The standard of care for the Córdoba patients was hydroxychloroquine and azithromycin, which is not the current standard of care in many countries. If the effect observed in the Córdoba study was associated with a synergistic interaction between calcifediol and hydroxychloroquine or azithromycin, or if drugs in the current standard of care have an antagonistic interaction with calcifediol, then the decrease in ICU admissions might not be seen in other patients. To the extent that the standard of care has improved since mid-April 2020 when the Córdoba study was completed, the bar is higher now, so any benefit of calcifediol on top of the improved standard of care could have lower effect size.

We note that the reduced ICU admissions might not specifically relate to COVID-19. Even before COVID-19 emerged, a meta-analysis of seven randomized trials of critically ill adult patients found that vitamin D supplementation was associated with reduced mortality compared to placebo and seemed safe [26]. Of course, such effects not specific to COVID-19 would not make the treatment any less valuable for COVID-19 patients.

Our knowledge of what factors determine severity of disease for COVID-19 are incomplete, so tests in other cohorts will be required to fully assess the generalizability of the study. We would expect that the patients most likely to be helped by calcifediol treatment are those with vitamin D deficiency. Although we do not know how prevalent deficiency was in the Córdoba cohort, we do know that it is very prevalent in the groups most at risk for COVID-19, so if this is the determining factor of effectiveness then we would expect the treatment to be associated with lower ICU admissions for a substantial fraction of COVID-19 patients.

**Conclusions**

We have shown with high confidence that the dramatic reduction in ICU admissions observed among hospitalized COVID-19 patients treated with calcifediol in the Córdoba study cannot be explained by imbalances in prognostic risk factors between the treated and control groups, the lack of blinding, or chance, and so is most likely associated with calcifediol treatment. Additional studies will be needed to determine how well these results generalize to other cohorts.

A concern about the Córdoba study is that 25OHD serum levels were not measured, so we do not know if the treatment was associated with a benefit only in patients who were deficient. A randomized controlled trial will be needed to determine whether calcifediol will benefit hospitalized COVID-19 patients who are not deficient. Including deficient patients in such a study could raise ethical concerns: since we know that vitamin D deficiency is harmful in general, that studies have found that high-dose vitamin D treatment can be safely administered to critically ill patients with low 25OHD serum levels [20], and we now have strong evidence that calcifediol is associated with a benefit in some hospitalized COVID-19 patients, the ethics of giving a placebo rather than treatment to a vitamin D deficient patient with this potentially fatal disease would need to be evaluated. It has been suggested that this ethical issue might be avoided by freezing baseline blood samples and only testing for deficiency at the end of the trial [27]. However, if failure to treat a deficient patient were determined to be unethical then the ethics of delaying testing in order to avoid treating the patients who need treatment might also need to be considered. While these issues are being worked out, the medical community should consider testing the vitamin D levels of all hospitalized COVID-19 patients, and taking remedial action for those who are deficient, to the extent that it can be done safely.

**Methods**
Proof of theorem

Theorem:  
In a randomized controlled study, let $p$ be the p-value of the study outcome, and let $q$ be the probability that the randomization distributes all prognostic risk factors combined sufficiently unevenly between the treatment and control groups that when controlling for these prognostic risk factors the outcome would no longer be statistically significant at the 95% level. Then $q < \frac{p}{0.05}$.

Proof:

Intuitively, the idea is that an extreme difference in outcomes (in this case, ICU admission) between the treated and control groups due to randomization producing an uneven distribution of prognostic risk factors between the treated and control groups is a special case of an extreme difference in outcomes due to chance, which is the p-value.

By definition, under the null hypothesis, the outcome for a patient will be the same if the patient is assigned to the treatment or control groups, so we can define an ICU patient to be a patient that will need admission to the ICU, regardless of which group the patient is assigned to. Let $UnbalancedICU$ be the event that the randomization assigns ICU patients to the treatment and control groups in a way that is at least as unbalanced as what was observed in the study. By definition $p = Probability(UnbalancedICU)$.

Let $MismatchedPrognostics$ be the event that a mismatch in prognostic risk factors between the treated and control groups is so large that when adjusted for it, the difference in ICU admissions between the two groups would no longer be statistically significant at the 95% level, i.e., $Probability(UnbalancedICU \text{ given } MismatchedPrognostics) > 0.05$.

Then by the definition of conditional probability we have

$$ p = Probability(UnbalancedICU) > Probability(UnbalancedICU \land MismatchedPrognostics) $$

$$ = Probability(MismatchedPrognostics) \times Probability(UnbalancedICU \text{ given } MismatchedPrognostics) $$

$$ > Probability(MismatchedPrognostics) \times 0.05 $$

So $Probability(MismatchedPrognostics) < \frac{p}{0.05}$.

Effects of imperfect blinding

Define a “blinded ICU patient” to be a patient in the treatment group who would have been admitted to the ICU if the study had been fully blinded. Let $N$ be the number of blinded ICU patients, and let $p$ be the probability that a blinded ICU patient would avoid ICU admission because of imperfect blinding. Then the probability of obtaining the observed result that exactly one of the 50 patients in the treatment group was admitted to the ICU is the binomial coefficient $N \times p^{N-1} \times (1-p)$. If we assume a uniform prior on $N$, then by Bayes theorem the posterior distribution for $N$ given that exactly one patient in this group required ICU admission is $C \times N \times p^{N-1} \times (1-p)$ for some constant $C$. Since the sum of the probabilities is 1, we find that

$$ 1 = \sum_{N=1}^{50} C \times N \times p^{N-1} \times (1-p). $$

Ignoring the negligible binomial coefficients for $N > 50$, we get

$$ 1 \approx C \times (1-p) \times \sum_{N=1}^{\infty} N \times p^{N-1} = C \times (1-p) \times \frac{1}{(1-p)^2} = \frac{C}{1} \quad \text{so} \quad C \approx 1-P \quad \text{and the distribution for } N \text{ is} $$

$$ N \times p^{N-1} \times (1-P). $$

For a given $N$, the p-value of $N$ out of 50 treated patients and 13 out of 26 control patients being admitted to the ICU is $h(N, 50, 26, 13 + N)$ where $h$ and its arguments are defined as in the R language phyper function, which gives the p-value for the hypergeometric distribution (the arguments are number drawn in specified category, total number in specified category, total number in other category, number drawn), so the expected p-value is \[ \sum_{N=1}^{50} N \times p^{N-1} \times (1-p)^2 \times h(N, 50, 26, 13 + N). \]

Using a standard root-finding algorithm, we found that the value of $P$ for which this p-value equals 0.05 is 0.794, which corresponds to a factor of \[ \frac{1}{1-0.794} = 4.865 \] decrease in the risk of requiring ICU admission.

Let $q_{\text{unblinded}}$ be the probability that the randomization distributes prognostic risk factors sufficiently unevenly that, when controlling for these prognostic risk factors and for the effects of imperfect blinding, the result would...
no longer be statistically significant at the 95% level. Applying our theorem, \( q_{\text{unblinded}} = 0.05 \) is equivalent to the p-value before adjusting for this uneven distribution being \( 0.05 \times 0.05 = 0.0025 \). We find that \( P \) would need to be 0.622 for the p-value to be 0.0025, which corresponds to a factor of \( \frac{1}{1-0.622} \) = 2.642 decrease in the risk of requiring ICU admission.

**Statistical significance in Table 1**
P-values in Table 1 for the difference between Córdoba and SOLIDARITY cohorts are one-sided binomial, based on percentages in the SOLIDARITY cohort. P-values in blue for the difference between calcifediol and non-calcifediol Córdoba patients were computed using the hypergeometric distribution; the ones in black are taken from Castillo et al Table 2 [21]. All p-values shown are nominal (no multiple hypothesis correction).

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| Poor prognosis risk factor | Group receiving Calcifediol (n=50) | Group without Calcifediol (n=26) | IC 95% | P   | SOLIDARITY (n=11266) | Córdoba Total (n=76) | Córdoba expected | Cór vs SOL P-val |
|---------------------------|------------------------------------|----------------------------------|-------|-----|----------------------|----------------------|-------------------|-----------------|
| ≥ 60 years                | 14 (28%)                           | 5 (19.23%)                       | -0.11 – 0.28 | 0.40 | 635(5.64%)           | 6(7.89%)             | 4                 | 0.257           |
| Previous lung disease     | 4 (8%)                             | 2 (7.69%)                        | -0.12 – 0.13 | 0.96 | 635(5.64%)           | 6(7.89%)             | 4                 | 0.257           |
| Previous Chronic kidney disease | 0                           | 0                                 |        |      | 2768(24.57%)         | 8(10.53%)            | 19                | 0.002           |
| Previous Diabetes mellitus | 3 (6%)                             | 5 (19.23%)                       | -0.30 – 0.03 | 0.08 | 2337(20.74%)         | 3(3.95%)             | 16                | <0.001          |
| Previous High blood pressure | 11 (24.19%)                     | 15 (57.69%)                       | -0.58 – -0.13 | 0.0023 |                      |                      |                   |                 |
| Previous Cardiovascular disease | 2 (4%)                         | 1 (3.85%)                        | -0.09 – 0.09 | 0.97 |                      |                      |                   |                 |
| Immunosuppressed & transplanted | 6 (12%)                        | 1 (3.85%)                        | -0.03 – 0.20 | 0.24 |                      |                      |                   |                 |
| At least one prognostic bad risk factor* | 24 (48%)                     | 16 (61.54%)                       | -0.37 – 0.10 | 0.26 |                      |                      |                   |                 |
| PaO2/FiO2 (mean +/-SD)     | 346.57 +/- 73.38                  | 334.62 +/- 66.33                 | -22.29 – 46.19 | 0.49 |                      |                      |                   |                 |
| C-reactive protein (mg/L) (mean +/-SD) | 82.93 +/- 62.74               | 94.71 +/- 63.64                  | -42.15 – 18.59 | 0.44 |                      |                      |                   |                 |
| LDH (U/L)(mean +/-SD)      | 308.12 +/- 83.83                  | 345.81 +/- 108.57                | -82.46 – 7.08  | 0.10 |                      |                      |                   |                 |
| D-Dimer (ng/mL) (mean +/-SD) | 650.92 +/- 405.61               | 1333.54 +/- 2570.50              | -360.29 – 1725.53 | 0.19 |                      |                      |                   |                 |
| Lymphocytes < 800/μL       | 10 (20%)                          | 6 (23.08%)                       | -0.16 – 0.23  | 0.75 |                      |                      |                   |                 |
| Ferritin (ng/mL) (mean +/-SD) | 691.04 +/- 603.54              | 825.16 +/- 613.95                 | -166.31 – 434.55 | 0.36 |                      |                      |                   |                 |
| IL-6 (22/48) (pg/mL) (mean +/-SD) | 28.88 +/- 75.05              | 19.54 +/- 19.45                 | -41.88 – 23.19 | 0.41 |                      |                      |                   |                 |
| Male                      | 27(54%)                           | 18(69.23%)                       | 0.15  | 6985(62.00%) | 45(59.21%) | 47 | 0.348             |
| <50 years*                | 18(36%)                           | 9(34.62%)                        | 0.56  | 3995(35.46%) | 27(35.53%) | 27 | 0.538             |
| 50-69*                    | 28(56%)                           | 16(61.54%)                       | 0.41  | 5125(45.49%) | 44(57.89%) | 35 | 0.020             |
| 70+*                      | 3(6%)                             | 1(3.85%)                         | 0.58  | 2146(19.05%) | 4(5.26%) | 14 | 0.001             |
Table 1. Prognostic factors for COVID-19 at baseline. Entries in black are taken from Castillo et al Table 2 [21], reproduced here for convenience. Entries in blue are comparison of the overall Córdoba cohort to hospitalized COVID-19 patients in the SOLIDARITY study [25], including the number and percent of patients with each characteristic measured in each study for the overall cohorts, expected number in the Córdoba study based on percentage in the SOLIDARITY study, and p-value for the difference. Numbers for “Male” are from Castillo et al Table 1. For age intervals matching those reported in the SOLIDARITY study (marked by *), the Córdoba counts were estimated using normal distributions with means and standard deviations matching those reported in the Córdoba study. One additional significant digit of the p-value for high blood pressure has been included because we make use of it in the text.