The influence of a capsule containing probiotic lactobacillus and prebiotic inulin (Yourgutplus+) on the duration and severity of symptoms among individuals with Covid-19.

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Abstract:

Background: Gut microfloral dysbiosis is known to affect the majority individuals suffering with a Covid-19 infection. This study evaluated whether a specific lactobacillus and inulin blend known as yourgutplus+, which aimed to improve gut health, could reduce the severity of early and chronic Covid-19 symptoms.

Methods: From May 2020 to May 2021, we evaluated 126 participants with Covid-19, with an average duration of symptoms of 108 days, who were given 30 days of this pre and probiotic capsule within the ongoing UK national Phyto-v study. Symptoms were recorded using the validated Cough Symptom Score, the Subjective Well-Being questionnaire and the Chandler fatigue questionnaire. The group was analysed as a whole and then subdivided into 40 (32%) in an early phase of infection (average symptoms 10 days before baseline) and the 86 (68%) in a chronic phase (average symptoms 120 days before trial baseline).

Results: Cough, fatigue and subjective well-being scores significantly improved over the 30 days in both the early and chronic phase cohorts. Participants who were more likely to have gut dysbiosis at trial entry, such as sedentary, hospitalised, older males with GI symptoms, had a statistically significantly better response to the probiotics. Gut symptoms improved in 25 of 31 (82%) who reported them at baseline. Two (1.5%) patients reported mild increased bloating and diarrhoea.

Discussion: Following this nutritional intervention, participants had a significant improvement in GI and non-GI symptoms resulting in a meaningful improvement in overall well-being. Although some participants with early disease would have improved spontaneously, such a rapid improvement in the majority who had been experiencing symptoms for over 6 months, was clinically relevant and welcomed, especially amoung those more likely to have pre-existing gut dysbiosis. Going forward, our research group are now evaluating whether intake of yourgutplus+, could also enhance antibody titres levels post Covid vaccination.

Key words: Probiotics, prebiotics, Covid-19, Gut-health, long-Covid symptoms, Yourgutplus+

Certification: This trial was approved by the Health Research Authority (REC reference: 20/YH/0164), sponsored and approved by Bedfordshire Hospitals NHS trust and its Research and Development committee. The Medicines and Health Regulatory Agency (MHRA) gave formally authority to proceed with the trial as no medical products license is required for food products.

Introduction and background

As emerging evidence from clinical studies and experience from managing patients with Covid-19 (Covid) unfolds, the links between severity of symptoms, mortality and gut microbial dysbiosis has become increasingly apparent [1,2,3,4,5] . Depleted healthy strains of commensal bacteria such as *Lactobacillus* have been reported in the majority of patients with Covid expressing gastrointestinal (GI) symptoms and especially those with persistent ongoing problems, coined long or chronic Covid [6,5,7,8,9,10,11] . Although most Covid cases have a mild self-limiting respiratory illness, individuals with co-morbidities and conditions, linked to poor gut health, such as being elderly, those living with obesity or diabetes do significantly worse [11, 12, 8,7] . The authors of these studies postulated microfloral dysbiosis contributes to symptoms via increased gut inflammation, impaired gut wall integrity which correspondingly leads to systemic inflammatory dysfunction, reduced immune surveillance leading to greater non-gut symptoms as well [4, 6, 8,5,11, 12 .] In this situation, overgrowth of less favourable gut bacteria have been found in the systemic circulation and within pulmonary aspirates leading to an increased inflammatory response, causing cough and breathlessness [13, 14, 15, 16,17] . Excess inflammatory cytokines and pulmonary exudates are a feature of acute respiratory distress syndrome (ARDS) following a viral infection, hence the recently coined term *cytokine storm* [13 14, 15, 16, 17] . The link between bowel dysbiosis and lung hyperinflammation has also been well documented in other chronic respiratory diseases including asthma and chronic bronchitis [18, 19, 20] .

In addition to dietary and behavioural measures, supplementary capsules are a convenient way to increase total intake of pro and prebiotics, as well as a way to spread their intake throughout the day. The most widely researched probiotics include lactic acid producing bacteria such as species of Lactobacillus, the colonisation of which is enhanced by concomitant intake with prebiotic soluble fibres such as inulin [21, 22] . Numerous interventional studies in humans and animals have shown they can help improve mircrofloral biodiversity, correct GI symptoms such as bloating and diarrhoea and improve Immune efficiency [23, 24, 25, 26, 27, 28] . Many of these studies, albeit most needing further confirmation, have shown they help modify a range of chronic diseases ranging from obesity, inflammatory bowel disease, diabetes, cardiovascular disease, hypertension, anxiety, depression, osteoporosis and dementia [21, 28, 29, 30, 31, 32, 33, 34, 35, 36 , 37] . More relevant to this study, intervention studies have shown regular intake of live probiotics, particularly Lactobacilli strains, shortened the incidence duration and severity of upper respiratory tract infections in several studies [38, 39, 40, 41, 42, 43] . This includes a summary of interventional studies published in the Cochrane Database which concluded that probiotics did reduce the number of symptomatic upper respiratory tract infections [43] . Another meta-analysis of small RCTs suggests that probiotics decreased the need for invasive mechanical ventilation due to development ARDS following viral pneumonia [44] .

The reported mechanism of action of probiotic bacteria is multifactorial [45, 46] . They encourage gut colonisation of anti-inflammatory strains which then out space pro-

inflammatory (Firmicutes*)* bacteria. They encourage the fermentation of otherwise poorly-digestible dietary carbohydrates into short-chain fatty acids (SCFA) such as butyrate. These have an important impact upon mucosal physiology as they are an idea source of energy for gut cells so help improve gut health and hence gut wall integrity [47, 48, 49, 50]. Probiotic bacteria also help the breakdown of polyphenols into more ready absorbed and more bioactive varieties [51, 52]. Higher serum levels of polyphenols and other phytochemicals are linked to lower systemic inflammation, a lower risks of chronic degenerative disease [49, 55, 52, 53, 55, 56, 57] and cancer [53, 54] .

As well as their positive influence on immune balance, probiotics have been found to have a range of other potential mechanisms of actions[45, 46] . They can enhance intracellular oxidative enzyme capacity and can help scavenge excess superoxide anions among patients with Covid[58]. This mechanism, in laboratory studies, was attributed to their ability to reduce oxidative stress via upregulating superoxide transferase and other anti-oxidant enzymes[60] . Excess oxidative stress is a linked to more aggressive pulmonary pathogenicity following Covid pneumonia[61,62]. Probiotics help increase vitamin D absorption and bioactivity [63, 64, 65, 66, 67, 68]. Low vitamin D is associated with higher levels of unregulated hyperinflammatory cytokine production and ultimately more severe respiratory Covid related symptoms [69, 70, 71, 72] . Finally, there are reports of direct anti-viral actions of lactic acid producing bacteria such as lactobacillus via the production of antiviral inhibitory metabolites following induction of the expression of genes involved in antiviral immunity [73, 74, 75, 76, 77].

The Kings app study has reported that individuals who have more symptoms initially, including bowel problems, were more at risk of long Covid [78] . In addition, people who took regular probiotics had a 14% lower risk of symptomatic Covid [79] . Many clinical trials are underway globally examining the role of pro and prebiotics in both prevention and treatment of Covid and some have reported benefits [1, 8, 58, 80, 81, 82, 83, 84]. Considering this background of evidence, this study aimed to examine whether their administration could reduce the severity and longevity of symptomatic Covid infection, particularly those with ongoing symptom, via their gut health promoting, immunomodulatory and anti-inflammatory and direct actions.

Methods:

This experimental trial was conducted at Bedford Hospital, part of the Addenbrooke's Hospital Cambridge University Trust Network. All participants (n=126, 70 male, 56 female) had one or more symptoms related to their Covid infection at the time of trial entry. Participants were recruited between May 2020 and May 2021, approached either at the local post Covid clinic, or from the daily Covid ward round or had contacted the trials unit themselves directly after hearing about the study via word of mouth. For this analysis we only included participants who were given a capsule containing a 5 species Lactobacillus

probiotic blend with inulin prebiotic derived from chicory for at least one month. The daily dose (from 2 capsules) was 200mg of Inulin and 10 Billion colony forming units (CFU's) of Lactobacillus plantarum (Lp90), Lactobacillus rhamnosus (LRa05), Lactobacillus bulgaricus (LB42), Lactococcus lactis (La61), Lactobacillus paracasei (LC86).

People were excluded if they had known sensitivities and allergies to the investigational foods, were immune-suppressed or were too ill to take oral capsules. The average age 53 years (range 16-82). Eighty three (66%) were smokers, 42 (34%) non-smokers. The average BMI was 28.7 Kg/m^2 , 47 (37.3%) were normal weight (BMI 18-<25 Kg/m^2), 79 (62.7%) were in the overweight or obese range (BMI >25 Kg/m^2) (30% overweight, 32.7% obese).

The average time from Covid diagnosis to trial entry was 108 days (range 2-467). The group was analysed as a whole and then subdivided into 40 (32%) in the early phase (EP) of infection (within 30 days of diagnosis) and 86 (68%) in a chronic phase (CP) of persistent symptoms (>30 days from diagnosis). Of the individuals in this chronic phase Covid group, the average time from diagnosis and trial entry was 120 days and for the individuals in the early phase this was 10 days.

Symptoms were recorded at trial entry and at 30 days 3 using validated questionnaires (The Cough Symptom Score, the Subjective Well-Being questionnaire and the Chandler fatigue questionnaire) [85,86,87]. All other symptoms were recorded using the NCI Common Toxicity Checklist.

Manufacture: The food supplement was made specifically for this trial by Park-acre, Lincoln, DN21 5TJ and The Oxford Health Company Ltd, Oxfordshire, OX26 5AH. Certified to conform to Good Manufacturing Practice (GMP - FSSC 22000, ISO 22000), UK and International food production laws [www.tohc.co.uk]. They are also certified organic by the Organic Food Federation. There in-house Research and Development department, for each batch tested for contamination with yeast, mould, E. coli, salmonella. They measured lead, arsenic, cadmium, mercury and pesticides then excluded batches which did not abide by international threshold guidance and law. A unit of the supplements are securely stored by the Trust Secretary and can be sent to any regulatory body at their request in the future.

Statistical analysis

The primary end points for this analysis were mean cough, fatigue and subjective well-being symptoms scores on one day one and day 30, for the whole cohort and then split into early phase and chronic phase groups. Statistical analysis was performed using IBM SPSS Statistics (IBM Corp., Amonk, NY, United States). All dependent variables were checked for normal distribution using Quantile-Quantile (Q-Q) Plots and were deemed plausible in all instances. All data was presented as mean ± SD with 95% confidence intervals (95%CI). A dependent paired samples t-test was used to assess the mean differences in cough, subjective wellbeing and fatigue scores at day 1 compared with day 30. The two-tailed alpha level was

set as P < 0.05. Cohen's d effect size were used to show the magnitude of change for each significant difference using the following thresholds; 0.2 – 0.49 small, 0.5 – 0.79 moderate, >0.8 large).

A predetermined subgroup determined which participants had the most benefit to the intervention by comparing the change in mean symptoms scores from baseline to day 30 between male versus (vs) female; normal weight versus overweight or obese; < 60 years vs > 60 years; ethnic group (White British vs other); Exercise levels (<3 hours a week v $\geq$ 3 hours a week); hospitalised vs not; history of bowel symptoms vs not, gastrointestinal symptoms at baseline vs not. An independent t-test was used to determine the differences in scores between normally distributed subgroups. A between groups one way analysis of variance (ANOVA) were used to determine differences for all other sub-group analysis. Assumptions of both tests were checked via the homogeneity of variance, which were not violated for any variables. In the instance of a significant main effect, a Bonferroni pairwise comparison was used to locate significant differences. The two-tailed alpha level was set as $p \leq 0.05$.

Results:

Formal symptoms scores: Tables 1 summarises the changes in formal cough, fatigue and well-being scores over the 30 days intervention for the whole cohort and separately for the patients who received probiotics in the acute and chronic phases of Covid (Table. 2).

Table 1: Changes in symptoms from day 1 to 30 days (all patients, n=126)

Symptom Score	Mean & SD (Day 1 vs 30)	Difference (Day 1 vs 30)	95%CI	p value
Cough	1.25 ± 1.99 vs 0.42 ± 1.01	0.83 ± 1.74	0.52 to 1.14	p< 0.001
Fatigue	21.37 ± 5.59 vs 16.57 ± 6.80	4.74 ± 6.85	3.58 to 6.01	p< 0.001
Subjective wellbeing	24.05 ± 8.47 vs 28.38 ± 6.95	4.11 ± 9.62	5.98 to -2.67	p< 0.001

Cough scores significantly decreased over the 30 days from a mean of 1.25 (± 1.99) to 0.42 (± 1.01) (t_{124} = 5.331, p < 0.001; 95%CI: 0.52 to 1.14, *d* = 0.48). Fatigue scores were significantly improved from a mean of 21.37 (± 5.59) to 16.57 (± 6.80), (t_{118} = 5.178, P < 0.001; 95%CI: 3.58 to 6.01, *d* = 1.36) by a large magnitude (*d* = 1.36)). Subjective wellbeing

demonstrated a significant improvement by a large magnitude from a mean of 24.05(± 8.47) to 28.38 (± 6.95) (t_{124} = 7.823, p < 0.001; 95%CI: -5.98 to -2.67, *d* = 1.10).

Table 2: Changes in Covid symptoms from day 1 to 30 days split into early and chronic cohorts

Symptom Scores	Mean & SD (Day 1 vs 30)	Difference (Day 1 vs 30)	95% CI	p value
Early Covid cohort (n=40) (symptoms developed within one month of trial entry (mean 14 days)				
Cough	1.84 ± 2.33 vs 0.61 ± 1.27	1.24 ± 2.33	0.42 to 2.07	p = 0.004
Fatigue	20.19 ± 5.69 vs 14.44 ± 4.53	4.70 ± 6.90	2.88 to 8.61	p< 0.001
Subjective wellbeing	24.45 ± 9.69 vs 30.94 ± 6.06	6.48 ± 10.39	10.17 to -2.8	p= 0.001
Chronic Covid cohort (n=96) Symptoms persistent > 1 month from trial entry (Mean = 120 days)				
Cough score	1.03 ± 1.82 vs 0.35 ± 0.89	0.67 ± 1.45	0.38 to 0.98	P< 0.001
Fatigue score	21.66 ± 5.55 vs 17.18 ± 7.20	4.70 ± 6.85	3.13 to 5.82	P< 0.001
Subjective wellbeing	23.99 ± 8.04 vs 27.51 ± 7.03	3.23 ± 9.20	5.33 to -.70	p< 0.001

* Early Covid = symptoms within 1 month of trial entry
+ Chronic Covid = Participants with symptoms for > 1 month prior to trial entry

Both fatigue and subjective wellbeing scores were significantly improved in the acute (fatigue: t_{39} = 4.120, P < 0.001, 95%: 2.88 to 8.61, *d* = 1.8; Subjective wellbeing: t_{39} = 3.585, P = 0.001, 95%: 2.80 to 10.17, *d* = 1.6) and chronic (fatigue: t_{95} = 6.618, P < 0.001, 95%: 3.13

to 5.82, *d* = 1.3; Subjective wellbeing: t_{95} = 3.840, P < 0.001, 95%: 0.70 to 5.33, *d* = 0.9) phase groups by a large magnitude. Furthermore, cough scores were significantly improved by a moderate effect size in the acute (t_{39} = 3.060, P = 0.004, 95%: 0.42 to 2.07, *d* = 0.6) phase group and by a small magnitude in the chronic (t_{95} = 4.472, P < 0.001, 95%: 0.38 to 0.98, *d* = 0.4) phase group.

Self-reported symptoms: The top 7 self-reported symptoms were fatigue, shortness of breath, pains, altered sense of smell, bowels symptoms, cough and headache (see Table 3). Table.4 (excluding cough and fatigue) summarised how these self-reported symptoms changed over the 30 days of the intervention with bowel symptoms improving the most.

Table 3. Self-reported symptoms at baseline (any grade of severity)

Symptom	Number (%)	Symptom	Number (%)
Fatigue	117 (92%)	Sore throat	7 (6%)
Breathlessness	53 (42%)	Anxiety or depression	7 (6%)
Joint, muscle or chest pains	43 (34%)	Altered hearing or vision	6 (5%)
Bowel symptoms, nausea	31 (25%)	Increased BP	6 (5%)
Cough	31 (25%)	Peripheral neuropathy	5 (4%)
Altered sense of smell	31 (25%)	Dizziness	5 (4%)
Headache	24 (19%)	Increased perspiration	4 (3%)
Muscle weakness	22 (17%)	Sneezing	4 (3%)
Fever / chills	18 (14%)	New onset asthma / asthma flare	4 (3%)
Poor appetite, nausea	14 (11%)	Altered voice / hoarseness	3 (2%)
Insomnia	10 (8%)	Hyperesthesia	3 (2%)
Heart palpitations	8 (6%)	Urinary problems	3 (2%)
Brain fog	8 (6%)	Weight loss	3 (2%)
Skin rash / Covid toes	8 (6%)	Period problems	2 (1%)

Table 4: Percentage of self-reported symptoms improving at 30 days other than cough and fatigue (reported above):

Symptoms	Number (%)	Symptom	Number (%)
Bowel symptoms	25 of 31 (82%)	Headache	3 of 24 (13%)
Sleep pattern	8 of 10 (80%)	Skin oiliness	1 of 6 (16%)
Brain fog or headache	3 of 8 (38%)	Asthma relief	1 of 4 (25%)
Breathlessness	11 of 53 (21%)	Sneezing	1 of 4 (25%)
Joint or chest pains	7 of 43 (16%)	Decreased palpitations	1 of 8 (12%)

Subgroup analysis

Subgroups were predetermined to evaluated whether some individuals had a greater or lesser benefit from the intervention. These subgroups included gender, body mass index (BMI) , age, ethnic group, exercise levels, whether hospitalised, whether they had a history of GI symptoms and whether they had new GI symptoms at trial entry. Although there was no difference in relative benefit between different ethnic groups or BMI levels, it appeared that males, those <60 years, those exercising more than 3 hours a week, those previously hospitalised, no prior indigestion, new or worse GI symptoms at trial entry had a statistically greater benefit for one or more measurable outcomes (see Table. 5)

Table 5: Subgroup analysis highlighting who got the greatest benefit from the intervention

Symptom scores	**Mean score change Day 1 to 30 SD (number)**	**Difference (95% CI)**	**P value**
	Gender Male (70) vs Female (56)		
Cough	1.1 ± 2.0 vs 0.5 ± 1.2	0.6 (0.1 to 1.6)	p=0.04
Fatigue	6.4 ± 7.2 vs 3.2 ± 6.2	3.2 (-0.6 to 0.5)	p=0.01
Subjective wellbeing	5.3 ± 10.6 vs 2.6 ± 8.0	2.7 (0.6 to 5.6)	p=0.10
	Age <60 years (89) vs ≥60 years (37)		
Cough	0.6 ± 1.8 vs 0.9 ± 1.7	0.3 (0.9 to 0.4)	p=0.46
Fatigue	1.6 ± 5.3 vs 6.4 ± 7.0	4.8 (7.2 to 2.5)	p<0.001
Subjective wellbeing	1.4 ± 9.8 vs 5.2 ± 9.4	3.8 (0.2 to 7.5)	p=0.04
	Ethnic group White British (92) v other (34)		
Cough	0.8 ± 1.8 vs 0.7 ± 1.7	0.1 (-0.8 to 0.5)	p=0.64
Fatigue	4.9 ± 7.1 vs 5.3 ± 6.5	0.4-2.4 to 3.3	p=0.74
Subjective wellbeing	4.2 ± 10.3 vs 3.8 ± 7.5	0.4 (-3.4 to 4.2)	p=0.82
	BMI Normal (46) vs OW and O (80)		
Cough	0.8 ± 1.5 vs 0.7 ± 1.9	0.1 (-0.8 ± 1.5)	p=1.00
Fatigue	2.7 ± 10.1 vs 4.7 ± 9.3	2 (2.6 to 4.7)	p=1.00
Subjective wellbeing	-4.2 ± 10.3 vs - 3.8 ± 7.5	0.4 (-2 to 4)	p=0.13
	Hospitalised Yes (79) vs No (47)		
Cough	0.9 ± 1.6 vs 0.7 ± 1.9	0.2 (-0.5 to 0.7)	p=0.77
Fatigue	6.1 ± 7.4. vs 2.9 ± 5.5	3.2 (0.9 to 5.6)	p<0.01
Subjective wellbeing	3.5 ± 9.0 vs 5.1 ± 10.5	1.6 (-2.0 to 5)	p=0.39
	Prior indigestion Yes (113) vs No (13)		
Cough	0.8 ± 1.7 vs 1.0 ± 2.1	0.2 (0.8 -1.2)	p=0.70
Fatigue	9.4 ± 7.9 vs 4.4 ± 6.6	5 (0.1 - 11.1)	p<0.01
Subjective wellbeing	9.1 ± 11.5 vs 3.5 ± 9.2	5.6 (8.9 - 1.1)	p=0.04
	New GI symptoms at baseline Yes (41) vs No (85)		

Cough	0.7 ± 1.9 vs 0.8 ± 1.7	0.1 (-0.5 to 0.8)	p=0.59
Fatigue	5.4 ± 7.0 vs 4.2 ± 6.8	1.2 (-1.5 to 3.8)	p=0.01
Subjective wellbeing	2.7 ± 10.1 vs 4.7 ± 9.3	2 (-5.6 to 1.6)	p=0.274
	Exercise 3 >hrs/wk (93) vs <3hrs/wk (33)		
Cough	0.5 ± 1.3 vs 1.6 ± 2.4	1.1 (0.2 - 1.9)	p=0.02
Fatigue	4.1 ± 6.4 vs 7.6 ± 7.8	3.5 (0.3 - 6.7)	p=0.03
Subjective wellbeing	2.7 ± 6.4 vs 8.0 ± 11.4	5.3 (-9.7 to -0.9)	p=0.02

Safety and adverse events: The assessment of safety was based on the frequency of adverse events reported by the investigator in the Case Report File. The level of adverse events attributable to the probiotics were very low (see table 7) with only two (1.5%) patients reported mild increased bloating and diarrhoea.

Table 6: Adverse events attributable to nutritional intervention

Adverse event	Number (Percentage of 126)	NCI toxicity (Severity)
Increased bloating	2 (1.5%)	1 (Mild)
Increased diarrhoea	2 (1.5%)	1 (Mild)
Increased indigestion	2 (1.5%)	1 (Mild)

At trial entry, it was also observed that, of the participants who were overweight or obese, 15% indicated they never exercised as opposed to 2% in the normal weight group. In the overweight or obese group, 15% ate meat less than 3 times a week compared to 28% in the normal weight group. Likewise, 15% of the overweight or obese participants added more than 2 spoons of sugar in their tea or coffee as opposed to 4% in the normal weight group.

Discussion:

This study highlights the wide variety of symptoms patients suffer following a Covid-19 infection and draws attention to the high prominence of those of gastro-intestinal (GI) origin. Self-reported GI symptoms of indigestion, bloating, nausea, diarrhoea and constipation were reported to have developed or increased from their usual level in over a quarter of patients at baseline. Previous publications have highlighted that patients with GI symptoms at presentation had worse non-GI symptoms, particularly fatigue, during their Covid episode, and had a greater risk of developing chronic on going symptoms[4, 5, 7, 8]. GI symptoms have been attributed to direct viral growth within the gut mucosa causing inflammation and dysbiosis in the gut bacterial flora[4, 5, 7, 8, 82, 8] .

This intervention, which aimed to improve gut health with a combination of lactobacillus probiotics and inulin prebiotics, demonstrated a clear improvement in fatigue, cough, subjective wellbeing and self-reported GI symptoms after their initiation. For patients

within the early phase of an infection (less than 30 days), this result was largely expected as most patients improve within a month of an acute infection, although the same cannot with said for participants with chronic persistent Covid-related symptoms[80,81]. In this cohort, participants had ongoing symptoms for an average of 120 days months pre-entry and therefore, improvement in symptoms seen within 30 days of the intervention, would unlikely to have happened spontaneously. This improvement was considered clinically relevant and welcomed by participants. This finding supports similar benefits from probiotic interventions, reported in patients, mainly with other respiratory tract viral infections, but more recently in patient with Covid[1,4, 8, 55, 75, 79, 78, 83]. Furthermore, it supports findings from the Kings app study which established a link between people who took probiotics and a lower risk of Covid[78] .

In terms of a subgroup differences, patients admitted to hospital had a greater improvement after this intervention compared to non-hospitalised patients. A possible explanation for this is that, gut dysbiosis is aggravated by treatments such as dexamethasone and antibiotics, often administered to patients during their hospital Covid management, so we suggest that an intervention to improve gut health has an even greater impact on these patients [88, 89] . Likewise, benefit from this intervention was greater in the older participants and those who exercised less, also factors known to be linked to less favourable gut microfloral profile [47, 91]. Males had statistically significantly better benefit to the intervention than females which is a clinically relevant factor as it has previously been reported that males have worse outcomes after Covid[90]. Our data, therefore, adds weight to the discussion that underlying variance in gut microbiota could be an explanation for this gender difference[92].

The mechanism of action of the probiotics, for non-GI symptoms, was not addressed in this study but the improvement in respiratory symptoms may be explained by improvement in the gut-lung axis highlighted in the introduction [13, 14, 15, 16 17] . The cause of fatigue, common during a viral infection and particularly Covid, is not certain. Some postulate there is an evolutionary advantage for fatigue within the innate immune related response as it reduces movement and interaction with other people and hence spread of the virus [93]. It is well known that poor gut health has a link to chronic fatigue [87, 94, 95, 96] . Previous interventions with lactobacillus supplements reduced fatigue related behaviour in laboratory animals [65]. In humans, there have been reported improvements in fatigue and memory following probiotic interventions which also demonstrated reductions in systemic inflammatory cytokines and improvements in gut integrity [65, 95, 96] . Some studies have shown a microbial-neuroendocrine relationship between certain dysbiotic flora species and the resultant adverse change in hormones and neurotransmitters such as acetylcholine and gamma-amino butyrate (GABA), serotonin and dopamine and that administration of lactobacillus probiotics helped reverse these changes [94, 95, 96, 97, 98, 99]. Although these are all a possible mechanisms, further research is required to establish whether these biochemical changes are responsible for the profound fatigue in those with long Covid.

Going forward, these data suggests that, in terms of Covid, greater emphasis should be placed on eating behaviour, nutritional factors and exercise which improve gut biodiversity. Various studies have shown these factors include stopping smoking, reducing processed sugar, exercising more, eating more live probiotic bacteria within yogurt, kefir, sauerkraut and kimchi, eating more fermentable soluble fibres such as inulin, oligosaccharides and beta-glucans found in chicory, artichoke, grains, beans and mushrooms and prebiotic polyphenols found in nuts, onions, fruit pomegranate and herbs [22, 53, 54, 89] . In addition, this study suggest that concentrating elements of these foods into capsules has a beneficial role. Nutritional supplement are certainly a convenient way to boost probiotic and prebiotic intake throughout the day. Many laboratory and some human studies that show probiotic capsules improve mirocrofloral biodiversity, improve immune efficiency, correct GI symptoms and modify a range of chronic diseases [21, 28, 34, 35, 36] . Other intervention trials have shown they can reduce the incidence of upper respiratory tract viral and flu like infections[38, 39, 40, 41, 42, 43, 44]. We believe, our data is one of the first to report the potential benefit for patients with symptoms post Covid.

In terms of safety, thousands of studies, have reported a high safety record amoung millions of healthy people who have consumed probiotic capsules for years, particularly the lactobacillus varieties [97, 98, 99, 100, 101, 102 , 103, 104, 105, 106]. More relevant to this study, the intake of lactobacillus probiotics has been shown to be particularly safe and beneficial amoung patients with several different medical condition including those on chemotherapy, those with irritable bowel syndrome and premature infants as well as elderly and even immunocompromised patients, hence a rationale for their inclusion in this study[105, 106, 107]. We add to this data by reporting this lactobacillus blend was safe in patients who had ongoing symptoms following a Covid infection. Only two patients discontinued their capsules because of an increase in bloating although it was not certain whether this was related to the intervention.

Data from this study would have been more robust if a randomised design was used, however, despite the high number of Covid cases in our hospital at the start of the trial, it became clear that participants would buy their own probiotics, over the counter, negating the any comparative data. Considering this factors and following feedback from patients, the trials committee decided to provide the probiotic to all participants, with the design limitations acknowledged.

Conclusion

This study highlights the wide range of symptoms people suffer following a Covid infection, in particular those related to GI tract. As it is known that patients with Covid with GI symptoms and other factors linked to gut dysbiosis have more severe and more persistent of symptoms, it would be wise to encourage lifestyle and nutritional factors which improve the gut microbiome. In addition, this study strongly suggests that this specific probiotic supplement, enhances recovery from Covid especially for individuals who

have symptoms or conditions suggestive of poor gut health. This blend of pro and prebiotics was safe and well tolerated, is now freely available as an over the counter supplement known as yourgutplus+ but it must be noted that it is classified as a food so cannot be licenced or prescribed by medical practitioners. Further research on this trial cohort will evaluate whether the addition of a phytochemical rich whole food supplement will further enhance the benefits of this intervention which will be reported separately. Going forward, our research group are now designing a study to evaluate whether yourgutplus+ could also enhance antibody titres post Covid vaccination.

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Declaration of interest
This was a non-commercial trial and no direct funding has been received from external organisations although the probiotics was supplied free of charge to the trials unit as mentioned above. The research team involved in the study were not being paid to recruit patients into the study, had no other financial incentives and have no connection with the manufactures. There are no intellectual patent issues on any of the investigational products as these are freely available over-the-counter. Information generated by the trial will be published in the public domain and the authors have no other conflict of interest.

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Supplementary information

Cough Symptom Score

DAYTIME COUGH

0 No cough during the day
1 Cough for one short period
2 Cough for more than two short periods
3 Frequent coughing, which did not interfere with usual daytime activities
4 Frequent coughing, which did interfere with usual daytime activities
5 Distressing coughs most of the day

NIGHT TIME COUGH

0 No cough during the night
1 Cough on waking only
2 Wake once or early due to cough
3 Frequent waking due to choughs
4 Frequent coughs most of the night
5 Distressing coughs preventing any sleep

Subjective Well-Being (SWB)

Overall, how satisfied are you with your life nowadays?

0 1 2 3 4 5 6 7 8 9 10

Not at all **Completely**

Overall, to what extent do you feel that the things you do in your life are worthwhile?

0 1 2 3 4 5 6 7 8 9 10

Not at all **Completely**

Overall, how happy did you feel yesterday?

0 1 2 3 4 5 6 7 8 9 10

Not at all **Completely**

Overall, how anxious did you feel yesterday?

0 1 2 3 4 5 6 7 8 9 10

Not at all **Completely**

We would like to know more about any problems you have had with feeling tired, weak or lacking in energy in the last month. Please answer ALL the questions by ticking the answer which applies to you most closely. If you have been feeling tired for a long while, then compare yourself to how you felt when you were last well. Please tick only one box per line.

	less than usual	**no more than usual**	**more than usual**	**much more than usual**
do you have problems with tiredness?				
do you need to rest more?				
do you feel sleepy or drowsy?				
do you have problems starting things?				
do you lack energy?				
do you have less strength in your muscles?				
do you feel weak?				
do you have difficulties concentrating?				
do you make slips of the tongue when speaking?				
do you find it more difficult to find the right word?				
	better than usual	**no worse than usual**	**worse than usual**	**much worse than usual**
how is your memory?				