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Hydroxychloroquine, nitazoxanide and ivermectin have similar effects in early COVID-19: a *head-to-head* comparison of the Pre-AndroCoV Trial.

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Abstract

Background: COVID-19 pandemic requires urgent responses in terms of identification of effective and safe therapies to reduce hospitalization, death, and post-COVID symptoms, while vaccines are not extensively available. Repurposing already existing medications for COVID-19 should be preferred over the development of new drugs due to their inherent advantages of well-established safety profile, familiarity, and cost. Although antiandrogens have strong plausibility to be effective against COVID-19, hydroxychloroquine, nitazoxanide and ivermectin gained unquestionable popularity due to their *in vitro* and *in vivo* direct or indirect antiviral activity, and preliminary observations of efficacy against COVID-19. The objective of the present open-label prospective observational study (the pre-AndroCoV trial) was to make a head-to-head comparative analysis between hydroxychloroquine, nitazoxanide and ivermectin, in terms of potential efficacy for COVID-19, combined with early COVID-19 detection, aiming to choose one of these three drugs to include in the AndroCoV randomized clinical trial (RCT).

Materials and methods: Participants were recruited from social media and referred from other medical centers. Patients confirmed for COVID-19 with positive rtPCR-SARS-CoV-2 with fewer than seven days of symptoms and four days of treatment were included. Patients were actively questioned for age, sex, body mass index (BMI), presence of approximately 40 existing diseases and regular use of 30 drug classes, and COVID-19 symptomatology. Hydroxychloroquine 400mg/day for five days, nitazoxanide 500mg twice daily for six days, or ivermectin 0.2mg/kg/day for three consecutive days was given in a quasi-random manner, in association with azithromycin 500mg/day for five days, and optional addition of vitamin C, vitamin D and zinc, and glucocorticoids and anticoagulants in case of signs of lung injury or higher risk for thrombosis, respectively. Patients were followed up for 60 days, including active questions on disease course and symptoms on Days 0, 1, 2, 3, 7, 14 and 30, and virtual medical visits on Days 0 and 14, and whenever symptoms got worse on in the presence of severe adverse effects.

Results: In total, 585 participants, including 270 females and 305 males, were included. Of these, 159, 357, and 110 patients received hydroxychloroquine, nitazoxanide, and ivermectin, respectively, with similar baseline characteristics and time-to-treat between them. The three groups had similar duration of positive rtPCR-SARS-CoV-2, clinical

disease duration and recovery speed. Of the 585 patients, none was hospitalized, needed mechanical ventilation, or died, and 1.5% persisted with symptoms after recovery.
Conclusion: Hydroxychloroquine, nitazoxanide and ivermectin seem to be similarly effective for overall clinical outcomes in COVID-19 when used before seven days of symptoms, and overwhelmingly superior compared to untreated COVID-19 population, even for those outcomes not influenced by placebo effect, at least when combined with azithromycin, and vitamin C, D and zinc in the majority of the cases. Between these drugs, nitazoxanide demonstrated the strongest broad *spectrum* antiviral activity, plausibility to act as an anti-COVID agent, and safety profile, at least at the time of the choice of the drug for the AndroCoV Trial.

Background

Coronavirus Disease 2019 (COVID-19) is a multi-systemic infection caused by the novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), that leads to a wide variety of symptoms in the upper and lower respiratory tract, gastrointestinal, neurological, and musculoskeletal systems, although unspecific symptoms in other systems may also appear (1-4).

In addition of the exponential risk with aging (when above 60 y/o), other major factors are correlated with worse prognosis in SARS-CoV-2: uncontrolled diabetes, hypertension, obesity, and increased sensitivity to androgens or androgenic levels or activity (4-11).

Unique and unprecedented combination between mechanisms of actions of during SARS-CoV-2 infection course hampers from conclusive detailing of its pathogenicity. Currently, the only undisputable *sine-quo-non* key proteins for SARS-CoV-2 infectivity are the angiotensin-converting enzyme-2 (ACE2) receptor and transmembrane serine protease 2 (TMPRSS2) (12-17).

While COVID-19 is yet to be fully understood, the understanding of the its time course is critical to detect windows of opportunity to propose and study promising effective therapies. Of the three stages of the natural course of COVID-19, the most relevant period is the first stage, when SARS-CoV-2 viral infection is the key event. Unlike the first stage, the second and third stages of COVID-19 progressively change its pathogenicity to the overreactive immunologic and inflammatory responses, leading to Acute Respiratory Distress Syndrome (ARDS) in many cases and to a hypercoagulability, pro-thrombogenic state in the majority of the cases. At these further stages, antiviral approaches become less relevant, and damage-control of the cytokine storm and potential thrombotic events become central.

In the apparent lack of evidence of effective therapies against COVID-19 during the first stage, detection or development of drugs that provide solid safety profile, sufficient efficacy and affordability for public health systems are key aspects that must be considered when hypothesizing novel molecules or novel use of current drugs for COVID-19.

Since the COVID-19 pandemic requires urgent answers, safe and potentially effective pharmacological approaches, repurposing already existing medications for COVID-19 should be preferred over the development of new drugs (18-22) due to inherent advantages of existing drugs, including well-defined safety profile, risks, and contraindications, the likely increased familiarity of their use by clinicians, including posology, expected effects, and management of adverse effects and complications, and potential favorable cost-effectiveness to justify their use in large scale without increased costs due to patents of newly-developed molecules. These aspects are of great importance when a massive number of subjects is intended to be treat in the course of the pandemic.

In this context, the clinical use prior to specific evidence of efficacy against COVID-19 has been accepted in the current lack of therapeutic options, particularly in patients at higher risk of development of severe COVID-19. Notwithstanding, the *off-label* use, also termed as compassionate use, should be restricted to drugs with low risk of complications, strong plausibility and preliminary clinical data indicating efficacy against COVID-19. Data on each drug as potentially effective for COVID-19 should be based following a specific logical sequence of the mechanisms of action that could theoretically provide benefits for COVID-19, when and how the drug could be used for COVID-19, and current specific data on COVID-19, if any.

Although antiandrogens have demonstrated potential efficacy to block SARS-CoV-2 entry (26) and extensive theoretical mechanistic plausibility to protect from COVID-19 (27,28), some drugs claimed to have direct or indirect antiviral activity were popularized as effective therapies when COVID-19 is in its earlier stages. Among these drugs, the most popular ones include hydroxychloroquine, nitazoxanide and ivermectin. While hydroxychloroquine is used as an antimalarial and has been extended to rheumatic diseases, nitazoxanide is used as a broad-spectrum antiparasitic and also as an antiviral for gastrointestinal viral infections, and ivermectin is also used as a broad-spectrum antiparasitic. In common, all these molecules have previously demonstrated mechanisms of action that could potentially mitigate SARS-CoV-2 infectivity and preliminary reports of benefits, despite the large controversial data (2).

In the absence of a clear evidence of superiority of one of these options for COVID-19 when safety and efficacy are analyzed altogether for a medical decision, a comparative study between hydroxychloroquine, nitazoxanide and ivermectin was performed. The objective was to detect superiority or non-inferiority of these drugs, aiming to drive the design of the randomized clinical trial (RCT) in terms of which, if any, of the three drugs would be used in the AndroCoV Trial, to be tested together with the already pre-established spironolactone and dutasteride as candidates for COVID-19, and whether full placebo-control or mix open-label placebo-control would be conducted in the AndroCoV Trial.

Materials and methods

Subject selection

Patients were recruited both direct- and indirectly through social media and referred from urgent units and outpatient clinics when suspected or confirmed for COVID-19 through rtPCR-SARS-CoV-2.

Case-detection screening for COVID-19 was based on the presence of at least one symptom from the upper-respiratory tract, musculoskeletal, cardiovascular, gastrointestinal and neurological systems, or any unspecific symptom, not only those limited to fever, shortness of breath, dry cough, anosmia or ageusia. Candidates suspected for COVID-19 underwent rtPCR-SARS-CoV-2 (Abbott RealTime SARS-CoV-2 Assay, Abbott, USA; or Cobas SARS-CoV-2, Roche, Switzerland), and included in the study in case of confirmation. Patients previously confirmed for COVID-19 were also included if they fulfilled criteria for inclusion in the study.

To participate in the present prospective observational study, confirmed subjects had to fill the following inclusion criteria: 1. 18 years old and above; 2. Less than seven days since the beginning of symptoms; 3. Less than five days from the confirmation of COVID-19; 4. Lack of use or use in less than 72 hours of hydroxychloroquine, nitazoxanide and ivermectin; 5. Lack of previous use of glucocorticoids in the last seven

days; and 5. Absence of clinical or radiological signs of progression to severe acute lung injury, including shortness of breath, oxygen saturation (SatO2) below 92%, and more than 25% of lungs affected in a chest computed tomography (CT) scan performed before entering in the study.

Patients included for the present analysis provided a written consent regarding the use of one of the three drugs: hydroxychloroquine, ivermectin or nitazoxanide, as experimental drugs for COVID-19, not officially approved nor having clinical evidence for COVID-19, following the approval of the Institutional Review Board (IRB) of the Ethics Committee of the National Board of Ethics Committee of the Ministry of Health, Brazil (CEP/CONEP: Parecer 4.173.074 / CAAE: 34110420.2.0000.0008), and as registered at ClinicalTrials.gov (Identifier: NCT04446429. Available at clinicaltrials.gov (https://clinicaltrials.gov/ct2/show/NCT04446429?term=NCT04446429&draw=2&rank=1).

Patient characterization

Patients were characterized for basal characteristics, including sex, age (years old), weight (Kg), height (m) and body mass index (BMI) (kg/m^2). Whether patients were married or had a partner living in the same room, and whether they had households or lived alone, were also questioned.

Patients were then actively questioned on personal history of myocardial infarction or cerebrovascular disease, and for existing diseases, including hypertension, congestive heart failure (CHF), lipid disorders (dyslipidemia, hypertriglyceridemia), type 2 diabetes *mellitus* (T2DM), prediabetes (self-reported, with exams checked), obesity (BMI > 30 kg/m^2), asthma, chronic obstructive pulmonary disorder (COPD), chronic kidney disease (CKD), liver fibrosis or cirrhosis, depression clinically diagnosed, anxiety-related disorders, attention deficit hyperactivity disorder (ADHD), narcolepsy, and related disorders, insomnia clinically diagnosed , frank or subclinical hypothyroidism, Hashimoto's disease, other autoimmune disorders (if any, these were specified), and previous and current cancer (except for prostate for males and breast for females).

For females, menopause, endometriosis, and current or previous breast, ovary, or endometrium cancer, and for males, hypogonadism, erectile dysfunction, benign prostate hyperplasia (BPH), and previous or current prostate cancer were also questioned.

Patients were also characterized for androgenic phenotypes. Females were analyzed for the existence of hyperandrogenic phenotypes, including polycystic ovary syndrome (PCOS), using at least two of the three Rotterdam criteria for PCOS (oligo- or amenorrhea, clinical or biochemical hyperandrogenism, and presence of more than 10 to 12 small cysts (2-9mm) peripherally distributed in the ovaries), idiopathic hirsutism (at least eight points in the Ferriman-Gallwey scale), androgenetic alopecia (AGA - confirmed by trichoscopy), or any state of evident hyperandrogenism, and were then classified as hyperandrogenic (HA) or non-hyperandrogenic (non-HA) females. Males were screened for the presence of male AGA, using the Norwood-Hamilton scale (stages I-VII), and classified as being AGA or non-AGA males.

Regular use of medications were also actively searched for hypertension and other heart- or vascular-related conditions, including selective and non-selective beta-blocker (carvedilol, nebivolol, metoprolol, propranolol, atenolol), angiotensin converting enzyme inhibitors (ACEi) (captopril, enalapril), angiotensin-2 receptor blockers (ARB) (valsartan, olmisartan, losartan…), loop diuretics (furosemide, as the only loop diuretic available in Brazil), thiazide diuretics (hydrochlorothiazide, indapamide), calcium channel blocker (CCB) (amlodipine, felodipine, nifedipine, nicardipine, verapamil, ditialzem), potassium-sparing diuretic (spironolactone, as the only K-sparing diuretic available in Brazil), statins (pitavastatin, rosuvastatin, atorvastatin, simvastatin, pravastatin), other lipid-lowering drugs (fibrates, ezetimibe, PCSK-9 inhibitors), aspirin, clopidogrel, direct Xa factor inhibitors (apibaxan, rivaroxaban), thrombin inhibitors (dabigatran), and enoxaparin; for T2DM and T1DM, obesity, and used as off-label therapies for prediabetes, metabolic syndrome, non-alcoholic fatty liver disease (NAFLD), insulin resistance and overweight, including biguanides (metformin), glucagon-like peptide-1 receptor analogues (GLP1Ra) (liraglutide, semaglutide, dulaglutide, exenatide), sodium-glucose cotransporter-2 inhibitors (SGLT2i) (dapagliflozin, canagliflozin, empagliflozin), dipeptidyl-peptidase 4 inhibitors (DPP4i) (vildagliptin, sitagliptin, saxagliptin, linagliptin), sulfolnylureas (glicazide, glipizide, glimepiride, glibenclazide), glitazones (pioglitazone), alpha-glucosidase inhibitor

(acarbose), insulin (of any type, fast, regular, or long duration), and lipase inhibitor (orlistat); use of hormonal replacement or treatment regimens, including levothyroxine for hypothyroidism, growth hormone (GH) for GH deficiency of the adult, testosterone for males, progesterone (P) alone, estradiol (E) alone,combined (E+P) therapy, oral contraceptives and other hormonal regimens for females, and aromatase inhibitors (anastrozole, letrozole) or selective estrogen receptor modulators (SERM) (tamoxifen) for hypogonadotrophic (central) hypogonadism in males) or hormone-sensitive breast cancer in females; central acting drugs, including hypnotics (zolpidem ("Ambien"), zopiclone, eszopiclone, ramelteon ("Rozerem")), selective serotonin reuptake inhibitors (SSRIs) (sertraline, fluoxetine, duloxetine, paroxetine, venlafaxine, desvenlafaxine, vortioxetine ("Brintellix"), escitalopram, citalopram), other anti-depressant and humor stabilizer drugs (bupropion, trazodone, agomelatine, amitriptyline, nortriptyline, topiramate, oxcarbamazepine), benzodiazepines (lorazepam, alprazolam, bromazepam, midazolam, diazepam, clonazepam), atypical Antipsychotics (olanzapine (Zyprexa), quetiapine (Seroquel), risperidone (Risperdal), clozapine, aripiprazole) and central nervous system (CNS) stimulants (lisdexamphetamine, methylphenidate, modafinile); and other drugs, including oral minoxidil, finasteride, dutasteride, and proton-pump inhibitors (PPI) (dexlansoprazole, esomeprazole, pantoprazole, omeprazole), not limited to these drugs, and supplementation with omega-3 (> 3g/day), vitamin D (> 1,000iu/day), zinc (> 15mg/day), biotin (> 500mcg/day), and vitamin C (> 500mg/day).

Bacillus Calmette-Guérin (BCG) vaccine for tuberculosis, pneumococcal vaccine received since 2017 and influenza vaccine received in 2020, current smoking (> 2 packs/week and > 10 pack-year history) and significant regular physical activity (> 150 minutes/week, moderate-to-vigorous - > 3.0 METs for > 1y) were questioned.

For the characterization of the clinical manifestations of COVID-19, patients were actively questioned for the presence, beginning, duration and intensity of one or more symptoms among the following: fever, 'feverish', dry cough, self-reported perception of 'sinusitis', self-reported perception of 'sore throat', rhinorrhea, hipo- or anosmia, dis- or ageusia, weakness, dizziness, fatigue, myalgia, arthralgia, upper back pain, lower back pain, respiratory-dependent thoracic pain, shortness of breath, diarrhea, nauseas, vomiting, abdominal pain, conjunctival hyperemia, pre-orbital pain, dry eyes and dry

mouth. Oxygen saturation (%), heart rate (bpm), and in high-risk patients, systolic and diastolic blood pressure (SBP and DBP, respectively) were measured.

Procedures

According to clinical manifestations, included participants were clustered into one of the following types of clinical presentation: 1. Anosmia-Ageusia dominance, with at least one of anosmia and ageusia, and less than two symptoms of dengue fever-like, URTI-like or GI infection-like clusters; 2. Dengue fever-like, with at least three of myalgia, arthralgia, upper back pain, conjunctival hyperemia or pre-orbital pain; 3. Upper respiratory tract infection (URTI) URTI-like, with at least two of nasal congestion or rhinorrhea, dry cough, self-reported perception of "sinusitis", or self-reported perception of "sore throat"; 4. Gastrointestinal (GI) infection-like, with at least two of diarrhea, nauseas, vomiting, or abdominal pain; 5. Mixed between types, when there are sufficient number of symptoms to fulfill criteria for at least two clusters; 6. Unspecific clinical presentation, when symptoms do not fulfill criteria for any cluster; or 7. Asymptomatic.

In addition, patients were grouped according to the use or non-use of drugs with antiandrogenic (AA) activity, including spironolactone, cyproterone and other androgen receptor (AR) antagonists, dutasteride, finasteride, GnRH analogues, and androgen-deprivation therapies (ADT).

Hydroxychloroquine was given at a dose of 400mg/day for five days, nitazoxanide was given at a dose of 500mg, twice a day, for six days, and ivermectin was given at a dose of 0.2mg/kg/day for three days. One of these drugs (exceptionally, two of them were used, except for the combination of ivermectin and nitazoxanide) was associated with azithromycin 500mg/day for five days.

After characterization and clinical clustering, in case participant had not started on any of the following drugs, one of them, between hydroxychloroquine, azithromycin or ivermectin was given. The drug was chosen in a quasi-random manner, *i.e.,* hydroxychloroquine tended to be avoided in patients at higher risk for heart complications, drugs with previous history of intolerance were avoided, and also

according to clinical judgement, availability, and individual medical history. Drugs were given in the following regimens: hydroxychloroquine - 400mg/day in a single daily dose for five days, nitazoxanide - 500mg twice a day for six days, and ivermectin - 0.2mg/kg/day in a single daily dose for three days. In some patients, more than one of the three drugs were used (combination of ivermectin and nitazoxanide was avoided for all patients). In these cases, patients were considered as taking the two of the three drugs prescribed. All these drugs were associated with azithromycin 500mg/day in a single daily dose for five days, independently of other combinations.

In case patients had already started on one of the three drugs, it was maintained, and adjustments in doses and inclusion of azithromycin were performed.

Optionally, vitamin D, vitamin C, zinc, Xa factor inhibitors (apibaxan or rivaroxaban), enoxaparin, glucocorticoids, colchicine, N-acetyl-cysteine and bromhexine were prescribed, according to clinical, biochemical or radiological abnormalities, including the addition of enoxaparin or a Xa factor inhibitor in patients with high risk for thrombosis and increased D-dimer, and glucocorticoids for increased ultrasensitive C-reactive protein (usCRP) or radiologically diagnosed lung injury affecting 25% or more of lungs. Dutasteride or spironolactone were openly prescribed in some cases as per the evidence of anti-COVID action, mechanistic plausibility, and in fully accordance with the approval of the IRB.

Patients had virtual medical visit in the Day 0 and 14, and in case of any adverse effect. They were followed up by the research team on a daily basis, with active questions regarding clinical manifestations, speed of recovery, and occurrence of new symptoms, on Days 1, 2, 3, 7, 14, 30 and 60.

Selected patients underwent commercially available automatized and standardized biochemical tests (COBAS, Roche, Switzerland) ultrasensitive C-reactive protein (usCRP) (serum; Latex-intensified immunoturbidimetry;, erythrocyte sedimentation rate (ESR) (mm/1h) (blood; automatized spontaneous sedimentation), ferritin (ng/mL) (serum; chemoiluminescence – CLIA;) - and D-dimer (ng/mL; plasma; immunoturbidimetry), with intra- and inter-assay below 3.5 and 4.5%, respectively, and chest computed tomography (CT) scan.

Clinical outcomes

Clinical outcomes were analyzed for overall patients and those not using AA. Multiple disease course and disease progression scales were employed. Time-to-treat, duration of positive rtPCR-SARS-CoV-2, duration of symptoms including and not including anosmia and ageusia, and loss of ability for everyday activities in Days 0, 3, 7 and 14 were the primary clinical course outcomes. Brescia COVID-19 Respiratory Severity Scale (from 0 to 4), hospitalization, mechanical ventilation, use of noradrenaline or dopamine, and death were the clinical progression outcomes.

Patients were also actively followed for 60 days, whenever any residual or relapsing symptom appeared, for the persistence or new-onset symptoms after COVID-19 rtPCR cure, for physical, mental, or both post-COVID symptoms. Assessed physical symptoms include easy tiredness, loss of physical performance not fully justified by the disease, lack of any progressive recovery in physical capacity, loss of libido, unjustified muscle pain, prolonged muscle recovery, arthralgia, development of autoimmune diseases, persistence of menstrual irregularity, decrease of male fertility (when compared to previously documented fertility rate), and new-onset gastrointestinal symptoms, not limited to these, when not justifiable by any other cause. Mental symptoms include brain fog, attention deficit hyperactive disorder (ADHD) -like manifestations, changes in daily activity patterns and cognitive abilities, and other mental manifestations unrelated to post-traumatic stress disorder (PTSD) or any anxiety state related to the process of COVID-19.

Data availability

Full raw data is publicly available at a repository (https://osf.io/cm4f8/).

Statistical analysis

Sample size was determined based on the assumptions that the sample size estimate for the chi-squared test will require 80% power to detect the difference in

proportions at p = 0.05, that 95% of subjects would complete the study, and based on the hospitalization and death rates between 3 and 20%, and 0.3 and 2.5%, respectively (5,29,30). Based on these assumptions, the minimum sample size was 354 subjects. The study would terminate earlier in case of unexpected outcomes and adverse effects that could justify its termination.

Data was provided for absolute number and percentages, and for both mean and median, and standard deviation (SD) and 95% confidence interval (95%CI), respectively. In order to avoid overestimation of our findings, we assumed that all data was non-normally distributed, and performed non parametric-based analyses. Nonparametric ANOVA (Kruskal-Wallis) was performed for all parameters and *post-hoc* adjusted Dunn's test was performed for subgroup analyses, whenever $p < 0.2$. All statistical tests were performed using XLSTAT version 22.4.1 (Microsoft, USA).

Results

Table 1. Number of patients per drug

Disease progression outcomes	**Overall (n = 585)**	**Overall females (n = 270)**	**Overall males (n = 315)**	**Non-HA females (n = 195)**	**HA females non-AA users (n = 67)**	**Non-AGA males (n = 192)**	**AGA males non-AA users (n = 71)**	**HA female AA users (*spiro*) (n = 8)**	**Non-AA users (non-HA + HA) (n = 262)**	**AGA male AA users (n = 52)**	**Male non-AA users (overall) (n = 263)**
Hydroxychloroquine	*159*	*91*	*68*	68	20	*42*	*10*	3	88	*16*	52
Nitazoxanide	*357*	*129*	*228*	*93*	*31*	*141*	*58*	*5*	124	*29*	199
Ivermectin	*110*	*64*	*46*	*45*	*18*	*30*	*7*	*1*	63	*9*	37

AA = antiandrogens; AGA = androgenetic alopecia; HA = hyperandrogenic

Patients' characteristics

Between June 15th and August 30th, in total, 585 participants, including 270 females and 305 males, were included in the present analysis. Of the 270 females, 195 were non-HA and did not use AA, 67 were HA and did not use AA, and eight used AA (in the present case, spironolactone), which means that among females, 262 were non-users of AA and eight were AA users. Of the 305 males, 192 did not present AGA (non-AGA) and did not use AA, 71 had AGA and did not use AA, and 52 had AGA and used

AA (in case, dutasteride). The dropout rate was eight (1.6%) for full follow-up and two (0.3%) for clinical and disease progression outcomes.

A total of 159 patients received hydroxychloroquine, 357 received nitazoxanide, and 110 received ivermectin. The number of patients receiving hydroxychloroquine, nitazoxanide, and ivermectin according to sex, androgenic phenotype, and use of anti-androgens was proportionally distributed, for overall, overall females, overall males, non-hyperandrogenic (non-HA) females, hyperandrogenic (HA) females, non-androgenetic alopecia (non-AGA) males, androgenetic alopecia (AGA) males, dutasteride (5ARi) users, non-dutasteride (no-5ARi) users (including AGA and non-AGA males), spironolactone users, and non-spironolactone users (including HA and non-HA females) (Table 1).

Baseline characteristics, including age and body mass index (BMI), were similar between groups (Table 2). Clinical presentation according to clinical clustering, including anosmia-ageusia dominance, dengue fever-like infection, upper respiratory tract infection (URTI) -like infection, gastrointestinal (GI) -like infection, mixed clusters, only unspecific symptoms, and percentage of asymptomatic patients were similar between hydroxychloroquine, nitazoxanide, and ivermectin (Table 3).

Additional drugs prescribed during COVID-19, including Xa factor inhibitors, enoxaparin, glucocorticoids, vitamin D, vitamin C and zinc, occurred in similar proportions between the three drugs (Table 4).

Tables 2. Baseline characteristics.

Baseline characteristics *(Mean ± SD)*	**Overall (n = 585)**	**Hydroxychloroquine (n = 159)**	**Nitazoxanide (n = 357)**	**Ivermectin (n = 110)**	***p-value*** **(overall)**
Age (y/o)	*42.4 ± 11.3*	*43.2 ± 10.8*	*43.2 ± 10.9*	*42.3 ± 10.0*	n/s
Height (m)	*1.70 ± 0.08*	*1.69 ± 0.09*	*1.71 ± 0.08*	*1.70 ± 0.08*	n/s
BMI (kg/m^2)	*25.7 ± 4.6*	*25.4 ± 4.5*	*25.9 ± 4.4*	*25.7 ± 4.6*	n/s

BMI = body mass index; n/s = non-significant.

Table 3. Clinical clustering

Clinical clustering *(Number and %)*	**Overall (n = 585)**	**Hydroxychloroquine (n = 159)**	**Nitazoxanide (n = 357)**	**Ivermectin (n = 110)**	**p-value (overall)**
Anosmia-Ageusia dominance	112 (19.1%)	*29 (18.3%)*	*78 (21.8%)*	19 (17.3%)	n/s
Dengue fever-like	142 (24.3%)	*45 (28.3%)*	*90 (25.2%)*	33 (30%)	n/s
URTI-like	189 (32.3%)	*49 (30.8%)*	*115 (32.2%)*	37 (34.8%)	n/s
GI infection-like	66 (11.3%)	*16 (10.1%)*	*34 (9.5%)*	12 (10.9%)	n/s
Mixed	58 (9.9%)	*13 (8.2%)*	*34 (9.5%)*	11 (10.0%)	n/s
Unspecific	100 (17.1%)	*24 (15.1%)*	*53 (14.8%)*	14 (12.7%)	n/s
Asymptomatic	78 (13.3%)	*23 (14.5%)*	*41 (11.5%)*	15 (13.6%)	n/s

URTI = upper respiratory tract infection; GI = gastrointestinal; n/s = non-significant

Table 4. Additional drugs and supplements used to treat COVID-19.

Additional drugs or supplements *(Number and %)*	**Overall (n = 585)**	**Hydroxychloroquine (n = 159)**	**Nitazoxanide (n = 357)**	**Ivermectin (n = 110)**	**p-value (overall)**
Dutasteride (specific use for COVID-19)	38 (6.5%)	*7 (4.4%) (5% of 140 non-AA users)*	*27 (7.6%) (8,4% of 323 non- AA users)*	4 (3.6%) *(4% of 100 non- AA users)*	n/s
Spironolactone (specific use for COVID-19)	298 (50.9%)	*86 (54.1%) (61.4% of 140 non-AA users)*	*146 (40.9%) (45.2% of 323 non- AA users)*	66 (60%) *(66% of 100 non- AA users)*	n/s
Xa factor inhibitors	64 (10.9%)	*30 (11.1%)*	*34 (10.8%)*	64 (10.9%)	n/s
Enoxaparin	42 (7.2%)	*23 (8.5%)*	*19 (6.0%)*	42 (7.2%)	n/s
Glucocorticoids	54 (10.3%)	*34 (12.6%)*	*20 (6.3%)*	54 (10.3%)	n/s
Vitamin C	100 (17.1%)	*57 (21.1%)*	*43 (13.6%)*	100 (17.1%)	n/s
Zinc	114 (19.5%)	*68 (25.2%)*	*46 (14.6%)*	114 (19.5%)	n/s
Vitamin D	499 (82.3%)	*221 (81.8%)*	*268 (88.2%)*	499 (82.3%)	n/s

AA = antiandrogens; n/s = non-significant

Clinical outcomes

Patients that received hydroxychloroquine, nitazoxanide, and ivermectin started treatment with similar intervals since first symptoms, had similar duration of positive rtPCR-SARS-CoV-2, and had similar time-to-remission, both including and not including anosmia and ageusia as symptoms to be resolved (Table 5). When only non-AA users

were considered, all parameters were slightly longer than overall patients, but similar between drugs (Table 6).

In terms of functionality for everyday activities, baseline impairment levels were similar between hydroxychloroquine, nitazoxanide and ivermectin, and had similar improvement speed between them, in both overall (Table 7) and non-AA users (Table 8).

Of the 585 patients enrolled in the observational study, none was hospitalized, needed mechanical ventilation, or died (Table 9).

As shown in Table 10, in total, nine patients (1.5%) persisted or developed long-term symptoms after COVID-19, including five females (1.9%) and four males (1.2%). Physical and mental symptoms affected 2.5% and 0.7%, and 0.3% and 0.9% of females and males, respectively. Four patients on hydroxychloroquine (2.5% of all patients that used hydroxychloroquine), one patient on nitazoxanide (0.2%) and five patients on ivermectin (4.5%) developed post-COVID syndrome, with no statistical differences between them. Median time-to-treat among post-COVID patients was 6.0 days (95% CI = 0.9), statistically higher than overall patients (p = 0.034).

Table 5. COVID-19 clinical outcomes in overall patients.

Clinical outcomes *(Mean ± SD)*	**Overall (n = 585)**	**Hydroxychloroquine (n = 159)**	**Nitazoxanide (n = 357)**	**Ivermectin (n = 110)**	**p-value (overall)**
Time-to-treat *(pairwise p-values)*	*2.9 ± 1.8*	*2.9 ± 1.8 (Median = 3; 95%CI = 0.1)*	*3.0 ± 1.8 (Median = 3; 95%CI = 0.1)*	*3.0 ± 1.8 (Median = 3; 95%CI = 0.1)*	n/s (0.98)
Duration of positive rtPCR (days) *(pairwise p-values)*	13.9 *± 6.0*	*13.6 ± 6.0 (Median = 14; 95%CI = 0.5)* *(p=0.48 vs Nit)*	*14.0 ± 5.9 (Median = 14; 95%CI = 0.5)* *(p=0.57 vs Ive)*	*13.6 ± 5.8 (Median = 14; 95%CI = 0.5)* *(p=0.99 vs Hyd)*	n/s (0.69)
Remission not including anosmia (days)	5.7 *± 4.6*	*5.8 ± 5.1* (*Median = 5; 95%CI = 0.4)* *(p=0.29 vs Nit)*	*6.0 ± 4.5* (*Median = 5; 95%CI = 0.4)* (p=0.84 vs Ive)	*6.1 ± 5.4* (*Median = 5; 95%CI = 0.4)* *(p=0.54 vs Hyd)*	n/s (0.57)

(pairwise p-values)					
Remission including anosmia (days) *(pairwise p-values)*	9.3 *± 7.3*	*8.6 ± 7.2 (Median = 7; 95%CI = 0.6)* *(p=0.23 vs Nit)*	*9.5 ± 7.2* (*Median = 8; 95%CI = 0.6)* *(p=0.65 vs Ive)*	*8.9 ± 7.0 (Median = 7; 95%CI = 0.6)* *(p=0.61 vs Hyd)*	n/s (0.49)

Nit = nitazoxanide; Ive = ivermectin; Hyd = hydroxychloroquine, n/s = non-significant
SD = standard deviation; 95%CI = 95% confidence interval

Table 6. COVID-19 clinical outcomes in non-antiandrogen users

Clinical outcomes *(Mean ± SD)*	**Overall non-AA users (n = 525)**	**Hydroxychloroquine (n = 140)**	**Nitazoxanide (n = 323)**	**Ivermectin (n = 100)**	**p-value (overall)**
Time-to-treat *(pairwise p-values)*	3.2 *± 1.7*	*3.3 ± 1.6 (Median = 3; 95%CI = 0.3)* (p=0.99 vs Nit)	*3.3 ± 1.6 (Median = 3; 95%CI = 0.3)* *(p=0.98 vs Ive)*	*3.3 ± 1.5 (Median = 4; 95%CI = 0.3)* (p=0.96 vs Hyd)	n/s (0.99)
Duration of positive rtPCR (days) *(pairwise p-values)*	14.6 *± 5.9*	*14.5 ± 5.9 (Median = 14; 95%CI = 1.1)* *(p=0.66 vs Nit)*	*14.7 ± 5.8 (Median = 14; 95%CI = 1.1)* *(p=0.56 vs Ive)*	*14.3 ± 5.7 (Median = 14; 95%CI = 1.1)* *(p=0.86 vs Hyd)*	n/s (0.81)
Remission not including anosmia (days) *(pairwise p-values)*	6.3 *± 4.5*	*6.6 ± 4.5* (*Median = 5.5; 95%CI = 0.9)* *(p=0.82 vs Nit)*	*6.5 ± 4.6* (*Median = 5; 95%CI = 0.9)* *(p=0.88 vs Ive)*	*6.8 ± 5.3* (*Median = 6 95%CI = 1.0)* (p=0.67 vs Hyd)	n/s (0.97)
Remission including anosmia (days) *(pairwise p-values)*	10.3 *± 7.0*	*9.8 ± 7.0 (Median = 8; 95%CI = 1.3)* *(p=0.40 vs Nit)*	*10.4 ± 7.0* (*Median = 9; 95%CI = 1.3)* *(p=0.64 vs Ive)*	*9.9 ± 6.7 (Median = 8.5; 95%CI = 1.3)* *(p=0.81 vs Hyd)*	n/s (0.68)

Nit = nitazoxanide; Ive = ivermectin; Hyd = hydroxychloroquine, n/s = non-significant
AA = antiandrogens; SD = standard deviation; 95%CI = 95% confidence interval

Table 7. Loss of ability to perform everyday activities due to COVID-19 in overall participants.

Loss of ability of everyday	**Overall (n = 585)**	**Hydroxychloroquine (n = 159)**	**Nitazoxanide (n = 357)**	**Ivermectin (n = 110)**	**p-value (overall)**

activities (%) *(Mean ± SD)*					
Day 0 *(pairwise p-values)*	11.1 ± 17.6 *(Median = 0; 95%CI = 1.4) [379 (64.8%) full functional capacity]*	*12.5 ± 17.9 (Median = 0; 95%CI = 1.4) (62.3% full functional capacity)* *(p=0.82 vs Nit)*	*12.2 ± 18.8 (Median = 0; 95%CI = 1.5) (63.9% full functional capacity)* (p=0.72 vs Ive)	13.4 ± 18.5 *(Median = 0; 95%CI = 1.5) (61.8%) full functional capacity)* *(p=0.87 vs Hyd)*	n/s (0.93)
Day 3 *(pairwise p-values)*	4.3 ± 10.9 *(Median = 0; 95%CI = 0.9) [479 (81.9%) full functional capacity]*	*4.9 ± 11.0 (Median = 0; 95%CI = 0.9) (79.9% full functional capacity)* (p=0.84 vs nit)	*4.8 ± 11.6 (Median = 0; 95%CI = 0.9) (80.9% full functional capacity)* (p=0.65 vs Ive)	5.6 ± 11.6 *(Median = 0; 95%CI = 0.9) (78.2% full functional capacity)* *(p=0.80 vs hyd)*	n/s (0.90)
vs Day 0 (*p*-value)	< 0.0001	*0.0017*	*< 0.0001*	0.012	
Day 7	*1.4 ± 5.7 (Median = 0; 95%CI = 0.5) [446 (93.0%) full functional capacity]*	*1.7 ± 5.4 (Median = 0; 95%CI = 0.5) (91.8% full functional capacity)*	*1.6 ± 6.2 (Median = 0; 95%CI = 0.5) (92.1% full functional capacity)*	*2.1 ± 6.7 (Median = 0; 95%CI = 0.5) (90.9% full functional capacity)*	n/s (0.98)
vs Day 0 (*p*-value)	< 0.0001	*< 0.0001*	*< 0.0001*	0.0001	
vs Day 3 (*p*-value)	n/s (0.084)	*0.062*	*0.0094*	n/s (0.098)	
Day 14	*0.2 ± 1.8 (Median = 0; 95%CI = 0.1) [578 (98.8%) full functional capacity]*	*0.1 ± 0.8 (Median = 0; 95%CI = 0.1) (99.4% full functional capacity)*	*0.2 ± 1.9 (Median = 0; 95%CI = 0.1) (98.9% full functional capacity)*	*0.1 ± 1.0 (Median = 0; 95%CI = 0.1) [578 (99.1% full functional capacity)*	n/s (0.99)
Day 30	*0 (Median = 0; 95%CI = 0) [585 (100%) full functional capacity]*	*0 (Median = 0; 95%CI = 0) (100% full functional capacity)*	*0 (Median = 0; 95%CI = 0) (100% full functional capacity)*	*0 (Median = 0; 95%CI = 0) (100% full functional capacity)*	n/s (1.0)

Nit = nitazoxanide; Ive = ivermectin; Hyd = hydroxychloroquine, n/s = non-significant
SD = standard deviation; 95%CI = 95% confidence interval

Table 8. Loss of ability to perform everyday activities due to COVID-19 in non-antiandrogen (AA) users.

Loss of ability of everyday activities (%) *(Mean ± SD)*	**Overall non-AA users (n = 525)**	**Hydroxychloroquine (n = 140)**	**Nitazoxanide (n = 323)**	**Ivermectin (n = 100)**	**p-value (overall)**
Day 0 *(pairwise p-values)*	*12.3 ± 18.2 (Median = 0; 95%CI = 3.5) [320 (60.9%) full functional capacity]*	*14.4 ± 18.5 (Median = 0; 95%CI = 3.5) (57.1% full functional capacity)* (p=0.67 vs Nit)	*13.5 ± 19.4 (Median = 0; 95%CI = 3.7) (60.1% full functional capacity)* (p=0.72 vs Ive)	15.0 ± 18.9 *(Median = 0; 95%CI = 3.6) (58% full functional capacity)* *(p=0.99 vs Hyd)*	n/s (0.89)

Day 3 *(pairwise p-values)*	4.9 *±11.4 (Median = 0; 95%CI = 2.2) [419 (79.8%) full functional capacity]*	*5.7 ±11.7 (Median = 0; 95%CI = 2.2) (77.1% full functional capacity)* (p=0.76 vs nit)	*5.3 ±12.2 (Median = 0; 95%CI = 2.3) (78.9% full functional capacity) (p=0.65)*	6.3 *±12.1 (Median = 0; 95%CI = 2.3) (75% full functional capacity) (p=0.86 vs hyd)*	n/s (0.88)
vs Day 0 (*p*-value)	<0.0001	*0.0006*	*< 0.0001*	0.0075	
Day 7	1.5 *±6.1 (Median = 0; 95%CI = 1.1) [484 (92.2%) full functional capacity]*	*2.0 ±6.4 (Median = 0; 95%CI = 1.2) (90.7% full functional capacity)*	*1.8 ±6.5 (Median = 0; 95%CI = 1.2) (91.3% full functional capacity)*	*2.3 ±7.0 (Median = 0; 95%CI = 1.3) (90%) full functional capacity)*	n/s (0.98)
vs Day 0 (*p*-value)	<0.0001	*< 0.0001*	*< 0.0001*	*< 0.0001*	
vs Day 3 (*p*-value)	n/s (0.068)	*0.046*	*0.006*	n/s (0.082)	
Day 14	0.2 *±1.9 (Median = 0; 95%CI = 0.4) [518 (98.7%) full functional capacity]*	*0.1 ±0.9 (Median = 0; 95%CI = 0.2) (99.3% full functional capacity)*	*0.2 ±2.0 (Median = 0; 95%CI = 0.4) (98.8% full functional capacity)*	*0.1 ±1.1 (Median = 0; 95%CI = 0.2) (99%) full functional capacity)*	n/s (0.99)
Day 30	< 0.0001	*0 (Median = 0; 95%CI = 0) (100% full functional capacity)*	*0 (Median = 0; 95%CI = 0) (100% full functional capacity)*	*0 (Median = 0; 95%CI = 0) (100% full functional capacity)*	n/s (1.0)

Nit = nitazoxanide; Ive = ivermectin; Hyd = hydroxychloroquine, n/s = non-significant
AA = antiandrogens; SD = standard deviation; 95%CI = 95% confidence interval

Table 9. COVID-19 progression outcomes.

Disease progression outcomes *(Number and %)*	**Overall (n = 585)**	**Hydroxychloroquine (n = 159)**	**Nitazoxanide (n = 357)**	**Ivermectin (n = 110)**	**p-value (overall)**
Brescia COVID-19 Respiratory Severity Scale (0-4)	*0*	*0*	*0*	*0*	n/s
Hospitalization	*0*	*0*	*0*	*0*	n/s
Mechanical ventilation	*0*	*0*	*0*	*0*	n/s
Noradrenaline/ dopamine	*0*	*0*	*0*	*0*	n/s
Death	*0*	*0*	*0*	*0*	n/s

n/s = non-significant

Table 10. Post-COVID mental and physical symptoms.

Post-COVID symptoms	**Overall (n = 585)**	**Overall females**	**Overall males**	**Non-HA**	**HA females (n = 67)**	**Non-AGA males**	**Male AGA**	**Female AA (n = 8)**	**Female non-AA users**	**Male AGA AA users**	**Male non-AA**

		(n = 270)	(n = 315)	females (n = 195)		(n = 192)	non-AA users (n = 71)		(overall; non-HA + HA) (n = 262)	(n = 52)	users(overall) (n = 263)
Physical symptoms											
Hydroxychloroquine (n = 159)	*3*	*3*	*0*	*2*	*1****	*0*	*0*	*0*	3	*0*	0
Nitazoxanide (n = 357)	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	0	*0*	0
Ivermectin (n = 110)	*4*	*3*	*1*	*1**	*2**/***	*0*	*1*	*0*	3	*0*	0
Overall [%]	*6 (7) [1.1%]*	*5 (6) [2.5%]*	*1 [0.3%]*	*3*	*3*	*0*	*1*	*0*	6 (5)	*0*	0
Mental symptoms											
Hydroxychloroquine (n = 159)	*1*	*0*	*1*	*0*	*0*	*0*	*1*	*0*	0	*0*	1
Nitazoxanide (n = 357)	*1*	*0*	*1*	*0*	*0*	*1*	*0*	*0*	0	*0*	1
Ivermectin (n = 110)	*3*	*2*	*1*	*1**	*1***	*1*	*0*	*0*	2	*0*	1
Overall [%]	*5 [0.8%]*	*2 [0.7%]*	*3 [0.9%]*	*1*	*1*	*2*	*1*	*0*	2	*0*	3
Overall symptoms											
Hydroxychloroquine (n = 159) [%]	*4 [2.5%]*	*0*	*1*	*2*	*1*	*0*	*1*	*0*	3	*0*	0
Nitazoxanide (n = 357) [%]	*1 [0.2%]*	*0*	*1*	*0*	*0*	*1*	*0*	*0*	0	*0*	1
Ivermectin (n = 110) [%]	*5 (7) [4.5%]*	*3 (5)*	*2*	*1 (2)*	*2 (3)*	*1*	*1*	*9*	3 (5)	*0*	1
Overall [%]	*9 (12) [1.5%]*	*5 (8) [1.9%]*	*4 [1.2%]*	*3 (4)*	*3 (4)*	*2*	*2*	*0*	6 (8)	*0*	3

*/**/*** = Same patient. Between parentheses = duplicated number of affected patients (patients are duplicated in case they had both physical and mental symptoms, or used more than one drug); outside parentheses = actual number of patients affected
AA = antiandrogens; HÁ = hyperandrogenic; AGA = androgenetic alopecia

Discussion

The present prospective observational study combined the early detection of COVID-19 by suspecting in the presence of any symptom, not restricted to those typically described to occur in COVID-19, nor those that appears later in the disease, including anosmia, ageusia, fever and shortness of breath, with an open-label therapy using some of the most popular drugs claimed to be effective against COVID-19, particularly during the first stage.

The large number of patients enrolled and treated (n = 585), with a virtual absence of dropout (0.3%), without occurrence of any hospitalization, mechanical ventilation, or

death, shows that the pronounced differences when compared to untreated COVID-19 course are unlikely to be due have occurred randomly. The present findings reinforce the hypothesis of the protective effective of early pharmacological approaches when COVID-19 is detected after its early clinical signs.

Between the three molecules evaluated, the lack of differences regarding all clinical outcomes, including disease duration, viral shedding, improvement speed, similar lack of progression to more severe stages, and post-COVID syndrome rates demonstrates the similarity of the efficacy against COVID-19 between hydroxychloroquine, nitazoxanide, and ivermectin, at least when combined with azithromycin, and when COVID-19 is treated until seven days after symptoms begin. The equal distribution of the drugs according to sex and androgenic phenotype, and the similar baseline characteristics between these three drugs reinforces the similarity between their ability to mitigate COVID-19.

The investigation of androgenic phenotypes and chronic use of AA is critical importance to determine COVID-19 disease course (2,9-11), since both have demonstrated to modulate the clinical presentation, including improvement of outcomes in COVID-19 in chronic use of AA (26). This finding is expected since TMPRSS-2, a cell surface protein that primes to the spike protein of SARS-CoV-2 and facilitates its cell entry, is strongly regulated by androgens, and is consequently underexpressed in chronic AA users. Hence, subgroup analyses of HA and non-HA females, AGA and non-AGA males, and AA users and non-users, *i.e.,* without the influence of suppressed or enhanced TMPRSS-2 expression, could lead to different results. However, when adjusted for androgenic expression and only non-AA users, results remained similar, which reinforces the similarity between the efficacy of hydroxychloroquine, nitazoxanide and invermectin.

Risk factors of COVID-19 severity, demonstrated by increased rates of hospitalization and death in these high-risk populations, seem to be mitigated by the early pharmacological intervention, regardless of which therapeutic option, when approached early in the disease. It means that the overrepresentation of obesity, hypertension, uncontrolled diabetes, male sex, elderly, and hyperandrogenism in more severe COVID-19 presentation is potentially revertible with the combination of early diagnosis and early pharmacological approach.

The apparently high rate of post-COVID syndrome, presented as a wide variety of physical and mental symptoms, that impair normal functionality (31-36), likely leading to a post-COVID public health issue, should become a key clinical outcome when evaluating and proposing interventions for patients with COVID-19. The persistence of symptoms or occurrence of new symptoms after COVID-19 cure in the population of the present study was as low as 1.5%, consistent throughout the different populations and drugs used, in contrast to the almost 90% of patients with manifestations after recovery, including 72.8% complaining of fatigue, when not pharmacologically approached during early COVID-19 (33).

For all outcomes, including the increasingly recognized post-COVID syndrome, early detection is likely the imperative aspect, confirming extensive previous descriptions and results (37). The lack of specificity of symptoms during the first days of COVID-19 makes its diagnosis challenging. Massive campaigns to educate and engage the population and healthcare workers to suspect of COVID-19 in the presence of any symptom, even unspecific ones, is highly recommended. In the spread use of masks, the likelihood of presenting URTI, cold, “flu”, sore throat, sinusitis, and infections caused by other viruses is low. Consequently, symptoms that resemble any of these types of infections are more likely due to COVID-19, and should be therefore suspected promptly.

Hydroxychloroquine, nitazoxanide, or ivermectin: which one to choose for the AndroCoV Trial?

In common, hydroxychloroquine, nitazoxanide and ivermectin have been used for a wide range of infectious and non-infectious diseases in the long-term, with apparently safe profile, lack of overwhelming risks, in large populations, even as preventive approaches to low-risk diseases. Considering that all these three molecules had sufficient safety to be used for lower-risk disorders, even in a preventive basis, particularly for nitazoxanide and ivermectin, it seems intuitive that their use for early COVID-19, when antiviral activity tends to be more efficient, would be recommended, at least until evidence shows otherwise. Also, in the present study, outcomes between the three drugs were similar, revealing a non-inferiority of any of them in terms of efficacy. The lack of

severe adverse effects and complications also reinforced their safety profile for COVID-19.

Despite the above-mentioned points, the choice between hydroxychloroquine, nitazoxanide and ivermectin occurred based on the safety strength level of safety, likelihood to present efficacy according to *in vitro* and *in vivo* results, and preliminary data from the present study and other reports, in accordance with recommendations on how to chose the correct drug for a RCT in COVID-19 (38). The choice was made without the full data, since the AndroCoV Trial (RCT) started one and half month later than the beginning of the observational study, when results were partial, although similar to the final ones presented herein.

By the time of the submission to the Ethics Committee, safety concerns had been raised for hydroxychloroquine regarding its alleged arrhythmogenic effects (39) and lack of response in previous studies (40,41), although almost none of the studies with hydroxychloroquine was performed in actual early mild COVID-19, before hospitalization. As per its mechanisms of actions, it is expected that hydroxychloroquine become less or not effective when used after seven days or when COVID-19 is severe enough to cause hospitalization, when second and third stages of the disease are undoubtfully installed, and antiviral approaches become less relevant. In addition, despite the conflicting findings on hydroxychloroquine cardiovascular safety, its long use as an antimalarial and an antirheumatic agent without clinical observations of cardiovascular complications, and a thorough analysis of previous data allowed the European Society of Cardiology (ESC) to release a statement saying that short-term use of hydroxychloroquine is indisputably safe (42), particularly when used in doses lower than 800mg per day.

Although ivermectin has demonstrated broad-spectrum antiviral activity in vitro (38,43,44), compared to nitazoxanide, it has weaker evidence of clinical antiviral activity. Questions regarding the minimum concentration of ivermectin needed for effective anti-SARS-CoV-2 action have been raised (38) were also considered against its use for the RCT.

Nitazoxanide demonstrated strong broad antiviral *in vitro* and *in vivo* activity (38,45,46), with clinical evidence of consistent antiviral activity in humans for a wide variety of virus families (46), with official indication for gastrointestinal viruses. Specific

anti-SARS-CoV-2 activity in vitro and in vivo has also been extensively demonstrated (46-48), and its plausibility as a promising therapy for COVID-19 found the strongest evidence between the three drugs, particularly when combined with azithromycin (48). Despite slightly less tolerable in terms of symptoms compared to hydroxychloroquine and ivermectin, intolerance rates are low.

Between the three drugs with excellent overall profiles, nitazoxanide was chosen to be the drug to be added in the AndroCoV RCT. However, we encourage clinical trials with all three drugs, because of their potential beneficial effects against COVID-19, at least when diagnosed before three to seven days of symptoms.

Limitations

This is an open label study without the use of placebo group as control, with weakens the findings, when compared to full placebo-control double-blind RCTs. However, unlike symptoms and self-reported recovery speed, hospitalization, mechanical ventilation, death and viral shedding duration are outcomes independent of placebo effects, and have demonstrated consistent improvement in all groups, and indisputable differences with the widely described clinical course of COVID-19.

The inclusion of glucocorticoids in some cases may have provided additional protection for those with signs of progression to second stage. Since glucocorticoids were added in similar proportions between drugs, it unlikely affected results of the comparative analysis between hydroxychloroquine, nitazoxanide and ivermectin. Conversely, it is uncertain whether its inclusion, as well as inclusion of azithromycin for all enrolled patients, and vitamin D, vitamin C and zinc for the majority of the patients played additional protective effects when compared to hydroxychloroquine, nitazoxanide or ivermectin alone.

Conclusion

Hydroxychloroquine, nitazoxanide and ivermectin seem to be equally effective for COVID-19 in terms of clinical disease duration, viral duration, avoidance of

hospitalization, mechanical ventilation and death, and to prevent post-COVID symptoms, at least when combined with azithromycin, vitamin C, vitamin D and zinc, with overwhelmingly differences, unlikely random, when compared to untreated COVID-19 population.

Between the three drugs, nitazoxanide was chosen for the AndroCoV randomized clinical trial due to its strong broad *spectrum* antiviral activity, plausibility to act as an anti-COVID agent, and safety profile.

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Conflict of interest statement

Authors declare no conflict of interest with any of the pharmacological interventions proposed by the present study.

Data availability

Full raw data is publicly available at a repository (https://osf.io/cm4f8/).

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