NLRP3 remmers ter voorkoming en vermindering van een cytokine storm en diffuse intravasale stolling (DIS) bij COVID-19 patienten

Juist door het gebrek aan medicamenteuze behandel opties, is het nu zaak om NLRP3 remmers goed te testen. NLRP3 remmers kunnen - mits in een vroeg stadium ingezet - mogelijk een cytokine storm en diffuse intravasale stolling (DIS) voorkomen of verminderen bij COVID-19 patienten. Een groep top-artsen en wetenschappers staat klaar om dit snel en professioneel op te pakken.

Virussen, zoals SARS-CoV, SARS-CoV-2 en influenza A en bacteriën overprikkelen het immuun systeem waardoor ze een cytokine storm kunnen veroorzaken. Een cytokine storm verergert de longontsteking en kan leiden tot nier- of zelfs multi-orgaan falen. Hierbovenop veroorzaakt hetzelfde overprikkelings mechanisme ook micro stolling, wat tot diffuse intravasale stolling (DIS) leidt. Dit verergert de COVID-19 symptomen nog verder.

Het aangeboren immuun systeem wordt gereguleerd door het NLRP3 eiwit. Remmen van NLRP3 verlaagd de productie van de cytokines die de storm veroorzaken en voorkomt ook stolling (DIS).

Onderzoek toont aan dat muizen, geïnfecteerd met SARS-CoV, die een NLPR3 remmer kregen toegediend minder ernstig ziek waren en een verbeterde overleving hadden. Vergelijkbare resultaten worden gezien bij muizen die geïnfecteerd zijn met Influenza A. Bij patienten met een ernstige bacteriële longontsteking, leidt NLRP3 remming tot een halvering van de sterfte.

In een Chinese RCT studie zag men bij COVID-19 patiënten die vroeg met een NLRP3 remmer behandeld worden, nauwelijks toename van de longontsteking. Men ziet juist sterke verbetering bij een meerderheid van de patiënten. Bij onbehandele patienten vind men bijna het omgekeerde resultaat (zie tabel rechts).

Groep	Aantal COVID-19 patiënten	Verergering long onsteking	>50% verbetering long ontsteking
Controle groep, aantal (%)	31	**9 (29.0%)**	5 (16.1%)
NLRP3 remmer, aantal (%)	31	2 (6,5%)	**19 (61.3%)**

Er zijn tenminste 4 geregistreerde geneesmiddelen die, als 'off-target' effect, ook NLRP3 remming geven:
- hydroxychloroquine (minder verergering longontsteking in vroege COVID-19 patienten)
- glyburide (halvering sterfte bij mensen met bacteriële longontsteking)
- azitromycine (remt NLRP3 activatie in celkweken en in proefdieren)
- colchicine (remt NLRP3 activatie in mensen)

Al deze middelen zijn veilig en hebben bekende en beheersbare bijwerkingen.

Daarnaast zijn er een aantal NLRP3 remmers die nog ontwikkeld worden (b.v. MCC950 en Dapansutrile). Van deze middelen weten we nog niet of deze veilig zijn.

Een andere strategie kan zijn om de verantwoordelijke cytokines te neutraliseren met anti-lichamen. Er zijn geen aanwijzingen dat je met deze anti-lichamen ook DIS kunt voorkomen.

Het is essentieel dat de behandeling vroeg wordt ingezet. NLRP3 remming heeft waarschijnlijk nauwelijks tot geen effect als de cytokine storm eenmaal 'geëxplodeerd' is en er een cascade van secundaire events is aangewakkerd. En juist op dit punt loopt het nu mis.

Alle experimenten worden (te) laat gestart, waardoor je een eventueel 'vroeg' effect mist. De eerste, zeer voorbarige en feitelijk onjuiste persberichten dat hydroxychloroquine niet zou werken gaan al rond. Ook een potentieel effect van b.v. Remsdesivir bij vroege patienten lijkt (in ieder geval door de pers) op deze manier in de kiem gesmoord te worden.

Anderzijds duren de meeste trials veel te lang (6-12 maanden) omdat er geen daadkrachtige organisatie achter zit. Dat dat sneller kan gaat Novartis nu laten zien. Helaas alleen bij 'late' COVID-19 patienten die al opgenomen zijn omdat ze zuurstof nodig hebben.

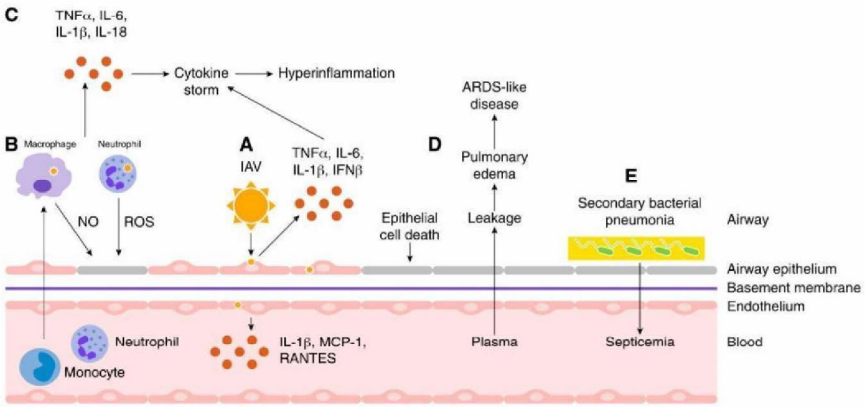
Daarom het voorstel om het 'kind niet met het badwater weg te gooien' en een goed en snel onderzoek in vroege COVID-19 patienten met hoog risico op ernstige ziekte uit te voeren.

Scientific background

A hyper inflammation-induced cytokine storm often accompanies severe viral or bacterial pneumonia. Viruses, e.g., SARS-CoV [1] or Influenza A [2], and bacteria [3] contain Pathogen Associated Molecular Patterns (PAMP's). SARS-CoV-2 most likely contains several DAMP's. Besides strong homology with the SARS-CoV PAMP's [4], SARS-CoV-2 also activates Interleukin (IL)-1β production in myeloid cells [5], and increased IL-1β is found in COVID-19 patients [6].

Such PAMP's trigger an innate immune response leading to the activation of NLRP3 inflammasomes [7]. Once activated, these inflammasomes produce the pro-inflammatory cytokines IL-1β and IL-18. IL-1β further promotes the expression of other proinflammatory cytokines such as tumor necrosis factor (TNF) α and IL-6 [8]. Activated NLRP3 inflammasomes also produce gasdermin-D, which creates pores in the cell membrane through which cytokines and other cellular compounds can be secreted [9]. Gasdermin-D also leads to pyroptosis, a novel inflammatory form of programmed cell death. Pyroptosis possibly plays a role in COVID-19 pathology [10]. It certainly does with other virus infections [29]

While NLRP3 activation plays an essential role in initiating an adaptive immune response, it becomes 'part of the problem' as the viral load increases over time. [11]. In severe Influenza A infections, in both humans and experimental animal models, viral load is associated with hyper inflammation or a cytokine storm. The high concentrations of proinflammatory cytokines such as IL-1β, IL-6, and TNF-α, cause damage to pulmonary tissue (leading to ARDS (see graph below)), and multiorgan failure [12]. NLRP3 activation also plays an important role in the pathology of lung fibrosis [30].



How a severe viral infection causes a cytokine storm. From: Hero turned villain: NLRP3 inflammasome-induced inflammation during influenza A virus infection

What is more, NLRP3 inflammasome activation can occur in thrombocytes. Production of IL-1β and gasdermin-D cause tissue factor (TF) release, disseminated intravascular coagulation (DIC), and thrombocytopenia [13]. Thrombocytopenia [14] and abnormal coagulation [15] are associated with poor prognosis in COVID19 patients.

The combination of a cytokine storm, (multi-) organ failure and DIC is also known as sepsis [31]. Septic shock is a subset of sepsis in which particularly profound circulatory, cellular, and metabolic abnormalities are associated with a higher risk of mortality than with sepsis alone. Both sepsis and septic shock can be found in severe COVID-19 patients [32].

Inhibition of NLRP3 inflammasome activation prevents hyper inflammation by lowering IL-1β and IL-6 [16, 17]. It also prevents/reduces DIC [18]. Administration of a specific NLRP3 inhibitor, seven days after intranasal inoculation with Influenza A virus (10^5 PFU) did significantly prolong their survical, but did not prevent death [19]. NF-κB activation is required, as priming step, before NLRP3 inflammasome activation can occur [20]. In mice infected with 10^5 PFU SARS-CoV, which

were treated with two NF-κB inhibitors, from day 1 to 4 a significant reduction in inflammation and lung pathology was found. More importantly, treatment increased the survival from 10% in untreated mice to 55% in treated mice [21]. In a sepsis mouse model, preventive administration of the NLRP3 inhibitor chloroquine increased survival from 20% to 60% [33]. In humans, infection with Burkholderia pseudomallei (Melioidosis) causes approximately 45% mortality [22]. In people who used Glyburide, anti-diabetic drug that also prevents NLRP3 activation, mortality was reduced with almost 50%. Colchicine, a drug used to threat gout also prevents NLRP3 inhibition. For this reason it has been studied in humans as a treatment for NLPR3 mediated atherosclerosis where it was found to have a positive effect on plaque reduction [47].

Currently, there is one prospective, randomized, and controlled study in 62 COVID-19 patients that had mild pneumonia (confirmed on CT). After randomization, 31 patients received hydroxychloroquine (HCQ) 400 mg/d for 5 days plus standard care [34]. The other 31 received standard care only. On day 6, exacerbation of the pneumonia was found in 19 out of 31 (29%) patients in the standard care only group. Four out of 31(12,9%) had a severe exacerbation. In the HCQ plus standard care treatment group, an exacerbation was found in only 2 out of 31 (6,5%) patients. No severe exacerbation was found in the treatment group. The amelioration of pneumonia was also determined. In the HCQ treatment group, 19 out of 31 patients saw an improvement of pneumonia (as determined by CT) of more than 50%. In the standard of care only control group, only 5 out of 31 (16,1%) patients saw such a significant improvement.

Lastly, an interesting observation: bats, who are considered as carriers of the SARC-CoV-2 virus, have a defective NLRP3 response against this virus [23]. Due to this, bats can survive SARS-CoV-2 infection and thereby function as a viral reservoir.

Non-linear feed-forward loops can cause explosive exarcerbation of disease
From bacterial sepsis, it is known that early treatment leads to a significant increase in survival [35]. Lethality is linked to an excessive innate immune response. It is hypothesized that a cytokine/ chemokine-driven feed-forward inflammatory circuit, caused by poorly contained and rapidly increasing numbers viruses in the 'innate phase' is responsible for the escalation of cytokine storm [36].

A systems analysis of the gene expression in influenza A infected mice identified a damaging feed-forward innate inflammatory circuit driven by cytokines/chemokines. This leads to a situation whereby neutrophils and monocytes amplify their own recruitment [37]. Such an explosive process also can explain why late intervention has little or no effect. It is no longer sufficient to stop the initial process that started this feed-forward loop. The authors also conclude that 'their feed-forward model suggested that a very non-linear process was involved in distinguishing between lethality and mild disease.' In other words, it only requires a relatively small step to go from mild to potentially lethal disease—for instance, pre-existing innate immune activation.

The point is that, in all the co-morbidities (hypertension, diabetes, high cholesterol, coronary artery disease, Alzheimer's, and atrial fibrillation) that significantly increase the risk for ventilation and morbidity in COVID-19 patients, NLRP3 inflammasomes all already activated [38-43]. Also, chronic activation of the innate immune system is a major characteristic of aging [44], another important COVID-19 risk factor.

Therefore it is hypothesized that the pre-existing NLRP3 inflammasome activation in these COVID-19 patients at risk contributes to the severity of the disease by accelerating the fast-forward loop. This then could explain why more patients require ventilation, and mortality is higher in the presence of one or more of these co-morbidities.

If the above (NLRP3 pre-activation and non-linear feed-forward loop) is correct, any intervention should be started as early as possible. What is more, negative results obtained with COVID-19 patients in which treatment is started in the 'explosive' phase (late-stage) of the disease should be interpreted with caution. It could well be that the effects of treatment in late-stage disease are so small that they are missed, especially when these results come from a retrospective, non-randomized clinical study in which they did not weigh in the more severe disease status of patients who received treatment [45,46].

There are two preclinical studies found where treatment is started after infection. In mice infected with a high dose Influenza A, late treatment (starting at day 7) prolonged survival but did not prevent death [19]. In mice infected with high dose SARS-CoV, treatment starting on day 1 increased survival from 10% to 55% [21]. This seems to underpin the importance of early treatment.

Repurposing drugs for the treatment of COVID-19
There are at least four registered medicines available that, as an off-target effect, inhibit NLRP3 inflammasome activation:

- Hydroxychloroquine prevents NLRP3 inflammasome activation in vitro [24]. Oral administration of 10 mg/kg hydroxychloroquine reduces NLRP3 mediated IL-1β production by more than 50% in a kidney injury mice model [25]. [It also prevents exacerbation pneumonia and ameliorates its resolution (on CT) in COVID-19 patients [34]
- Glyburide inhibits NLRP3 in vitro [26] and in humans [22].
- Azithromycin (AZT) inhibits NLRP3 in vitro. In a mouse model where NLRP3 activation is induced by ischemia/reperfusion, oral administration of 3,8 mg/kg AZT gave a statistically significant reduction of IL-1β of approximately 40% [27].
- Colchicine: based on its NLRP3 inhibiting effect in humans, a large randomized clinical trial in early-stage COVID-19 patients is initiated in Canada [48]. Patients will be treated at home.

Also, there are at least two specific NLRP3 inhibitors under clinical development:

- Inflazome develops 2 NLRP3 inhibitors. Both are have already passed phase I testing:
  - Somalix (https://inflazome.com/news/45)
  - Inzomelid (https://inflazome.com/news/46)
- Olatec develops the specific NLRP3 inhibitor Dapansutrile, which is already in phase II [28]

Another strategy could be to neutralize the cytokines, or their receptor, that are responsible for the cytokine storm:

- Anakinra is an anti-IL1R antibody that is registered for the treatment of rheumatoid arthritis and several rare auto-inflammatory diseases
- Canakinumab is an anti-IL1b antibody that is registered for the treatment of a rare-autoinflammatory disease
- Tocilizumab (RoActemra) is an anti-IL6 antibody that is registered for the treatment of rheumatoid arthritis

There is no scientific evidence that one of these neutralizing antibodies, also prevents or reduces pyroptosis and/or DIC.

Prospective, double-blinded, placebo-controlled, randomized clinical trial in early-stage COVID-19 patients at riks
The combination of the evidence above, especially in this time of crisis, where no treatments for COVID-19 are available, warrant a thorough and swift RCT in which the potential beneficial role of NLRP3 inhibitors in early-stage COVID-19 'at risk' is determined.

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