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

A.Vitiello – F.Ferrara - C.Pelliccia - G.Granata - R. La Porta

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 immune reaction can damage tissue and eventually kill people.

Evidence shows that blocking such cytokine storms can be effective, so trials are underway to test drugs that act by reducing the cytokine response, such as tocilizumab and sarilumab that bind interleukin 6 (IL-6), or anikinra that is interleukin 1 (IL-1) receptor antagonist. However, other drugs that block the cytokine cascade may also be considered. In this article we describe the scientific and molecular motivation for the use of drugs that act by modulating the inflammatory system in patients affected by 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 result favorable its use, with low cost and good tolerability.

Introduction

In December 2019, a cluster of pneumonia cases, caused by a new identified coronavirus (SARS-CoV-2), occurred in Wuhan, China. The SARS-CoV-2 is a β-coronavirus, which is enveloped non-segmented positive-sense RNA virus and can lead to severe and potentially fatal respiratory tract infections. It was found that the genome sequence of SARS-CoV-2 shares 79.5% identity to SARS-CoV. Based on virus genome sequencing results and evolutionary analysis, bat has been suspected as natural host of virus origin, and SARS-CoV-2 might be transmitted from bats via unknown intermediate hosts to infect humans. It is clear now that SARS-CoV-2 could use angiotensin-converting enzyme 2 (ACE2), the same receptor as SARS-CoV, to infect humans.

Clinical characteristics of patients infected with SARS-CoV-2

Based on current 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. It is highly transmissible in humans, especially in the elderly and people with underlying diseases. Most adults or children with SARS-CoV-2 infection have mild flu-like symptoms and some patients are in critical condition because they rapidly develop acute respiratory distress syndrome, respiratory failure, multiple organ failure, even deaths. (1-12)

The most common clinical manifestations are fever (88.7%), cough (67.8%), fatigue (38.1%), sputum production (33.4%), dyspnea (18.6%), sore throat (13.9%) and headache (13.6%). In addition, some patients experienced gastrointestinal symptoms, with diarrhoea (3.8%) and vomiting (5.0%).

Clinical manifestations are as reported earlier, fever and cough, dominant symptoms, upper respiratory symptoms and gastrointestinal symptoms are rare, suggesting differences in viral tropism compared to SARS-CoV, MERS-CoV and influenza. In the results of laboratory tests, most patients have a normal or decreased white blood cell count and lymphocytopenia. But in severe patients, neutrophil count, D-dimer, blood urea and creatinine levels are significantly higher and the lymphocyte count continues to decrease. In addition, the inflammatory factors IL-6, IL-10, IL-1 tumor necrosis factor-α (TNF-α) increase, indicating the immune status of the patients. Data showed that patients have higher plasma levels than IL-2, IL-7, IL-10, granulocyte colony stimulation factor

(GCSF), 10 kD interferongamma-induced protein (IP-10), monocyte chemoattractant-1 protein (MCP-1), macrophage inflammatory protein 1-α (MIP-1α), and TNF-α. (13-20)

Inflammatory cytokine storm in patients with severe SARS-CoV-2

Cytokine storm (CS) refers to the excessive and uncontrolled release of pro-inflammatory cytokines. CS syndrome can be caused by a variety of diseases, including infectious diseases, rheumatic diseases and cancer immunotherapy. Clinically, it commonly 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 the circulation. In coronavirus pneumonia, SARS-CoV-2 accompanied by rapid virus replication, a large number of infiltrations of inflammatory cells and CS that led to acute lung lesions, acute respiratory distress syndrome (ARDS) and death. The accumulation of evidence revealed that a proportion of patients with severe SARS-CoV-2 have a high cytokinic profile resembling CS in SARS and MERS. Studies have reported the level of inflammatory factors in patients with SARS-CoV-2, cytokine levels in 41 hospitalized patients (including 13 in intensive care and 28 non-in intensive care patients), IL-1, IL-7, IL-8, IL-9, IL-10, fibroblast growth factor (FGF), granulocyte-macrophage colony stimulating factor (GM-CSF), IFNγ, granulocyte colony stimulating factor (G-CSF), interferon-γ-inducible protein (IP10), the monocyte chemoattractant protein (MCP1), inflammatory macrophage protein 1 alpha (MIP1A), platelet-derived growth factor (PDGF), tumor necrosis factor (TNFα), vascular endothelial growth factor (VEGF) were increased, including IL-2, IL-7, IL-10, G-CSF, IP10, MCP1, MIP1A, TNFα were higher in severe patients. In particular, there was no pronounced difference in serum IL-6 level in intensive care and non-in intensive care patients. However, in another retrospective multicentre cohort study, the same study group reported a significant increase in IL-6 level in the non-SARS-CoV-2 survivor group compared to the survivors. Several other reports confirmed the rise in IL-6 levels in severely ill patients with SARS-CoV-2. In severely ill patients with SARS-CoV-2, although patients with lymphocytopenia, lymphocytes were activated. A study analyzed lymphocyte subsets and cytokines in 123 patients, all patients had lymphocytopenia, the percentage of CD8 + T cell reduction was 28.43% and 61.9% in the mild and severe groups respectively, and the reduction of NK cells was 34.31% and 47.62% in the mild and severe groups respectively.(44-72) In addition, serum levels of IL-6 in the severe group were significantly higher than in the mild group. In addition, HLA-DR expression in CD4 + and CD8 + cells was increased, CD4 + CCR4 + CCR6 + Th17 cells were also increased, and cytotoxic particles such as perforin and granolysin were highly expressed in CD8 + T cells. It is possible that CS aggravates lung damage as well as leads to other fatal complications. At this stage, the markers of systemic inflammation therefore appear to be extremely high. Therefore, blocking CS and to known when to start anti-inflammatory therapy is essential to reduce the mortality rate of SARS-CoV-2. This result is consistent with the characteristics of the so-called "primary cytokine storm" induced by viral infection which were mainly produced by alveolar macrophages, epithelial cells and endothelial cells, rather than those observed in the "secondary cytokine storm" induced by several sub-sets of T lymphocytes activated in the final stage of viral infection or by a complication of therapies involving T cells.

There are two possible reasons for the destruction of the immune system in patients with SARS-CoV-2, lymphocytes directly invaded by the virus or indirectly damaged by CS. Since we know that SARS-CoV-2 infects target cells through the angiotensin 2 conversion enzyme (ACE2), while there was no ACE2 expression on lymphocytes, we assume that the lymphocytes were probably destroyed by CS. (73-125)

Colchicine

Colchicine is used for the treatment of gout, Behçet's disease. prevention of pericarditis and familial Mediterranean fever, Sweet's syndrome, scleroderma, amyloidosis and cirrhosis of the liver. It is

taken orally. Perhaps the most effective results of colchicine treatment have been obtained in family Mediterranean fever prophylaxis. The scientific hypothesis of the use of colchicine in SARS-CoV-2 is based on the antiinflammation properties of the drug. And recently published data on colchicine seems to suggest a potential synergic in treating at different level trigger point the cythokine 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 vitro microtubules. In particular, its anti-inflammatory effect has been attributed to its breakdown of microtubules into neutrophils, thus inhibiting their migration to chemotactic factors. 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 (WBCs) 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 cytokinic cascade reduction in SARS-CoV-2 patients is the inhibition of IL-1, IL-6 and IL-18 interfering with the inflammatory protein complex NLRP3,6-8 which is increasingly recognized for its role in acute coronary syndrome, crystal-induced gout and especially in recurrent idiopathic pericarditis. (Figure 1)
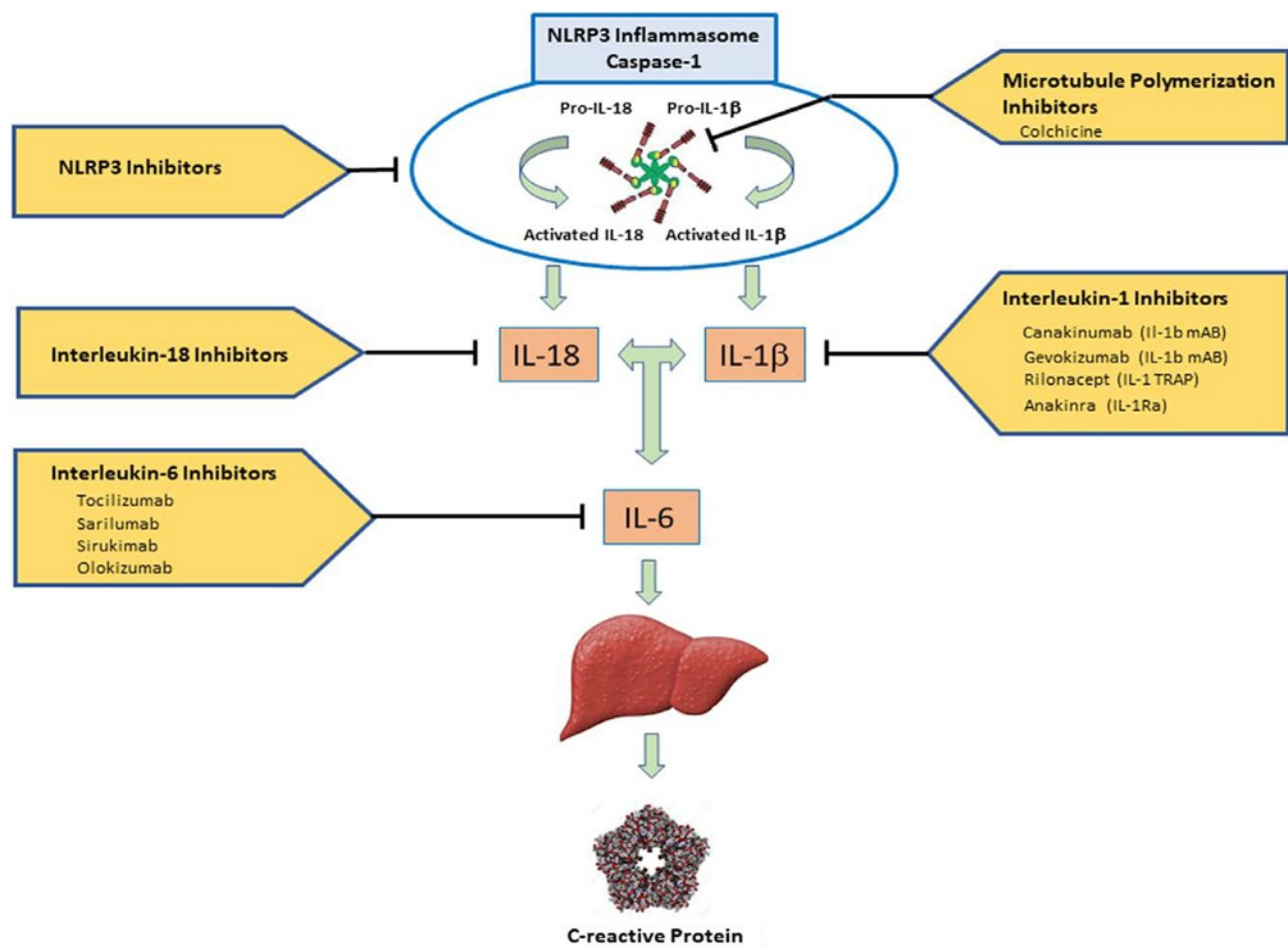

Figure 1. *Potential therapeutic targets in the NLRP3 inflammasome to interleukin-1 to interleukin-6 to C-reactive protein (CRP) signaling pathway*

Row	Study Title	Conditions
1	Colchicine Coronavirus SARS-CoV2 Trial (COLCORONA)	-Corona Virus Infection
2	The GReek Study in the Effects of Colchicine in Covid-19	-Corona Virus Disease 19 (SARS-Cov 2)
3	Colchicine Efficacy in COVID-19 Pneumonia	-Coronavirus Infections -Pneumonia, Viral
4	The ECLA PHRI COLCOVID TRIAL	- SARS-Cov-2

Table 1: *Trials on going with colchicine in SARS-Cov-2 patients.(Clinicaltrials.gov)*
Colchicine accumulates in white blood cells and affects them in various ways: by decreasing motility, loosening (especially chemotaxis) and adhesion. Then, the colchicine inhibits the production of superoxide anions in response to urate crystals, interrupts the degranulation of mast cells and lysosome, inhibits the release of glycoproteins that promote the chemotaxis of synovial cells and neutrophils. It is important to note that previous studies have shown that viroporine E, a component of the coronavirus associated with SARS (SARS-CoV), forms Ca2C-permeable ion channels and activates inflammation of NLRP3. In addition, another viroporin 3a was found 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, which are formed in response to warning signals. Several clinical trials are currently underway to study the efficacy of colchicine in patients infected with SARS-Cov-2, as shown in the table 1. (144-183)

Conclusion

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 / 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 cytokinic 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 IL6 and IL 1, bringing more benefits, it is also a low cost and if used at the right doses with a good tolerability profile. In addition there is a fundamental aspect to add, biological drugs (such as tocilizumab, sarilumab etc..) can, with reference to the RCP of the drugs, cause with a 'common' frequency secondary infections of the respiratory tract and

therefore compromise paradossally the clinical situation of patients infected 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 and the clinical and pharmacovigilance data, the risk of upper respiratory tract infections may not be an issue. However, given the viral nature of SARS-CoV-2, and considering a substantial impairment of the host's immune system in severe cases, it is essential to balance the risk/benefit ratio before starting anti-inflammatory therapy. In addition, early anti-inflammatory treatment initiated at the right time is of paramount importance and should be tailored to the individual patient to achieve the most nevertheless, this would be an interesting area for future research favourable effects from clinicians derived from ongoing trials will answer our questions.

BIBLIOGRAPHY

1) Ware LB, Matthay MA. *The acute respiratory distress syndrome.* N Engl J Med 2000;342:1334–49.

2) Baggiolini M, Walz A, Kunkel SL. *Neutrophil-activating peptide-1/ interleukin-8: a novel cytokine that activates neutrophils.* J Clin Invest 1989;84:1045–9.

3) Standiford TJ, Rolfe MW, Kunkel SL, et al. *Macrophage inflammatory protein-1: expression in interstitial lung disease.* J Immunol 1993;151:2852–63.

4)Suter PM, Suter S, Girardin E, Roux-Lombard P, Grau GE, Dayer JM. *High bronchoalveolar levels of tumor necrosis factor and its inhibitors, interkeukin-1, interferon, and elastase, in patients with respiratory distress syndrome after trauma, shock, or sepsis.* Am Rev Respir Dis 1992;145:1016–22.

5) Siler TM, Swierkosz JE, Hyers TM, Fowler AA, Webster RO. *Immunoreactive interleukin-1 in bronchoalveolar lavage fluid of high-risk patients and patients with the adult respiratory distress syndrome.* Exp Lung Res 1989;15:881–94.

6)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.

7)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.

8)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.

9)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.

10)Arend WP. *Interleukin-1 receptor antagonist.* Adv Immunol 1993;54:167–227.

11)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.

12)Sims JE. *IL-1 and IL-18 receptors, and their extended family.* Curr Opin Immunol 2002;14:117–22.

13)Jensen LE, Muzio M, Mantovani A, Whitehead AS. *IL-1 signaling cascade in liver cells and the involvement of a soluble form of the IL-1 receptor accessory protein.* J Immunol 2000;164:5277–86.

14)Pugin J, Ricou B, Steinberg KP, Suter PM, Martin TR. *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.

15)Pugin J, Verghese G, Widmer MC, Matthay MA. *The alveolar space is the site of intense inflammatory and profibrotic reactions in the early phase of ARDS.* Crit Care Med 1999;27:304–12.

16)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.

17)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.

18)Fiorentino DF, Bond MW, Mosmann TR. *Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones.* J Exp Med 1989;170:2081–95.

19)Fiorentino DF, Zlotnik A, Mosmann TR, Howard M, O'Garra A. *IL-10 inhibits cytokine production by activated macrophages.* J Immunol 1991;147:3815–22.

20)Bogdan C, Vodovotz Y, Nathan C. *Macrophage deactivation by interleukin-10.* J Exp Med 1991;174:1549–55.

21)Ralph P, Nakoinz I, Sampson-Johannes A. *IL-10, T lymphocyte inhibitor of human blood cell production of IL-1 and tumor necrosis factor.* J Immunol 1992;148:808–14.

22)Dickensheets HL, Freeman SL, Smith MF, Donnelly RP. *Interleukin-10 upregulates tumor necrosis factor receptor type-II (p75) gene expression in endotoxin-stimulated human monocytes.* Blood 1997;90:4162–71.

23)Wanidworanum C, Strober W. *Prdominant role of tumor necrosis factor-alpha in human monocyte IL-10 synthesis.* J Immunol 1993;151:6853–61.

24)Armstrong L, Millar AB. *Relative production of tumour necrosis factor alpha and interleukin-10 in adult respiratory distress syndrome.* Thorax 1997;52:442–6.

25)Fumeaux T, Pugin J. *Role of interleukin-10 in the intracellular sequestration of human leukocyte antigen-DR in moncocytes during septic shock.* Am J Respir Crit Care Med 2002;166:1475–82.

26)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.

27)Bloom BR, Bennett B. *Mechanism of a reaction in vitro associated with delayed-type hypersensitivity.* Science 1966;153:80–2.

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

29)Bernhagen J, Calandra T, Mitchell RA, et al. *MIF is a pituitaryderived cytokine that potentiates lethal endotoxaemia.* Nature 1993;365:756–9.

30)Roger T, David J, Glauser MP, Calandra T. *MIF regulates innate immune responses through modulation of Toll-like receptor-4.* Nature 2001;414:920–4.

31)Mitchell RA, Liao H, Chesney J, et al. *Macrophage migration inhibitory factor (MIF) sustains macrophage pro-inflammatory function by inhibition p53: regulatory role in the innate immune response.* Proc Natl Acad Sci USA 2002;99:345–50.

32)Yang EA. *HMG-1 rediscovered as a cytokine.* Shock 2001;4:247– 53.

33)Abraham E, Arcaroli J, Carmody A, Wang H, Tracey KJ. *HMG-1 as a mediator of acute lung inflammation.* J Immunol 2000;165: 2950–4.

34)Sappington PL, Yang R, Yang H, Tracey KJ, Delude RL, Fink MP. *HMGB1 B box increases the permeability of Caco-2 enterocytic monolayers and impairs intestinal barrier function in mice.* Gastroenterology 2002;123:790–802.

35)Fowler AA, Hyers TM, Fisher BJ, Bechard DE, Centor RM, Webster RO. *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.

36)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.

37)Weiland JE, Davis WB, Holter JF, Mohammed JR, Dorinsky PM, Gadek JE. *Lung neutrophils in the adult respiratory distress syndrome: clinical and pathophysiolgic significance.* Am Rev Respir Dis 1986;133:218–25.

38)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.

39)Repine JE, Beehler CJ. *Neutrophils and adult respiratory distress syndrome: two interlocking perspectives in 1991.* Am Rev Respir Dis (1991);144:251–2.

40)Steinberg KP, Milberg JA, Martin TR, Maunder RJ, Cockrill BA, Hudson LD. *Evolution of bronchoalveolar cell populations in the adult respiratory distress syndrome.* Am J Respir Crit Care Med 1994;150:113–22.

41)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.

42)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.

43)Maunder RJ, Hackman RC, Riff RE, Albert RK, Springmeyer SC. *Occurrence of the adult respiratory distress syndrome in neutropenic patients.* Am Rev Respir Dis 1987;133:313–6.

44)Rinaldo JE, Borovetz H. *Deterioration of oxygenation and abnormal lung microvascular permeability during resolution of leukopenia in patients with diffuse lung injury.* Am Rev Respir Dis 1985;131:579– 83.

45)Rinaldo JE. *Mediation of ARDS by leukocytes. Clinical evidence and implications for therapy.* Chest 1986;89:590–3.

46)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.

47)Chollet-Martin S, Montravers P, Gibert C, et al. *Subpopulation of hyperresponsive polymorphonuclear neutrophils in patients with adult respiratory distress syndrome: role of cytokine production.* Am Rev Respir Dis 1992;146:990–6.

48)Cochrane CG, Spragg RG, Revak SD, Cohen AB, McGuire WW. *The presence of neutrophil elastase and evidence of oxidation activity in bronchoalveolar lavage fluid of patients with adult respiratory distress syndrome.* Am Rev Respir Dis 1983;127:S25–7.

49)Lee CT, Fein AM, Lippman M, Holtzman H, Kimbel P, Weinbaum G. *Elastolytic activity in pulmonary lavage fluid from patients with the adult respiratory distress syndrome.* N Engl J Med 1981;304:192–6.

50)Li G Fan Y Lai Y Han T Li Z Zhou P Pan P Wang W Hu D Liu X Zhang Q Wu J. *Coronavirus infections and immune responses.* J Med Virol. 2020 Apr;92(4):424-432. doi: 10.1002/jmv.25685. Epub 2020 Feb 7.

51)Conti P Ronconi GCaraffa A Gallenga CE Ross RFrydas I Kritas SK. *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.* Masters PS, Perlman, S (2013) Coronaviridae. In: Knipe DM, Howley P (eds) Fields Virology. Lippincott Williams and Wilkins, Philadelphia, PA, pp 825–858.

52)Siddell SZJ, Snijder EJ. *Coronaviruses, toroviruses, and arteriviruses, vol. 1.* London: Hodder Arnold; 2005.

53)Peck KM, et al. *Coronavirus host range expansion and Middle East respiratory syndrome coronavirus emergence: biochemical mechanisms and evolutionary perspectives.* Annu Rev Virol. 2015;2(1):95–117. doi: 10.1146/annurev-virology-100114-055029.

54)Su S, et al. *Epidemiology, genetic recombination, and pathogenesis of coronaviruses.* Trends Microbiol. 2016;24(6):490–502. doi: 10.1016/j.tim.2016.03.003.

55)Weiss SR, Navas-Martin S. *Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus.* Microbiol Mol Biol Rev. 2005;69(4):635–664. doi: 10.1128/MMBR.69.4.635-664.2005.

56)Heugel J, et al. *Coronavirus-associated pneumonia in previously healthy children.* Pediatr Infect Dis J. 2007;26(8):753–755. doi: 10.1097/INF.0b013e318054e31b.

57)Kuypers J, et al. *Clinical disease in children associated with newly described coronavirus subtypes.* Pediatrics. 2007;119(1):e70–e76. doi: 10.1542/peds.2006-1406.

58)Drosten C, et al. *Identification of a novel coronavirus in patients with severe acute respiratory syndrome.* N Engl J Med. 2003;348(20):1967–1976. doi: 10.1056/NEJMoa030747.

59)Kuiken T, et al. *Newly discovered coronavirus as the primary cause of severe acute respiratory syndrome.* Lancet. 2003;362(9380):263–270. doi: 10.1016/S0140-6736(03)13967-0.

60)Peiris JS, et al. *Coronavirus as a possible cause of severe acute respiratory syndrome.* Lancet. 2003;361(9366):1319–1325. doi: 10.1016/S0140-6736(03)13077-2.

61)van Boheemen S et al (2012) *Genomic characterization of a newly discovered coronavirus associated with acute respiratory distress syndrome in humans.* MBio 3(6)

62)Zaki AM, et al. *Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia.* N Engl J Med. 2012;367(19):1814–1820. doi: 10.1056/NEJMoa1211721.

63)Perlman S, Netland J. *Coronaviruses post-SARS: update on replication and pathogenesis.* Nat Rev Microbiol. 2009;7(6):439–450. doi: 10.1038/nrmicro2147.

64)Wong CK, et al. *Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome.* Clin Exp Immunol. 2004;136(1):95–103. doi: 10.1111/j.1365-2249.2004.02415.x. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

65)Zhang Y, et al. *Analysis of serum cytokines in patients with severe acute respiratory syndrome.* Infect Immun. 2004;72(8):4410–4415. doi: 10.1128/IAI.72.8.4410-4415.2004. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

66)Cameron MJ, et al. *Human immunopathogenesis of severe acute respiratory syndrome (SARS)* Virus Res. 2008;133(1):13–19. doi: 10.1016/j.virusres.2007.02.014.

67)Cameron MJRL, Xu L, Danesh A, Bermejo-Martin JF, Cameron CM, Muller MP, Gold WL, Richardson SE, Poutanen SM, Willey BM, DeVries ME, Fang Y, Seneviratne C, Bosinger SE, Persad D, Keshavjee S, Louie M, Loeb MB, Brunton J, McGeer AJ, Kelvin DJ. *Interferon-mediated immunopathological events are associated with atypical innate and adaptive immune responses in patients with severe acute respiratory syndrome.* J Virol. 2007;81(16):8692–8706. 10.1128/JVI.00527-07.

68)Huang KJ, et al. *An interferon-gamma-related cytokine storm in SARS patients.* J Med Virol. 2005;75(2):185–194. doi: 10.1002/jmv.20255.

69)Theron M, et al. *A probable role for IFN-gamma in the development of a lung immunopathology in SARS.* Cytokine. 2005;32(1):30–38. doi: 10.1016/j.cyto.2005.07.007.

70)Lau SK, et al. *Delayed induction of proinflammatory cytokines and suppression of innate antiviral response by the novel Middle East respiratory syndrome coronavirus: implications for pathogenesis and treatment.* J Gen Virol. 2013;94(Pt 12):2679–2690. doi: 10.1099/vir.0.055533-0.

71)Chu H et al (2015) *Middle East respiratory syndrome coronavirus efficiently infects human primary T lymphocytes and activates the extrinsic and intrinsic apoptosis pathways.* J Infect Dis 213(6):904–14

72)Tynell J, et al. *Middle East respiratory syndrome coronavirus shows poor replication but significant induction of antiviral responses in human monocyte-derived macrophages and dendritic cells.* J Gen Virol. 2016;97(2):344–355. doi: 10.1099/jgv.0.000351.

73)Zhou J, et al. *Active replication of Middle East respiratory syndrome coronavirus and aberrant induction of inflammatory cytokines and chemokines in human macrophages: implications for pathogenesis.* J Infect Dis. 2014;209(9):1331–1342. doi: 10.1093/infdis/jit504.

74)Scheuplein VA, et al. *High secretion of interferons by human plasmacytoid dendritic cells upon recognition of Middle East respiratory syndrome coronavirus.* J Virol. 2015;89(7):3859–3869. doi: 10.1128/JVI.03607-14.

75)Kim ES, et al. *Clinical progression and cytokine profiles of Middle East respiratory syndrome coronavirus infection.* J Korean Med Sci. 2016;31(11):1717–1725. doi: 10.3346/jkms.2016.31.11.1717.

76)Min CK, et al. *Comparative and kinetic analysis of viral shedding and immunological responses in MERS patients representing a broad spectrum of disease severity.* Sci Rep. 2016;6:25359. doi: 10.1038/srep25359.

77)Roberts A, et al. *Aged BALB/c mice as a model for increased severity of severe acute respiratory syndrome in elderly humans.* J Virol. 2005;79(9):5833–5838. doi: 10.1128/JVI.79.9.5833-5838.2005.

78)Day CW, et al. *A new mouse-adapted strain of SARS-CoV as a lethal model for evaluating antiviral agents in vitro and in vivo.* Virology. 2009;395(2):210–222. doi: 10.1016/j.virol.2009.09.023.

79)Narayanan K, et al. *Severe acute respiratory syndrome coronavirus nsp1 suppresses host gene expression, including that of type I interferon, in infected cells*. J Virol. 2008;82(9):4471–4479.

80)Herold S, et al. *Lung epithelial apoptosis in influenza virus pneumonia: the role of macrophage-expressed TNF-related apoptosis-inducing ligand.* J Exp Med. 2008;205(13):3065–3077. doi: 10.1084/jem.20080201.

81)Hogner K, et al. *Macrophage-expressed IFN-beta contributes to apoptotic alveolar epithelial cell injury in severe influenza virus pneumonia.* PLoS Pathog. 2013;9(2):e1003188. doi: 10.1371/journal.ppat.1003188.

82)Rodrigue-Gervais IG, et al. *Cellular inhibitor of apoptosis protein cIAP2 protects against pulmonary tissue necrosis during influenza virus infection to promote host survival.* Cell Host Microbe. 2014;15(1):23–35. doi: 10.1016/j.chom.2013.12.003. Zhao J, et al. T cell responses are required for protection from clinical disease and for virus clearance in severe acute respiratory syndrome coronavirus-infected mice. J Virol. 2010;84(18):9318–9325. doi: 10.1128/JVI.01049-10. Kim KD, et al. *Adaptive immune cells temper initial innate responses. Nat Med. 2007;13(10):1248–1252.* doi: 10.1038/nm1633.

83)Palm NW, Medzhitov R. *Not so fast: adaptive suppression of innate immunity.* Nat Med. 2007;13(10):1142–1144. doi: 10.1038/nm1007-1142b.

84)Zornetzer GA, et al. *Transcriptomic analysis reveals a mechanism for a prefibrotic phenotype in STAT1 knockout mice during severe acute respiratory syndrome coronavirus infection.* J Virol. 2010;84(21):11297–11309. doi: 10.1128/JVI.01130-10.

85)Page C, et al. *Induction of alternatively activated macrophages enhances pathogenesis during severe acute respiratory syndrome coronavirus infection.* J Virol. 2012;86(24):13334–13349. doi: 10.1128/JVI.01689-12.

86)Gralinski LE, et al. *Genome wide identification of SARS-CoV susceptibility loci using the collaborative cross.* PLoS Genet. 2015;11(10):e1005504. doi: 10.1371/journal.pgen.1005504.

87)Drosten C, et al. *Clinical features and virological analysis of a case of Middle East respiratory syndrome coronavirus infection.* Lancet Infect Dis. 2013;13(9):745–751. doi: 10.1016/S1473-3099(13)70154-3.

88)Lew TW, et al. *Acute respiratory distress syndrome in critically ill patients with severe acute respiratory syndrome.* JAMA. 2003;290(3):374–380. doi: 10.1001/jama.290.3.374.

89)iang Y, et al. *Characterization of cytokine/chemokine profiles of severe acute respiratory syndrome.* Am J Respir Crit Care Med. 2005;171(8):850–857. doi: 10.1164/rccm.200407-857OC.

90)Reghunathan R et al (2005) *Expression profile of immune response genes in patients with Severe Acute Respiratory Syndrome.* BMC Immunology 6:2.

91)R. Channappanavar, S. Perlman. *Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology* Semin. Immunopathol., 39 (2017), pp. 529-539 CrossRefView

92)B.G. Chousterman, F.K. Swirski, G.F. Weber. *Cytokine storm and sepsis disease pathogenesis* Semin. Immunopathol., 39 (2017), pp. 517-528 CrossRefView Record in ScopusGoogle Scholar

93)C. Huang, Y. Wang, X. Li, L. Ren, J. Zhao, Y. Hu, et al. *Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China* Lancet, 395 (2020), pp. 497-506 ArticleDownload PDFView Record in ScopusGoogle Scholar

94)P. Conti, G. Ronconi, A. Caraffa, C.E. Gallenga, R. Ross, I. Frydas, et al. *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* J. Biol. Regul. Homeost. Agents, 34 (2020), 10.23812/ CONTI-E Google Scholar

95)F. Zhou, T. Yu, R. Du, G. Fan, Y. Liu, Z. Liu, et al. *Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study* Lancet (2020), 10.1016/s0140-6736(20)30566-3 Google Scholar

96) Q. Ruan, K. Yang, W. Wang, L. Jiang, J. Song. *Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China* Intensive Care Med. (2020), 10.1007/s00134-020-05991-x Mar 3 Google Scholar

97) N. Chen, M. Zhou, X. Dong, J. Qu, F. Gong, Y. Han, et al. *Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study* Lancet, 395 (2020), pp. 507-513 ArticleDownload PDFView Record in ScopusGoogle Scholar

98) C. Wu, X. Chen, Y. Cai, J. Xia, X. Zhou, S. Xu, et al. *Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China* JAMA Intern. Med. (2020), 10.1001/jamainternmed.2020.0994 Google Scholar

99) S. Wan, Q. Yi, S. Fan, J. Lv, X. Zhang, L. Guo, et al. *Characteristics of lymphocyte subsets and cytokines in peripheral blood of 123 hospitalized patients with 2019 novel coronavirus pneumonia (NCP)* medRxiv (2020), 10.1101/2020. 02.10.20021832 Google Scholar

100) Z. Xu, L. Shi, Y. Wang, J. Zhang, L. Huang, C. Zhang, et al. *Pathological findings of COVID-19 associated with acute respiratory distress syndrome* Lancet Respir. Med. (2020), 10.1016/S2213-2600(20)30076-X Google Scholar

101) H.K. Siddiqi, M.R. Mehra. *COVID-19 illness in native and immunosuppressed states: a clinical-therapeutic staging proposal* J. Heart Lung Transplant. (2020), 10.1016/j. healu n.2020.03.012 Google Scholar

102) National Health Commission of the People's Republic of ChinaThe Diagnosis and Treatment Plan for 2019-nCoV (The Seventh Trial Edition) http://www.nhc.gov.cn/yzygj/s7653p/202003/46c9294a7dfe4cef80dc7f5912eb1989.shtml (accessed 4 March 2020)

103) C. Qin, L. Zhou, Z. Hu, S. Zhang, S. Yang, Y. Tao, et al. *Dysregulation of immune response in patients with COVID-19 in Wuhan, China* Clin. Infect. Dis. (2020), 10.1093/cid/ciaa248

104) X.H. Yao, T.Y. Li, Z.C. He, Y.F. Ping, H.W. Liu, S.C. Yu, et al. *A pathological report of three COVID-19 cases by minimally invasive autopsies Chin*. J. Pathol., 49 (2020), Article E009, 10.3760/cma.j.cn112151-20200312-00193 Google Scholar
105) X. Guo, P. Thomas. *New fronts emerge in the influenza cytokine storm Semin.* Immunopathol., 39 (2017), pp. 541-550 CrossRefView Record in ScopusGoogle Scholar

106) A. Shimabukuro-Vornhagen, P. Gödel, M. Subklewe, H.J. Stemmler, H.A. Schlößer, M. Schlaak, et al. *Cytokine release syndrome* J. Immunother. Cancer, 6 (2018), p. 56 Google Scholar

107) C. Chen, X.R. Zhang, Z.Y. Ju, W.F. He. *Advances in the research of cytokine storm mechanism induced by Corona virus disease 2019 and the corresponding immunotherapies* Chin. J. Burns, 36 (2020), Article E005, 10.3760/cma.j.cn501120-20200224-00088 View Record in ScopusGoogle Scholar

108) T.W. Auyeung, J.S. Lee, W.K. Lai, C.H. Choi, H.K. Lee, J.S. Lee, et al. *The use of corticosteroid as treatment in SARS was associated with adverse outcomes: a retrospective cohort study* J. Inf. Secur., 51 (2005), pp. 98-102 ArticleDownload PDFView Record in ScopusGoogle Scholar

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

110)J.Benhamou, Ori-Michael; Geva, Shahar; Jacobs, Miriam; Drew, Jonathan; Waldman, Maor; Kalchiem-Dekel, *The Use of Colchicine in Respiratory Diseases* Or Current Respiratory Medicine Reviews, Volume 9, Number 5, 2013, pp. 300-304(5)

111)Front. Pharmacol., 16 August 2019 | https://doi.org/10.3389/fphar.2019.00908 The Association Between Usage of Colchicine and Pneumonia: A Nationwide, Population-Based Cohort Study

112)Shekelle PG, Newberry SJ, FitzGerald JD, Motala A, O'Hanlon CE, Tariq A, et al. (January 2017). *"Management of Gout: A Systematic Review in Support of an American College of Physicians Clinical Practice Guideline"*. Annals of Internal Medicine. **166** (1): 37–51. doi:10.7326/M16-0461. PMID 27802478.

113)*"Colchicine Monograph for Professionals"*. Drugs.com. American Society of Health-System Pharmacists. Retrieved 27 March 2019.

114)Schachner, Lawrence A.; Hansen, Ronald C. (2011). *Pediatric Dermatology E-Book*. Elsevier Health Sciences. p. 177. ISBN 9780723436652.

115)Hutchison, Stuart J. (2009). *Pericardial Diseases: Clinical Diagnostic Imaging Atlas with DVD*. Elsevier Health Sciences. p. 58. ISBN 9781416052746.

116)*"Colchicine for acute gout: updated information about dosing and drug interactions"*. National Prescribing Service, Australia. 14 May 2010. Archived from the original on 30 June 2012. Retrieved 14 May 2010.

117)British national formulary : BNF 76 (76 ed.). Pharmaceutical Press. 2018. pp. 1085–1086. ISBN 9780857113382.

118)Dalbeth N, Lauterio TJ, Wolfe HR (October 2014). *"Mechanism of action of colchicine in the treatment of gout"*. Clinical Therapeutics. **36** (10): 1465–79. doi:10.1016/j.clinthera.2014.07.017. PMID 25151572.

119)Chen LX, Schumacher HR (October 2008). *"Gout: an evidence-based review"*. Journal of Clinical Rheumatology. **14** (5 Suppl): S55-62. doi:10.1097/RHU.0b013e3181896921. PMID 18830092.

120)*"Colcrys (colchicine, USP) tablets 0.6 mg. Drug Approval Package"*. US Food and Drug Administration. 17 February 2010. Retrieved 19 August 2018.

121)*"Information for Healthcare Professionals: New Safety Information for Colchicine (marketed as Colcrys)"*. U.S. Food and Drug Administration.
122)Laubscher T, Dumont Z, Regier L, Jensen B (December 2009). *"Taking the stress out of managing gout"*. Canadian Family Physician. **55** (12): 1209–12. PMC 2793228. PMID 20008601.

123)van Echteld I, Wechalekar MD, Schlesinger N, Buchbinder R, Aletaha D (August 2014). *"Colchicine for acute gout"*. The Cochrane Database of Systematic Reviews. **8** (8): CD006190. doi:10.1002/14651858.CD006190.pub2. PMID 25123076.

124)Terkeltaub RA, Furst DE, Bennett K, Kook KA, Crockett RS, Davis MW (April 2010). *"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 and Rheumatism. **62** (4): 1060–8. doi:10.1002/art.27327. PMID 20131255.

125)*"Colchicine"*. Drugs.com. 1 January 2017. Retrieved 19 August 2018.

126)Shekelle, Paul G.; Newberry, Sydne J.; FitzGerald, John D.; Motala, Aneesa; O'Hanlon, Claire E.; Tariq, Abdul; Okunogbe, Adeyemi; Han, Dan; Shanman, Roberta (2017). *"Management of gout: A systematic review in support of an American College of Physicians Clinical Practice Guideline"*. Annals of Internal Medicine. **166** (1): 37–51. doi:10.7326/M16-0461. ISSN 0003-4819. PMID 27802478.

127)Qaseem, Amir; Harris, Russell P.; Forciea, Mary Ann (2017). *"Management of acute and recurrent gout: A Clinical Practice Guideline From the American College of Physicians"*. Annals of Internal Medicine. **166** (1): 58–68. doi:10.7326/M16-0570. ISSN 0003-4819. PMID 27802508.

128)Cocco G, Chu DC, Pandolfi S (December 2010). *"Colchicine in clinical medicine. A guide for internists"*. European Journal of Internal Medicine. **21** (6): 503–8. doi:10.1016/j.ejim.2010.09.010. PMID 21111934.

129)Alabed S, Cabello JB, Irving GJ, Qintar M, Burls A (August 2014). *"Colchicine for pericarditis" (PDF)*. The Cochrane Database of Systematic Reviews. **8** (8): CD010652. doi:10.1002/14651858.CD010652.pub2. PMID 25164988.

130)Portincasa P (2016). *"Colchicine, Biologic Agents and More for the Treatment of Familial Mediterranean Fever. The Old, the New, and the Rare"*. Current Medicinal Chemistry. **23** (1): 60–86. doi:10.2174/0929867323666151117121706. PMID 26572612.

131)Lennerz C, Barman M, Tantawy M, Sopher M, Whittaker P (December 2017). *"Colchicine for primary prevention of atrial fibrillation after open-heart surgery: Systematic review and meta-analysis" (PDF)*. International Journal of Cardiology. 249: 127–137. doi:10.1016/j.ijcard.2017.08.039. PMID 28918897.

132)*"CDC - The Emergency Response Safety and Health Database: Biotoxin: Cochicine"*. Centers for Disease Control and Prevention, US Department of Health and Human Services. Retrieved 31 December 2015.

133)Finkelstein Y, Aks SE, Hutson JR, Juurlink DN, Nguyen P, Dubnov-Raz G, Pollak U, Koren G, Bentur Y (June 2010). *"Colchicine poisoning: the dark side of an ancient drug"*. Clinical Toxicology. 48 (5): 407–14. doi:10.3109/15563650.2010.495348. PMID 20586571.

134)Matt Doogue (2014). *"Colchicine – extremely toxic in overdose" (PDF)*. Christchurch and Canterbury District Health Board, New Zealand. Retrieved 23 August 2018.
135)Graham W, Roberts JB (March 1953). *"Intravenous colchicine in the management of gouty arthritis" (PDF)*. Annals of the Rheumatic Diseases. 12 (1): 16–9. doi:10.1136/ard.12.1.16. PMC 1030428. PMID 13031443.

136)*"Colcrys (colchicine). Summary review for regulatory action" (PDF)*. Center for Drug Evaluation and Research, US Food and Drug Administration. 30 July 2009. Retrieved 19 August 2018.

137)Hartung EF (September 1954). *"History of the use of colchicum and related medicaments in gout; with suggestions for further research" (PDF)*. Annals of the Rheumatic Diseases. 13 (3): 190–200. doi:10.1136/ard.13.3.190. PMC 1006735. PMID 13198053. (free BMJ registration required)

138)Pelletier and Caventou (1820) *"Examen chimique des plusieurs végétaux de la famille des colchicées, et du principe actif qu'ils renferment. [Cévadille (veratrum sabadilla) ; hellébore blanc (veratrum album) ; colchique commun (colchicum autumnale)]"* (Chemical examination of several plants of the meadow saffron family, and of the active principle that they contain.) Annales de Chimie et de Physique, 14 : 69-81.

139)Geiger, Ph. L. (1833) *"Ueber einige neue giftige organische Alkalien" (On some new poisonous organic alkalis)* Annalen der Pharmacie, 7 (3) : 269-280; colchicine is discussed on pages 274-276.

140)Cerquaglia C, Diaco M, Nucera G, La Regina M, Montalto M, Manna R (February 2005). *"Pharmacological and clinical basis of treatment of Familial Mediterranean Fever (FMF) with colchicine or analogues: an update"*. Current Drug Targets. Inflammation and Allergy. 4 (1): 117–24. doi:10.2174/1568010053622984. PMID 15720245. Archived from the original on 2008-12-11. Retrieved 2019-07-06.

141)Karst KR (21 October 2009). *"California Court Denies Preliminary Injunction in Lanham Act Case Concerning Unapproved Colchicine Drugs"*.

142)Kesselheim AS, Solomon DH (June 2010). *"Incentives for drug development--the curious case of colchicine"*. The New England Journal of Medicine. 362 (22): 2045–7. doi:10.1056/NEJMp1003126. PMID 20393164.

143)*"FDA orders halt to marketing of unapproved single-ingredient oral colchicine"*. 30 September 2010.

144)*"40 CFR Appendix A to Part 355, The List of Extremely Hazardous Substances and Their Threshold Planning Quantities"*. LII / Legal Information Institute. Retrieved 2018-03-11.
145)Leete E (1963). *"The biosynthesis of the alkaloids of Colchicum: The incorporation of phenylalaline-2-C14 into colchicine and demecolcine"*. J. Am. Chem. Soc. 85 (22): 3666–3669. doi:10.1021/ja00905a030.

146)Dewick PM (2009). *Medicinal natural products: A biosynthetic approach*. Wiley. pp. 360–362.

147)Maier UH, Meinhart HZ (1997). *"Colchicine is formed by para para phenol-coupling from autumnaline"*. Tetrahedron Lett. 38 (42): 7357–7360. doi:10.1016/s0040-4039(97)10011-9.