Original Article

The Greek study in the effects of colchicine in COvid-19 complications prevention (GRECCO-19 study): Rationale and study design

Spyridon G. Deftereos [1, *], Gerasimos Siasos [1], Georgios Giannopoulos [2],
Dimitrios A. Vrachatis [1, 3], Christos Angelidis [1], Sotiria G. Giotaki [1], Panagiotis Gargalianos [4],
Helen Giamarellou [1, 5], Charalampos Gogos [6], Georgios Daikos [1, 7], Marios Lazanas [8],
Pagona Lagiou [1], Georgios Saroglou [1, 9], Nikolaos Sipsas [1], Sotirios Tsiodras [1],
Dimitrios Chatzigeorgiou [10], Nikolaos Moussas [4], Anastasia Kotanidou [1], Nikolaos Koulouris [1],
Evangelos Oikonomou [1], Andreas Kaoukis [2], Charalampos Kossyvakis [2], Konstantinos Raisakis [2],
Katerina Fountoulaki [11], Mihalis Comis [11], Dimitrios Tsiachris [4], Eleni Sarri [1],
Andreas Theodorakis [12], Luis Martinez-Dolz [13], Jorge Sanz-Sánchez [3, 13], Bernhard Reimers [3],
Giulio G. Stefanini [3], Michael Cleman [14], Dimitrios Filippou [1], Christoforos D. Olympios [11],
Vlasios N. Pyrgakis [2], John Goudevenos [15], George Hahalis [6], Theofilos M. Kolettis [15],
Efstathios Iliodromitis [1], Dimitrios Tousoulis [1, *], Christodoulos Stefanadis [1, 4, *]

[1] Medical School, National & Kapodistrian University of Athens, Athens, Greece
[2] General Hospital of Athens "G.Gennimatas" Hospital, Athens, Greece
[3] Humanitas Clinical and Research Institute, Rozzano, Milan, Italy
[4] Athens Medical Center, Athens, Greece
[5] Therapeutirion Hygeia, Athens, Greece
[6] Medical School, University of Patras, Patra, Greece
[7] Mitera Hospital, Athens, Greece
[8] Metropolitan General Hospital, Athens, Greece
[9] Metropolitan Hospital, Athens, Greece
[10] Hellenic Air Force General Staff
[11] General Hospital of Elefsina "Thriasio", Elefsina, Greece
[12] Missolonghi General Hospital "Chatzikosta", Missolonghi, Greece
[13] Hospital Universitario y Politécnico La Fe, Valencia, Spain
[14] Yale University School of Medicine, New Haven, CT, USA
[15] University of Ioannina, Ioannina, Greece

ARTICLE INFO

ABSTRACT

Objective: Colchicine has been utilized safely in a variety of cardiovascular clinical conditions. Among its potential mechanisms of action is the non-selective inhibition of NLRP3 inflammasome which is thought to be a major pathophysiologic component in the clinical course of patients with COVID-19. GRECCO-19 will be a prospective, randomized, open-labeled, controlled study to assess the effects of colchicine in COVID-19 complications prevention.

Methods: Patients with laboratory confirmed SARS-CoV-2 infection (under RT PCR) and clinical picture that involves temperature >37.5 oC and at least two out of: i. sustained coughing, ii. sustained throat pain, iii. Anosmia and/or ageusia, iv. fatigue/tiredness, v. PaO2<95 mmHg will be included. Patients will be randomised (1:1) in colchicine or control group.

Results: Trial results will be disseminated through peer-reviewed publications and conference presentations.

* Corresponding author. Spiridon Deftereos, Rimini 1, Attikon Hospital, National and Kapodistrian University of Athens, Greece.
E-mail address: spdeftereos@gmail.com (S.G. Deftereos).
Peer review under responsibility of Hellenic Society of Cardiology.
* Equal contribution.

Conclusion: GRECCO-19 trial aims to identify whether colchicine may positively intervene in the clinical course of COVID-19. (ClinicalTrials.gov Identifier: NCT04326790).

1. Introduction

The COVID-19 pandemic crisis has literally altered the way scientific knowledge is to be discovered and diffused. It is our strong belief that moving into this new era, we should also implement a paradigm shift in research: we should move from open-data to open-research-ideas. In fact, in a time when reliable "cross-checked" results are needed in a time-efficient manner, multiple-input multimodal collaborative projects (a medical research GitHub, if one dares the simile) may be the answer. Therefore, we opted to publish the rationale and design of the present study prior to obtaining its first results.

2. Background

Colchicine is a drug administered for years in various patient settings. It is a classic treatment for acute pericarditis[1]. Moreover, in the recent ESC guidelines it is suggested that it may be administered for the prevention of post-pericardiotomy syndrome[1] and in a consensus document it is suggested that it may be used for prevention of atrial fibrillation recurrence after cardiac surgery or ablation procedures[2]. Beyond these, colchicine has been administered in numerous research protocols with various clinical settings (including acute myocardial infarction) showing a favorable safety profile and promising results[3,4].

As far as patients with COVID-19 are concerned, first reports indicate that myocardial injury is frequently present. In a study from Wuhan, China, in 191 patients, cardiac necrosis biomarkers' elevation was found to be an independent predictor of need for mechanical respiratory support[5]. In another study from Wuhan, which included 150 patients with COVID-19, 7% of deaths was attributed in myocarditis with circulatory collapse, while in 33% myocarditis had played a crucial role in the final adverse outcome[6].

Patients with COVID-19 often develop acute respiratory distress syndrome and/or acute lung injury (ARDS/ALI). Fulminant pulmonary inflammation provokes diffuse alveolar injury resulting in pulmonary infiltrations and, clinically, in acute respiratory failure and it has been reported that myopericarditis is observed in a substantial proportion of hospitalized patients[5].

In experimental models it has been shown that inflammasome NLRP3 is a major pathophysiological component in the development of ARDS/ALI[7–10], while structural dry-lab models have shown that in the new SARS-CoV-2 proteins such as viroporins E, 3a and 8A play a substantial role in viral replication and pathogenetic sequelae[11]. Additionally, there are data supporting that these three proteins provoke the activation of inflammasome NLP3[12–16].

Furthermore, SARS-CoV-2 entry in cells is dependent on the connection of viral proteins S with cellular receptors and activation of viral proteins by proteases of host-cells[17,18]. Therefore, factors that may have an effect on clathrin-mediated endocytosis (a procedure that is – in part – regulated by microtubules remodeling[19]) would potentially decelerate viral infection of cells[20].

Colchicine is a lipid-soluble alkaloid[21], which after oral administration is absorbed in the jejunum and Ileum with maximum plasma concentrations achieved after 1-2 hours (after a single p.o.

dose). Maximum anti-inflammatory effect develops within a time frame of 24-48h. This time period is required for drug accumulation in granulocyes and monocytes, in which concentrations are several times higher than plasma concentrations[22]. Indeed, it remains there for several days after last administration[23].

Colchicine binds to unpolymerized tubulin heterodimers, forming a stable complex that effectively inhibits microtubule dynamics upon binding to microtubule ends[24]. Moreover, colchicine is a non-selective inhibitor of NLRP3 inflammasome[25]. While initially it has been thought of merely as an inhibitor of microtubule polymerization and leucocyte infiltration, it is now presumed that a significant part of colchicine anti-inflammatory action is attributed to inhibition of the NLRP3 inflammasome[26]. Colchicine inhibits inflammasome on two levels: it inhibits P2X7 receptor activation and ASC polymerization, thereby inhibiting interaction between pyrin-like domains[27]. Additionally, colchicine suppresses the transport of mitochondria and subsequent approximation of ASC to NLRP3, indicating that microtubules mediated the transport of mitochondria to create optimal sites for activation of the NLRP3 inflammasome[28]. Colchicine has been shown to limit IL-1b production as a response to various NLRP3 inflammasome inducers in a dose-dependent fashion. For example, in the setting of acute coronary syndrome, colchicine was effective in suppressing interleukin IL-1b, IL-18 and IL-6, which was attributed to inflammasome inhibition[29,30].

In terms of side-effects, colchicine has been safely administered to patients with myocardial infarction both in the acute phase and later on, in the chronic post-myocardial infarction period[4,31].

3. Research hypothesis

Based on the aforementioned data, the question which arises is whether colchicine, administered in a relatively low dose, could potentially have an effect the patients' clinical course by limiting the myocardial necrosis and pneumonia development in the context of COVID-19. If present, this effect would be attributed to its potential to inhibit inflammasome and (less probably) to the process of SARS-CoV-2 endocytosis in myocardial and endothelial respiratory cells.

4. Patient population

Patients with laboratory confirmed SARS-CoV-2 infection (under RT PCR) and clinical picture that involves body temperature >37.5 oC and at least two out of the: i. sustained coughing, ii. sustained throat pain, iii. anosmia and/or ageusia, iv. fatigue/tiredness, v. PaO2<95 mmHg.

5. Exclusion criteria

Pregnancy; known hypersensitivity to colchicine; known hepatic failure; eGFR<20 ml/min; clinical estimation that the patient will require mechanical respiratory support in less than 24 hours; any clinical estimation of the attending physician under which the patient shall be excluded; QTc >450 msec.

6. Study design

Prospective, randomized, open labeled, controlled study. Patients will be randomized in two groups (A and B). Patients of group-A will be treated under optimal treatment based on the algorithm proposed in Greece (as per Ministerial Committee decisions) while in group-B patients on top of this colchicine will be also administered.

6.1. Treatment Arm

On top of usual medical treatment, a loading dose (p.o) of colchicine 1.5 mg (followed 60 min later by 0.5 mg if no adverse gastrointestinal effects are observed) will be administered followed by 0.5 mg colchicine twice a day (except for patients weighting <60 kg in whom 0.5 mg colchicine will be administered once daily). In the case of concurrent treatment with azithromycin as per local protocol then the loading dose of colchicine will be reduced to 1 mg and the maintenance dose will remain unchanged.

6.2. Control Arm

Usual medical treatment only.

7. Endpoints

BIOCHEMICAL "PHASE"

- Time for CRP levels that exceeds > 3xUNL
- Difference in maximum high-sensitivity troponin within 10 days from treatment

8. Clinical "PHASE"

8.1. Primary

Time to clinical deterioration (criterion: 2 levels in WHO R&D Blueprint scale[32–34]).

8.2. Secondary

- Percentage of patients who will require mechanical ventilation
- Mortality
- Function Lung Tests in healed patients

9. Sample size

For the clinical phase time-dependent univariate COX regression analysis (power 0.8, alpha 0.05) a total of 180 patients was found to be required to detect a hazard reduction of 50% (i.e. hazard ratio = 2 for the group A)[35].

Due to the pandemic crisis, data will be analyzed every 20 patients who complete the study.

10. Ethics and dissemination

Local Ethics approval has already been obtained in Greece and submission is in process in other participating countries. Trial results will be disseminated through peer-reviewed publications and conference presentations.

11. Trial registration number

ClinicalTrials.gov Identifier: NCT04326790

12. Conclusion

GRECCO-19 trial aims to identify whether colchicine may positively intervene in the clinical course of COVID-19.

Conflict of interest

No conflict of interest exists.

Acknowledgements

References

1. The 2015 ESC Guidelines on the diagnosis and management of pericardial diseases. PubMed - NCBI https://www.ncbi.nlm.nih.gov/pubmed/26547486. Accessed March 25, 2020.
2. Calkins H, Hindricks G, Cappato R, et al. HRS/EHRA/ECAS/APHRS/SOLAECE expert consensus statement on catheter and surgical ablation of atrial fibrillation: Executive summary. *J Arrhythmia*. 2017;33(5):369–409. https://doi.org/10.1016/j.joa.2017.08.001, 2017.
3. Hemkens LG, Ewald H, Gloy VL, et al. Cardiovascular effects and safety of long-term colchicine treatment: Cochrane review and meta-analysis. *Heart*. 2016;102(8):590–596. https://doi.org/10.1136/heartjnl-2015-308542.
4. Tardif JC, Kouz S, Waters DD, et al. Efficacy and safety of low-dose colchicine after myocardial infarction. *N Engl J Med*. 2019;381(26):2497–2505. https://doi.org/10.1056/NEJMoa1912388.
5. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. March 2020. https://doi.org/10.1016/s0140-6736(20)30566-3.
6. Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive Care Med*. March 2020. https://doi.org/10.1007/s00134-020-05991-x.
7. Grailer JJ, Canning BA, Kalbitz M, et al. Critical Role for the NLRP3 Inflammasome during Acute Lung Injury. *J Immunol*. 2014;192(12):5974–5983. https://doi.org/10.4049/jimmunol.1400368.
8. Li D, Ren W, Jiang Z, Zhu L. Regulation of the NLRP3 inflammasome and macrophage pyroptosis by the p38 MAPK signaling pathway in a mouse model of acute lung injury. *Mol Med Rep*. 2018;18(5):4399–4409. https://doi.org/10.3892/mmr.2018.9427.
9. Jones HD, Crother TR, Gonzalez-Villalobos RA, et al. The NLRP3 inflammasome is required for the development of hypoxemia in LPS/mechanical ventilation acute lung injury. *Am J Respir Cell Mol Biol*. 2014;50(2):270–280. https://doi.org/10.1165/rcmb.2013-0087OC.
10. Dolinay T, Kim YS, Howrylak J, et al. Inflammasome-regulated cytokines are critical mediators of acute lung injury. *Am J Respir Crit Care Med*. 2012;185(11):1225–1234. https://doi.org/10.1164/rccm.201201-0003OC.
11. Castaño-Rodriguez C, Honrubia JM, Gutiérrez-Álvarez J, et al. Role of Severe Acute Respiratory Syndrome Coronavirus Viroporins E, 3a, and 8a in Replication and Pathogenesis. *MBio*. 2018;9(3). https://doi.org/10.1128/mBio.02325-17.
12. Nieto-Torres JL, DeDiego ML, Verdiá-Báguena C, et al. Severe acute respiratory syndrome coronavirus envelope protein ion channel activity promotes virus fitness and pathogenesis. *PLoS Pathog*. 2014;10(5). https://doi.org/10.1371/journal.ppat.1004077. e1004077.
13. Nieto-Torres JL, Verdiá-Báguena C, Jimenez-Guardeño JM, et al. Severe acute respiratory syndrome coronavirus E protein transports calcium ions and activates the NLRP3 inflammasome. *Virology*. 2015;485:330–339. https://doi.org/10.1016/j.virol.2015.08.010.
14. Shi C-S, Nabar NR, Huang N-N, Kehrl JH. SARS-Coronavirus Open Reading Frame-8b triggers intracellular stress pathways and activates NLRP3 inflammasomes. *Cell death Discov*. 2019;5(1):101. https://doi.org/10.1038/s41420-019-0181-7.
15. Siu K-L, Yuen K-S, Castaño-Rodriguez C, et al. Severe acute respiratory syndrome coronavirus ORF3a protein activates the NLRP3 inflammasome by promoting TRAF3-dependent ubiquitination of ASC. *FASEB J*. 2019;33(8):8865–8877. https://doi.org/10.1096/fj.201802418R.
16. Chen I-Y, Moriyama M, Chang M-F, Ichinohe T. Severe Acute Respiratory Syndrome Coronavirus Viroporin 3a Activates the NLRP3 Inflammasome. *Front Microbiol*. 2019;10(JAN):50. https://doi.org/10.3389/fmicb.2019.00050.
17. Walls AC, Park Y-J, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell*. March 2020. https://doi.org/10.1016/j.cell.2020.02.058.

18. Morales L, Oliveros JC, Fernandez-Delgado R, tenOever BR, Enjuanes L, Sola I. SARS-CoV-Encoded Small RNAs Contribute to Infection-Associated Lung Pathology. *Cell Host Microbe*. 2017;21(3):344−355. https://doi.org/10.1016/j.chom.2017.01.015.

19. Kaksonen M, Roux A. Mechanisms of clathrin-mediated endocytosis. *Nat Rev Mol Cell Biol*. 2018;19(5):313−326. https://doi.org/10.1038/nrm.2017.132.

20. Stebbing J, Phelan A, Griffin I, et al. COVID-19: combining antiviral and anti-inflammatory treatments. *Lancet Infect Dis*. February 2020. https://doi.org/10.1016/S1473-3099(20)30132-8.

21. Cerquaglia C, Diaco M, Nucera G, La Regina M, Montalto M, Manna R. Pharmacological and clinical basis of treatment of Familial Mediterranean Fever (FMF) with colchicine or analogues: An update. *Curr Drug Targets - Inflamm Allergy*. 2005;4(1):117−124. https://doi.org/10.2174/1568010053622984.

22. Chappey ON, Niel E, Wautier JL, et al. Colchicine disposition in human leukocytes after single and multiple oral administration. *Clin Pharmacol Ther*. 1993;54(4):360−367. https://doi.org/10.1038/clpt.1993.161.

23. Molad Y. Update on colchicine and its mechanism of action. *Curr Rheumatol Rep*. 2002;4(3):252−256. https://doi.org/10.1007/s11926-002-0073-2.

24. Deftereos S, Giannopoulos G, Papoutsidakis N, et al. Colchicine and the heart: pushing the envelope. *J Am Coll Cardiol*. 2013;62(20):1817−1825. https://doi.org/10.1016/j.jacc.2013.08.726.

25. Toldo S, Abbate A. The NLRP3 inflammasome in acute myocardial infarction. *Nat Rev Cardiol*. 2018;15(4):203−214. https://doi.org/10.1038/nrcardio.2017.161.

26. Leung YY, Yao Hui LL, Kraus VB. Colchicine-Update on mechanisms of action and therapeutic uses. *Semin Arthritis Rheum*. 2015;45(3):341−350. https://doi.org/10.1016/j.semarthrit.2015.06.013.

27. Marques-da-Silva C, Chaves MM, Castro NG, Coutinho-Silva R, Guimaraes MZP. Colchicine inhibits cationic dye uptake induced by ATP in P2X2 and P2X7 receptor-expressing cells: implications for its therapeutic action. *Br J Pharmacol*. 2011;163(5):912−926. https://doi.org/10.1111/j.1476-5381.2011.01254.x.

28. Misawa T, Takahama M, Kozaki T, et al. Microtubule-driven spatial arrangement of mitochondria promotes activation of the NLRP3 inflammasome. *Nat Immunol*. 2013;14(5):454−460. https://doi.org/10.1038/ni.2550.

29. Martínez GJ, Robertson S, Barraclough J, et al. Colchicine Acutely Suppresses Local Cardiac Production of Inflammatory Cytokines in Patients With an Acute Coronary Syndrome. *J Am Heart Assoc*. 2015;4(8). https://doi.org/10.1161/JAHA.115.002128. e002128.

30. Robertson S, Martínez GJ, Payet CA, et al. Colchicine therapy in acute coronary syndrome patients acts on caspase-1 to suppress NLRP3 inflammasome monocyte activation. *Clin Sci (Lond)*. 2016;130(14):1237−1246. https://doi.org/10.1042/CS20160090.

31. Deftereos S, Giannopoulos G, Angelidis C, et al. Anti-Inflammatory Treatment With Colchicine in Acute Myocardial Infarction: A Pilot study. *Circulation*. 2015;132(15):1395−1403. https://doi.org/10.1161/CIRCULATIONAHA.115.017611.

32. WHO | Coronavirus disease (COVID-2019) R&D. https://www.who.int/blueprint/priority-diseases/key-action/novel-coronavirus/en/. Accessed March 25, 2020.

33. Wang Y, Fan G, Salam A, et al. Comparative Effectiveness of Combined Favipiravir and Oseltamivir Therapy Versus Oseltamivir Monotherapy in Critically Ill Patients With Influenza Virus Infection. *J Infect Dis*. December 2019. https://doi.org/10.1093/infdis/jiz656.

34. Cao B, Wang Y, Wen D, et al. A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19. *N Engl J Med*. March 2020. https://doi.org/10.1056/NEJMoa2001282.

35. Chow S, Shao J, Wang H. No Title. In: *Sample Size Calculations in Clinical Research*. 2nd Ed. Chapman & Hall/CRC Biostatistics Series.; 2008.