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Effectiveness of a multidrug therapy consisting of Ivermectin, Azithromycin, Montelukast, and Acetylsalicylic acid to prevent hospitalization and death among ambulatory COVID-19 cases in Tlaxcala, Mexico

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ABSTRACT

Objective: There is an urgent need for effective treatments to prevent or attenuate lung and systemic inflammation, endotheliitis, and thrombosis related to COVID-19. This study aimed to assess the effectiveness of a multidrug-therapy consisting of Ivermectin, Azithromycin, Montelukast, and Acetylsalicylic acid ("TNR4" therapy) to prevent hospitalization and death among ambulatory COVID-19 cases in Tlaxcala, Mexico.

Design and methods: A comparative effectiveness study was performed among 768 confirmed SARS-CoV-2 cases aged 18–80 years, who received ambulatory care at the Ministry of Health of Tlaxcala. A total of 481 cases received the TNR4 therapy, while 287 received another treatment (comparison group). All participants received home visits and/or phone calls for clinical evaluation during the 14 days after enrollment.

Results: Nearly 85% of cases who received the TNR4 recovered within 14 days compared to 59% in the comparison group. The likelihood of recovery within 14 days was 3.4 times greater among the TNR4 group than in the comparison group. Patients treated with TNR4 had a 75% and 81% lower risk of being hospitalized or death, respectively, than the comparison group.

Conclusions: TNR4 therapy improved recovery and prevented the risk of hospitalization and death among ambulatory COVID-19 cases.

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Introduction

The novel coronavirus SARS-CoV-2 is characterized by significant morbidity and mortality (Jean et al., 2020; Li et al., 2020), with no proven prevention or treatment therapies (Baud et al., 2020). In some cases, SARS-CoV-2 can generate an exaggerated immune response that is characterized by a decreased quantity of lymphocytes, increased cytokine expression, as well as a greater risk of cardiovascular complications due to endotheliitis in several organs (Varga et al., 2020) and Disseminated Intravascular Coagulation (DIC), which are both highly correlated with COVID-19 deaths (Tang et al., 2020).

The impaired and overactive immune responses in COVID-19 patients result from cytokine dysregulation, which causes tissue inflammation and subsequent severe lung and systemic inflammation (Li et al., 2020). Recent research has shown that severe COVID-19 cases are associated with an increase in inflammatory cytokines such as IL-1β, IL-6, IL-8, IL-12, IFN-γ, GM-CSF, TNF-α (Huang et al., 2020), and lymphopenia, which is characterized by a reduction in the number of CD4+ and CD8+ T cells (Guan et al., 2020; Huang et al., 2020). Moreover, SARS-CoV-2 infection causes endotheliitis in several organs due to the host inflammatory response (Tang et al., 2020). Cardiovascular complications are a significant risk since SARS-CoV-2 infects the host using the angiotensin-converting enzyme 2 (ACE2) receptor, which is present in the lungs, heart, kidneys, intestines, and endothelial cells (Tang et al., 2020).

The role of the immune system must be examined to better understand the molecular mechanisms of SARS-CoV-2 pathogenesis and to evaluate therapeutic intervention strategies that target the immune system response and complications due to endotheliitis in several organs. For this reason, therapies should focus on stabilizing the endothelium while tackling viral replication, particularly with anti-inflammatory anti-cytokine drugs, ACE inhibitors, and statins (Anderson et al., 1995; David et al., 2002; Fang et al., 2020; Feldmann et al., 2020; Flammer et al., 2008; Taddei et al., 1998).

Despite the urgent need for effective COVID-19 treatment strategies, there is no consensus about what specific treatments are best. The most common treatments for COVID-19 have been anti-inflammatory agents, antiviral therapies, and other medications with proven efficacy against viruses (Hydroxychloroquine, Chloroquine, and Ivermectin) (Poduri et al., 2020; Caly et al., 2020; Gupta et al., 2020; Tsang et al., 2020). Other treatments include antibiotics such as Azithromycin (Bleyzac et al., 2020; Jean et al., 2020), Tocilizumab, which may have a clinical benefit because of its anti-IL-6 nature, cellular therapy (Mesenchymal stem cells), and immunotherapy (Convalescent plasma therapy). Combination therapies, such as a triple combination of Interferon beta-1b, Lopinavir-Ritonavir, and Ribavirin (Hung et al., 2020), or Hydroxychloroquine plus Azithromycin, have also shown promising clinical improvement (Tsang et al., 2020).

Antiviral therapy has been used to deter viruses from entering cells and prevent them from multiplying or moving to other cells (Abd El-Aziz and Stockand, 2020). The most commonly used antivirals include Lopinavir/Ritonavir (Cao et al., 2020), Remdesivir (Beigel et al., 2020; Wang et al., 2020), and Favipiravir (Cai et al., 2020). Hydroxychloroquine and Chloroquine have been used in combination with Remdesivir to treat hospitalized patients with COVID-19 (Poduri et al., 2020), and a Chinese study found that Chloroquine was able to inhibit the exacerbation of pneumonia (Gao et al., 2020).

Antibiotics have mainly been used to treat secondary bacterial infections (Abd El-Aziz and Stockand, 2020). However, Azithromycin has also shown anti-inflammatory and immunomodulatory effects against viral infections (Bleyzac et al., 2020; Jean et al., 2020). During the acute phase of SARS-CoV-2 infection, Azithromycin may regulate and reduce the production of pro-inflammatory cytokines such as interleukin 1 beta, 6, 8, 10, 12, and TNF alpha. In the resolution phase, this drug had demonstrated an increase in neutrophil apoptosis and oxidative stress. Azithromycin has also shown beneficial effects against other coronavirus types by decreasing viral proliferation (Bleyzac et al., 2020; Pani et al., 2020). Since Azithromycin works favorably in the lung parenchyma, it can act prophylactically to prevent co-infection by bacterial agents and SARS-CoV-2. Like Ivermectin, Azithromycin has also demonstrated anti-inflammatory activity and can be a useful treatment during the mild and moderate stages of SARS-CoV-2 infection (Bleyzac et al., 2020; Damle et al., 2020).

Ivermectin is another promising therapeutic option that can effectively limit infections caused by RNA viruses and is highly capable of reducing viral replication through protein inhibition. In clinical studies, Ivermectin has resulted in a viral clearance of SARS-CoV-2 from between 93% to 98% in cells (Caly et al., 2020; Gupta et al., 2020). Ivermectin produces a modulation of the host's immune system by increasing C-reactive protein levels, reducing the production of TNF alpha and interleukin 1–6, as well as the activation of neutrophils. It has also shown an anti-inflammatory effect at the lung parenchyma level by reducing the production of IgE and IgG1 cytokines in bronchial cells. Since Ivermectin can reduce viral replication and the release of pro-inflammatory cytokines, this drug has produced a good response in the non-severe clinical stages of COVID-19 (Gupta et al., 2020; Portmann-Baracco et al., 2020).

Montelukast is a potent cysteinyl leukotriene receptor antagonist that reduces protein expression of IL-4, IL-5, and IL-13 in the lungs and exerts its anti-inflammatory effect by suppressing T-helper type-2 cytokines (Fidan and Aydoğdu, 2020). Montelukast may have an immune-modulatory role and inhibitory effect on bradykinin, as well as a bronchoconstrictive effect, secondary to the interaction of the SARS-CoV-2 virus and the receptors of the ACE2 (Almerie and Kerrigan, 2020; Fidan and Aydoğdu, 2020). Moreover, some studies have detected a significant reduction in SARS-CoV-2 infection among elderly patients with asthma treated with Montelukast (Bozek and Winterstein, 2020).

The cytokine storm triggered by SARS-CoV-2 infection causes a state of hypercoagulability and platelet hyperaggregation that can lead to DIC, venous and arterial thrombosis, and microvascular thrombosis (Bianconi et al., 2020). Acetylsalicylic acid (ASA) has been used as an anti-inflammatory and antithrombotic medication in patients with severe COVID-19. A clinical trial involving antiplatelet therapy that included ASA and clopidogrel found an improved ventilation/perfusion ratio in COVID-19 patients with severe respiratory failure (Viecca et al., 2020).

Currently, there is a pressing need for effective treatments that can prevent or attenuate lung and systemic inflammation, as well as endotheliitis and thrombosis, to prevent the severity of health complications related to COVID-19. This study aimed to assess the effectiveness of a multidrug therapy: "TNR4" (Ivermectin, Azithromycin, Montelukast, and ASA), on the health recovery, risk of hospitalization, and death, in a sample of ambulatory COVID-19 cases in Tlaxcala, Mexico.

Material and methods

Study design, population, and identification of COVID-19 cases

A comparative effectiveness study was performed among patients with laboratory-confirmed SARS-CoV-2 infection at the Ministry of Health of Tlaxcala. Cases were reported through the Epidemiological Surveillance System for Viral Respiratory Diseases and the National System for Epidemiological Surveillance

(Gobierno de México, 2020; Secretaría de Salud-Gobierno de México, 2020; Secretaría de Salud, 2013).

Incident SARS-CoV-2 cases were identified from the following sources: (1) individuals with respiratory symptoms who sought healthcare attention by calling an emergency 911 number; (2) individuals who were in contact with a positive COVID-19 case and were visited at home; and (3) individuals with respiratory symptoms of COVID-19 who sought medical attention at primary healthcare units or at public hospitals of the Ministry of Health, Mexican Institute of Social Security (IMSS), Institute for Social Security and Services for State Workers (ISSSTE), or private healthcare providers.

Suspected COVID-19 cases were evaluated by medical personnel from primary healthcare units or epidemiologists from public hospitals, using a COVID-19 early identification/triage. Persons with acute respiratory illness and sudden onset of at least one of the following symptoms: cough, sore throat, shortness of breath, or fever [$\geq$38 °C (measured) or history of fever (subjective)] irrespective of admission status were considered suspected cases. Other criteria included having at least one of the following during the 14 days prior to the onset of symptoms: (1) close contact with a confirmed or probable case of SARS-CoV-2 infection; (2) travel to areas with local transmission of SARS-CoV-2; (3) worked in or attended, a health care facility where patients with SARS-CoV-2 infections were being treated; or (4) was admitted with severe pneumonia of unknown etiology (World Health Organization, 2020).

From May 11 to September 9, 2020, a total of 6798 laboratory-confirmed COVID-19 cases were reported in the state of Tlaxcala, and 3399 of these cases were treated at the Tlaxcala Ministry of Health. Of these cases, 1147 were eligible ambulatory patients who presented mild or moderate symptoms of COVID-19 and were invited to participate in this study. Patients excluded from this study were those who refused to participate (n = 251), who were under 18 years or older than 80 years (n = 44), and those who initiated the treatment on the same day or one day before they were hospitalized or died (n = 84). The study population consisted of 768 COVID-19 patients who accepted to be clinically evaluated for 14 days, 481 accepted to receive the TNR4 therapy. The other 287 patients agreed to the clinical follow-up but did not receive the TNR4 therapy because they had already been offered another treatment, they had self-medicated for cold and flu symptoms or were asymptomatic, so they did not think any further treatment was necessary (Figure 1).

Study participants completed a questionnaire and accepted follow-up visits at home and/or phone calls for clinical evaluation during 14 days. Sociodemographic characteristics, risk factors, and other health conditions (smoking, obesity, and pregnancy), as well as comorbidities, were identified. The study protocol and procedures were approved by the institutional review boards and the Tlaxcala State Ministry of Health ethics committee.

Assessment of effectiveness and safety of TNR4 therapy and therapies used by the comparison group

The TNR4 multidrug therapy (*New Therapy for Recovery* of COVID-19 infection, four *medications*; in Spanish: *Terapia Nueva para la Recuperación* en la infección por COVID-19, *4 medicamentos*) was given to SARS-CoV-2 patients who were initially treated as outpatients. TNR4 consists of four drugs administered orally to COVID-19 cases with mild or moderate symptoms: (1) Ivermectin, 12 mg single dose; (2) Azithromycin 500 mg for 4 days; (3) Montelukast, 60 mg on the first day and then 10 mg between days 2–21; and (4) acetylsalicylic acid, 100 mg for 30 days. All four medications are approved by the Federal Commission for Protection against Health Risks (COFEPRIS, in Spanish) (Comisión Federal para la Protección contra Riesgos Sanitarios, 2016).

The effectiveness of TNR4 and the therapies used in the comparison group was measured utilizing next health outcomes: percentage of patients who completely recovered within the first 14 days after their symptoms began and risk of hospitalization or death. Participants were also asked if they experienced any undesirable side effects from the TNR4 medications.

Statistical analysis

Differences between the TNR4 and comparison group were determined based on sociodemographic variables, occupation, the prevalence of comorbidities, smoking, pregnancy, and average days elapsed between the onset of symptoms and therapy initiation. For continuous variables, we calculated the difference between means using linear regression, and for categorical variables, we obtained the difference between proportions using chi–squared tests.

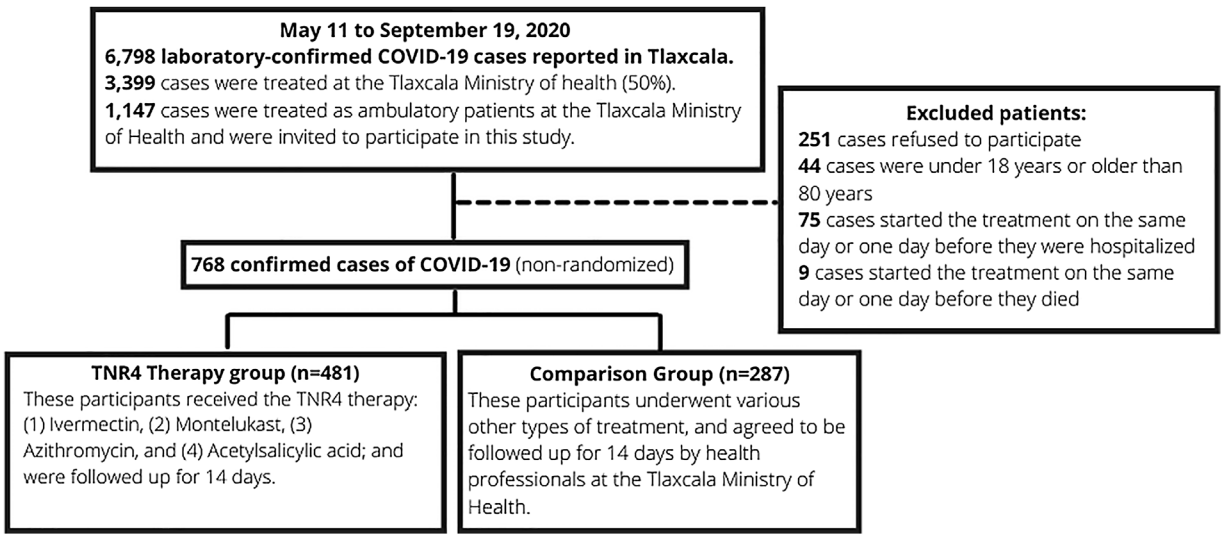

Figure 1. The study population of laboratory-confirmed cases of COVID-19 who received ambulatory treatment at the Tlaxcala Ministry of Health, Mexico.

The difference of proportions was also performed to compare the percentage of COVID-19 patients who recovered their health within 14 days after the onset of symptoms between TNR4 *versus* the comparison group. These analyses were adjusted by age, sex, occupation, and comorbidities and were additionally stratified by comorbidities, age groups (in tertiles), and sex.

We also compared the likelihood of occurrence of specific health outcomes, *e.g.*, recovery within the first 14 days after the onset of symptoms, hospitalization, and death, in the TNR4 and the comparison groups. These probabilities were estimated using multivariate logistic regression models and 95% Confidence Intervals (CI's), which were adjusted by age, sex, comorbidities, and occupation.

Finally, we assessed the percentage of undesirable side effects associated with the TNR4 medications and any other medications used by the patients who were primarily treated with TNR4. Additionally, we identified the type of medications or therapies used by participants who did not accept the TNR4 therapy. All statistical analyses were performed using Stata SE version 15.0 software.

Results

Table 1 compares the sociodemographic variables, occupation, comorbidities, and average days from onset of symptoms and therapy initiation between both treatment groups. Participants in the TNR4 group were 4.9 years younger than those in the comparison group, were 10.3% less likely to be male, had 7.1% lower prevalence of any comorbidity, and were 8.4% more likely to be health workers ($p \leq 0.050$, in all cases).

Table 2 shows a comparison between the COVID-19 cases treated with TNR4 *versus* the comparison group in terms of days to recovery, percentage of patients who fully recovered within 14 days, and percentage of patients who had to be hospitalized or died. These results are presented stratified by comorbidities, older age group (49–80 years), sex (males), and occupation (health workers). The TNR4 group had a significantly higher percentage of patients who recovered by day 14 (84.4%) than the comparison group (58.9%). Moreover, a significantly higher percentage of TNR4 patients fully recovered after 14 days, compared with the other forms of treatment in the following sub-groups: patients with comorbidities (TNR4 74.6% *versus* comparison group 41.2%), older participants between 49–80 years (TNR4 76.4% *versus* comparison group 45.4%), in males (TNR4 85.4% *versus* comparison group 57.8%), and among health workers (TNR4 86.5% *versus* comparison group 60.5%) (p values ≤ 0.001, in all groups).

The percentage of hospitalized participants was significantly lower among those who took TNR4 therapy than in the comparison group. This was observed in all sub-groups (p values ≤ 0.010, in all groups). The percentage of participants who died was also significantly lower among the TNR4 treatment than in the comparison group for all subgroups: patients with comorbidities, older participants, males, and health workers ($p \leq 0.027$ in all groups).

The likelihood of the three outcomes of interest: fully recovering, being hospitalized, or dying within 14 days after the onset of symptoms, are reported in Table 3. All analyses were stratified by comorbidities, age, sex, and occupation. In the total study population, participants in the TNR4 group had a 3.4 times greater likelihood of making a full recovery than those in the comparison group. The same result was observed in the study sub-groups: a 3.5-fold higher likelihood of recovery among people with comorbidities; a 3.0 and 4.0 times greater probability of fully recovering in the younger and older age groups; and a 3.9 and 1.9 higher likelihood of recovery among males and health workers ($p \leq 0.005$ in all groups, except for health workers group).

Table 1
Characteristics of COVID-19 cases treated with TNR4 *versus* the comparison group in Tlaxcala, Mexico.

Sociodemographics, comorbidities, smoking, and pregnancy	Type of treatment				*p*-value
	TNR4(n = 481)		**Comparison group**[a] (n = 287)		
	Frequency	Mean (±SD) or Percentage	Frequency	Mean (±SD) or Percentage	
Age, years (±SD)	481	41.3 (±13.5)	287	46.2 (±14.8)	0.000
Sex					
Males	226	47.5%	161	57.8%	0.002
Occupation					
Housewife	95	19.7%	55	19.2%	0.843
Farmer, driver, merchant, worker	80	16.6%	55	19.2%	0.373
Employees of public/private institutions	100	20.8%	67	23.3%	0.406
Health care workers	104	21.6%	38	13.2%	0.004
Other professionals: teachers, engineers, lawyers, etc.	34	7.1%	21	7.3%	0.897
Unemployed or retired	9	1.9%	13	4.5%	0.033
Students	23	4.8%	8	2.8%	0.174
Other jobs	36	7.5%	30	10.5%	0.156
Comorbidities or health condition					
At least one comorbidity	185	38.5%	131	45.6%	0.050
Hypertension	45	9.4%	37	12.9%	0.125
Type 2 Diabetes	35	7.3%	30	10.6%	0.126
Overweight/obesity	64	13.3%	43	15.0%	0.516
CVD, COPD, CKD, and Immunosuppression	6	1.3%	4	1.4%	0.863
Asthma	7	1.5%	4	1.4%	0.945
Smoking	20	4.2%	5	1.7%	0.068
Other diseases and pregnancy	8	1.7%	8	2.8%	0.291
Days between onset of symptoms and therapy initiation, mean (±SD)	481	7.2 (±3.5)	287	7.1 (±3.0)	0.481

The difference between means and *p*-values were estimated from linear regression, and proportions were evaluated using the chi2 test. A *p*-value ≤0.05 was considered to be significant.
CVD: Cardiovascular Diseases.
COPD: Chronic Obstructive Pulmonary Disease.
Chronic Kidney Disease: CKD.
[a] Comparison group: participants who did not accept the TNR4 therapy because they were asymptomatic, were already taking another treatment, or they had self-medicated for cold and flu. However, they agreed to take part in the follow-up portion of the study.

Table 2
Health outcomes observed in COVID-19 cases treated with TNR4 *versus* comparison group, stratified by comorbidities, older age-group, sex, and health workers.

HEALTH OUTCOMES	**All participants** (n = 787)		*p*-value	**Patients with comorbidities** (n = 316)		*p*-value	**Older patients: 49–80 years** (n = 256)		*p*-value	**Males** (n = 387)		*p*-value	**Health workers** (n = 142)		*p*-value
	TNR4 (n = 481)	**Comparison group**[a] (n = 287)		**TNR4** (n = 185)	**Comparison group**[a] (n = 131)		**TNR4** (n = 137)	**Comparison group**[a] (n = 119)		**TNR4** (n = 226)	**Comparison group**[a] (n = 161)		**TNR4** (n = 104)	**Comparison group**[a] (n = 38)	
Days between the onset of symptoms and health recovery, mean (±SD)	10.5 (±5.8)	11.5 (±4.6)	0.016	11.6 (±7.4)	12.1 (±4.9)	0.548	10.4 (±5.7)	11.2 (±4.9)	0.317	10.6 (±6.0)	11.3 (±4.7)	0.233	10.1 (±4.8)	13.2 (±3.7)	0.001
Percentage of recovered patients within 14 days, all population (n = 575/768)	84.4% n = 406/481	58.9% n = 169/287	0.000	74.6% n = 138/185	41.2% n = 54/131	0.000	76.4% n = 105/137	45.4% n = 54/119	0.000	85.4% n = 193/226	57.8% n = 93/161	0.000	86.5% n = 90/104	60.5% n = 23/38	0.001
Patients who were hospitalized (regardless of whether they were intubated or died) (n = 133/768)	9.1% n = 44/481	31.0% n = 89/287	0.000	15.1% n = 28/185	46.6% n = 61/131	0.000	19.0% n = 26/137	42.9% n = 51/119	0.000	7.5% n = 17/226	29.8% n = 48/161	0.000	4.8% n = 5/104	18.4% n = 7/38	0.010
Patients who died (n = 67/768) (regardless of whether they were hospitalized or intubated)	3.1% n = 15/481	18.1% n = 52/287	0.000	6.5% n = 12/185	29.0% n = 38/131	0.000	10.2% n = 14/137	34.5% n = 41/119	0.000	3.5% n = 8/226	21.1% n = 34/161	0.000	1.0% n = 1/104	7.9% n = 3/38	0.027

Differences between means were estimated from linear regression, and differences between proportions were evaluated using the chi2 test. A *p*-value $\leq$0.05 was considered to be significant.
Proportions and means were adjusted by age, sex, comorbidities, and occupation. A *p*-value $\leq$0·05 was considered significant.

[a] Comparison group: participants who did not accept the TNR4 treatment because they were asymptomatic, were already taking another treatment, or they had self-medicated for cold and flu. However, they agreed to take part in the follow-up portion of the study.

Table 3
Likelihood of recovery, hospitalization, or death during the first 14 days after the onset of COVID-19 symptoms among cases treated with TNR4 *versus* those in the comparison group.

Type of treatment	**Recovery within 14 days after onset of symptoms** Yes (n = 575) Odds Ratio (95% CI; *p*-value)	**Hospitalization within 14 days after onset of symptoms** Yes (n = 133) Odds Ratio (95% CI; *p*-value)	**Death within 14 days after onset of symptoms** Yes (n = 67) Odds Ratio (95% CI; *p*-value)
[a]**Comparison group**, all participants (n = 287)	Reference	Reference	Reference
TNR4, all participants (n = 481)	**3.4** (2.38–4.96; p = 0.000)	**0.25** (0.16–0.38; p = 0.000)	**0.19** (0.10–0.36; p = 0.000)
[a]**Comparison group** with comorbidities (n = 131)	Reference	Reference	Reference
TNR4 with comorbidities (n = 185)	**3.5** (2.12–5.78; p = 0.000)	**0.24** (0.14–0.41; p = 0.000)	**0.22** (0.10–0.47; p = 0.000)
[a]**Comparison group**, cases between 18–35 years (n = 77)	Reference	Reference	Reference
TNR4, cases between 18–35 years (n = 181)	**3.0**(1.38–6.39; p = 0.005)	**0.23** (0.09–0.60; p = 0.003)	–
[a]**Comparison group**, cases between 36–48 years (n = 91)	Reference	Reference	Reference
TNR4, cases between 36–48 years (n = 163)	**3.6** (1.94–6.58; p = 0.000)	**0.15** (0.06–0.33; p = 0.000)	**0.06** (0.01–0.48; p = 0.008)
[a]**Comparison group,** cases between 49–80 years (n = 119)	Reference	Reference	Reference
[a]**TNR4**, cases between 49–80 years (n = 137)	**4.0** (2.25–7.08; p = 0.000)	**0.32** (0.18–0.58; p = 0.000)	**0.22** (0.11–0.45; p = 0.000)
[a]**Comparison group**, males (n = 161)	Reference	Reference	Reference
TNR4, males (n = 226)	**3.9** (2.27–6.62; p = 0.000)	**0.24** (0.12–0.45; p = 0.000)	**0.18** (0.08–0.43; p = 0.000)
[a]**Comparison group**, health workers (n = 38)	Reference	Reference	Reference
TNR4, health workers (n = 104)	**1.9** (0.88–3.99; p = 0.102)	**0.34** (0.09–1.28; p = 0.111)	**0.26** (0.02–3.55; p = 0.311)

95% Confidence Intervals (CIS) were estimated with multivariate logistic regression models adjusted by age, sex, comorbidities, and occupation.
[a] Comparison group: participants who did not accept the TNR4 treatment because they were asymptomatic, were already taking another treatment, or they had self-medicated for cold and flu. However, they agreed to take part in the follow-up portion of the study.

Among the total study population, the risk of being hospitalized was 75% lower among participants treated with TNR4 (OR: 0.25). Patients with comorbidities also had a 76% lower risk of hospitalization (OR: 0.24); younger (77%) and older (68%) participants had a lower risk as well (OR: 0.23 and 0.32, respectively); as did males and health workers who had a 76% and 66% lower risk of being hospitalized (OR: 0.24 and 0.34, respectively) than those in the comparison group (p values ≤ 0.000 in all groups, except for health workers group).

Patients treated with TNR4 had an 81% lower risk of death among the total study population (OR: 0.19). Patients with comorbidities also had a 78% lower risk of dying (OR: 0.22); the risk was also 94% and 78% lower among middle-aged and older participants (OR: 0.06 and 0.22, respectively); and an 82% lower risk was observed among males (OR: 0.18), concerning the comparison group (p values were ≤ 0.008 in all groups).

Of the 481 COVID-19 patients who received the TNR4 treatment, 90.3% took all four drugs, 8.3% took three, and 1.4% took one medication. Some common undesirable side effects from the four TNR4 medications were reported by 354 participants, and most participants did not experience any side effects from any of the drugs. The most frequently reported adverse effects from the combined medications were: gastrointestinal issues such as abdominal pain, nausea, vomiting, diarrhea, constipation, diarrhea and constipation (5.9%), headache and dizziness (3.1%), asthenia, fatigue, and confusion (1.4%), and urticarial and itchy feeling (0.9%).

Most patients who received the TNR4 medications did not use any other treatment (89.8%); however, some of them reported taking the following medications: 2.71% used non-steroidal anti-inflammatory drugs (NSAIDs), 1.90% took medications to treat cold and flu symptoms, 0.9% used antibiotics, and 0.74% used antivirals.

The COVID-19 patients who did not receive the TNR4 treatment reported the following: 19% did not take any medications, 61.4% used NSAIDs, 14.4% combined antibiotics with NSAIDs or corticosteroids, and 5.2% took antiviral drugs along with NSAIDs or corticosteroids.

Discussion

The results of our study indicate that the combination of Ivermectin, Azithromycin, Montelukast, and Acetylsalicylic acid, significantly increases the likelihood of full recovery within 14 days after the onset of symptoms and decreases the risk of hospitalization or death among ambulatory cases of COVID-19 in the state of Tlaxcala, Mexico. The participants who received the TNR4 treatment had a significantly lower prevalence of comorbidities. A higher proportion of them was younger, male, and healthcare professionals than the comparison group, which may have also influenced the improved health outcomes we observed in this group.

There is limited clinical evidence regarding the effectiveness of multidrug treatments that can significantly improve health outcomes in COVID-19 patients (Ortega et al., 2020; Sanders et al., 2020). A descriptive metanalysis of 689 trials conducted in 2020 reports that the most frequently used treatments were antivirals (n = 144), antimalarials (n = 112), Hydroxychloroquine alone (n = 110), antibiotic/antiparasitic drugs (n = 34), and trials with Azithromycin (n = 28) (Janiaud et al., 2020). No published studies have investigated the combined effect of the four drugs used in the TNR4 treatment to the best of our knowledge, but others have examined at least two of the medications we tested as part of this study. For example, a Randomized Clinical Trial (RCT) conducted in Brazil among 504 cases of mild to moderate COVID-19 reported that patients treated with Hydroxychloroquine alone or with Azithromycin during 15 days showed no clinical improvement; instead, they reported more adverse effects in the treatment groups than among patients who received the standard care (Cavalcanti et al., 2020). In contrast, a Chinese study found that Chloroquine effectively stopped the exacerbation of pneumonia, improving lung imaging and shortening the disease course, compared with the control treatment (Gao et al., 2020). However, this medication's use is limited due to its cardiovascular toxicity and other safety concerns (Tsang et al., 2020). A retrospective cohort study of 1438 hospitalized patients with COVID-19 in New York, USA, reported that the likelihood of death among patients treated with Hydroxychloroquine alone was 1.08, for Azithromycin alone it was 0.56, and for a combination of both medications, it was 1.35. However, these results were not statistically significant (Rosenberg et al., 2020). In contrast, a multicenter retrospective study in Michigan evaluated 2541 hospitalized patients with COVID-19 and found a significant survival benefit of 66% among those who received Hydroxychloroquine alone and 71% among those who were treated with

Hydroxychloroquine plus Azithromycin, compared to those who did not receive either drug (Arshad et al., 2020).

Recent studies that examined the efficacy of Ivermectin have shown antiviral activity for many viral infections (Jabeen et al., 2020). A retrospective analysis of consecutive patients with COVID-19 from four hospitals in Florida, USA, compared 173 patients who received Ivermectin plus Hydroxychloroquine, or Hydroxychloroquine with Azithromycin, *versus* 103 patients who received usual care plus Hydroxychloroquine, or Hydroxychloroquine with Azithromycin. All-cause mortality was lower in the Ivermectin group than in the usual care (OR 0.27; p = 0.03), and the proportion of patients who were successfully extubated was higher in the Ivermectin group (36%) than in the usual care (15%) (p = 0.07) (Rajter et al., 2020). Our study results indicate that patients who received the TNR4 treatment had an 81% lower risk of death, while even older patients and those with comorbidities reduced their risk by 78%. The IDEA (Ivermectin, Dexamethasone, Enoxaparin, and Aspirin) Study, which investigated 167 mild to severe COVID-19 patients from Argentina, found that none of the mild or moderate cases of COVID-19 who received the experimental treatment were hospitalized. Only one patient died (0.59%) (Carvallo et al., 2020). By comparison, the TNR4 therapy resulted in higher mortality, with 67 patients who died (8.7%) from a total of 768 participants. The use of ASA among patients with COVID-19 has been associated with reduced thrombo-inflammation and a lower risk of clinical complications and mortality. A recent systematic review reports that ASA has demonstrated anti-inflammatory, antithrombotic effects and a significant ASA-mediated antiviral activity against DNA and RNA viruses, including different human coronaviruses (Bianconi et al., 2020).

To date, it is still unclear whether multidrug therapies against COVID-19 infection can provide additional benefits over monotherapy since drug-drug interaction may further worsen the clinical outcomes of COVID-19 patients, especially among those with comorbidities (Awortwe and Cascorbi, 2020; Back et al., 2020). In this context, our study has some important strengths and weaknesses that should be considered. First, we were unable to identify the specific therapeutic effect of each TNR4 drug against COVID-19 since they were not administered separately; however, participants were asked to report how many drugs they took and if they experienced any side effects from TNR4 therapy: nearly 90% of the participants indicated no undesirable effects from any of the four drugs. Second, our study focused on ambulatory patients, so we could not collect specific clinical measures such as oxygen saturation, radiographic findings, laboratory studies, and bacteriology or virology cultures. Nonetheless, we did obtain detailed information regarding the use of TNR4 or other pharmacological drugs, including the time of drug intake, undesirable effects, symptoms, and health recovery from all study participants. Each COVID-19 patient was contacted through a daily phone call or home visit to reduce the probability of introducing information biases and to compare the survival and health outcomes among study groups. Hospitalization and mortality information was obtained and verified from the Epidemiological Surveillance System's national database for Viral Respiratory Diseases (Gobierno de México, 2020).

Conclusion

Our findings indicate that the use of the TNR4 multidrug therapy can improve the recovery and reduce the risk of more severe disease among ambulatory cases of COVID-19. However, future research efforts are required to design and conduct RCTs to further assess the effectiveness of TNR4 in the context of different treatment schemes and healthcare settings.

Conflict of interest

The authors declare no conflict of interest in this article.

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Ethical approval

Approval for this study was granted by the Ministry of Health of the Tlaxcala state, Mexico (#CEI02092020).

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