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Letter to the Editor

The influence of renin angiotensin aldosterone system (RAAS), endothelial nitric oxide synthase (eNOS) and erythropoietin (EPO) on COVID-19 complications

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ABSTRACT

Certain aspects of the renin-angiotensin-aldosterone system (RAAS) have eluded deserved attention such as the role of erythropoietin (EPO) and nitric oxide (NO) both of which appear to significantly modulate COVID-19 disease course. Furthermore, renin-angiotensin-aldosterone system (RAAS) and endothelial NO synthase (eNOS) genetic polymorphisms additionally impact on EPO and NO homeostasis and have extensive implications on pharmacological disease management.

We read with interest the Review of Agustin and colleagues [1]. We wish to comment on certain aspects of the renin-angiotensin-aldosterone system (RAAS) that have eluded deserved attention [2]. That the angiotensin-converting enzyme (ACE) 2 is the obligate receptor for the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is self-evident in view of the evolutionary perspective of protection in the young. Following binding of SARS-CoV-2 to ACE2, the chronicle of a battle foretold unravels. SARS-CoV-2-mediated downregulation of ACE2, removes the protective arm of the RAAS and increases angiotensin II (Ang II) and aldosterone [1]. Elevated Ang II and aldosterone are both master regulators of erythropoietin (EPO) homeostasis and engage host humoral and tissue RAAS to induce EPO oversecretion that we posit is protective against SARS-CoV-2 infection in children and young adults [2–4]. Due to lower ACE2 expression in children, this SARS-CoV-2-mediated ACE/ACE2 imbalance, swiftly and potently calls upon an innate immune response motif with EPO as the main protagonist mediating a phylogenetically preserved, tissue protective mechanism mitigating tissue injury and pathogen invasion at an early age [2]. An age-dependent EPO elevation, dissociated from anemic stimuli, protects the young brain from prematurity, kernicterus, and malaria [2]. Malaria's protective genetic determinants, such as the ACE D-allele, the ACE2 T-allele, or hemoglobin E (HbE)/beta thalassemia and other hemoglobinopathies, all appear to converge into a common augmentation of EPO secretion, especially at an early age [2]. The ineffective erythropoiesis in hemoglobinopathies, and the elevated Ang II, coded and augmented by RAAS genetic polymorphisms, all ensure persistent and elevated, systemic and tissue EPO levels to elicit EPO's non-erythropoietic tissue protective actions [2]. Unrelated to the level of anemia, thalassemic children under 5 years of age have more than 6-fold EPO increase compared with 10 years of age, and younger children with malaria under 2.5–3 years of age similarly had significant and up to 2-fold increase in EPO compared with children over 5 years of age [5,6]. High plasma and cerebrospinal fluid levels of EPO are associated with reduced risk of neurological sequelae in children with cerebral malaria [7]. Elevated EPO mRNA levels (2·6 times) were recently reported in nasopharyngeal swab samples of adult SARS-CoV-2 patients with asymptomatic or mild coronavirus disease 2019 (COVID-19), as compared to controls [8]. Most of EPO effects are linked to increased endothelial nitric oxide (NO) synthase (eNOS) activity in endothelial cells where increased NO-generation and bioavailability exerts microbicidal and cardiovascular protective effects [9]. EPO-mediated eNOS activation and increased NO bioavailability may in fact counteract the endotheliitis and eNOS activity impairment induced by SARS-CoV-2 [10]. In addition, increased NO bioavailability may halt infection at an early stage by inhibiting i) palmitoylation and fusion of the SARS-CoV-1/2 spike protein to ACE2, and ii) early production of viral RNA, processes critical in controlling membrane fusion and virion infectivity [11]. Palmitoylation of SARS-CoV-2 S protein is critical in controlling membrane fusion and virion infectivity [12]. Later in the course of SARS-CoV-2 infection, EPO-induced NO-increases may be cardio-, and reno-protective as previously demonstrated [13].

Additional protective EPO effects in children are the increased numbers of $CD71^{+}$ erythroid cells (CEC), absent in healthy adults but abundant in pregnancy and the very young, that are able to prevent a hyperimmune reaction to pathogens in early life [14]. Furthermore, EPO mediates reduction of auto- and alloantibody (AA) formation while its binding to T cell-expressed EPO-receptor (EPOR) inhibits Th17 cell induction preventing collateral damage and autoimmune pathology [2].

It is, however, evident that children can get infected by SARS-CoV-2, and a few may progress to multisystem inflammatory syndrome in children (MIS-C) while older adults may progress to acute respiratory distress syndrome (ARDS) and cytokine storm [2]. EPO protection override possibly occurs through a combination of blunted EPO secretion and reduced NO-generation and bioavailability. EPO levels are modulated by RAAS genetic polymorphisms [15]. eNOS genetic variants also alter NO-generation and bioavailability [16,17]. Interactions between the two have pleiotropic results [16,17]. In children, EPO falls with increasing age but malaria-protective alleles (ACE D-/ACE2 T-alleles) may counteract eNOS loss-of-function polymorphisms and extend SARS-CoV-2 protection further towards a higher age [2]. When a tipping point is reached, unopposed augmented RAAS hyperactivity turns detrimental and can progress to MIS-C due to loss of NO protection. In

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adults, blunted EPO secretion may result from hypertension with widespread use of RAAS inhibitors, obesity, diabetes with ensuing hyperglycemia, glucotoxicity, diabetic hyporeninemic hypoaldosteronism, autonomic neuropathy, hypogonadism with low testosterone, chronic and acute inflammation through Ang II induced interleukin (IL)-6 increase [18]. All of these factors collectively and separately can inhibit renal EPO secretion [18]. Furthermore, elevated Ang II increases plasminogen activator inhibitor (PAI-1) and IL-6 levels but reduces α-Klotho and increases fibroblast growth factor (FGF) 23, that contribute to additional EPO inhibition [2]. eNOS and RAAS polymorphisms are both associated with numerous cardiovascular and metabolic comorbidities and independently or synergistically influence cardiovascular pathology and pharmacological interventions [19]. Their concurrent occurrence in the presence of the above co-morbidities can set the scene for a particularly detrimental COVID-19 course with cytokine storm and autoimmunity [1]. Finally, FGF23 and Ang II interactions are reported to augment their mutually detrimental cardiovascular effects through eNOS inhibition [2,20,21]. This pattern can potentially explain the downregulated EPO and eNOS levels observed in critically ill adult COVID-19 patients and related ARDS [22]. Additionally, ACE D allele potentiated, pyroptosis-induced Ang II type 1 receptor autoantibodies (AT1-AA) correlating significantly with IL-6 and systolic blood pressure have been described in hypertension, pre-eclampsia and COVID-19 [23]. AT1-AA could mediate persistent proinflammatory Ang II effects unresponsive to RAAS pharmacological inhibition and confound treatment effects [24].

An evolution-congruent strategy, restoring the integrity of the RAAS-EPO pathway and endogenous EPO secretion as well as supporting a healthy NO-generation by using EPO-neutral antihypertensive treatments, NO donor modalities, fluvoxamine/fluoxetine, statins while concurrently employing rhEPO and/or EPO analogs will break this vicious circle of RAAS hyperstimulation [2,18,20,25,26]. Knowledge of patients' genetic status regarding RAAS and eNOS polymorphisms as well as awareness of their pharmacogenetic implications in cardiovascular drug therapy along with recognition of AT1-AA presence will help decide which pharmacological interventions will benefit which patient groups best. The most potent Ang II type 1 receptor blocker, olmesartan, significantly increased NO generation by 30% in all eNOS polymorphic variants but especially in the homozygous minor variant genotypes 894TT/786CC [27]. Novel compounds like NP-6A4 appear as an ideal adjuvant drug candidate for EPO mediated tissue protection and mitigation of cytokine storm [28]. Future research should include mendelian randomizations, but to date and to the authors' knowledge, such clinical trials do not yet exist.

CRediT authorship contribution statement

Konstantinos I. Papadopoulos: Investigation, Conceptualization, Validation, Writing – original draft, Writing – review & editing. **Warachaya Sutheesophon:** Conceptualization, Validation, Writing – review & editing. **Tar-Choon Aw:** Validation, Writing – review & editing, All authors have approved the manuscript and accept responsibility to submit for publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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