Cytokine storm and colchicine potential role in fighting SARS-CoV-2 pneumonia

Antonio Vitiello,1 Francesco Ferrara,1 Chiara Pelliccia,2 Giovanni Granata,1 Raffaele La Porta4
1Usl Umbria 1, Perugia; 2Usl Umbria 2, Terni; 1Asl Salerno, Salerno; 4Asur Marche, Ancona, Italy

ABSTRACT

For some patients with SARS-CoV-2, the worst clinical damage is not caused by the virus itself, but by an overactive inflammatory state. In fact, in some people the immune system goes into overdrive and launches a large-scale assault on the tissue known as cytokine storm. This excessive inflammatory/immune reaction can damage tissue and eventually kill people. Evidence shows that blocking such cytokine storms can be effective and trials are under way to test drugs that act by reducing cytokine response, such as tocilizumab and sarilumab which bind interleukin 6 (IL-6), or anakinra which is the interleukin 1 receptor antagonist (IL-1). However, other drugs that block the cytokine cascade can also be considered. In this article we describe the scientific and molecular motivation for the use of drugs that act by modulating the hyperactive inflammatory system in severe patients suffering from SARS-CoV-2, considering in particular an old drug that has been in use for many years for other therapeutic indications such as colchicine, and that could be favorable to its use, with low cost and good tolerability.

Introduction

In December 2019, a group of pneumonia cases occurred in Wuhan, China, caused by a newly identified coronavirus (SARS-CoV-2). This coronavirus rapidly spread to China and other countries causing a global pandemic. SARS-CoV-2 is a β-coronavirus, virus that can lead to serious and potentially fatal respiratory tract infections. It was found that the genomic sequence of SARS-CoV-2 shares 79.5% of the identity with the SARS-CoV responsible for the epidemic in 2003. It is now known that SARS-CoV-2 could use the angiotensin 2 conversion enzyme (ACE2), the same receptor as SARS-CoV, to infect humans and penetrate cells.

Clinical characteristics of patients infected with SARS-CoV-2

Based on ongoing epidemiological investigations, the incubation period of the virus is 1-14 days, mostly 3-7 days. SARS-CoV-2 is contagious during the latency period. Based on the knowledge acquired during these months the infection has been divided into three phases, the first asymptomatic or with mild symptoms, the second and third characterized by hyperactive inflammatory state responsible for lung lesions and that in some patients can rapidly develop acute respiratory distress syndrome, respiratory failure, multiple organ failure, and even death.

The most common clinical manifestations recorded in the three stages of infection are fever, cough, fatigue, dyspnea, sore throat. In addition, some patients experience gastrointestinal symptoms such as diarrhea and vomiting.

Elderly people or those with underlying diseases (e.g. hypertension, chronic obstructive pulmonary disease, diabetes, cardiovascular disease) are at greater risk of rapidly developing acute respiratory distress syndrome, septic shock, metabolic acidosis and clotting dysfunction, which quickly lead to death. Laboratory tests vary depending on the stages of infection, in the most severe stages neutrophil count, D-dimer, blood urea and creatinine are significantly higher and...
Inflammatory cytokine storm in patients with severe SARS-CoV-2

Phase two and three of the infection are characterized by a hyperactive inflammatory state that can result in a cytokine storm (CS). CS refers to the excessive and uncontrolled release of pro-inflammatory cytokines. The syndrome can be caused by a variety of diseases, including infectious diseases, rheumatic diseases and cancer immunotherapy such as CAR-T. Clinically, it presents as systemic inflammation, multiple organ failure and elevated inflammatory parameters. In infectious diseases, CS usually originates from the infected focal zone, spreading throughout the body through circulation. The accumulation of evidence has revealed that a percentage of patients with severe SARS-CoV-2 have a high cytokine profile similar to CS. Studies have reported the level of inflammatory factors in patients with SARS-CoV-2 during CS, cytokine levels in 41 hospitalized patients (including 13 intensive care and 28 non-intensive care patients), IL-1, IL-7, IL-8, IL-9, IL-10, fibroblast growth factor (FGF), granulocyte and macrophage colony stimulating factor (GM-CSF), IFNγ, tumor necrosis factor (TNFα), vascular endothelial growth factor (VEGF) were significantly increased, including IL-2, IL-7, IL-10, G-CSF, TNFα were higher in severe patients. Several other studies confirmed increased levels of IL-6 in severe SARS-CoV-2 patients. One study analyzed lymphocyte subsets and cytokines in 123 patients, all patients had lymphocytopenia, the percentage of reduction in CD8+ T lymphocytes was 28.43% and 61.9% in mild and severe groups respectively, and the reduction in NK cells was 34.31% and 47.62% in mild and severe groups respectively.

In addition, serum levels of IL-6 in the severe group were significantly higher than in the mild group. It is possible that CS aggravates lung damage and leads to other fatal complications. At this stage, the markers of systemic inflammation therefore appear to be extremely high. Therefore, blocking CS and knowing when to start anti-inflammatory therapy is essential to reduce the mortality rate of SARS-CoV-2.

Probably in phase one of the infection the inflammatory/immune response is important to fight the virus, in phase two and three a multi-organ systemic inflammation is probably responsible for the worsening of the health condition, in these phases, therefore, it could be useful to act with CS blocking agents.6-20

Therapeutic approaches to reduce cytokine storm

Based on the above, new strategies to attenuate inflammatory responses are likely to improve clinical outcomes in SARS-CoV-2 patients in stages two and three of infection. Here we describe the agents that have the potential to reduce inflammation and CS virus-induced. Undoubtedly, antiviral and supportive treatments are very important. The CS is relatively common in severe cases and often leads to fatal lung lesions, anti-inflammatory therapy can help prevent these damages. As we know, there is a variety of anti-inflammatory and immunomodulatory drugs, including nonsteroidal anti-inflammatory drugs, glucocorticoids, chloroquine/hydroxychloroquine, immunosuppressants, cytokine inflammatory antagonists (such as IL-6 inhibitor monoclonal antibodies, TNF inhibitors, IL-1 inhibitors, Janus kinase inhibitors). Evidence suggests that the use of immunomodulatory agents reduces systemic inflammation before causing multi-organ dysfunction. At this stage, the use of corticosteroids may also be justified in combination with the use of cytokine inhibitors such as tocilizumab (IL-6 inhibitor) or anakinra (IL-1 receptor antagonist). However, is there a dilemma of anti-inflammatory and immunomodulatory therapy? If so, when and at what doses? All these questions are still the subject of intense debate and do not reach a consensus. The main concern is that anti-inflammatory/immunomodulatory drugs, such as corticosteroid, may delay the elimination of the virus and increase the risk of secondary infections, especially in those with compromised immune systems. Second, biological agents targeting pro-inflammatory cytokines can only inhibit a specific inflammatory factor, and therefore may not be very effective in stopping CS in SARS-CoV-2, where other cytokines may be of significant importance. Finally, the time window of anti-inflammatory treatment is very important. According to the evidence, severe patients usually suffered a sharp deterioration in 1-2 weeks after the onset of infection, early initiation of anti-inflammatory therapy in this extremely short time window is likely to achieve a favorable response to treatment and avoid a worsening of the condition. During the SARS epidemic in 2003, glucocorticoid was the main drug of immunomodulatory therapy. However, some studies have shown no beneficial ef-
fect with glucocorticoid in SARS-Cov-2 infection leading to deterioration of the disease. In addition, there is no evidence of randomized clinical trials supporting glucocorticoid treatment for SARS-CoV-2. However, some evidence indicates that the benefit of glucocorticoid use is likely to outweigh the adverse effect. Among the interleukin inhibitors that have shown some evidence of efficacy there are IL-6 inhibitors. Tocilizumab is an antibody that binds specifically to IL-6 thus blocking its signaling and inflammatory mediated response. Tocilizumab is widely used in rheumatic diseases such as rheumatoid arthritis and on August 30, 2017, Tocilizumab was approved in the United States for severe life-threatening cytokine release syndrome caused by chimeric immunotherapy of T cell antigen receptors (CAR-T).

Recent trials demonstrate the efficacy of Tocilizumab in the treatment of phase two or three patients with SARS-CoV-2. Together with basic anti-virus treatment, Tocilizumab was applied to 20 patients 400 mg once intravenously. Within a few days, the fever returned to normal and the other symptoms improved significantly. The opacity of the lung lesion on Tocilizumab scans was absorbed in 90.5% of patients. In addition, the percentage of peripheral lymphocytes returned to normal in 52.6% of patients. Their data suggest that Tocilizumab could be an effective treatment in severe SARS-CoV-2. So far, several clinical studies have been recorded on the safety and efficacy of Tocilizumab in the treatment of severe SARS-CoV-2 pneumonia in adult hospitalized patients. To date, several clinical trials are under way in Italy to test its efficacy and safety in SARS-CoV-2 patients.

Chinese health authorities have approved the use of this interleukin 6 inhibitor drug in SARS-CoV-2 patients. Sarilumab belongs to the same class. It is a human monoclonal interleukin 6 receptor antibody for the treatment of rheumatoid arthritis, it has the same pharmacodynamic profile as tocilizumab and also shares the same tolerability profile, and the common side effects that occur in 1-10% of treated patients are lung tract infections, which could be an unfortunate factor for treatment in SARS-CoV-2 patients. Emapalumab is an anti-interferon-gamma antibody (IFNγ) used for the treatment of hemophagocytic lymphohistiocytosis (HLH). HLH causes excessive secretion of IFN-γ which contributes to the pathogenesis of the disease. Emapalumab binds and neutralizes IFN-γ, preventing it from inducing pathological effects Anakinra is a biopharmaceutical drug used for the treatment of rheumatoid arthritis. It is a recombinant and slightly modified version of the human interleukin 1 receptor antagonist protein. It also appears to be effective in the treatment of macrophage activation syndrome (MAS), a form of cytokine storm. A study is currently under way to study the efficacy and safety of Emapalumab and Anakinra in reducing hyperinflammation and respiratory distress in patients with SARS-CoV-2 infection. Chloroquine (CQ) and hydroxychloroquine (HCQ) are first-line drugs for the treatment and prophylaxis of malaria and are also used for the treatment of autoimmune diseases, including rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). Some studies have reported that QC/HCQ have a broad spectrum of antiviral effects on a variety of different viruses, such as human immunodeficiency virus (HIV), Marburg virus, and SARS-CoV-1. QC and HCQ may interfere with the binding of viral particles to the receptor of their cell surface or with the pH-dependent endosome-mediated viral input of wound viruses to inhibit the viral cycle. They may also interfere with post-translational modification of viral proteins or compromise proper viral protein maturation through pH modulation. In addition, CQ and HCQ can regulate the immune system by affecting cell signaling and the production of pro-inflammatory cytokines. Although QC or HCQ are often used for the treatment of rheumatic diseases due to its immunomodulatory and anti-inflammatory effects, the benefit in the treatment of SARS-CoV-2 can be attributed primarily to its antiviral effects. Recently, CQ and HCQ have been demonstrated in several studies to reduce the viral load of SARS-CoV-2 and shorten the duration of viremia. Whether their immunomodulatory effect also plays a role in the treatment of SARS-CoV-2 still requires further investigation.\textsuperscript{21-44}

Colchicine

One drug that could perhaps add value to counteract SARS-CoV-2 CS is colchicine.

Colchicine is used for the treatment of gout, Behçet’s disease, prevention and treatment of pericarditis and family Mediterranean fever, Sweet syndrome, scleroderma, and amyloidosis. Perhaps the most effective results of colchicine treatment have been obtained in family prophylaxis of Mediterranean fever. The scientific hypothesis of the use of colchicine in SARS-CoV-2 is based on the anti-inflammatory properties of the drug and on the already widely demonstrated efficacy of familial Mediterranean fever and in pericarditis, considering that these diseases also demonstrate a phase of hyperactivation of the inflammatory system. Recently published data on colchicine seem to suggest a potential synergism in the treatment at different trigger point levels of cytokine storm. In fact, colchicine acts by decreasing inflammation through multiple mechanisms. The main mechanism of action is to bind the tubulin molecule and thus inhibit its polymerization. In particular, its anti-inflammatory effect has been attributed to the decomposition of microtubules into neutrophils thus inhibiting their
migration. Furthermore, colchicine can also alter the distribution of adhesion molecules on the surface of both neutrophils and endothelial cells, leading to a significant inhibition of the interaction between white blood cells and endothelial cells interfering with their transmigration. Therefore, there is growing evidence that the anti-inflammatory effect of colchicine is multifaceted. Probably the main mechanism of action for cytokine reduction in patients with SARS-CoV-2 is the inhibition of IL-1, IL-6 and IL-18 interfering with the inflammatory protein complex NLRP3, a factor increasingly recognized for its role especially in recurrent idiopathic pericarditis and Mediterranean fever (Figure 1).

In addition, colchicine accumulates in white blood cells and affects them in various ways: by decreasing motility, loosening chemotaxis and adhesion, it inhibits the production of superoxide anions, interrupts the degranulation of mast cells. It is important to note that studies have shown that viroporin E, a component of the SARS-associated coronavirus (SARS-CoV), forms Ca2C-permeable ion channels and activates NLRP3 inflammation. In addition, another viroporin 3a has been shown to induce activation of NLRP3 inflammation. The mechanisms are unclear. Colchicine counteracts the increased inflammation of NLRP3, thus reducing the release of IL-1b and a number of other interleukins, including IL-6, the added value of this drug compared to IL-1 or IL-6 inhibitors is that it acts upstream of the cascade of cytokines and not only on one route, moreover at standard doses it shows a good tolerability profile. Several clinical trials are currently underway to study the efficacy of colchicine in patients with SARS-CoV-2, as shown in Table 1. In particular, two trials are underway in Italy to evaluate the efficacy and safety of colchicine in SARS-Cov-2 patients, the first Colchicine Counteracting Inflammation in COVID-19 Pneumonia (ColCOVID-19) and the second entitled Treatment with colchicine of patients affected by Covid-19: a pilot Study.44-68

Table 1. Ongoing trials with colchicine in SARS-Cov-2 patients (Clinicaltrials.gov).

| Study title                                                                 | Conditions                                      |
|---------------------------------------------------------------------------|-------------------------------------------------|
| Colchicine Coronavirus SARS-CoV2 Trial (COLCORONA)                        | Coronavirus infection                            |
| The GReek Study in the Effects of Colchicine in Covid-19                  | Coronavirus disease 19 (SARS-CoV-2)              |
| Colchicine Efficacy in COVID-19 Pneumonia                                 | Coronavirus infections pneumonia viral          |
| The ECLA PHRI COLCOVID TRIAL                                             | SARS-Cov-2 infection                            |
| Colchicine Counteracting Inflammation in COVID-19 Pneumonia (ColCOVID-19) | SARS-Cov-2                                      |
| Treatment with colchicine of patients affected by Covid-19: a pilot Study | SARS-Cov-2                                      |

Figure 1. Potential therapeutic targets in the NLRP3 inflammasome and IL-1, IL-18, IL-6.
Conclusions

Inflammation is an indispensable part of an effective immune response, without which it is difficult to successfully eliminate an infectious agent. The inflammatory response begins with the initial recognition of a pathogen, which then mediates the recruitment of immune cells, eliminates the pathogens and ultimately leads to tissue repair and return to homeostasis. However, some viruses such as SARS-CoV-2 induce an excessive and prolonged cytokine response, known as cytokine storms, which results in high morbidity and mortality due to immunopathology. Therefore, therapeutic interventions targeting these pro-inflammatory cytokines and chemokines could be useful to improve undesirable inflammatory responses. In addition, since high viral titers in the early and later stages of infection are strongly related to the severity of the disease in humans, strategies to control viral load and attenuate the inflammatory response may be useful. In conclusion, SARS-CoV-2 is a viral infectious disease that mainly manifests itself in fever and pneumonia, and antiviral therapies are certainly the mainstream, but we believe that treatments that reduce the cytokine response may be effective especially for more severe cases. In this way, biological agents targeting pro-inflammatory cytokines can only inhibit a specific inflammatory factor, and therefore may not be very effective in stopping CS in SARS-CoV-2 where other cytokines may be of significant importance. The colchicine could result in a therapeutic treatment that acts upstream of the cytokine cascade in IL-6 and IL-1, bringing more benefits, it is also low-cost and if used at the right doses with a good tolerability profile. Furthermore, there is a fundamental aspect to add, biological drugs (such as tocilizumab, sarilumab etc.) can, with reference to the RCP and evidence of the drugs, cause with a common frequency secondary infections of the respiratory tract and, therefore, paradoxically compromise the clinical situation of patients infected with SARS-CoV-2, therefore clinical evidence is needed to clarify their possible use and on which target of SARS-CoV-2 patients. For colchicine, however, with reference to the RCP, clinical study and pharmacovigilance data, the risk of upper respiratory tract infections may not be an issue. However, data on the viral nature of SARS-CoV-2 and considering substantial damage to the host’s immune system in severe cases, it is essential to balance the risk/benefit before starting anti-inflammatory therapy. Furthermore, the early anti-inflammatory treatment started at the right time is of fundamental importance and should be adapted to the individual patient to get the most out of it, however this would be an interesting area for future research, and data deriving from ongoing clinical studies will respond to our questions.

References

1. Ware LB, Matthay MA. The acute respiratory distress syndrome. N Engl J Med 2000;342:1334-49.
2. Goodman RB, Strieter RM, Martin DP, et al. Inflammatory cytokines in patients with persistence of the acute respiratory distress syndrome. Am J Respir Crit Care Med 1996;154:602-11.
3. Park WY, Goodman RB, Steinberg KP, et al. Cytokine balance in the lungs of patients with acute respiratory distress syndrome. Am J Respir Crit Care Med 2001;164:1896-903.
4. Donnelly SC, Strieter RM, Reid PT, et al. The association between mortality rates and decreased concentrations of interleukin-10 and interleukin-1 receptor antagonist in the lung fluids of patients with the adult respiratory distress syndrome. Ann Intern Med 1996;125:191-6.
5. Arend WP, Joslin FG, Thompson RC, Hannum CH. An IL-1 inhibitor from human monocytes: production and characterization of biologic properties. J Immunol 1989;143:1851-8.
6. Arend WP. Interleukin-1 receptor antagonist. Adv Immunol 1993;54:167-227.
7. Burger D, Chicheportiche R, Giri JG, Dayer JM. The inhibitory activity of human interleukin-1 receptor antagonist is enhanced by type II interleukin-1 soluble receptor and hindered by type I interleukin-1 soluble receptor. J Clin Invest 1995;96:38-41.
8. Pugin J, Ricou B, Steinberg KP, et al. Pro-inflammatory activity in bronchoalveolar lavage fluids from patients with ARDS, a prominent role for interleukin-1. Am J Respir Crit Care Med 1996;153:1850-6.
9. Olman MA, White KE, Ware LB, et al. Microarray analysis indicates that pulmonary edema fluid from patients with acute lung injury mediates inflammation, mitogen gene expression, and fibroblast proliferation through bioactive interleukin-1. Chest 2002;121:69S-70S.
10. Li XY, Donaldson K, Brown D, MacNee W. The role of tumor necrosis factor in increased airspace epithelial permeability. Am J Respir Cell Biol 1995;13:185-95.
11. Ertel W, Scholl FA, Gallati H, et al. Increased release of soluble tumor necrosis factor receptors into blood during clinical sepsis. Arch Surg 1994;129:1330-7.
12. Goldie AS, Fearon KCH, Ross JA, et al. Natural cytokine antagonists and endogenous antiendotoxin core antibodies in sepsis syndrome. JAMA 1995;274:172-7.
13. Van der Poll T, Jansen J, van Leenen D, et al. Release of soluble receptors for tumor necrosis factor in clinical sepsis and experimental endotoxemia. J Infect Dis 1993;168:955-60.
14. Abraham E, Glauser MP, Butler T, et al. P55 tumor necrosis factor receptor fusion protein in the treatment of patients with severe sepsis and septic shock: a randomized controlled multi-center trial. JAMA 1997;277:1531-8.
15. Armstrong L, Millar AB. Relative production of tumour necrosis factor alpha and interleukin-10 in adult respiratory distress syndrome. Thorax 1997;52:442-6.
16. Fumeaux T, Pugin J. Role of interleukin-10 in the intracellular sequestration of human leukocyte antigen-DR in monocytes during septic shock. Am J Respir Crit Care Med 2002;166:1475-82.
17. Bernhagen J, Calandra T, Bucala R. Regulation of the immune response by macrophage migration inhibitory factor: biological and structural features. J Mol Med 1998;76:151-61.

18. Nathan CF, Karnovsky ML, David JR. Alterations of macrophage functions by mediators from lymphocytes. J Exp Med 1971;133:1356-76.

19. Nathan CF, Remold HG, David JR. Characterization of a lymphocyte factor which alters macrophage function. J Exp Med 1973;137:275-88.

20. Donnelly SC, Haslett C, Reid PT, et al. Regulatory role for macrophage migration inhibitory factor in acute respiratory distress syndrome. Nat Med 1997;3:320-3.

21. Calandra T, Bernhagen J, Metz CN, et al. MIF as a glucocorticoid-induced modulator of cytokine production. Nature 1995;377:68-71.

22. Fowler AA, Hyers TM, Fisher BJ, et al. The adult respiratory distress syndrome. Cell populations and soluble mediators in the airspaces of patients at high risk. Am Rev Respir Dis 1987;136:1225-31.

23. McGuire WW, Spragg RG, Cohen AM, Cochrane CG. Studies on the pathogenesis of the adult respiratory distress syndrome. J Clin Invest 1982;69:543-53.

24. Weiland JE, Davis WB, Holter JF, et al. Lung neutrophils in the adult respiratory distress syndrome: clinical and pathophysiologic significance. Am Rev Respir Dis 1986;133:218-25.

25. Martin TR, Pistorese BP, Hudson LD, Maunder RJ. The function of lung and blood neutrophils in patients with the adult respiratory distress syndrome: implications of the pathogenesis of lung infections. Am Rev Respir Dis 1991;144:254-62.

26. Parsons PE, Fowler AA, Hyers TM, Henson PM. Chemotactic activity in bronchoalveolar lavage fluid from patients with adult respiratory distress syndrome. Am Rev Respir Dis 1985;132:490-3.

27. Ognibene FP, Martin SE, Parker MM, et al. Adult respiratory distress syndrome in patients with severe neutropenia. N Engl J Med 1986;315:547-51.

28. Maunder RJ, Hackman RC, Riff RE, et al. Occurrence of the adult respiratory distress syndrome in neutropenic patients. Am Rev Respir Dis 1987;135:313-6.

29. Zimmerman GA, Renzetti AD, Hill HR. Functional and metabolic activity of granulocytes from patients with adult respiratory distress syndrome. Am Rev Respir Dis 1983;127:290-300.

30. Li G, Fan Y, Lai Y, et al. Coronavirus infections and immune responses. J Med Virol 2020;92:424-32.

31. Comi P. Induction of pro-inflammatory cytokines (IL-1 and IL-6) and lung inflammation by Coronavirus-19 (COVI-19 or SARS-CoV-2): anti-inflammatory strategies. In: Knipe DM, Howley P (eds.), Fields Virology. Philadelphia, PA: Lippincott Williams and Wilkins; 2018. pp. 275-84.

32. Yam LY, Lau AC, Lai FY, et al. Wong Corticosteroid treatment of severe acute respiratory syndrome in Hong Kong J. Inf. Secur 2007;54:28-39.

33. Chen RC, Tang XP, Tan SY, et al. Treatment of severe acute respiratory syndrome with glucocorticoids: the Guangzhou experience. Chest 2006;129:1441-52.

34. Rhodes A, Evans LE, Alhazzani W, et al. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock: 2016. Intensive Care Med 2017;43:304-77.

35. Chan KW, Wong VT, Tang SCW. COVID-19: an update on the epidemiological, clinical, preventive and therapeutic evidence and guidelines of integrative Chinese-Western medicine for the management of 2019 novel coronavirus disease Am J Chin Med 2020;1:26.

36. Russell CD, Millar JE, Baille JK. Clinical evidence does not support corticosteroid treatment for 2019-nCoV lung injury. Lancet 2020;395:473-5.

37. Barnard DL, Day CW, Bailey K, et al. Evaluation of immunomodulators, interferons and known in vitro SARS-coV inhibitors for inhibition of SARS-coV replication in BALB/c mice. Antivir Chem Chemother 2006;17:275-84.

38. FDA Approves Gamifant® (emapalumab-lzsg), the First and Only Treatment Indicated for Primary Hemophagocytic Lymphohistiocytosis (HLH). Business Wire. Business Wire, Inc. Retrieved 21 November 2018.

39. Singh JA, Hossain A, Tanjong Ghogomu E, et al. Biologics or tofacitinib for rheumatoid arthritis in incomplete responders to methotrexate or other traditional disease-modifying anti-rheumatic drugs: a systematic review and network meta-analysis. Cochrane Datab Syst Rev 2016;(5): CD012183.

40. Kumar A, Liang B, Aarthy M, et al. Hydroxychloroquine inhibits Zika virus NS2B-NS3 protease. ACS Omega 2018;3:18132-41.

41. Wang LF, Lin YS, Huang NC, et al. Hydroxychloroquine-inhibited dengue virus is associated with host defense machinery. J Interf Cytokine Res 2015;35:143-56.

42. Akpovwa H. Chloroquine could be used for the treatment of filoviral infections and other viral infections that emerge or emerged from viruses requiring an acidic pH for infectivity. Cell Biochem Funct 2016;34:191-6.

43. Vincent MJ, Bergeron E, Benjannet S, et al. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. Virol J 2005;2:69.

44. Dong L, Hu S, Gao J. Discovering drugs to treat coronavirus disease 2019 (COVID-19). Drug Discov Ther 2020;14:58-60.

45. Benhamou J, Ori M, Shahar G, et al. The use of colchicine in rheumatoid arthritis: a systematic review and network meta-analysis. Cochrane Datab Syst Rev 2013;9:300-4.

46. Tsai T-L, Wei JC-C, Wu Y-T, et al. The association between usage of colchicine and pneumonia: a nationwide, population-based cohort study. Front Pharmacol 2019;10:908.

47. Shekelle PG, Newberry SJ, FitzGerald JD, et al. Management of gout: a systematic review in support of an American College of Physicians Clinical Practice Guideline. Ann Intern Med 2017;166:37-51.

48. Hutchison SJ. Pericardial diseases: clinical diagnostic imaging Atlas with DVD. Amsterdam: Elsevier Health Sciences; 2009. p. 58.

49. National Prescribing Service, Australia. Colchicine for acute gout: updated information about dosing and drug interactions; 14 May 2010. Archived from the original on 30 June 2012. Retrieved 14 May 2010 British national formulary: BNF 76 (76 ed.). Pharmaceutical Press; 2018. pp. 1085-1086.

50. Dalbeth N, Lauterio TJ, Wolfe HR. Mechanism of action of colchicine in the treatment of gout. Clin Ther 2014;36:1465-79.
51. Chen LX, Schumacher HR. Gout: an evidence-based re-
view. J Clin Rheumatol 2008;14:S55-62.
52. U.S. Food and Drug Administration. Colcrys (colchicine, USP) tablets 0.6 mg. Drug Approval Pack-
age; 17 February 2010; Retrieved 19 August 2018. Available from: https://www.accessdata.fda.gov/
drugsatfda_docs/nda/2009/022352_colcrys_toc.cfm
53. U.S. Food and Drug Administration. Information for Healthcare Professionals: New Safety Information for Colchicine (marketed as Colcrys); 07 October 2015. Available from: https://www.fda.gov/drugs/postmarket-drug-safety-information-patients-and-providers/colchicine-marketed-colcrys-information
54. Laubscher T, Dumont Z, Regier L, Jensen B. Taking the stress out of managing gout. Canad Family Phys 2009;55:1209-12.
55. van Echteld I, Wechalekar MD, Schlesinger N, et al. Colchicine for acute gout. Cochrane Datab Syst Rev 2014;8:CD006190.
56. Terkeltaub RA, Furst DE, Bennett K, et al. High versus low dosing of oral colchicine for early acute gout flare: Twenty-four-hour outcome of the first multicenter, randomized, double-blind, placebo-controlled, parallel-group, dose-comparison colchicine study. Arthritis Rheum 2010;62:1060-8.
57. Alabed S, Cabello JB, Irving GI, et al. Colchicine for pericarditis. Cochrane Datab Syst Rev 2014;8:CD010652.
58. Portincasa P. Colchicine, Biologic Agents and More for the Treatment of Familial Mediterranean Fever. The Old, the New, and the Rare. Curr Med Chem 2016;23:60-86.
59. Lennerz C, Barman M, Tantawy M, et al. Colchicine for primary prevention of atrial fibrillation after open-heart surgery: Systematic review and meta-analysis. Int J Cardiol 2017;249:127-37.
60. Centers for Disease Control and Prevention (CDC). The Emergency Response Safety and Health Database: Biotoxin: Colchicine. Centers for Disease Control and Prevention, US Department of Health and Human Serv-
ices; Retrieved 31 December 2015. Available from: https://www.cdc.gov/niosh/ershdb/emergencyresponse-card_29750016.html
61. Finkelstein Y, Aks SE, Hutson JR, et al. Colchicine poi-
sioning: the dark side of an ancient drug. Clin Toxicol 2010;48:407-14.
62. Doogee M. Colchicine - extremely toxic in overdose; 2014. Christchurch and Canterbury District Health Board, New Zealand; Retrieved 23 August 2018 Available from: https://bpac.org.nz/BPJ/2014/september/docs/BPJ63-safer-prescribing.pdf
63. Graham W, Roberts JB. Intravenous colchicine in the management of gouty arthritis. Ann Rheum Dis 1953;12:16-9.
64. US Food and Drug Administration. Colcrys (colchicine). Summary review for regulatory action. Center for Drug Evaluation and Research; 30 July 2009; Retrieved 19 August 2018. Available from: https://www.fda.gov/drugs/postmarket-drug-safety-information-patients-and-providers/colchicine-marketed-colcrys-information
65. Hartung EF. History of the use of colchicum and related medicaments in gout; with suggestions for further re-
search. Ann Rheum Dis 1954;13:190-200.
66. Pelletier and Caventou. Examen chimique des plusieurs végétaux de la famille des colchicées, et du principe acit qu'ils renferment. [Cévadille (veratrum sabadilla); hellébore blanc (veratrum album) ; colchique commun (colchicum autumnale)]. (Chemical examination of se-
veral plants of the meadow saffron family, and of the active principle that they contain.) Ann Chimie Physique 1820;14:69-81.
67. Geiger L. Ueber einige neue giftige organische Alkalien. (On some new poisonous organic alkalis). Ann Pharmacie 1833;7:269-80.
68. Cerquaglia C, Diaco M, Nucera G, et al. Pharmacological and clinical basis of treatment of Familial Mediterranean Fever (FMF) with colchicine or analogues: an update. Curr Drug Targets. Inflamm Allergy 2005;4:117-24.