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1 **Supportive therapy during COVID-19: the proposed mechanism of short-**
2 **chain fatty acids to prevent cytokine storm and multi-organ failure**

3
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14
15 **Abstract**

16 The world is currently facing the COVID-19 pandemic that is taking a heavy toll on several
17 countries. While many infected patients have a good prognosis, in some cases the progression
18 can be serious and even lead to death. The commonly seen complications are a cytokine storm
19 and multi-organ failure that require intensive care. The mortality of critically ill patients
20 depends on age, sex, immune state or co-morbidities. There is an urgent need to discover a
21 biomarker to identify early on patients at risk of developing serious complications and to find
22 an effective treatment that could prevent disease progression and critical states. Recent
23 investigations have pointed to the possible contribution of intestinal dysbiosis to the
24 pathophysiology of COVID-19. Herein, we hypothesize that butyrate, a short-chain fatty acid

25 initially produced by the gut microbiota, could be administered as supportive therapy to prevent
26 immune system activation and disease progression.

27

28 **Keywords**

29 COVID-19, cytokine storm, multi-organ failure, intestinal dysbiosis, short-chain fatty acids,
30 butyrate

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BACKGROUND

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54 The coronavirus disease (COVID-19) pandemic for which the first case was reported in
55 December 2019 in Wuhan (China), is a highly contagious and life-threatening viral infection.
56 For the period from December 2019 to June 2021, it has already affected more than 176 million
57 people and caused more than 3.8 million deaths worldwide according to the World Health
58 Organization (1).

59

Clinical presentation

61 The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which is responsible for
62 COVID-19 is a new member of the *Coronaviridae* family. Transmission mostly occurs via
63 respiratory droplets and aerosols from person-to-person. Once in the respiratory tract, the virus
64 attaches to its specific receptor site, the angiotensin I converting enzyme 2 (ACE-2) and its
65 entry into cells then requires transmembrane protease serine 2 (TMPRSS2) expressed by
66 pulmonary epithelial cells. The virus penetrates host cells for replication followed by viral
67 particles assembling and release of the new virions (2).

68 The clinical spectrum of COVID-19 ranges from asymptomatic, moderate, severe illnesses, up
69 to critical states. The most frequent symptoms are fever, dry cough, dyspnea and fatigue that
70 can be associated to myalgia, rhinorrhea, vomiting and cephalgia. Most of the infected patients
71 have a good prognosis but in some cases, progression can become severe, possibly leading to
72 death. The commonly seen complications in critically ill patients are acute respiratory distress
73 syndrome (ARDS), sepsis, disseminated intravascular coagulation, acute liver or kidney
74 injuries and pulmonary embolism. In rare cases, multisystem inflammatory syndrome,

75 rhabdomyolysis, autoimmune hemolytic anemia and neurological complications have been
76 observed (3,4). The mortality of critically ill patients depends on age (more than 65 years old),
77 sex (male), co-morbidities (*e.g.*, metabolic syndrome, diabetes, cerebrovascular, cardiac or
78 pulmonary diseases) and immune state (5,6). Furthermore, SARS-CoV-2 associated risk is
79 increased with respect to seasonal influenza since its transmission rate is nearly three times
80 higher (7,8). [Fortunately, influenza vaccine is associated with a better clinical outcomes and
81 presents no harmful effect on COVID-19 susceptibility \(9\).](#)

82 Severe prognosis is correlated to the so-called “cytokine storm” which is frequently observed
83 in critically ill patients. Such an event is defined by a huge increase of pro-inflammatory
84 cytokines (namely interleukin (IL)-1 β , IL-6, IL-12, interferon (IFN)- α/β , tumor necrosis factor
85 (TNF)- α) and chemokines (namely C-C motif chemokine ligand (CCL)2, CCL3, CCL5). A
86 well-coordinated innate immune response is the first line of defense against pathogens and plays
87 a crucial role in the prevention of SARS-CoV-2 spreading (10). However, dysregulated and/or
88 excessive immune responses have been shown to fail at clearing the virus and in turn, can even
89 contribute to the pathogenesis of ARDS as well as multi-organ failure (3). The excessive
90 production of cytokines results in tissular infiltration of inflammatory cells (*e.g.*, macrophages
91 and neutrophils); which favors (i) the alteration of the alveolar-capillary barrier, (ii) lung tissue
92 damage (vascular leakage, alveolar oedema) and (iii) hypoxia associated with ARDS (11). The
93 cytokine storm also plays a central role in extrapulmonary multi-organ failure (heart, kidney
94 and liver) (12). Moreover, decrease in natural killer (NK) and T cell populations ascribed to
95 lymphocytopenia have been also observed and correlated to disease severity (13,14). The
96 cytokine storm promotes tissue homing of immune cells and thrombosis. Moreover, a post-
97 COVID syndrome leading to multi-organ sequelae is associated to immunologic aberrations
98 and inflammatory damages. This syndrome encompass a spectrum of pulmonary, hematologic,
99 cardiovascular, neuropsychiatric, renal, endocrine, dermatologic and gastrointestinal injuries.

100

101 ***Current pharmacological treatment of the cytokine storm***

102 Since the beginning of the pandemic, many antiviral agents have been used or are still under
103 clinical evaluation for COVID-19 (e.g., lopinavir-ritonavir or darunavir-cobicistat
104 combinations, remdesivir, favipiravir, camostat mesylate-nafamostat) (15). However, once
105 immunologic complications occur, the use of standalone antiviral treatment is not sufficient. It
106 should be combined with anti-inflammatory treatments to manage the cytokine storm as well
107 as anti-coagulative drugs. Regarding the cytokine storm mechanism, the current therapeutic
108 approaches consist in: (i) supplementing with IFN- λ to potentiate innate immunity, (ii) using
109 immunomodulators (e.g., corticosteroids, intravenous immunoglobulins) to restore immune
110 balance, (iii) inhibiting cytokine productions (e.g., IL-1 or IL-6 antagonists, TNF blockers, IFN-
111 α/β inhibitors, ulinastatin, oxidase phospholipids, sphingosine-1-phosphate receptor 1 agonists
112 and stem cell therapy), (iv) scavenging cytokines (hemofiltration), (v) inhibiting mononuclear
113 macrophage recruitment and action (toll-like receptor (TLR) 7 antagonist and C-C motif
114 chemokine receptor type (CCR) 2 blockers), and/or (vi) strengthening the vascular barrier by
115 activating the endothelial Slit-Robot4 signal pathway (12,15,16).

116 The period of treatment administration for SARS-CoV-2 is crucial. Indeed, an early exposure
117 of the aforementioned strategies may inhibit the onset of the host's immune response, for
118 example if drugs are administered too early. However, the best strategy is to act before the
119 production of pro-inflammatory cytokines. For instance, montelukast commonly used in the
120 treatment of persistent asthma has recently been proposed for COVID-19 treatment in an
121 intensive care unit (17). Montelukast can inhibit cytokine production through the inhibition of
122 the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) signaling pathway,
123 which down-regulates pro-inflammatory mediators. This approach seems promising since the

124 blockade of cytokine production might be efficient in preventing pro-inflammatory
125 consequences.

126

127

THE HYPOTHESIS

128

129 In the present article, we aim to propose a new strategy dedicated to block the immune system
130 overactivation. It is worth mentioning that after virus recognition, macrophages polarize
131 towards the pro-inflammatory M1 phenotype. M1 macrophages actively produce pro-
132 inflammatory cytokines which in turn drive viral replication and facilitate spreading of
133 pathogens in the patient and tissue damage (18). Therefore, a very promising therapeutic
134 alternative could be to modulate the differentiation of macrophages in order to orient them
135 towards the anti-inflammatory M2 phenotype.

136

Indirect proof of macrophages M1/M2 ratio skewing in critically ill COVID-19 patients

138 Macrophages are major regulators of inflammatory response and also play a central role in host
139 defense (19). They are featured by an important cell heterogeneity and plasticity. Indeed, in
140 reaction to microenvironmental stimuli, macrophages can adopt different functional programs.
141 Macrophages can either differentiate through a pro-inflammatory (M1) or anti-inflammatory
142 (M2) phenotype (18). M1 macrophage polarization is mediated by IFN- γ , TNF- α or
143 lipopolysaccharides stimulation. M1 transformation hallmarks are both the inducible nitric
144 oxide synthase (iNOS) expression and the pro-inflammatory cytokines IL-1 β , IL-6, IL-12, IFN-
145 γ , TNF- α and chemokines CCL2, CCL3, CCL4, CCL5, CCL8 production. This leads to
146 pathogens destruction for which the time-window of inflammatory processes usually ranges
147 from hours to days, but it has been shown that it can last for months or years, resulting in non-
148 resolving inflammation. On the other hand, M2 macrophage polarization involves other

149 interleukins stimulation (namely IL-4 and IL-13) and promotes up-regulation of genes
150 associated to tissue repair and remodeling (e.g., *arginase 1 (arg1)*) (18). M2 macrophages exert
151 their anti-inflammatory response *via* some specific cytokines like IL-1, IL-13, tumor growth
152 factor (TGF)- β and chemokines CCL1, CCL2, CCL13 (18). Therefore, M1 and M2
153 macrophages can easily be distinguished by their cytokine profiles as well as the expression of
154 cell surface markers.

155 M1/M2 macrophages balance is finely regulated by specific signaling pathways. NF- κ B and
156 signal transducer and activator of transcription (STAT) 1 signaling pathways lead to M1
157 phenotype, while STAT3, STAT6 and PPAR γ signaling pathways favor the M2 phenotype (19).
158 An imbalance between these two phenotypes has been already observed in chronic
159 inflammatory diseases such as asthma, chronic obstructive pulmonary disease or
160 atherosclerosis (20,21).

161 According to cytokines and chemokines profiles at the systemic level of critically ill patients
162 (e.g., IL-1 β , IL-6, IL-12, TNF- α and CCL5), it has been suggested that macrophages involved
163 in the cytokine storm have the pro-inflammatory M1 phenotype (22). However, such an
164 observation still needs to be validated in clinical samples.

165

166 ***Intestinal dysbiosis, macrophages polarization and arguments for supportive therapy with***
167 ***short-chain fatty acids***

168 Clinical characteristics of patients who develop severe forms of COVID-19 consistently suggest
169 the contribution of intestinal dysbiosis. These patients are those with specific risk factors in
170 relation to unhealthy gut microbiome status (23). Moreover, COVID-19 infection itself is
171 associated to the alteration of the gut microbiome characterized by an enrichment of
172 opportunistic organisms and depletion of beneficial commensals (24,25). Therefore, there is a
173 link between gut microbiota alterations and the pathophysiology of COVID-19. Maintaining a

174 healthy microbiome status is a possible strategy to tame COVID-19 severity. Gut microbiota is
175 known as a key factor of host immune system homeostasis and its composition has been found
176 depleted with bacteria associated to immunomodulatory effects in some COVID-19 patients
177 (24). Furthermore, intestinal dysbiosis has been linked to the decrease of main pro-resolving
178 mediators. *Faecalibacterium prausnitzii*, a butyrate-producing bacteria, has been found
179 depleted in critically ill patients (26). This has also been associated to a pro-inflammatory state
180 featured by (i) a decrease of regulatory T cells and (ii) polarization of macrophages into the M1
181 phenotype (27).

182 Short-chain fatty acids (SCFAs) belong to the pro-resolving mediators that participate in the
183 dynamic host-microbiome network to regulate immune response. SCFAs are produced by the
184 gut microbiota from the anaerobic fermentation of indigestible polysaccharides such as dietary
185 fibers and resistant starches. Acetate, propionate and butyrate are key since they ensure the
186 communication between the host and the microbiome, playing an important role in host
187 homeostasis (28). SCFAs can act on various immune cells in the gut to inhibit inflammation
188 through multiple mechanisms. For instance, they modulate the differentiation of regulatory T
189 cells or inhibit histone deacetylase (HDAC). They can also specifically activate some G protein-
190 coupled receptors (GPCRs, *e.g.*, FFAR2, FFAR3) in order to enhance the intestinal barrier
191 function and to modulate the immune system. Moreover, butyrate mainly triggers the metabolic
192 shift in macrophages towards anti-inflammatory M2 phenotype *via* the inhibition of HDAC3
193 (28). In other words, SCFAs may provide a better control of the inflammatory response,
194 suggesting that their therapeutic properties are worth exploring.

195

196 ***Focus on the systemic anti-inflammatory effects of butyrate***

197 SCFAs are first absorbed by intestinal epithelial cells and are then metabolized in the liver.
198 Only a small fraction reaches the systemic circulation. However, it has been suggested that

199 systemic SCFAs are protective against allergic asthma (29). Butyrate can inhibit adhesion of
200 eosinophils to the endothelium and is known to improve allergen-induced inflammation in mice
201 (29). Moreover, HDAC inhibitors are protective against vascular inflammation and
202 atherosclerosis by modulating the endothelial function and production of pro-inflammatory
203 cytokines, particularly with butyrate. SCFAs also improve kidney function after acute kidney
204 injury induced by ischemia-reperfusion (28). Butyrate has been thoroughly evaluated as HDAC
205 inhibitor and pointed as the most pharmacologically active of SCFA (30). HDAC inhibition by
206 butyrate promotes the inhibition of lipopolysaccharide-induced production of nitric oxide (*via*
207 iNOS) and pro-inflammatory cytokines (IL-6, IL-12) release (30). More interestingly, butyrate
208 has shown inhibition of NF- κ B signaling pathway and facilitates the expression of anti-
209 inflammatory cytokines such as IL-10 in mononuclear cells and neutrophils (31). Recently, it
210 has been shown in gut epithelial organoids and in some gastric cells lines that butyrate can
211 downregulate genes essential for SARS-CoV-2 infection (*Ace2*, *Tmprss2*) and can also
212 upregulated other antiviral pathways (32,33). Altogether, all these studies provide strong
213 evidence that SCFA and especially butyrate exhibit a worth-investigating anti-inflammatory
214 action that might be used to prevent or alter the course of cytokine storm in critically ill COVID-
215 19 patients.

216

217 ***Butyrate supplementation for preventing the cytokine storm***

218 Similar to the observations made on experimental models of viral infection (e.g., Influenza A),
219 we propose that the inflammatory response during COVID-19 involves an increased M1/M2
220 macrophage phenotype ratio (34). The M1 phenotype is likely to be associated to the activation
221 of the NF- κ B pathway and the pro-inflammatory cytokine production (e.g., IL-6, IL-12, TNF-
222 α). This could at least partially explain the observed excessive cytokine release. Therefore, a
223 relevant alternative is to restore the M1/M2 balance ratio by favoring M2 macrophage

224 differentiation. Butyrate supplementation is expected to help polarize macrophages into M2
225 phenotype, which in turn might decrease pro-inflammatory cytokine production. Butyrate is
226 proposed to inhibit the NF- κ B pathway by preventing proteasomal degradation of the NF- κ B
227 inhibitor, namely I κ B. Ji et al. elegantly demonstrated that butyrate facilitates M2 macrophage
228 polarization by HDAC inhibition and STAT6 signaling pathway activation. Furthermore, they
229 observed an upregulation of M2 markers such as *arg1* and *found in inflammatory zone 1 (fizz1)*
230 (35). Furthermore, butyrate is known to prevent excessive neutrophil recruitment into the
231 airways via a GPCR-dependent receptor and by an alteration of the CXCL1 synthesis, which is
232 a neutrophil chemoattractant produced by macrophages (34). In other words, butyrate
233 supplementation could also limit inflammatory cell infiltration in the airways, which in turn
234 might reduce tissue and vascular disruption. In addition, butyrate also stimulates hematopoiesis
235 by favoring the development of macrophage progenitors with the M2 phenotype (36). Through
236 the expression of the transcription factor *forkhead box protein P3 (foxp3)* butyrate also
237 promotes regulatory T cells, which modulate the immune system and secrete anti-inflammatory
238 cytokines (IL-10). Butyrate is also known to limit the thrombo-inflammation phenomenon by
239 activating the tissue-plasminogen activator (t-PA), thus likely to play a role in anti-coagulation
240 COVID-19 strategies (**Figure 1**).

241

242 **EVALUATION OF THE HYPOTHESIS**

243

244 ***Supportive therapeutic strategy with SCFA***

245 The cytokine storm plays a critical role in COVID-19 pathophysiology depending on
246 macrophage activation. Supplementation with SCFAs and especially butyrate could help to
247 modulate the immune system activation early on. Our idea is to systematically associate SCFAs
248 serum profiling, cytokine dosage and/or M1/M2 macrophages ratio determination in each

249 patient positive for COVID-19 at the early stages of the disease. In face of the absence of
250 validated borderlines of these tests in specific cohort of COVID-19 patients, investigations will
251 be performed according to limits routinely applied for other pathologies. This might help to
252 detect intestinal dysbiosis and identify patients at risk of developing severe immune
253 complications that could benefit from this supportive therapy. In addition to clinical
254 characteristics, these parameters could also serve to elaborate a strong predictive score of
255 COVID-19 progression and to initiate early butyrate supplementation as a supportive treatment
256 in such detected vulnerable patients (**Figure 2**).

257

258 *Preclinical proof-of-concept of butyrate as supportive therapy*

259 Recently, butyrate treatment shows beneficial effects in influenza-induced mice by preventing
260 excessive neutrophil recruitment into the airways (34). These results have driven the hypothesis
261 that butyrate could serve as a supportive therapy during COVID-19 infection. Preclinical
262 investigations performed with gut epithelial organoids or gastric cell lines have shown down-
263 regulation of SARS-CoV-2 entry genes under butyrate treatment (32,33). Another level of
264 proof-of-concept could be reached by investigating the effects of butyrate supplementation in
265 a relevant mouse model of SARS-CoV-2 infection. This could be achieved with mice
266 expressing human ACE2 enzyme, inoculated with SARS-CoV-2 that develop ARDS within
267 five days (37).

268

269 *Clinical trial design*

270 Our hypothesis could be tested through a randomized double-blind prospective trial. This trial
271 would enroll patients tested positive for SARS-CoV-2 by reverse transcription polymerase
272 chain reaction (RT-PCR) and who are at risk of progressing to severe COVID-19 (older patients
273 with confirmed co-morbidities like heart disease, hypertension, chronic respiratory disease,

274 obesity, diabetes). After signing the informed consent, patients would be randomized to either
275 “butyrate” or “placebo” groups. Patients would be proposed either butyrate capsules or placebo
276 on a daily basis and for a fortnight. A remote telehealth monitoring service for clinical events
277 would be performed from diagnosis to the final outcome. This would include the use of
278 validated specific and general health questionnaires to document disease progression. Blood
279 samples would be collected before treatment initiation and at 7 and 14 days after, for SCFA
280 profiles and cytokine dosage. The primary endpoint could be a combined score aggregating
281 clinical adverse events, severity markers, sequential organ failure assessment, the need and
282 duration of hospitalization, time until virus negativation or death due to SARS-CoV-2 infection
283 **(Figure 3)**.

284

285

CONCLUSION

286

287 The cytokine storm acts as a critical factor in the occurrence of severe-to-death patients with
288 COVID-19. This event is mostly defined by the unbalanced increase of circulating pro-
289 inflammatory cytokines, which in turn may lead to multi-organ failure. To counterpose this
290 phenomenon, we propose an early identification of patients at risk by investigating
291 macrophages polarization. The decision to supplement with butyrate will be made on the basis
292 of SCFA profiles, cytokine dosage and M1/M2 macrophage ratio. SCFAs and mainly butyrate
293 can be considered as pro-resolving mediators due to their already known role in the immune
294 response modulation. It is important to note that pro-resolving mediators have the major
295 advantage of not blocking the initial immune response but rather downregulating the process to
296 resolve inflammation, which is crucial during COVID-19.

297

298

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307 **Consent statement/Ethical approval**

308 Not required

309

310 **Conflict of interest**

311 There are no competing interests to declare.

312

313

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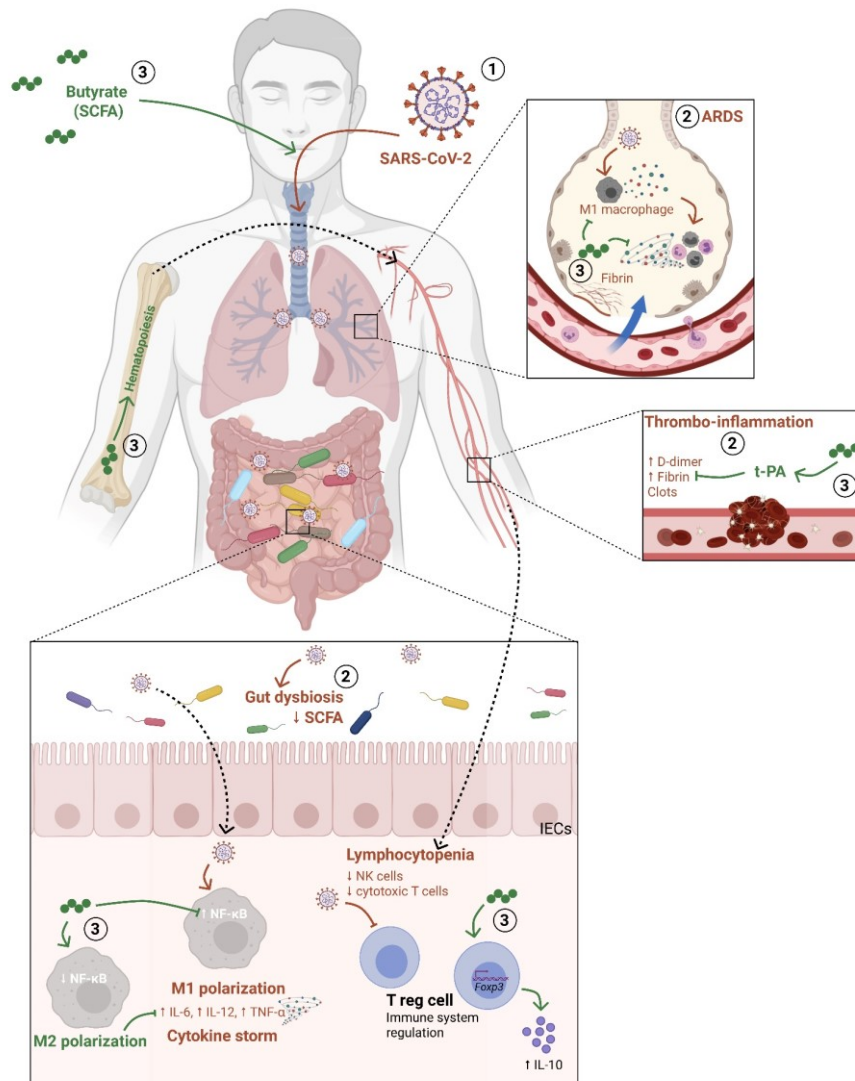
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439 **Figure 1: The proposed mechanism of short-chain fatty acids to prevent cytokine storm**
 440 **and multi-organ failure**

441 (1) SARS-CoV-2 enters and colonizes the host organism mainly through the airways. (2)

442 Intestinal dysbiosis is found in patients with severe forms of COVID-19. Lymphocytopenia is

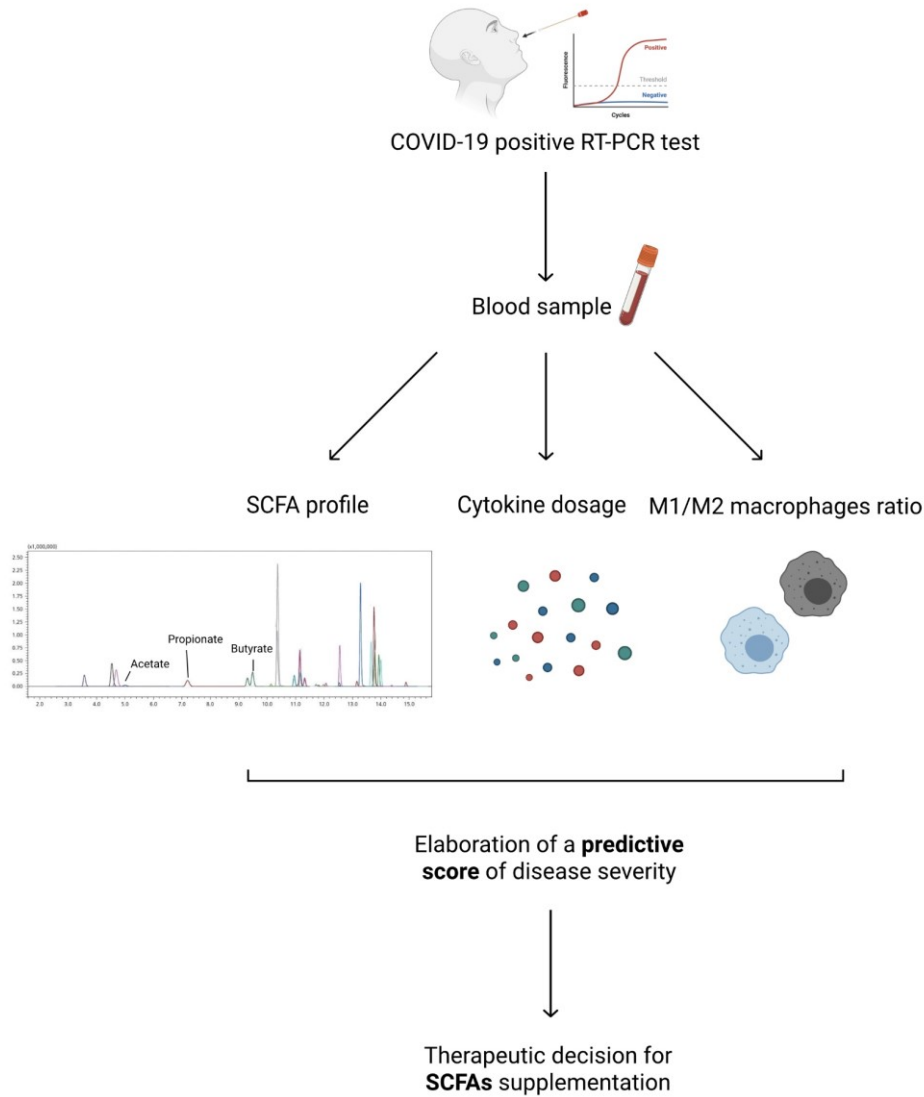
443 also observed and SARS-CoV-2 inhibits regulatory T cells and leads to activation of the NF-

444 κB signalling pathway which promotes the cytokine storm. (3) Supplementation with butyrate

445 could help to limit the cytokine storm through the inhibition of the NF-κB pathway and

446 promotion of anti-inflammatory M2 macrophages. Moreover, butyrate could restrain the

447 thrombo-inflammation phenomenon by activating the t-PA. Created with Biorender.com



448

449 **Figure 2: Path for the decision of supportive therapy with SCFA**

450 From the blood sample of a person tested positive for COVID-19, we propose to perform

451 plasma SCFA profiling, relevant cytokine dosage and M1/M2 macrophages ratio at early stages

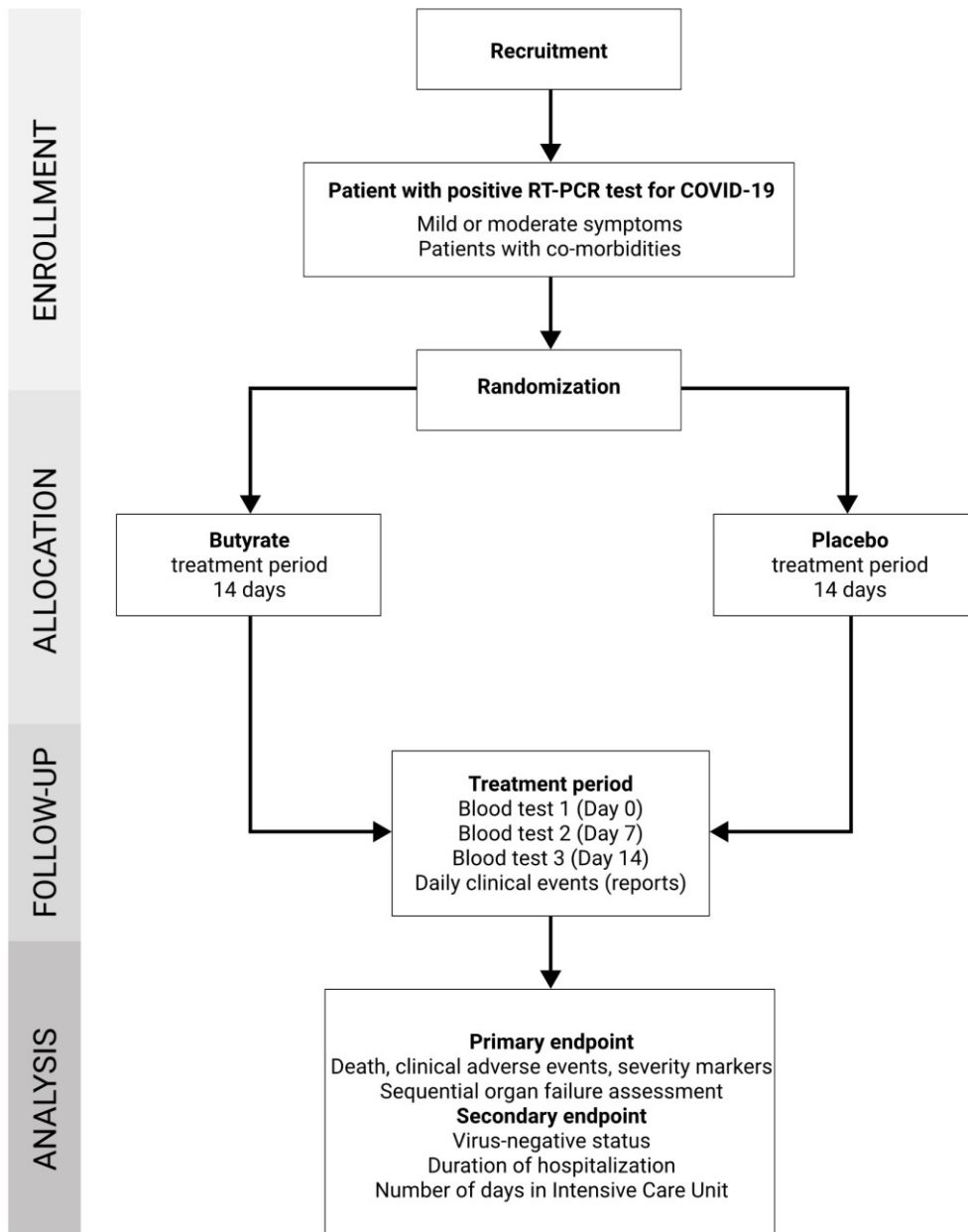
452 of the disease. These data will serve as inputs to build a predictive score of disease severity or

453 progression and to decide SCFAs supplementation.

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458 **Figure 3: Flowchart of the proposed clinical trial**

459 This clinical trial will enrol patients positive for COVID-19 (RT-PCR test) at mild to moderate
 460 stage who have co-morbidities. After randomization, blood samples will be collected at
 461 different endpoints for SCFA profile and cytokine dosage. The primary endpoint could combine
 462 clinical adverse events, disease severity markers, sequential organ failure assessment, the need
 463 and duration of hospitalization, time until virus negativation or death due to SARS-CoV-2
 464 infection.