A theory on SARS-CoV-2 susceptibility:
low sensitivity of tlr7-signalling as a mechanistic explanation for the increased risk of obese and elderly COVID-19 patients

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Conti and Younes have pointed to a connection between lower tlr7 expression in men and their increased vulnerability towards a severe form of COVID-19 infection (1). I had started to investigate the same idea and think that I have discovered a wealth of additional supporting information that renders the hypothesis plausible and, if correct, could establish lower activity/sensitivity of tlr7-signalling prior to infection as a common molecular characteristic of several high-risk patient groups.

As Conti and Younes have already pointed out, in the current COVID-19 epidemic about twice as many men seem to die as women. This has been attributed to lifestyle in our mass media, but this would not explain a ratio that is pretty uniform across societies. In addition, the same quantitative imbalance between male and female deaths was already observed for the 2003 SARS outbreak (2), which was caused by a closely related Coronavirus.

For SARS, the receptor ACE2, encoded on the X-chromosome, was investigated as a possible reason for the gender difference. But there is no established link between different ace2-alleles and disease outcome, making this explanation less likely.

However, tlr7 (toll-like receptor 7) is also encoded on the X-chromosome, and it is a key regulator of pathogen detection and the resulting innate immune response. Tlr7 detects viral RNA in the endosome and then stimulates the production of inflammatory cytokines and interferons. It is a key component of our immune response to infection by RNA-viruses. SARS-CoV-2 is such an RNA virus.

Importantly, tlr7 is one of few genes that evade X-chromosome silencing in immune cells (3). It is known that this biallelic expression of tlr7 in immune cells of women can have physiological consequences, which can be positive, but also negative, as manifest

in female predisposition to systemic lupus erythematosus (SLE). The higher gene dosage of *tlr7* has been shown to be the reason for increased female susceptibility to this autoimmune disease.

Tlr7 has been described to bind and respond strongly to SARS-RNA (4). It is altogether plausible that higher *tlr7* expression is the reason why women are better equipped to deal with SARS-CoV-2.

If this is correct, a prediction is that patient groups with lower *tlr7* expression or lower sensitivity of *tlr7*-signalling should be more prone to develop a severe form of COVID-19.

There are patient groups who are emerging as carrying increased risk; obese patients and old people with a senescent immune system. One characteristic that obese and old people have in common is their physiological state of low-level, chronic inflammation. I speculated that this could lead to a common molecular characteristic with men: low *tlr7* expression or low sensitivity of *tlr7*-signalling, and analyzed the literature for supporting or contradictory evidence.

There is crosstalk between obesity and the innate immune system. Obesity is characterised by a chronic state of low inflammation (for a review see 5). A role of inflammation in the development of obesity-induced metabolic syndrome and insulin resistance via the pro-inflammatory cytokine TNF- α was already suggested in the 1990s and resident immune cells in the adipose tissue are responsible for that response.

While particularly *tlr4* has been examined for its role in the development of obesity-induced insulin resistance, it is interesting to appreciate that there is a high prevalence of insulin resistance and metabolic syndrome in SLE patients (6), indicating that also

chronic inflammation triggered by overactive tlr7-signalling increases the risk to develop metabolic syndrome. The increased prevalence of insulin resistance and metabolic syndrome in SLE patients is not linked to obesity.

Revelo et al. (7) have shown that in high-fat-diet (HFD) fed mice metabolic parameters worsen through mechanisms including activation of macrophages in visceral adipose tissue and expansion of plasmacytoid dendritic cells in the liver. HFD-fed mice lacking tlr7 and tlr9, however, showed reduced metabolic inflammation and improved glucose homeostasis. Treatment of HFD-fed mice with inhibitors against tlr7 and 9 improved metabolic disease. The authors conclude that obesity leads to metabolic inflammation via the activation of tlr7 and 9.

Hanna Kazazian et al. (8) have come to a very similar conclusion. Tlr8-deficient mice, which develop spontaneous lupus-like disease due to increased tlr7-signalling by dendritic cells, show an aggravated SLE pathogenesis when fed with a high-fat-diet. Taken together these two studies show that obesity leads to a chronic, low-level activation of the tlr7 receptor.

There is also a link between immune senescence and tlr7. It is well established that there are age-related changes in immune system function which shows a functional decline in its ability to respond to new pathogens during aging. This functional decline includes the innate immune system and Metcalfe et al. (9) found that peripheral blood mononuclear cells isolated from old individuals showed a delayed response to stimulation with tlr4, tlr7/8 and RIG-1 agonists compared to corresponding cells from young adults. This alone might explain the lower initial response and thus the higher vulnerability of old people towards SARS-CoV-2 infection.

On the other hand, serum levels of inflammatory cytokines increase with age. This age-related, systemic, chronic inflammation is called inflammaging. Increased levels of circulating inflammatory mediators such as interleukin-6 (IL-6) and C-reactive protein (CRP), which increase in an age-dependent manner, are indicators of inflammaging.

While diverse underlying reasons for inflammaging have been discussed, it is interesting that tlr7-activation has been implied in it. Levels of miR-146a are significantly increased in senescent cells that have a senescence associated secretory phenotype. miR-146a is a well-known negative regulator of tlr-signalling acting downstream of MyD88 by decreasing signalling through IRAK1 and TRAF-6. However ectopic localization of miR-146a in the extracellular space triggers an innate immune response, via - interestingly! - tlr7 (10). It has been suggested that it is the chronic tlr-activation by endogenous tlr ligands in the absence of pathogen infection - such as miR-146a activation of tlr7? - which contributes to the phenotype of “inflammaging” in old people.

Thus, a chronic, low level activation of tlr7 could link obesity and “inflammaging”, and thus SARS-CoV-2 sensitive groups. Over time, however, chronic low-level tlr7 activation should result in acquired tolerance to tlr7-signalling, similar to the homologous immune tolerance which Michaelis et al. (11) have observed upon repeated tlr7 stimulation with the tlr7 agonist R848 (resiquimod).

Thus, I hypothesize that high-risk patients - obesity, inflammaging - have in common that there is a chronic, low-level activation of tlr7 by intrinsic substrates. This leads to desensitization of tlr7-signalling and, more generally, to a state of tlr-tolerance prior to infection. Upon SARS-CoV-2 infection the induced activation of tlr7-signalling in high-risk patient groups can be expected to be weaker and delayed and consequently these

patients will show a weaker or delayed initial innate antiviral response immediately after infection. In men, this is then exacerbated further by the naturally lower expression level of tlr7.

It is tempting to speculate that another high-risk group, people who are exposed to continuous air pollution, might also show decreased sensitivity towards SARS-CoV-2 induced activation of tlr7-signalling by adaptive responses to chronic stimulation of the innate immune system (Figure 1), possibly by heterologous PAMP tolerance reciprocal to the R848-induced cross-tolerance towards tlr4-signalling observed by Michaelis et al. If so, people exposed to continuous air pollution would also be characterized by a state of acquired tlr-tolerance, and thus by a physiological state, where the signal of a new viral infection is initially not detected (see the figure for the proposed mechanistic model).

The proposed mechanistic model allows several predictions to be made. First, the gender difference in developing a severe SARS-CoV-2 infection should be independent of age, i.e. prepubertarian boys should be more susceptible than prepubertarian girls, as the different sensitivity would be a primary consequence of different tlr7 expression levels and not a secondary consequence due to sex hormone regulation.

Also, untreated SLE patients, where the activity of tlr7-signalling is high, should show a stronger initial innate immune response against infection by SARS-CoV-2, which should protect them. However, such patients will be hard to find, as the treatment against SLE typically focuses on suppressing the exaggerated, pathogenic tlr7-signalling. For example, hydroxychloroquine, which is frequently used in SLE-patients, reduces tlr7-signalling in SLE-patients by inhibiting autophagy and thus preventing the delivery of extracellular tlr7-substrates to endosomal tlr7.

While protection from viral infection is probably not present in human SLE-patients, where their respective immunosuppressing treatments would more than counteract their inherently overactive innate immune system, experiments in untreated lupus-prone mice are in line with the proposed model. Lupus-prone MRL/MpJ-Fas(lpr) mice did show accelerated viral clearance upon influenza infection (12), and thus demonstrated a beneficial role of high tlr7-activity in the initial phase of virus infection. But the same mouse model also showed a severe disadvantage of high tlr7-activity after viral clearance since the lupus-prone mice had severe complications during the contraction and resolution phase of the infection with widespread pulmonary inflammation. This points to a positive effect of highly active tlr7-signalling during the early stages of infection, but to a negative effect during the late stages. What is the underlying reason for the negative effect of highly active tlr7-signalling during the late stage of infection, even after the virus has been cleared? It may be that the continued tlr7-activity in the lupus-prone mice after virus clearance prevents their immune system from realizing that the virus is indeed gone and that it is now time to contract and resolve.

If the innate immune system of obese people and elderly people reacted to the establishment of a severe SARS-CoV-2 infection (due to an initially laggard response of tlr7-signalling as suggested by this model) by restoring normal sensitivity to tlr7-signalling after the initially delayed recognition of the viral infection, then they would find themselves in a situation which is similar to the lupus-prone mice of reference 12: at a later stage of the infection tlr7-signalling would be overactive in obese and old SARS-CoV-2 patients, as not only the viral RNA, but also the chronic intrinsic tlr7-substrates would signal to tlr7, since the tlr7-pathway has regained normal sensitivity (to better fight the virus). Tlr7-signalling in obese and elderly patients would even continue after virus

clearance and the innate immune system would effectively continue fighting a virus that is no longer there.

Thus, a prediction of the model is that at a later stage of the infection, resensitization of the tlr7-signalling pathway has the effect that obese and elderly patients suffer from exaggerated tlr7-signalling – much like SLE-patients in a flare.

It is interesting to note that the symptoms which have been described for COVID-19 patients show a striking similarity to the symptoms of SLE patients: a tendency for blood-clotting, cardiovascular involvement, kidney involvement, involvement of the central nervous system including the peculiar feature loss of smell, gastrointestinal involvement as seen by patients experiencing diarrhea, effects on the eye... – all of these symptoms have not only been described in severe COVID-19 cases, they have also been described as symptoms of SLE. This suggests that severe COVID-19 cases have something in common with SLE-patients and this theory proposes that it is overactive tlr7-signalling at the late stage of COVID-19.

Thus, severe cases of COVID-19 might be treatable in a way akin to SLE patients suffering from a flare. It should be worthwhile to consider repurposing of the approved SLE-medications for COVID-19 use! For the use of hydroxychloroquine, the model would predict that it should not be used as an antiviral agent in the early stages of an infection - where any positive effects on viral replication would be counteracted by its interference with a proper innate antiviral immune response - but rather be used to suppress the negative effects of tlr7-signalling during the contraction and resolution phase of the infection and thus with a mechanistic logic that is very similar to its use in SLE patients.

The proposed mechanistic model also confirms the significance of the early phase of SARS-CoV-2 infection for disease progression. If obese people and people of old age have a significantly higher risk than others to develop severe COVID-19 because of an initially weak and/or delayed antiviral response, then therapeutic interventions that aim at improving the initial response should have the most visible effects and antiviral therapy should thus start very early for at risk patients. This is in line with what others have suggested previously, however based on a different logic.

Zhao et al. (13) observe a protective prophylactic effect of the tlr7/8 agonist R848 (resiquimod) (and tlr-agonists in general) in aged mice from SARS-CoV-1 infection. A clinical trial with the tlr3 agonist PrEP-001 administered as a nasal powder prior to exposure to influenza-A (14) reduced the number of individuals with clinical illness and attenuated severity and duration of infection without compromising seroconversion and was well tolerated.

tlr-agonists have up to now not led to approved prophylactics against viral infection, but this is probably due to trying to shoehorn them into the context of continuous dosage regimens and hoping that their administration would give prolonged protection (which will not happen because repeated tlr-agonist administration induces tlr-tolerance). Based on the results from Zhao (13) and Malcolm (14) one would predict that if tlr-agonists are used in the proper context, such as in a one-time rescue prophylactic treatment - for example by providing PrEP-001 to yet asymptomatic inhabitants of an old people care facility where a very first case with Covid-19 symptoms has just been detected - they should be beneficial by attenuating severity and duration of infection in those whose innate immune system was boosted just in time.

If tlr-agonists proved protective against SARS-CoV-2 if administered immediately prior to virus exposure, and if attempts to develop a vaccine against SARS-CoV-2 should fail, then tlr-agonists might provide an option - albeit suboptimal - to immunization of selected parts of the population, for example by protecting a group with already high resistance against the virus, like people under the age of 40, even further by tlr-agonist administration prior to controlled virus exposure. While this approach would still raise serious ethical and political questions, it might be less undesirable than allowing SARS-CoV-2 to spread through an unprotected population or shutting down societies for months or years.

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Figure: Reduced activity/sensitivity of the tlr7-signalling pathway at the time of SARS-CoV-2 infection as a mechanistic link between high-risk patient groups

NET: Neutrophil extracellular traps