NOS+SARS-CoV-2 Pathogenesis

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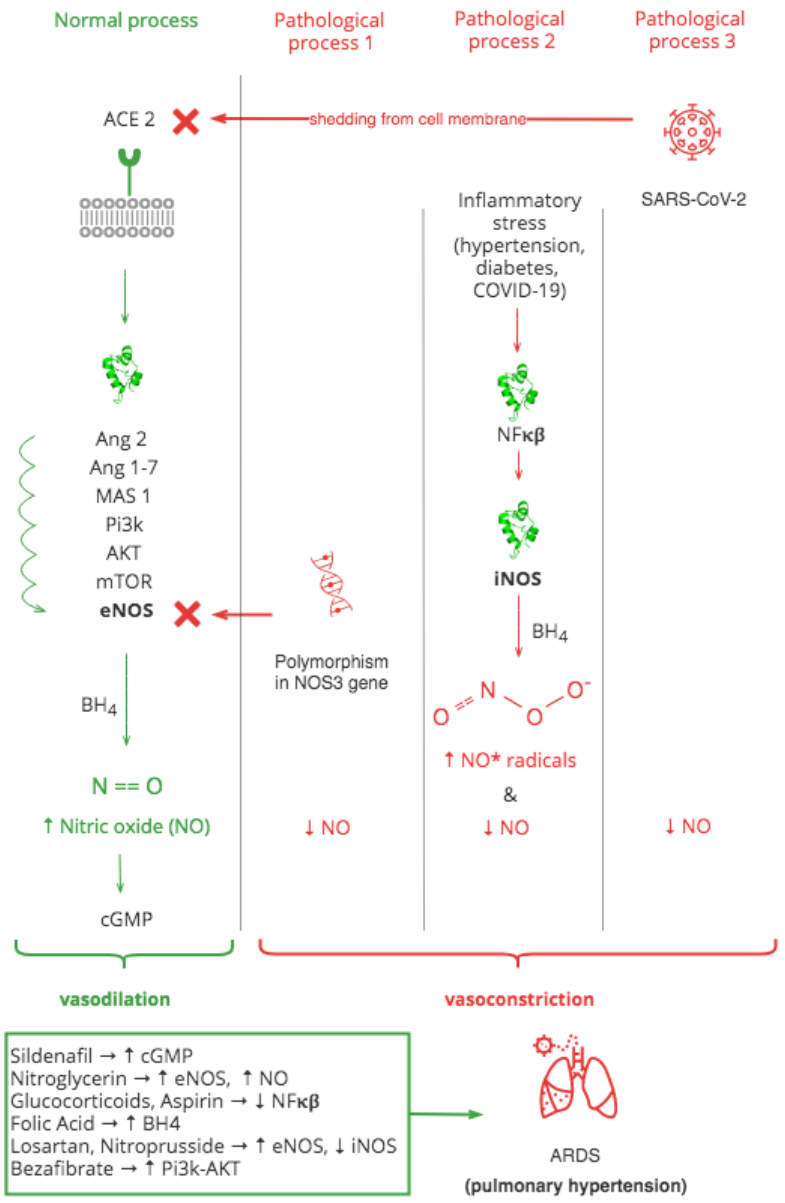
Graphical Abstract

COVID-19 aggravates already compromised NO production in cohort with NOS3 polymorphism. Management of eNOS/iNOS ratio and NO level can prevent development of severe ARDS.



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Abstract

OBSERVATION. As of March 2020, according to WHO status reports, there is an observable disproportion in COVID-19-related mortality rates throughout global population, even for geo-, socio-, politico-, economically similar and *close* countries (www.who.int/docs/default-source/coronaviruse/situation-reports/20200324-sitrep-64-covid-19.pdf). Namely, mortality rate in Italy is 21x higher that in Germany.

HYPOTHESIS. Genetic and epigenetic factors specific to Northern Italian population influence outcome of a disease due to overlap of viral and background conditions' pathways. Reported previously [4,5] NOS3 gene (eNOS) polymorphism in Northern Italian population reflects hereditary insufficiency of basal NO production.

METHODS. Domain expert provided starting points (*SARS-CoV-2, polymorphism, italian, ards. ...*) to trigger chain reaction-like facts extraction and reasoning by machine operating on publications available in Pubmed and PMC. Then extracted facts and presynthesized steps were validated by expert to form (1) evidence-supporting dataset and final (2) normal, (3) pathological and (4) drug-induced pathways.

RESULTS.

1. Normal Angiotensin converting enzyme 2 (ACE2) >> endothelial nitric oxide synthase (eNOS) pathway
2. Model of integral NOS+SARS-CoV-2 pathogenesis and its subprocesses:
   2.1. Pathological impact of SARS-CoV-2.
   2.2. Contribution of NOS3 polymorphism to NO deficiency.
3. Considerations for ARDS management. Key point is maintenance of iNOS/eNOS balance.

NOS+SARS-CoV-2 pathogenesis provides partial explanation of a data that is observed on epidemiological level.

Research data is available at https://doi.org/10.6084/m9.figshare.12021336

Points of consideration in management of NOS+SARS-CoV-2 pathogenesis

Appropriate therapy should be prescribed by a doctor considering anamnesis. Points below are relevant as a part of pathogenetic treatment of a NOS3 polymorphic cohort with insufficiency of eNOS activity.

iNOS/eNOS balance

iNOS/eNOS regulation is a part of physiological response to essentially any pathogen. iNOS produces nitrogen radicals to kill pathogen at early stages of a response [30,31]. At the same time, free radicals damage host's cells thus worsening course of disease [25]. eNOS is responsible for recovery via production of nitric oxide (NO) with vasodilative, antioxidative, antifibrotic and antiproliferative effects [1,2,3].

SARS-CoV-2, as any infection in general, stimulates iNOS via non-specific inflammatory response (NF-kb) [6], simultaneously inhibits eNOS pathway by downregulation of ACE2 as a specific response and therefore depleting the rest of NO production [8,9].

Also, iNOS prevalence aggravates reduction in NO production by limiting availability of BH_4 for eNOS because *BH_4 is an essential cofactor for both iNOS and eNOS* [7].

Contributing factors

Older people have increased iNOS/eNOS ratio [26].

Estrogen stimulates eNOS expression [28,29]. It conforms with gender-related difference in hospitalization [27].

Comorbidity [39] as any pathological process has inflammatory component inducing iNOS transcription [37] and consequently decreased eNOS. [35,40,41]

The best approach to prevent iNOS activation is controlled management of comorbidity such as hypertension and diabetes.

Feedback Loop Trap

Typically, infection-related hypoxia leads to pulmonary hypertension due to iNOS prevalence which is balanced then with opposite action of eNOS.

Lungs of patients with background conditions (genetic susceptibility and/ or comorbidity which shift balance toward iNOS) *maintain rigidity of vessels*. It develops inability to manage their respiratory function and leads to ARDS.

In case of any attempt to ventilate them mechanically, such patients develop ventilator-associated lung injury (VALI) without any mechanism of recovery thus trapping into vicious circle [21,22,23,24].

Lack of NO is a core of a problem of a NOS3-polymorhic cohort. Sustained vasoconstriction of pulmonary vessels counteracts any aggressive respiratory- and hemodynamic management. In order to mediate gas exchange, vessels should be relaxed with NO.

Medicines with relevant mechanism of action

Drug	Key function relevant to NOS+SARS-CoV-2 pathogenesis
Nitroglycerin	Activates eNOS [13]
Sildenafil	Inhibits PDE5 which otherwise degrades cGMP. The latest mediates vasodilation by NO [10,11,12]
Ionomycin	Activates eNOS [36]
Bezafibrate	Stimulates eNOS [34]
Losartan	Inhibits iNOS and sustains normal eNOS [17]
Nitroprusside	Inhibits iNOS [33]
Folic Acid	Exogenous source of BH4 [16]
Glucocorticoids	Inhibits iNOS [14]
Aspirin	Inhibits iNOS [15]
Atorvastatin	Inhibits iNOS [26]
Aminoguanidine	Inhibits iNOS [38]

Treatment that targets ACE2

Drugs that up-regulate eNOS expression via up-regulation of ACE2 expression should be prescribed with caution, because ACE2 acts as an entrance gate for SARS-CoV-2 too.

ARBs vs iACE

Still debatable. Angiotensin (Ang) II type 1 receptor blockers (ARBs) are preferred over angiotensin converting enzyme (ACE) inhibitors, because ARBs positively regulate eNOS and negatively regulate iNOS expression while the ACE inhibitors positively regulate both eNOS and iNOS expression or have no impact at all [18,19, 20].

Exogenic NO

Can be considered in critical condition. At the same time, exogenic supply downregulates own eNOS expression in long term [32] thus to be avoided in recovery phase.

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Appendix A. Selected citations

Subset of facts that were used in pathways synthesis. Full dataset is located at https://doi.org/10.6084/m9.figshare.12021336.v1

However, NO can also abate the oxidation chemistry mediated by reactive oxygen species such as H2O2 and O2- that occurs at physiological levels of NO. In addition to the antioxidant chemistry, NO protects against cell death mediated by H2O2, alkylhydroperoxides, and xanthine oxidase. The attenuation of metal/peroxide oxidative chemistry, as well as lipid peroxidation, appears to be the major chemical mechanisms by which NO may limit oxidative injury to mammalian cells. [1]
These results provide evidence for a novel antiproliferative effect of NO and ANP in HASMCs mediated through cGMP-dependent and cGMP-independent mechanisms. [2]
The additive effects of L−arginine and IFN could be due to a synergism of both compounds by increasing NO concentration, which can act as an antifibrotic agent but also as a cytoprotective compound. [3]
Moreover, to appraise the role of hypertension in the association among eNOS variants and cardiovascular damage, we compared the prevalence of eNOS genotype in the hypertensives and normotensives both with cardiovascular damage (table (table5).5). Hypertensives with cardiovascular damage were characterized by a greater prevalence of G894T polymorphism ($p < 0.001$) and introne 4 mutations ($p < 0.05$) than normotensives. [4]
The present study provides evidence that the Glu(298)-->Asp and T(786)-->C polymorphisms of the eNOS gene are associated with the presence and severity of angiographically defined CAD in the Italian population and that those individuals carrying both eNOS variants simultaneously might have a higher risk of developing CAD. [5]
The binding of NF-κB to the promoter of the iNOS gene was enhanced in the 25 mM glucose group compared with the 5.5 mM glucose group or the 25 mM glucose + 100 μL PDTC group, and this difference was statistically significant ($P < 0.05$).[6]
Because BH4 is an essential cofactor for production of NO by both iNOS and endothelial nitric oxide synthase (eNOS), these results suggest that iNOS may reduce production of NO by eNOS by limiting availability of BH4.[7]
Notably, experimental SARS-CoV infections of wild-type mice in vivo resulted in considerably reduced ACE2 expression in the lungs (Fig. 2b) suggesting that reduced ACE2 expression might have a role in SARS-CoV–mediated severe acute lung pathologies. [8]
However,binding of the SARS viral spike protein to ACE2 does trigger enzyme internalization, down-regulating activity from the cell-surface. [9]
In the hand vein, sildenafil administration increased sensitivity to local nitroglycerin. [10]
It was observed that animals treated with sildenafil citrate showed a highly significant increase in NO and a decrease in PDE level, but the histological architecture of the cardiomyocytes did not show much change other than a slightly elongated and swollen nucleus. [11]
Administration of sildenafil in patients with ARDS decreased mean pulmonary arterial pressure from 25 to 22 mmHg ($P = 0.022$) and pulmonary artery occlusion pressure from 16 to 13 mmHg ($P = 0.049$). [12]
These data demonstrated that eNOS is phosphorylated at Ser-1177 in the rats and mice treated with nitroglycerin, indicating nitroglycerin-induced eNOS activation. [13]
iNOS promoter activity induced by IL-1beta was inhibited by dexamethasone and the inhibitory effect was reversed by HDAC inhibitor trichostatin A. [14]
High doses of aspirin inhibited iNOS protein expression in BVSMCs and decreased NF-kappa B mobilization. [15]
In the 5 and 10 nmol/l FA treatment groups, FA was found to significantly increase the levels of BH4 (10.56±3.86 and 11.23±2.1919 pmol/g vs 6.32+2.87 nmol/g; $P<0.05$ vs. control) and NO production (37.86±12.34 nmol/l, 38.45±11.23 nmol/l vs 26.21±9.24 nmol/l; $P<0.001$ vs. paired Hcy group), but reduce the levels of Hcy (132.87±29.67 and 140.87±26.76 nmol/l vs. 165.23±30.56 nmol/l; $P<0.05$ vs. Hcy group). [16]
Losartan pretreatment significantly suppressed an increase in inducible nitric oxide synthase (iNOS) and sustained normal levels of eNOS expression 24h after MCAO-

R injury. Phosphorylated eNOS and Akt levels were much lower than those in the sham group at 24h after MCAO-R, suggesting that losartan pretreatment significantly preserved eNOS phosphorylation in response to the activated Akt. [17]
Both eNOS and iNOS expression seemed to increase during perindopril treatment. These results suggest that suppression of angiotensin II formation in the vascular wall and increased expression of eNOS and iNOS during ACE inhibition may be beneficial in reversing endothelial dysfunction in patients with cardiovascular disease. [18]
Captopril did not affect eNOS protein expression (Figure 3). There were no significant changes in inducible NOS (iNOS) protein expression within the groups (Figure 4).[19]
Moreover, olmesartan significantly increased the cardiac ACE2 expression level compared to that in Wistar Kyoto rats and SHRSP treated with a vehicle.[20]
In ARDS mice, the expression of the angiogenesis-related marker CD31 and eNOS was downregulated.[21]
Acute respiratory distress syndrome (ARDS) is associated with increased superoxide (O2•−) formation in the pulmonary vasculature and negation of the bioavailability of nitric oxide (NO).[22]
Our results show significantly higher serum NO levels in ARDS survivors compared to ARDS non-survivors, ($p < 0.05$). We conclude that higher serum levels of NO are strongly associated with better clinical outcomes, including increased survival.[23]
We observed the significant expression of iNOS, IL-6, and IL-8 only in the ARDS group. Meanwhile, NOx (the sum of NO2- + NO3-) was elevated in the BALF supernatant, and IL-6 and IL-8 levels in both the BALF supernatant and the serum were also elevated in the ARDS group.[24]
Tissue specific increases in iNOS production in the chicken appear to be associated with a higher degree of disease severity in H5N1 infection in chickens when compared to ducks.[25]
Comparisons between young, middle, and old control rats showed that the senescent phenotype was enhanced in intima and media ($p<0.01$), and that MDA, calcium-independent NOS activity, and iNOS increased with age ($p<0.01$), whereas endothelium-dependent relaxation, SOD, NO, calcium-dependent NOS activity, eNOS, the eNOS/iNOS ratio, and SIRT1 declined with age ($p<0.01$). Compared with old controls, long-term administration of atorvastatin to old rats inhibited the senescent phenotype ($p<0.05$), improved endothelium-dependent relaxation ($p<0.05$ or 0.01), decreased MDA ($p<0.01$), increased SOD, NO, eNOS, and SIRT1 expression ($p<0.01$), and inhibited iNOS expression (not detectable) in aged rat aortas. The results indicate that the long-term administration of atorvastatin improves age-related endothelial dysfunction in aged rats via inhibition of the senescent phenotype, amelioration of oxidative stress, and normalization of eNOS/iNOS imbalance. [26]
By Jan 2, 2020, 41 admitted hospital patients had been identified as having laboratory-confirmed 2019-nCoV infection. Most of the infected patients were men (30 [73%] of 41); less than half had underlying diseases (13 [32%]), including diabetes (eight [20%]), hypertension (six [15%]), and cardiovascular disease (six [15%]). [27]
Female and estrogen-treated male mice show greater resistance to pneumococcal pneumonia, seen as greater bacterial clearance, diminished lung inflammation, and better survival. [28]
E2 induced increases in eNOS protein levels and phosphorylated eNOS (eNOS(p)). [29]
Our results demonstrated that NO specifically inhibits the replication cycle of SARS CoV, most probably during the early steps of infection, suggesting that the production of NO by iNOS results in an antiviral effect. However, the production of NO should be adjusted to exert antiviral rather than damaging effects. [30]
The level of MD during first stage was higher than that of recovery stage and the MDA level of recovery stage was higher than that of follow-up stage, contact group, and health control group ($P<0.01$). The content of NO_(2)~(-)/NO_(3)~(-) during early stage was higher than that of other grups, and the NO_(2)~(-)/NO_(3)~(-) contents of recovery stage, follow-up stage were higher than that of contact grup and health control group ($P<0.01$), respectivley. The mean of iNOS during early stage was highest than that of other stages ($P<0.01$) and the mean of recovery stage was higher than that of contact group ($P<0.05$), there were no difference in iNOS activity among and other groups ($P>0.05$). The patholgical injuury in pathogenesis of SARS is rleated to free radicals. [31]
Inhalation of NO at 20 ppm early after birth decreases eNOS gene transcription, protein expression and enzyme activity. [32]

Preincubation with NO donors sodium nitroprusside (SNP 1-1000 μM) or S-nitroso-acetyl-penicillamine (SNAP 1-1000 μM), or with the heavy metals cadmium chloride (10-40 μM), bismuth citrate, or ranitidine bismuth citrate (10-3000 μM) inhibited iNOS activity in a concentration dependent manner. [33]

Firstly, in cultured bovine aorta endothelial cells (BAEC), bezafibrate significantly upregulated eNOS at protein, mRNA levels and NO production, respectively, in a concentration-dependent fashion (50-200muM). [34]

In the lungs of the control subjects, nitric oxide synthase was expressed at a high level in the vascular endothelium of all types of vessels and in the pulmonary epithelium. In contrast, little or no expression of the enzyme was found in the vascular endothelium of pulmonary arteries with severe histologic abnormalities (i.e., plexiform lesions) in patients with pulmonary hypertension. The intensity of the enzyme immunoreactivity correlated inversely with the severity of histologic changes. There was an inverse correlation between the arterial expression of the enzyme and total pulmonary resistance in patients with plexogenic pulmonary arteriopathy ($r = -0.766$, $P = 0.004$). [35]

Our findings indicate that 4-day and 2-wk posthypoxic rats exhibit persistent pulmonary hypertension, likely due to maintained arterial remodeling and polycythemia associated with prior exposure to CH. Furthermore, arterial dilation to ionomycin was augmented in lungs from each experimental group compared with controls. Finally, arterial eNOS immunoreactivity and whole lung eNOS mRNA levels remained elevated in posthypoxic animals. These findings suggest that altered vascular mechanical forces or vascular remodeling contributes to enhanced EDNO-dependent arterial dilation and upregulation of arterial eNOS in various models of established pulmonary hypertension. [36]

In vitro iNOS expression was increased ($P=0.006$) and phosphorylated vasodilator-stimulated phosphoprotein was decreased in skin from hypertensive humans ($P=0.04$). These data suggest that iNOS is upregulated in essential hypertensive humans and contributes to reduced NO-dependent cutaneous vasodilation. [37]

However, the effect of aminoguanidine was predominantly mediated by inhibition of iNOS activity, thereby reducing peroxynitrite formation. We propose that the development of a more specific and potent inhibitor of iNOS might be beneficial in preventing pathological conditions such as the essential hypertension. [38]

The elderly and those with underlying disorders (i.e., hypertension, chronic obstructive pulmonary disease, diabetes, cardiovascular disease), developed rapidly into acute respiratory distress syndrome, septic shock, metabolic acidosis hard to correct and coagulation dysfunction, even leading to the death [48] (lower panel, Fig. 1). [39]

Any decrease in nitric oxide generation, as has been postulated to occur in essential hypertension, could have substantial effects on blood pressure and tissue blood flow. [40]

The results of this study cumulatively indicate that gene therapy with human eNOS decreased fructose-induced hypertension and insulin resistance in rats and suggest potential signaling pathways that mediate these effects. [41]