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Efficacy of *Lactococcus lactis* strain plasma (LC-Plasma) in easing symptoms in patients with mild COVID-19: protocol for an exploratory, multicentre, double-blinded, randomised controlled trial (PLATEAU study)

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ABSTRACT

Introduction The COVID-19 pandemic has been a major concern worldwide; however, easily accessible treatment options for patients with mild COVID-19 remain limited. Since the oral intake of *Lactococcus lactis* strain plasma (LC-Plasma) enhances both the innate and acquired immune systems through the activation of plasmacytoid dendritic cells (pDCs), we hypothesised that the oral intake of LC-Plasma could aid the relief or prevention of symptoms in patients with asymptomatic or mild COVID-19.

Methods and analysis This is an exploratory, multicentre, double-blinded, randomised, placebo-controlled trial. This study was initiated in December 2021 and concludes in April 2023. The planned number of enrolled subjects is 100 (50 subjects×2 groups); subject enrolment will be conducted until October 2022. Patients with asymptomatic or mild COVID-19 will be enrolled and randomly assigned in a 1:1 ratio to group A (oral intake of LC-Plasma-containing capsule, 200 mg/day, for 14 days) or group B (oral intake of placebo capsule, for 14 days). The primary endpoint is the change in subjective symptoms measured by the severity score. Secondary endpoints include SARS-CoV-2 viral loads, biomarkers for pDC activation, serum SARS-CoV-2-specific antibodies, serum cytokines, interferon and interferon-inducible antiviral effectors and the proportion of subjects with emergency room visits to medical institutions or who are hospitalised.

Ethics and dissemination The study protocol was approved by the Clinical Research Review Board of Nagasaki University, in accordance with the Clinical Trials Act of Japan. The study will be conducted in accordance with the Declaration of Helsinki, the Clinical Trials Act, and other current legal regulations in Japan. Written informed consent will be obtained from all the participants. The results of this study will be reported in journal publications.

Trial registration number Japan Registry of Clinical Trials (registration number: jRCTs071210097).

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This is a randomised controlled trial to assess the efficacy of *Lactococcus lactis* strain plasma (LC-Plasma) in preventing the onset and attenuation of symptoms in patients with asymptomatic or mild COVID-19.
⇒ This study evaluates the significance of plasmacytoid dendritic cell-related immune responses, including interferon production, clearance of symptoms and prevention of COVID-19 progression.
⇒ This study is conducted during the wave where the SARS-CoV-2 variant Omicron BA1 is dominant.
⇒ This is an exploratory study, due to the lack of previous clinical evidence that evaluated the effect of LC-Plasma intake in patients with COVID-19.
⇒ Other limitations include the subjective endpoint as the primary endpoint and generalisability since this study will be conducted only in Japan among Japanese patients.

INTRODUCTION

The COVID-19 pandemic is a major concern worldwide. In Japan, a cumulative total of 2.92 million polymerase chain reaction-positive cases have been confirmed, and 18 387 deaths have been reported by the Ministry of Health, Labour and Welfare in Japan as of 28 December 2021.[1] Although the extensive introduction of SARS-CoV-2 vaccines has effectively decreased the frequency of COVID-19-related severe diseases, some breakthrough infections due to new SARS-CoV-2 variants are currently emerging threats.[2 3]

Approximately 90% of patients with COVID-19 have mild disease and symptoms in Japan.[4] From a public health perspective, the management of asymptomatic or mild cases is

particularly important because these patients move around freely and may be unaware that they are infected with SARS-CoV-2, causing the spread of infection. Recently, monoclonal antibody therapies, such as casirivimab/imdevimab antibody cocktails, and novel oral antiviral agents, such as molnupiravir, have been approved for mild-to-moderate COVID-19.[5–7] These treatments are associated with a reduced risk of hospitalisation or death in patients with mild-to-moderate COVID-19.[8 9] However, these new drugs are not easily accessible and are restricted by the government for limited use in patients with COVID-19 with high-risk backgrounds.

Lactococcus lactis strain plasma (LC-Plasma; eg, *L. lactis* subsp. *lactis* JCM 5805) is a lactic acid bacterium that directly activates the plasmacytoid dendritic cells (pDCs) and induces type I and III interferons (IFNs) through toll-like receptor 9 stimulation.[10 11] The pDCs are known to act as key regulators of antiviral immunity.[12] Activation of the pDCs induces two antiviral immune responses: (1) production of IFN-alpha/beta that directly inhibit viral replication as part of the innate immune response[13] and (2) subsequent activation of adaptive T cell/B cell-mediated acquired immune response.[14] IFN production is a critical immune response that occurs rapidly during the early phase of SARS-CoV-2 infection.[15] One reason elderly patients with COVID-19 have more severe disease and fatality than those of younger adults is insufficient IFN production in the early phase of infection. Early IFN production by the host accelerates the decrease in SARS-CoV-2 viral load and suppresses progression to severe disease.[16] This suggests that a novel treatment that activates the innate immunity may help to prevent or suppress the onset of COVID-19 symptoms. A previous study reported that healthy volunteers who ingested LC-Plasma showed activated pDCs with increased activity of IFNs by peripheral blood mononuclear cells on exposure to inactivated influenza virus and that onset of flu-like symptoms was prevented.[13] LC-Plasma treatment also increased the proportion of antigen-specific $CD8^+$ T cells[14] whose coordination with $CD4^+$ T cells is important for preventing the exacerbation of COVID-19.[17] These findings suggest that intake of LC-Plasma prevents and/or attenuates COVID-19 symptoms through the activation of both innate and acquired immune responses. Further, LC-Plasma has the advantage of being a natural and safe product according to previous reports.[18 19]

Thus, LC-Plasma is a safe and easy-to-use option for treating asymptomatic or mild COVID-19. The present study aimed to evaluate the efficacy of LC-Plasma capsules in preventing the onset and attenuating symptoms of COVID-19 in patients with asymptomatic or mild infection.

METHODS AND ANALYSIS

Study design and setting

The 'efficacy of *Lactococcus lactis* strain *PL*asmA *To EA*se symptoms in patients with coronavir*Us* disease 2019 (PLATEAU) study: a multicenter, double-blinded, randomized placebo-controlled trial' was initiated in December 2021 following the approval by the Clinical Research Review Board of Nagasaki University in November 2021 (approval number: CRB20-027). This study was registered with the Japan Registry of Clinical Trials (registration number: jRCTs071210097) in December 2021 prior to study initiation. Patients have been enrolled since December 2021; enrolment will culminate in October 2022, and the study is scheduled to end in April 2023. This study is being conducted at seven medical institutions in Japan: Nagasaki University Hospital, Nagasaki Harbor Medical Center, Japanese Red Cross Nagasaki Genbaku Hospital, Saiseikai Nagasaki Hospital, Juko Memorial Nagasaki Hospital, Kouseikai Hospital and Nagasakikita Tokushukai Hospital. As shown in figure 1, eligible patients will be asked to participate in this study, and informed consent will be obtained prior to registration/randomisation. After the written consent is obtained from the eligible patients, they will be enrolled and randomised into group A (intake of LC-Plasma-containing capsule) or group B (intake of placebo capsule). Group A patients will be treated with 200 mg/day of heat-killed LC-Plasma, which includes at least 4.0×10^{11} cells.

Formulation of test capsules

An LC-Plasma-containing capsule comprises 50 mg heat-killed LC-Plasma, 127.3 mg dextrin and 2.7 mg calcium stearate. A placebo capsule contains 177.3 mg dextrin and 2.7 mg calcium stearate. Subjects will orally ingest four test capsules (LC-Plasma-containing capsules or placebo capsules) once daily. The study controller has confirmed that the two types of test capsules cannot be distinguished based on taste, appearance or smell.

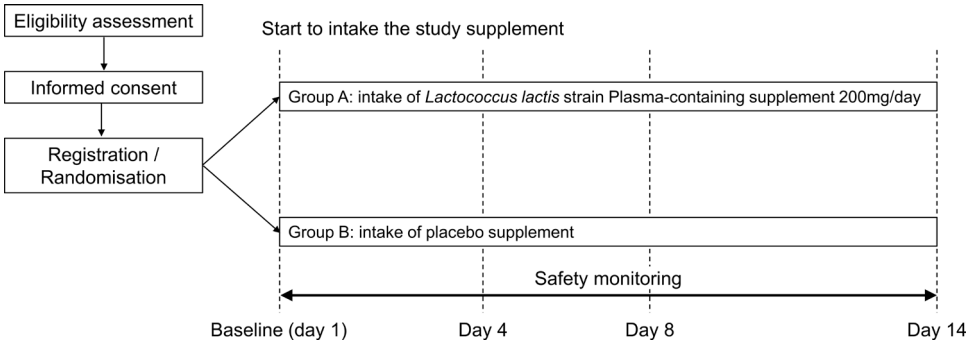

Figure 1 Study design and flow of recruitment, randomisation, study intervention and observation.

Eligibility criteria

Patients who are SARS-CoV-2 positive with asymptomatic or mild COVID-19 disease, staying at isolation facilities for patients with COVID-19 in Nagasaki City, Nagasaki, Japan are eligible for this study. The detailed inclusion criteria are as follows: (1) subjects who are SARS-CoV-2 positive; (2) whose arterial oxygen saturation (SpO_2) is 96% or higher; (3) who are in the age group 20–65 years; (4) who do not refuse to disclose their vaccination status; (5) who can stay at isolation facilities for patients with COVID-19 designated by Nagasaki City; and (6) who give their written consent to participate in the study. Subjects who meet any of the following exclusion criteria will be excluded from participation: (1) obese (body mass index (BMI) ≥30 kg/m^2); (2) subjects with strong dyspnoea, chest pain or hemosputum; (3) a history of COVID-19; (4) being treated or planning to be treated with neutralising antibody drugs for SARS-CoV-2; (5) being treated with immunosuppressive agents, antirheumatic agents, corticosteroids or immunoglobulin preparations; (6) subjects administered oral intestinal regulators; (7) taking one or more beverage or food containing LC-Plasma or yogurt that contains *Lactobacillus delbrueckii* subsp. *bulgaricus* daily; (8) who are pregnant, possibly pregnant or breast feeding; (9) participating in other clinical trials; (10) requiring legal representation for giving consent; or (11) with other conditions that the responsible investigator or subinvestigators deem inappropriate for study participation.

Recruitment and consent

The informed consent document (see online supplemental material) will be provided to the candidates who meet all inclusion criteria and none of the exclusion criteria to provide a comprehensive explanation of this study. Written consent will be obtained from all the participants. After obtaining consent, candidates will be provisionally enrolled in this study. Additional candidates will be enrolled in this study when they enter isolation facilities as described above.

Random allocation

After obtaining informed consent, eligible participants will be randomly assigned in a 1:1 ratio to group A (intake of LC-Plasma-containing capsule, 200 mg/day) or group B (intake of placebo capsule). The randomisation sequence will be generated using a computer-based dynamic allocation method with a minimisation procedure to balance allocation factors (age: less than 50 years or 50 years or older; SARS-CoV-2 vaccination status; and use of anti-SARS-CoV-2 agents).

Blinding and anonymisation

This study will be conducted as a double-blinded trial. All parties are blinded, including the investigators, participants, manufacturer of test capsules, core laboratories and biostatisticians. A central registration number will be used to identify subjects for anonymisation. The manufacturer of test capsules marked LC-Plasma-containing capsules and placebo capsules with specific characters, such as X and Y, and the person in charge of assignment will complete a corresponding table of characters and central registration numbers. The computer-based allocation system will provide a central registration number linked to each subject during registration/assignment. Unblinding will be conducted only in emergency cases, such as the occurrence of serious adverse events (SAEs).

Study intervention and observation

Subjects will be assigned to a group and orally administered test capsules for 14 days. All subjects will be subsequently observed for 14 days (observation points are days 1, 4, 8 and 14). During the observation period, subjects will be restricted to use systemic antiviral agents (except topical antiviral agents) or intestinal regulators. For palliative care for COVID-19, temporary use of antitussive agents, expectorants, general cold medications, antipyretic analgesic (acetaminophen only) and antidiarrheal agents are allowed, but not for routine use. In addition, during the observation period, addition, discontinuation, switch or dose change of any medical agents or supplements will be recorded. Furthermore, during the observation period, the subjects will be asked to refrain from consuming lactic acid bacteria-containing foods, such as yogurt. Since all the subjects will stay in the designated isolation facilities for COVID-19 until day 8, investigators in the isolation facilities will confirm the intake of medication and foods and supplements. At the entry in the isolation facilities, investigators will check the belongings of the subjects whether they have any medical agents or supplements with them. During the stay in the isolation facilities, only designated foods will be supplied to the subjects, avoiding yogurt or other lactic acid bacteria-containing foods. After the discharge from the isolation facilities, the patients will be asked to record the medication or the intake of any supplements every day on the study diary until day 14, and the investigators will ask the patients about the medication or intake of other supplements by interview. The intervention will be discontinued if the subjects meet any of the discontinuation criteria: consent withdrawal, worsening of the primary disease, complications, adverse events (AEs) requiring discontinuation of the study intervention, remarkably poor adherence to the test capsules regimen, or any other condition that investigators deem appropriate for discontinuation of the study intervention.

Table 1 shows the schedule of assessments to be performed at each observation point, including the mandatory and optional assessments. The observation items are as follows: (1) subjects' background characteristics (sex, height, weight, BMI, presence or absence of onset of COVID-19, estimated infection day, onset day of COVID-19 if symptomatic), and SARS-CoV-2 vaccination status (timing, number of vaccinations, vaccine type, and last day of vaccination); (2) severity of COVID-19 assessed by severity classification according to COVID-19 infectious

Table 1 Schedule of assessments performed at each observation point

Observation items	Consenting/ Registration	Day 1*	Day 4	Day 8	Day 14	Discontinuation
1. Eligibility	○					
2. Patients' characteristics	○					
3. Severity of COVID-19 pneumonia	○		○	○		△
4. Blood test 1		○	○	○		△
5. Blood test 2		○	○	○		△
6. PCR test for SARS-CoV-2		○	○	○		△
7. Medication information		○	○	○		△
8. Vital sign		←	←	○	→	△
9. Adherence of test capsules		←	←	○	→	△
10. Subjective symptoms		←	←	○	→	△
11. Adverse events	←	←	○	→	→	

○ mandatory, △ optional.
*Observation of day 1 can be conducted simultaneously with registration before the initiation of intake of the test capsules.

disease treatment guidelines published by the Ministry of Health, Labour and Welfare in Japan[20] (mild: $SpO_2 \geq 96\%$, no respiratory symptoms or only coughing without dyspnoea, and without pneumonia findings, moderate I: $93\% < SpO_2 < 96\%$, with dyspnoea, and with pneumonia findings, moderate II: $SpO_2 \leq 93\%$ and oxygen administration required, and severe: admission to intensive care unit or ventilator required); (3) blood test 1: differential count of leukocytes (neutrophil, lymphocyte, eosinophil, monocyte and basophil), platelet, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase, blood urea nitrogen, serum creatinine, lactate dehydrogenase, C reactive protein, ferritin, and HbA1c; (4) blood test 2: pDCs activation markers (human leucocyte antigen DR and CD86, cytokines (interleukin-6 and monocyte chemoattractant protein-1), SARS-CoV-2-specific antibodies (immunoglobulin M and G), expression of IFN and IFN-inducible antiviral effector genes in peripheral blood mononuclear cells (PBMC), and immune cell analysis (T cells and B cells); (5) real-time PCR test for SARS-CoV-2 by nasopharyngeal swabs; (6) medication information; (7) vital signs (body temperature, pulse, SpO_2 and frequency of breath); (8) adherence of test capsules; (9) patients' subjective symptoms measured by the severity score[21] and visual analogue scale (VAS); and (10) AEs/SAEs.

Outcomes

The primary endpoint of this study is the change in subjective symptoms measured using the severity score[21] and VAS. Subjective symptoms (cough, shortness of breath, fatigue, headaches, anosmia, dysgeusia and anorexia) are assessed using a 4-point Likert scale (not affected=0 points, little effect=1 point, affected=2 points and severely affected=3 points), and the total severity score is calculated as the sum of scores for these seven subjective symptoms. The severity score questionnaire is shown in online supplemental table 1).

Secondary endpoints are as follows: (1) change or per cent change in viral load of SARS-CoV-2; (2) change or per cent change in biomarkers for pDC activation (HLA-DR, CD86); (3) change or per cent change in SARS-CoV-2-specific antibodies (immunoglobulin G and M); (4) change or per cent change in blood cytokines (IL-6, MCP-1); (5) change or per cent change in IFN-or IFN-inducible antiviral effectors; and (6) proportion of subjects who visit the emergency room and are hospitalised.

Data collection, data management and monitoring

A case report form will be used for data collection. Data collection and management will be carried out by third party entities to avoid bias. Data will be managed by Soiken, the Data Management Group (Data Centre). To ensure quality, the study will be monitored by Soiken, the Monitoring Group.

Safety evaluation

During the study, the investigators will constantly monitor for any AEs during regular medical checkups. All related AEs, including study agent side effects, abnormal clinical laboratory test values, and untoward medical occurrences, will be reported and documented. If AEs meet the following criteria, they are referred to as SAEs based on the ICH E2A, ICH E2D, and the 'Ethical Guidelines for Medical and Health Research Involving Human Subjects'[22]: AEs that result in (1) death; (2) life-threatening hospitalisation; or (3) extension of hospitalisation; (4) persistent or significant disability or incapability; (5) medically important or critical condition; (6) AEs that are equivalently severe to criteria (1)–(5); or (7) AEs that cause congenital abnormality or birth

defects. AEs will be followed until the normalisation or recovery to a level that is not considered an AE.

Sample size calculation

The severity score was used in a case series study to estimate the patients' subjective symptoms.[21] When the total severity score from this previous study was translated using a calculation method mentioned in the outcomes section of the present study, the total score on days 0 and 14 was 11±5.3 and 1.7±2.3, respectively, and the change from day 0 to day 14 was –9.3±6.0.

Since this study will enrol patients who are asymptomatic or have mild COVID-19, we assumed that the severity score of cough at baseline (day 1) will be 0–1 and that of other symptoms will be 0 in this study. Therefore, the mean total severity score at baseline predicted in this study is 0.5. We also assumed that the severity score will not worsen in group A (ie, change in the total severity score=0). Since a recent meta-analysis reported that 48.9% of initially asymptomatic patients with COVID-19 became symptomatic,[23] we assumed that the severity score in group B will worsen to a level of half of the severity score at day 0 reported in the case series study mentioned above,[21] meaning that the total severity score would be 5.5 (ie, change in the total severity score=5). Since it is expected that the degree of subjective symptoms varies widely among patients when asymptomatic subjects become symptomatic, we assumed that the SD in the change in the severity score will be 7.0, which is slightly larger than that in the case series study mentioned above.[21] Under these assumptions, the minimum sample size required to achieve a significance of 0.05 from a 2-sided test with a statistical power of 90% was determined to be 42 subjects for both groups or a total of 84 subjects. We estimated the dropout rate to be 15%; thus, the planned enrolment was set at 100 subjects, with 50 in each group.

Statistical analysis

All tests will be two-sided, and a $p<0.05$ will be considered statistically significant. As this is an exploratory trial, multiplicity will not be adjusted for all endpoints. A statistical analysis plan was developed before the database lock. All statistical analyses will be conducted by independent biostatisticians.

Three analysis sets are defined in this study; the full analysis set (FAS) includes all patients who will be registered in this study. However, patients with severe protocol violations, such as registration without obtaining consent or registration outside of the enrolment period, will be excluded from the FAS. The per-protocol set excludes the patients with a protocol violation, such as violation of the eligibility criteria, use of prohibited or restricted concomitant treatments, or poor adherence to the test capsules (less than 75% or more than 120%). The safety analysis set includes all patients who will be registered in this study who receive at least one dose of the test capsules.

Patient characteristics at baseline will be presented as frequencies and proportions for categorical data and summary statistics (number of patients, mean, SD, minimum, first quartile, median, third quartile and maximum) for continuous data. Patient characteristics will be compared using the χ^2 test or Fisher's exact test for categorical data and the two-sample t-test or Wilcoxon rank-sum test for continuous variables.

The primary endpoint of this study is the change in subjective symptoms measured by the severity score.[21] The total score of the severity score is calculated by summarising the severity scores of seven symptoms, and the summary statistics of change in the total score of the severity score from baseline to day 14 will be calculated. Analysis of covariance (ANCOVA) will be conducted to test the null hypothesis that the change in the total score of the severity score from baseline to day 14 is the same in both groups. The allocation factors age (less than 50 years or 50 years or older), vaccination status for SARS-CoV-2 (vaccinated or unvaccinated), use of anti-SARS-CoV-2 agent (presence or absence of anti-SARS-CoV-2 agent use) and the total score of the severity score at baseline will be used as covariates in the ANCOVA. The sensitivity analysis will be performed using the mixed-effects model for repeated measures with an unstructured covariance structure with treatment groups, time (day), interactions between treatment groups and time, allocation factors, and the total score of the severity score at baseline as fixed effects, and subjects as random effects. If the calculation results do not converge, compound symmetry will be used. The VAS score will be analysed similar to the analysis of severity score.

For the secondary endpoints, summary statistics (number of patients, mean, SD, minimum, first quartile, median, third quartile and maximum) for measurements, changes from baseline, and percentage changes from baseline will be calculated for continuous data. Frequencies and proportions will be calculated for categorical data. Two-sample t-test or Wilcoxon rank-sum test for intergroup comparisons of continuous data, one-sample t-test or Wilcoxon signed-rank test for intragroup comparisons of continuous data and the χ^2 test or Fisher's exact test for intergroup comparisons of categorical data will be performed.

For safety endpoints, summary statistics for the frequency of AEs will be calculated for each group, and Fisher's exact tests will be performed for intergroup comparisons.

Patient and public involvement

Patients and the public were not involved in the conception or planning of this study and will not be involved in the execution, analysis and evaluation.

DISCUSSION

This is the first randomised controlled trial to estimate the efficacy of LC-Plasma in preventing the onset and attenuating the symptoms of patients with asymptomatic or mild COVID-19. Since the intake of LC-Plasma enhances both

the innate[13] and acquired immune responses[14] through the activation of pDCs,[10 11] we hypothesise that the intake of LC-Plasma contributes to the prevention of the onset and attenuation of the severity of COVID-19. Easy-access treatment options for patients with mild COVID-19 are limited; thus, results of this study may contribute to the treatment of asymptomatic or patients with mild COVID-19. This study is also noteworthy because it evaluates the significance of pDC-related immune responses, including IFN production, clearance of symptoms and prevention of COVID-19 progression.

The safety of LC-Plasma has been previously confirmed. Long-term intake tests (50 mg daily, 12 weeks, or 150 mg daily, 4 weeks)[18] and an excessive intake test (250 mg daily, 4 weeks)[19] of LC-Plasma reported no safety concerns. In addition, LC-Plasma-containing yogurt, beverages and supplements have been commercially available since 2012, and no health hazards associated with LC-Plasma-containing products have been reported. This suggests the safety of the LC-Plasma-containing supplements used in this study (200 mg daily for 14 days). Molnupiravir, the first approved oral antiviral agent for high-risk patients with COVID-19, has common side effects, including diarrhoea, nausea, dizziness and headache within 14 days of the last dose, and is restricted in pregnant women because it affects the development of the fetus.[24 25] Non-elderly and non-high-risk patients with mild COVID-19 rarely progress to severe disease; therefore, it is reasonable to choose safer drugs that sufficiently control their symptoms.

This study has several limitations. First, this is an exploratory study due to the lack of previous clinical evaluation of the effects of LC-Plasma intake in patients with COVID-19. The target number of enrolled patients in this study was calculated from results of a case series study estimating subjective symptoms in non-hospitalised patients with COVID-19 treated with the histamine-2 receptor antagonist famotidine.[21] Since this case series evaluated subjective symptoms in only 10 patients, the calculated mean and SD of the results might not reflect the actual patients' subjective symptoms. Second, the primary endpoint in this study is patients' subjective symptoms as reported by subjects themselves. Therefore, biases such as responder bias, non-responder bias and volunteer bias cannot be completely avoided. However, the effect of bias is minimised using a double-blinded study design. Third, the possibility of the effect of confounding factors is not completely eliminated. One possible confounding factor is the usage of any virus inhibitors, such as Paxlovid. However, this study does not exclude patients who are treated or are planned to be treated with anti-SARS-CoV-2 agents, because of the following two reasons: (1) at the planning and initiation of this study, none of the anti-SARS-CoV-2 agents had been approved in Japan and (2) the authors had expected that once the anti-SARS-CoV-2 agent is approved in Japan, it will be widely used for patients with asymptomatic or mild COVID-19. If the patients who used or planned to use the anti-SARS-CoV-2 agent are excluded, it might be hard to enrol patients in this study. Therefore, patients who used or planned to use anti-SARS-CoV-2 agents are not excluded from this study; instead, the use of anti-SARS-CoV-2 agents is designated as one of the allocation factors to achieve balance between the groups and will be used as one of the covariates in the ANCOVA. Another possible confounding factor is sex, which might also influence the severity of COVID-19. However, because of the sample size, the authors determined the maximum number of allocation factors as three. In particular, considering the impact of the confounding factors on the results in this study, the authors designated age, SARS-CoV-2 vaccination status and use of anti-SARS-CoV-2 agents as the allocation factors, and not sex. Since the authors expected that consuming lactic acid bacteria-containing foods could also be a confounding factor, the subjects will be asked to refrain from consuming yogurt. However, consumption of cheese, which also contains lactic acid bacteria, is not clearly prohibited in this study, as the amount of lactic acid bacteria is less than that in yogurt, and cheese is not commonly consumed daily in Japan. Furthermore, the possibility of the effect of unknown confounding factors was not completely eliminated, although known (expected) confounding factors (age, SARS-CoV-2 vaccination status, and use of anti-SARS-CoV-2 agents) are designated as the allocation factors to achieve balance between the groups. Other unknown confounding factors might also be balanced between groups, since this was a randomised-controlled trial. Fourth, this study will be conducted in medical institutions in Japan and will enrol only Japanese patients. These constraints could limit the generalisability of this study. Further larger-scale, international clinical trials are required in the future.

Ethics and dissemination

This study and its protocol were approved by the Clinical Research Review Board of Nagasaki University (approval no. CRB20-027) in accordance with the Clinical Trials Act of Japan. The study will be conducted in accordance with the Declaration of Helsinki, the Clinical Trials Act, and other current legal regulations in Japan. If any amendments to the protocol are required, the amended protocol will be resubmitted for investigation and approval by the Clinical Research Review Board of Nagasaki University before the implementation of this study according to the amended protocol. Written informed consent will be obtained from all participants after a full explanation of the study. Any health hazards caused by this study will be compensated by clinical research insurance. The results of this study will be disseminated through medical conferences and journal publications.

Datasets generated and/or analysed during this study will not be publicly available because of the absence of a statement in the study protocol and the informed consent documents enabling data sharing with a third party after the end of the study. Data sharing has not been approved by the certified review board.

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Contributors KazY, RT, KJ and DF contributed to the study conception and design, drafted the protocol and supervised the revision. NH, KatY, TI and KI provided intellectual input to improve the study design and to revise the protocol. HM supervised the conception and design of the study. All authors have read and approved the final manuscript.

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Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by Clinical Research Review Board of Nagasaki University Reference number CRB21-009. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

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