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Original Research Article

Effect of spirulina on risk of hospitalization among patients with COVID-19: the TOGETHER randomized trial

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A B S T R A C T

Background: Algae-derived nutraceuticals, such as spirulina, have been reported to have biological activities that may minimize clinical consequences to COVID-19 infections.

Objectives: This study aimed to determine whether spirulina is an effective treatment for high-risk patients with early COVID-19 in an outpatient setting.

Methods: The TOGETHER trial is a placebo-controlled, randomized, platform trial conducted in Brazil. Eligible participants were symptomatic adults with a positive rapid test for SARS-CoV-2 older than 50 y or with a known risk factor for disease severity. Patients were randomly assigned to receive placebo or spirulina (1 g twice daily for 14 d). The primary end point was hospitalization defined as either retention in a COVID-19 emergency setting for >6 h or transfer to tertiary hospital owing to COVID-19 at 28 d. Secondary outcomes included time-to-hospitalization, mortality, and adverse drug reactions. We used a Bayesian framework to compare spirulina with placebo.

Results: We recruited 1126 participants, 569 randomly assigned to spirulina and 557 to placebo. The median age was 49.0 y, and 65.3% were female. The primary outcome occurred in 11.2% in the spirulina group and 8.1% in the placebo group (odds ratio [OR]: 1.24; 95% credible interval: 0.84, 1.86). There were no differences in emergency department visit (OR: 1.21; 95% credible interval: 0.81, 1.83), nor time to symptom relief (hazard ratio: 0.90; 95% credible interval: 0.79, 1.03). Spirulina also not demonstrate important treatment effects in the prespecified subgroups defined by age, sex, BMI, days since symptom onset, or vaccination status.

Conclusions: Spirulina has no any clinical benefits as an outpatient therapy for COVID-19 compared with placebo with respect to reducing the retention in an emergency setting or COVID-19–related hospitalization. There are no differences between spirulina and placebo for other secondary outcomes. This trial was registered at clinicaltrials.gov as NCT04727424.

Keywords: Brazil, SARS-CoV-2, repurposed drugs, outpatients, spirulina

Abbreviations: ITT, intention-to-treat; OR, odds ratio; WURSS, Wisconsin Upper Respiratory Symptom Survey.

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Introduction

Algae-derived nutraceuticals have been proposed as being of potential benefit for the management of COVID-19 infections [1]. Traditionally used as an approved food coloring agent, *Spirulina* spp and its' associated extracts have been reported to have biologic activity in multiple domains of potential relevance to COVID-19 infections. For example, among anemic senior citizens, who did not have COVID-19, spirulina supplement administration was associated with improvements to both complete cell count and indoleamine 2,3-dioxygenase enzyme activity [2], a marker of immune function. Other in-human assessments of spirulina on immune function have noted improvements to interferon-γ expression [3], while in vitro evidence suggests potential activity with respect to angiotensin converting enzyme and angiotensin converting enzyme 2 modulation [4], reducing extracellular signal-regulated kinase 1–mediated nucleotide-binding oligomerization domain–containing, leucine-rich repeat (LRR)-containing, and pyrin domain–containing protein 3 inflammasome activation [5], and antiviral activity against SARS-CoV-2 infection [6].

Despite these promising mechanistic characteristics, randomized, controlled evidence on spirulina for the management of COVID-19 is sparse. Previous randomized evidence on spirulina is limited to a publication of 104 patients in critically ill patients with COVID-19, where authors reported improvements in biochemical assessments of coagulation and lymphocytopenia [7]. Accordingly, an evidence gap exists for the use of spirulina among outpatients for clinical outcome measures.

Given the promising preclinical data and biochemical clinical data on spirulina, it is important to understand its potential role in managing COVID-19–associated morbidity and mortality. As it is an approved coloring agent for food products, it would be anticipated to have a favorable safety profile and is readily available at scale and at comparatively affordable price. As such, should spirulina demonstrate meaningful clinical improvement for COVID-19, a substantial source of morbidity and mortality globally, it may represent an affordable and scalable tool for disease management.

Accordingly, we have conducted a randomized controlled trial to evaluate the efficacy of spirulina relative to placebo plus standard-of-care for the prevention of COVID-19–associated hospitalization or emergency department visitation among outpatients.

Methods

Trial design and oversight

The TOGETHER trial is a randomized, placebo-controlled adaptive platform trial that has been established to investigate the efficacy of repurposed treatments for patients with COVID-19 at high risk of hospitalization [8]. Since initiation (2 June, 2020), the TOGETHER trial has enrolled patients at 12 cities across Brazil, South Africa, and Canada. Details on the conduct of the trial are provided in Supplemental Appendix 1**.** The full trial protocol and statistical analysis plan (SAP) have been previously published [8] and are also available with the full-text online article in Supplemental Appendix 1. This article details the efficacy and safety results of spirulina and its matched-placebo control, enrolled between September 2022 and September 2023. Results of other treatment arms have been reported previously [9–12].

The principal investigators (GR and EJM) had full access to all the trial data and can verify the accuracy and entirety of the data and for the fidelity of the trial to the protocol. The sponsors had no role in the design and conduct of the trial; the collection, management, analysis, or interpretation of the data; the preparation, review, or approval of the manuscript; or the decision to submit the manuscript for publication.

Ethics

Ethical approval followed the CEP-CONEP approval process (CONEP approval number: 41174620.0.1001.5120). Certification of Brazilian ethics approvals were submitted to the Hamilton Integrated Research Ethics Board (HiREB) at McMaster University for final review and approval (HiREB project number: 13390). Trial registration is held at clinicaltrials.gov number NCT04727424.

Study eligibility

Upon presentation to one of the trial outpatient care clinics, potential participants were screened by the study team (from Cardresearch) against eligibility criteria. Key inclusion criteria were as follows: *1*) age 18 y or older; *2*) presenting within 7 d of symptom onset to an outpatient care setting with an acute clinical condition consistent with COVID-19; *3*) positive rapid test (Panbio rapid antigen for SARS-CoV-2; Abbott Laboratories); and *4*) participants had ≥1 high-risk criterion for deterioration including age of 50 y, diabetes mellitus, hypertension requiring medication(s) for treatment, cardiovascular disease, lung disease, smoking, obesity (BMI >30 kg/m^2), organ transplant, chronic kidney disease (stage IV) or receiving dialysis, immunosuppressive therapy (10 mg prednisone daily or equivalent), cancer diagnosis within 6 mo, or receiving cancer chemotherapy. Further inclusion/exclusion criteria are documented in the study protocol [8].

If a patient met the above eligibility criteria, study personnel obtained written in-person informed consent and performed a rapid antigen test for SARS-CoV-2 (Panbio). Before randomization, study personnel collected the following data: demographics, medical history, comorbidities, concomitant medications, information on exposure to index case, and WHO clinical progression scale [13].

Randomization and interventions

At the point of randomization to an assigned study arm, an unblinded pharmacist at the coordinating center received a text message, who responded to the site with a medication letter and randomization number, concealing allocation of treatment from the administering sites. Randomization was generated by computer sequence. All site staff, trial team members, and patients were unaware of treatment assignments. Block randomization was used, with a block size of 10 and with stratification according to participant age (<50 and ≥50 y).

All patients received routine standard-of-care treatment provided by local sites. Patients received either spirulina, at a dose of 2 tablets, 500 mg twice a day each (for a today of 1 g twice a day) for 14 d, or matched-placebo for 14 d, beginning of the day of randomization.

End point measures

Our primary end point was a composite end point of COVID-19–related hospitalization, defined as referral to a tertiary hospital setting owing to the progression of COVID-19 or admission to a COVID-19 emergency setting for >6 h (based on local standards of operating care), both within 28 d of randomization. Because many patients who would ordinarily have been hospitalized were prevented from admission owing to hospital overcapacity during peak waves of COVID-19, the composite end point was developed to measure both

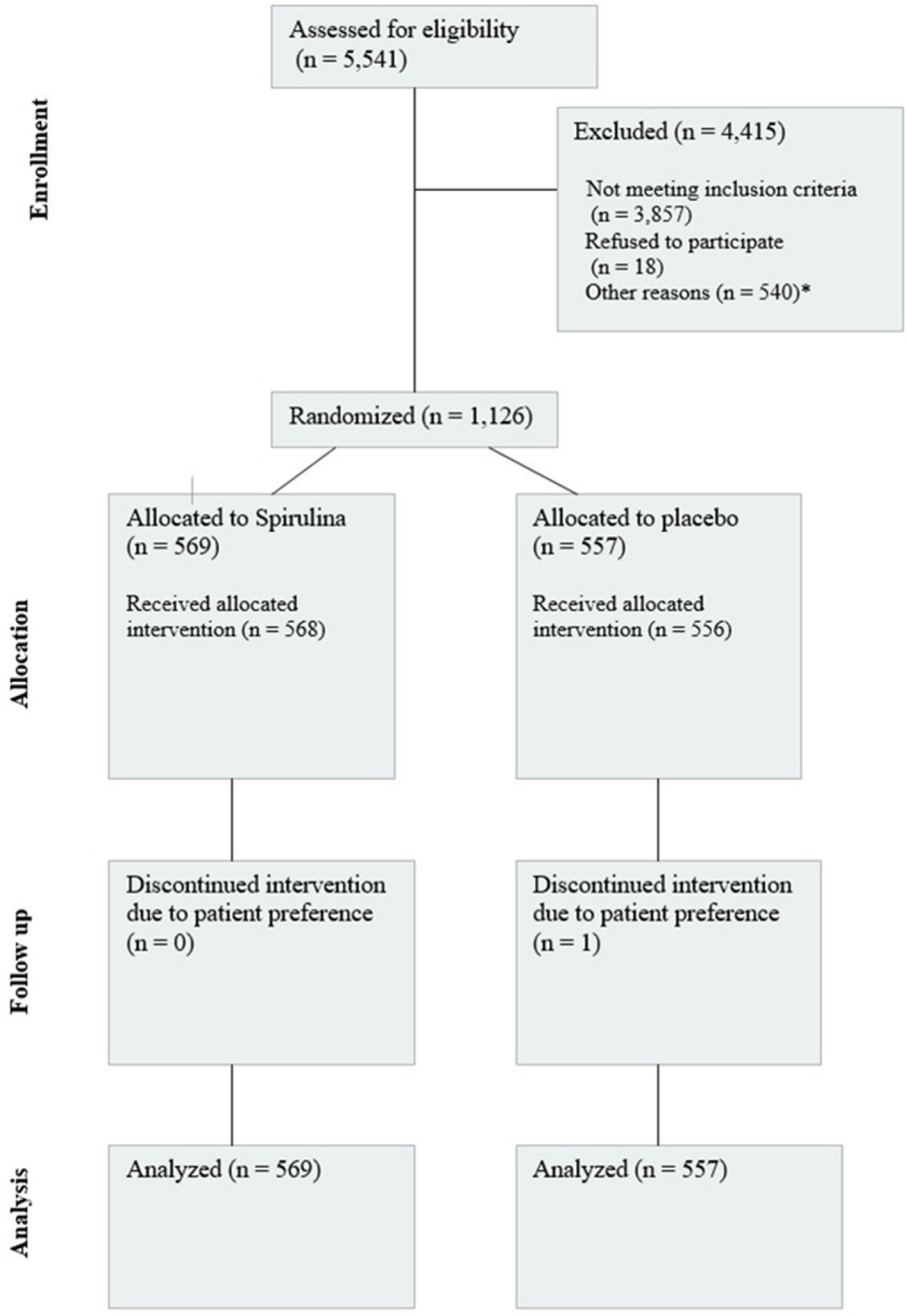

FIGURE 1. CONSORT diagram of patient flow. *540 patients were included in the TOGETHER trial but were assigned to famotidine, the topic of a separate manuscript.

hospitalization and a proxy for hospitalization, retention in a COVID-19 emergency hospital setting.

Secondary outcomes included time from randomization to hospitalization or emergency department visit for COVID-19, hospitalization for COVID-19, time from randomization to hospitalization for COVID-19, hospitalization for any cause, visitation of an emergency department for COVID-19–related symptoms, time from randomization to self-reported symptom-relief, Wisconsin Upper Respiratory Symptom Survey (WURSS)-21 score [14], and day 28 EQ-5D-5L utility index [15]. Adverse events were recorded at all patient contact dates, with grading informed by the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events [16].

Trial procedures

Local health care staff conducted outcome assessment on days 1, 2, 3, 4, 5, 7, 10, 14, 28, and 60. This was done through mixed means of in-person contact, telephone contact, and WhatsApp video-conference contact. Outcome data were collected regardless of whether participants took their assigned study medication. In cases of adverse events, unscheduled visits outside of scheduled activities could occur any time.

In adherence to the local regulatory requirements, all serious and nonserious adverse events were reported to the study personnel. Reportable adverse events included serious adverse events, adverse events resulting in discontinuation of study medication, and adverse events deemed to possibly be related to study medication.

TABLE 1
Demographics and clinical characteristics of the randomly assigned participants[1]

	Placebo ($n = 557$)	Spirulina ($n = 569$)	Overall ($N = 1126$)
Sex			
Female	358 (64.3)	377 (66.3)	735 (65.3)
Male	198 (35.5)	191 (33.6)	389 (34.5)
Missing	1 (0.2)	1 (0.2)	2 (0.2)
Race			
Mixed race	551 (98.9)	562 (98.8)	1113 (98.8)
Unknown	6 (1.1)	4 (0.7)	10 (0.9)
Black or African American	0 (0)	3 (0.5)	3 (0.3)
Time since onset of symptoms (d)			
0–3	424 (76.1)	433 (76.1)	857 (76.1)
4–7	128 (23.0)	134 (23.6)	262 (23.3)
Missing	5 (0.9)	2 (0.4)	7 (0.6)
Age (y)			
≤50	280 (50.3)	285 (50.1)	565 (50.2)
>50	275 (49.4)	283 (49.7)	558 (49.6)
Missing	2 (0.4)	1 (0.2)	3 (0.3)
Age (y)			
Mean (SD)	48.0 (15.7)	48.7 (16.1)	48.3 (15.9)
Median (Min, Max)	49.0 (18.0, 92.0)	50.0 (18.0, 94.0)	49.0 (18.0, 94.0)
Missing	2 (0.4)	2 (0.4)	4 (0.4)
SARS-CoV-2 status			
Negative	2 (0.4)	0 (0)	2 (0.2)
Positive	554 (99.5)	568 (99.8)	1122 (99.6)
Missing	1 (0.2)	1 (0.2)	2 (0.2)
Doses of COVID-19 vaccine			
1 dose	16 (2.9)	19 (3.3)	35 (3.1)
2 doses	94 (16.9)	86 (15.1)	180 (16.0)
3 doses	182 (32.7)	211 (37.1)	393 (34.9)
4 doses	191 (34.3)	183 (32.2)	374 (33.2)
Missing data	74 (13.3)	70 (12.3)	144 (12.8)
Hypertension	215 (38.6)	246 (43.2)	461 (40.9)
Cardiovascular disease	10 (1.8)	5 (0.9)	15 (1.3)
Lung disease	5 (0.9)	1 (0.2)	6 (0.5)
Asthma	45 (8.1)	41 (7.2)	86 (7.6)
Type 1 diabetes	8 (1.4)	9 (1.6)	17 (1.5)
Type 2 diabetes	64 (11.5)	76 (13.4)	140 (12.4)
Obesity	210 (37.7)	202 (35.5)	412 (36.6)
Cancer	10 (1.8)	5 (0.9)	15 (1.3)

[1] Values are *n* (%) unless specified.

Statistical analysis

The details on sample size determination and statistical analyses are provided in the protocol and the SAP [8]. This platform trial is adaptive and allows for flexible sample size based on prespecified interim analyses. The sample size was determined based on primary end point (composite end point of COVID-19–related hospitalization within 28 d). The maximum sample size of 681 patients per arm was chosen to detect a relative risk reduction of 37.5%, assuming a control event rate of 15% with 80% power at 0.05 2-sided type I error rate for a pairwise compa rison against the placebo. As recruitment continued during the interim analysis results being evaluated by the independent data safety monitoring committee, 1126 patients (557 for placebo and 569 for treatment) were enrolled for the evaluation of spirulina.

Baseline characteristics are reported as counts and percentages or, for continuous variables, as medians with interquartile ranges. We applied a Bayesian framework to analyze the efficacy end points and report the posterior probabilities of superiority. Posterior probability for the efficacy of spirulina with regard to the primary outcome was calculated with the use of a Bayesian logistic regression model. The modified intention-to-treat (ITT) population included all patients who had received spirulina or placebo for ≥24 h before a primary-outcome event (i.e., if events occurred before 24 h after randomization, the patient was not counted in this analysis). The matched-placebo population included only the patients who had received a 10-d oral placebo. Exploratory subgroup effects were assessed in accordance with the prespecified SAP.

We assessed time-to-event outcomes using a Bayesian Cox proportional-hazards model, binary outcomes using logistic regression, and continuous outcomes using mixed-effect linear regression (for the WURSS-21 analysis) or Tobit regression (for the EQ5-5D-5L utility index analysis). Adverse events and severe adverse events were analyzed using a Bayesian negative-binomial regression for count data.

All models were adjusted for age, sex, and BMI, while day-28 EQ-5L-5D utility index model was adjusted for baseline utility index. We determined subgroups a priori. These included age (<50 and ≥50 y), sex, symptom onset (≤3 or >3 d), SARS-CoV-2 vaccination status, and obesity (BMI ≤30 or >30 kg/m^2). We assessed subgroup effects according to the preplanned SAP. We applied the Instrument to assess the Credibility of Effect Modification Analyses tool for subgroup credibility [17].

All presented comparisons were limited to the concurrently randomly assigned population. No imputation was performed for missing data due to very small missingness. Complete case analysis was conducted throughout. Statistical analyses were conducted with the use of R software (R Studio), version 4.3.0.

Results

During the study period, 5541 potential participants were screened for inclusion. Of the 1126 participants enrolled and randomly assigned, 569 were randomly assigned to spirulina and 557 to the concurrent placebo arm, consisting of a mixture of patients receiving 10-d and 14-d oral placebo (Figure 1). Of the 557 patients assigned to receive placebo, 292 were assigned a 14-d oral placebo (spirulina matched) and 265 were assigned a 10-d oral placebo (famotidine matched). A summary of patient baseline demographics is provided in Table 1. The median age of the patients was 49 y (range, 18–94 y), and 735 patients (65.3%) were female. Almost all patients identified as being of mixed race [1113 (98.8%)]. The mean (±SD) number of days with COVID-19 symptoms before randomization was 2.5 ± 1.5. A summary of individual outcomes is provided in Supplemental Table 1.

Primary outcome

In the ITT population, a primary outcome event occurred in 63 of 569 patients (11.2%) in the spirulina group, compared with 50 of 557 patients (9.1%) in the placebo group (odds ratio [OR]: 1.24; 95% Bayesian credible interval: 0.84, 1.86; posterior probability of superiority to placebo: 14.3%) (Table 2). Similar results were observed in the modified ITT population (57 of 563 patients in the spirulina group, compared with 50 of 557 patients in the placebo group, had a primary-outcome event; OR: 1.12; 95% Bayesian credible interval: 0.74, 1.68) (Supplemental Table 2), and in the matched-placebo analysis that included only the 292 patients who received a 14-d oral placebo, ignoring the 10-d placebo patients matched to a separate study arm not reported in this manuscript (63 of 569 patients in the spirulina group, compared with 29 of 292 patients in the placebo group; OR: 1.14; 95% Bayesian credible interval: 0.71, 1.83) (Supplemental Table 3). A summary of the posterior OR distribution of the ITT, modified mITT,

TABLE 2
Primary, secondary, and safety outcomes of spirulina compared with placebo in the TOGETHER trial for the intention-to-treat population

Outcome	Spirulina ($n = 569$)	Placebo ($n = 557$)	Effect measure	Estimated treatment effect (95% Bayesian credible interval)	Posterior probability of superiority to placebo (%)
Primary outcome					
Hospitalization or emergency department visit for COVID-19, *n* (%)	63 (11.2)	50 (9.1)	OR	1.24 (0.84, 1.86)[1]	14.3
Secondary outcomes					
Time from randomization to hospitalization or emergency department visit for COVID-19	—	—	HR	1.24 (0.85, 1.81)[2]	13.1
Hospitalization for COVID-19, *n* (%)	3 (0.5)	2 (0.4)	OR	1.58 (0.25, 12.33)[1]	32.2
Time from randomization to hospitalization for COVID-19	—	—	HR	1.54 (0.24, 12.09)[2]	32.0
Hospitalization for any cause, *n* (%)	4 (0.7)	3 (0.5)	OR	1.68 (0.40, 8.01)[1]	24.8
Emergency department visit for COVID-19, *n* (%)	58 (10.2)	47 (8.4)	OR	1.21 (0.81, 1.83)[1]	18.0
Time from randomization to symptom relief	—	—	HR	0.90 (0.79, 1.03)[2]	6.6
WURSS-21 score	—	—	MD/d	0.01 (−0.02, 0.03)[3]	31.6
Day 28 EQ-5D-5L utility index (mean ± SD)			MD	−0.01 (−0.04, 0.02)[4]	25.4
Adverse events					
Adverse event during treatment period, *n* (%)	66 (11.6)	69 (12.4)	RR	0.91 (0.64, 1.29)[5]	70.4
Severe adverse event during treatment period, *n* (%)	12 (2.1)	13 (2.3)	RR	0.90 (0.40, 2.03)[5]	59.8
Grade 1	6 (1.1)	12 (2.2)	—	—	—
Grade 2	47 (8.3)	44 (7.9)	—	—	—
Grade 3	9 (1.6)	9 (1.6)	—	—	—
Grade 4	3 (0.5)	3 (0.5)	—	—	—
Grade 5	0 (0.0)	1 (0.2)	—	—	—

Note that the event rates reported (in parenthesis) are Bayesian posterior median event rates and not raw proportions.
Abbreviations: HR, hazard ratio; MD, mean difference; OR, odds ratio; RR, relative rate; WURSS, Wisconsin Upper Respiratory Symptom Survey.
[1] Adjusted OR from a logistic regression.
[2] Adjusted HR from a Cox model.
[3] Adjusted coefficient of the time × treatment interaction term in a mixed-effect linear regression model with log-transformed response and subject random effect.
[4] Adjusted MD from a Tobit regression.
[5] Adjusted RR from a negative-binomial regression.

and matched-placebo analyses are shown in Figure 2. In the ITT population, hospitalization accounted to 6.2% (7 of 113) of the composite primary end point. Median time to primary outcome event was 6 d (interquartile range, 4–12 d) after randomization.

Secondary outcomes

No significant differences between spirulina and placebo groups were noted among any of the observed secondary outcomes (Table 2). No need for mechanical ventilation was recorded throughout the efficacy window of 28 d. One death of a 55-y-old patient randomly assigned to the placebo group was registered on 16 January, 2023, 44 d after randomization, with an unknown cause of death. Another death of a 65-y-old patient occurred 59 d after their randomization to spirulina, with an unknown cause of death.

Three cases of Covid-19–related hospitalization were observed in the spirulina group within 28 d after randomization, compared with 2 cases in the placebo group (OR: 1.58; 95% Bayesian credible interval: 0.25, 12.33). The analysis on time-to-hospitalization for COVID-19 showed a hazard ratio of 1.54 (95% Bayesian credible interval: 0.24, 12.09). The median time to recovery was 10 d in the spirulina group, compared with 9.5 d in the placebo group (hazard ratio: 0.90; 95% Bayesian credible interval: 0.79, 1.03). Time-to-hospitalization or emergency department visit owing to COVID-19 is presented in Supplemental Figure 1, and time to COVID-19 symptom relief is presented in Supplemental Figure 2. Neither analysis demonstrated a significant difference between the 2 study populations.

With respect to patient-reported outcomes, no benefit was evident for the treatment with spirulina when analyzing the results of the WURSS-21. A daily mean difference between the spirulina and placebo arms of 0.01 (Bayesian credible interval: −0.02, 0.03) was estimated. In the analysis of the EQ-5D-5L questionnaire, a mean difference between spirulina and placebo arms of −0.01 (Bayesian credible interval: −0.04, 0.02) was observed. WURSS-21 change score overtime is presented in Supplemental Figure 3.

Subgroup analyses

In prespecified subgroup analyses, we found no evidence of treatment benefits with spirulina compared with placebo in any of the subgroups defined according to patient age, sex, BMI, days since symptom onset, or vaccination status (Figure 3). Analysis of the modified ITT population is provided in Supplemental Table 2. An analysis of the matched-placebo population only is presented in Supplemental Table 3. No significant differences were noted relative to the primary analysis population in either of these 2 subpopulations.

Discussion

In this large, randomized platform trial, spirulina treatment did not show important treatment effects in reducing the proportion of patients hospitalized or visiting the emergency department in comparison with placebo. Similarly, comparisons on any of secondary clinical, patient-reported, or safety outcomes did not show results that would indicate beneficial treatment effects of spirulina in comparison with those of placebo.

Our evidence differs from previous studies. Although previous studies have provided preliminary evidence of the ability for spirulina

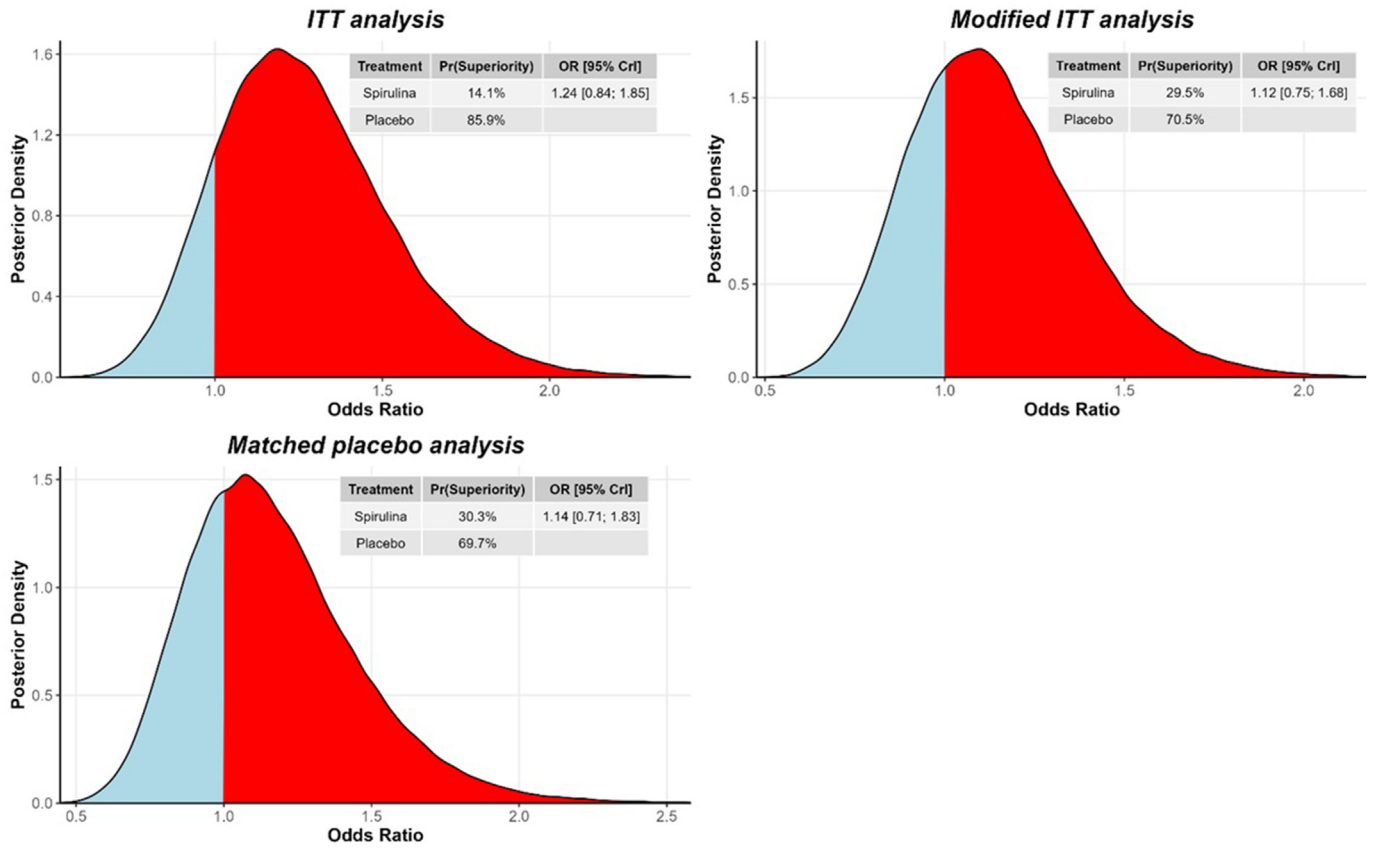

FIGURE 2. Primary analysis and posterior odds ratio distribution of spirulina vs. placebo for hospitalization or emergency department visit owing to COVID-19. The posterior probability of spirulina superiority is represented by the light blue area. CrI, credible interval; ITT, intention-to-treat; OR = odds ratio; Pr = probability odds ratio (calculated from an adjusted logistic regression on age, sex, and BMI).

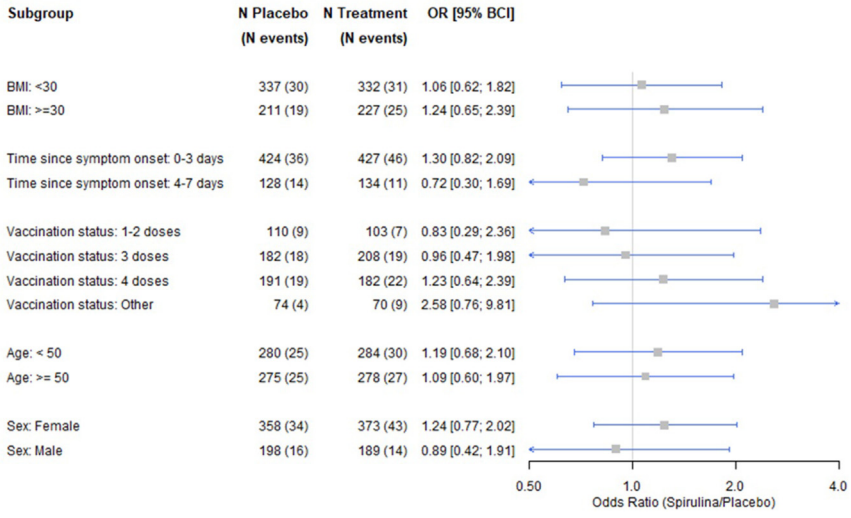

FIGURE 3. A forest plot demonstrating subgroup analyses of spirulina vs. placebo in the TOGETHER trial for prevention of COVID-19 hospitalization or emergency setting observation. Odds ratio was calculated from an adjusted logistic regression on age, sex, and BMI, excluding the subgroup variable. BCI, Bayesian credible interval.

to act upon relevant biochemical parameters, our study showed no benefits of spirulina as a treatment option for COVID-19 in an outpatient setting. It is important to contextualize the findings from this large, randomized trial in context with the previous biochemical studies. Previous studies on spirulina have identified moderate variability in diagnostic performance of routinely collected laboratory values [18], particularly when correlating among patients with differing clinical severity [19]. However, it is important to note the possibility of variable correlation between biochemical markers and clinical outcomes for patients with COVID-19.

Critically, before our study, no randomized evidence among outpatient populations for spirulina has been published. Other therapeutic agents have been known to demonstrate a wide variety of treatment effects conditional on COVID-19 severity. For example, evidence suggests that therapeutic-dose heparin is favorable among patients with moderate severity COVID-19 when compared with pharmacologic thromboprophylaxis and that this effect is reversed among patients with severe illness [20]. Even among therapeutic agents consistently used, relative dosage for optimal treatment effects can vary depending on disease severity [21]. As such, although our data contradict earlier randomized data on outcomes for patients with severe COVID-19, it is not possible to determine whether this could be attributable to differences in disease severity, outcome types measured (which can differ in severe COVID-19 trials), or other between-population differences observed or unobserved [7]. Additionally, insufficient evidence exists regarding any dose–response relationships, and this study was unable to operationalize a pharmacokinetic/pharmacodynamic element into the current design. There may be plausible lags in dose–response relationships as seen in other nutraceutical products, and therefore when interpreting the results of this study, it is important to consider them only in the context of the evaluated outcome window and dose regimen. Further, with respect to generalizability, it is important to consider the context of this trial. Our study was performed among a Brazilian population for whom the interaction of treatment effect modifiers, prognostic factors, and standards of care may not translate directly to in other populations. It is also critical to consider temporal impacts—the COVID-19 pandemic has been through multiple waves of infections, with differing strains of virus that can influence the associated morbidity and mortality of infections. As such, when reviewing this evidence and considering the applications to alternative geographies and at other points in time, reviewers should consider these potential impacts.

Limitations

Our study was focused on near-term prevention of hospitalization or emergency department visitation among outpatients. We are unable to determine whether spirulina-treated participants may demonstrate any benefit with respect to long-term complications of COVID-19 infection. However, evidence does exist to indicate that long COVID may be associated with severity of initial infection [22]. As our current findings suggest that severity (as measured by hospitalization and emergency department visitation) are unaffected by spirulina treatment, as are duration of symptoms, the mechanism through which spirulina treatment may derive benefit are uncertain. It is highly questionable whether spirulina is a viable candidate for long-term management of continued COVID-19 symptoms in light of our existing data. Our study is also limited by the aforementioned lack of pharmacokinetic/pharmacodynamic data, as well as a lack of information on viral strain among included patients.

In conclusion, in outpatients with COVID-19, spirulina does not appear to improve patient outcomes when compared with placebo.

Author Contributions

The authors' responsibilities were as follows – GR, EAdSMS, DCMS, OH, JIF, KT, EJM: conceived and designed the study; GR, LCMS, TSF, LLFR, MICS, LBR, DCMS, EAdSMS, APFGA, ADdFN, VHdSC, CB, EDC, RO, PL, OH, JIF, LD, EJM: performed acquisition, analysis, or interpretation of data; GR, DCMS, PL, OH, JIF, LD, EJM: drafted the manuscript; GR, LT, LCMS, TSF, LLFR, MICS, LBR, DCMS, EAdSMS, APFGA, ADdFN, VHdSC, CB, EDC, RO, PL, OH, LD, EJM: critically reviewed the manuscript for important intellectual content; GR, PL, OH, EJM: performed statistical analysis; GR, LCMS, TSF, LLFR, MICS, LBR, JIF, LD, EJM: were responsible for administrative and technical of material support; GR, TSF, LLFR, MICS, LBR, DCMS, KT, EJM: supervised the study; and all authors: read and approved the final manuscript.

Conflict of interest

The authors report no conflicts of interest.

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Data availability

Data described in the manuscript, code book, and analytic code will be made available upon request pending reasonable request to the primary and corresponding authors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajcnut.2024.06.016.

References

[1] S.K. Ratha, N. Renuka, I. Rawat, F. Bux, Prospective options of algae-derived nutraceuticals as supplements to combat COVID-19 and human coronavirus diseases, Nutrition 83 (2021) 111089, https://doi.org/10.1016/j.nut.2020.111089.

[2] C. Selmi, P.S.C. Leung, L. Fischer, B. German, C.Y. Yang, T.P. Kenny, et al., The effects of spirulina on anemia and immune function in senior citizens, Cell. Mol. Immunol. 8 (3) (2011) 248–254, https://doi.org/10.1038/cmi.2010.76.

[3] T. Hirahashi, M. Matsumoto, K. Hazeki, Y. Saeki, M. Ui, T. Seya, Activation of the human innate immune system by spirulina: augmentation of interferon production and NK cytotoxicity by oral administration of hot water extract of *Spirulina platensis*, Int. Immunopharmacol. 2 (4) (2002) 423–434, https://doi.org/10.1016/S1567-5769(01)00166-7.

[4] G. Aiello, Y. Li, G. Boschin, C. Bollati, A. Arnoldi, C. Lammi, Chemical and biological characterization of spirulina protein hydrolysates: focus on ACE and DPP-IV activities modulation, J. Funct. Foods. 63 (2019) 103592, https://doi.org/10.1016/j.jff.2019.103592.

[5] S. Chei, H.J. Oh, J.H. Song, Y.J. Seo, K. Lee, K.J. Kim, B. Y Lee, Spirulina maxima extract prevents activation of the NLRP3 inflammasome by inhibiting ERK signaling, Sci. Rep. 10 (1) (2020) 2075, https://doi.org/10.1038/s41598-020-58896-6.

[6] D. Garcia-Ruiz, E. Villalobos-Sánchez, D. Alam-Escamilla, D. Elizondo-Quiroga, In vitro inhibition of SARS-CoV-2 infection by dry algae powders, Sci. Rep. 12 (1) (2022) 17101, https://doi.org/10.1038/s41598-022-22148-6.

[7] Z. Javid, H. Santos, M. Norouzi, M. Taghavi, M. Hatami, M. Nazari, et al., The effects of *Spirulina platensis* supplementation on COVID-19 severity in critically ill patients: a randomized clinical trial, ResearchSquare, 2022, https://doi.org/10.21203/rs.3.rs-2382997/v1.

[8] G. Reis, E.A. dos Santos Moreira Silva, D.C.M. Silva, K. Thorlund, L. Thabane, G.H. Guyatt, et al., A multi-center, adaptive, randomized, platform trial to evaluate the effect of repurposed medicines in outpatients with early coronavirus disease 2019 (COVID-19) and high-risk for complications: the TOGETHER master trial protocol, Gates Open Res 5 (2021) 117, https://doi.org/10.12688/gatesopenres.13304.1.

[9] G. Reis, E.A. dos Santos Moreira-Silva, D.C.M. Silva, L. Thabane, A.C. Milagres, T.S. Ferreira, et al., Effect of early treatment with fluvoxamine on risk of emergency care and hospitalisation among patients with COVID-19: the TOGETHER randomised, platform clinical trial, Lancet Glob. Health 10 (1) (2022) e42–e51, https://doi.org/10.1016/S2214-109X(21)00448-4.

[10] G. Reis, E.A.D.S. Moreira Silva, D.C. Medeiros Silva, L. Thabane, G. Singh, J.J.H. Park, et al., Effect of early treatment with hydroxychloroquine or lopinavir and ritonavir on risk of hospitalization among patients with COVID-19: the TOGETHER randomized clinical trial, JAMA Netw. Open 4 (4) (2021) e216468, https://doi.org/10.1001/jamanetworkopen.2021.6468.

[11] G. Reis, E.A.S.M. Silva, D.C.M. Silva, L. Thabane, A.C. Milagres, T.S. Ferreira, et al., Effect of early treatment with ivermectin among patients

with COVID-19, N. Engl. J. Med. 386 (18) (2022) 1721–1731, https://doi.org/10.1056/NEJMoa2115869.
[12] G. Reis, E.A. dos Santos Moreira Silva, D.C. Medeiros Silva, L. Thabane, A. Cruz Milagres, T.S. Ferreira, et al., Effect of early treatment with metformin on risk of emergency care and hospitalization among patients with COVID-19: the TOGETHER randomized platform clinical trial, Lancet Reg. Health Am 6 (2022) 100142, https://doi.org/10.1016/j.lana.2021.100142.
[13] WHO Working Group on the Clinical Characterisation and Management of COVID-19 infection, A minimal common outcome measure set for COVID-19 clinical research, Lancet Infect. Dis. 20 (8) (2020) e192–e197, https://doi.org/10.1016/S1473-3099(20)30483-7.
[14] R.L. Brown, C.N. Obasi, B. Barrett, Rasch analysis of the WURSS-21 dimensional validation and assessment of invariance, J. Lung Pulm. Respir. Res. 3 (2) (2016) 00076, https://doi.org/10.15406/jlprr.2015.03.00076.
[15] M. Herdman, C. Gudex, A. Lloyd, M. Janssen, P. Kind, D. Parkin, et al., Development and preliminary testing of the new five-level version of EQ-5D (EQ-5D-5L), Qual. Life Res. 20 (10) (2011) 1727–1736, https://doi.org/10.1007/s11136-011-9903-x.
[16] US Department of Health Human Services, National Institute of Allergy and Infectious Diseases; Division of AIDS, DAIDS table for grading the severity of adult and pediatric adverse events, corrected version 2.1. [July 2017] [Internet]. Available from: https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf, 2020. (Accessed 15 July 2024).
[17] S. Schandelmaier, M. Briel, R. Varadhan, C.H. Schmid, N. Devasenapathy, R.A. Hayward, et al., Development of the Instrument to assess the Credibility of Effect Modification Analyses (ICEMAN) in randomized controlled trials and meta-analyses, CMAJ 192 (32) (2020) E901–E906, https://doi.org/10.1503/cmaj.200077.
[18] M.T. Shenoy, P.K. Mohanty, K. Suganthy, J.K. Manavalan, H. Alexander, Utility of biochemical markers in predicting severe COVID-19: experience from a tertiary hospital in South India, EJIFCC 33 (2) (2022) 131–144.
[19] A. Niraula, N. Baral, M. Lamsal, M. Bataju, S. Thapa, Potential role of biochemical markers in the prognosis of COVID-19 patients, SAGE Open Med 10 (2022) 20503121221108613, https://doi.org/10.1177/20503121221108613.
[20] E.C. Goligher, P.R. Lawler, T.P. Jensen, V. Talisa, L.R. Berry, E. Lorenzi, et al., Heterogeneous treatment effects of therapeutic-dose heparin in patients hospitalized for COVID-19, JAMA 329 (13) (2023) 1066–1077, https://doi.org/10.1001/jama.2023.3651.
[21] B.S. Blette, A. Granholm, F. Li, M. Shankar-Hari, T. Lange, M.W. Munch, et al., Causal Bayesian machine learning to assess treatment effect heterogeneity by dexamethasone dose for patients with COVID-19 and severe hypoxemia, Sci. Rep. 13 (1) (2023) 6570, https://doi.org/10.1038/s41598-023-33425-3.
[22] F. Ceban, S. Ling, L.M. Lui, Y. Lee, H. Gill, K.M. Teopiz, et al., Fatigue and cognitive impairment in post-COVID-19 syndrome: a systematic review and meta-analysis, Brain Behav Immun 101 (2022) 93–135, https://doi.org/10.1016/j.bbi.2021.12.020.