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Measurement of serum ACE status may potentially improve the diagnosis of SARS-CoV-2 infection

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ABSTRACT

The severity of SARS-CoV-2 infection is associated with underlying cardiovascular or pulmonary pathological conditions. The fatality rate of this typical pneumonia has superseded the two previous coronavirus epidemics combined. Thus far, comprehensive diagnosis of SARS-CoV-2 remains essential for effective screening, detection, and disease monitoring. This allows employment of different life-saving interventions to lower the spread and mortality, whilst the development of labelled therapeutics is underway. In this perspective, the measurement of Angiotensin-converting enzyme (ACE) status is perceived as a potential prognostic biomarker for SARS-CoV-2 patients. This notion is based on the observation that SARS-CoV-2 infection via attachment to Angiotensin-converting enzyme-2 (ACE2) receptor, downregulates ACE2 expression. Thus leading to the inability to efficiently counter-regulate the damaging effects of its homolog; ACE. The perspective is further strengthened by the recommendations of therapeutics that attenuate the conversion of Angiotensin I to a vasoconstrictor; Angiotensin II as an effective treatment of SARS-CoV-2 infection. In addition, other off-labelled used drugs target the latter; restoration of multiple organ failure and or cytokine storm inhibition. Therefore, this suggests that ACE may be strongly implicated in the pathogenesis of SARS-CoV-2.

Introduction

Similarities in the genomic and amino acid sequences of the severe acute respiratory syndrome coronavirus (SARS-CoV) spike (S) glycoprotein with the SARS-CoV-2 S presents a critical avenue towards understanding the pathogenesis of SARS-CoV-2 [1,2]. The SARS-CoV and SARS-CoV-2 have different binding affinities to Angiotensin-converting enzyme-2 (ACE2) presumably because of the eight strictly conserved sequences discovered on 144 SARS-CoV-2 genome sequences [2]. Previous comprehensive *in vitro* and *in vivo* studies on the pathogenesis and lethality of SARS-CoV have established that the binding of the SARS-CoV S glycoprotein to ACE2 receptor, downregulates ACE2 expression [3]. This phenomenon is seemingly enhanced in SARS-CoV-2 due to conserved sequences and the insertion of polybasic furin cleavage sites on the Spike Glycoprotein [2].

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Downregulation of ACE2 leaves the action of its homolog: ACE, unopposed [3]. The catalytic activity of ACE activates the vasoinactive Angiotensin I into a potent vasoconstrictor, Angiotensin II (Ang-II) [4]. Ang-II disrupts all cells in the vascular wall and is considered as a hallmark in vascular disease development [5]. Overproduction of Ang-II as a result of ACE hyperactivation triggers vascular inflammation [6].

ACE triggered pro-inflammatory cytokines

The severity of SARS-CoV-2 infection is associated with a variety of co-morbidities; cardiovascular diseases, diabetes, kidney injury, and secondary bacterial infections [7]. These disorders are characterized by a significantly low ACE2 and elevated ACE concentration [8]. The emergence of the so-called cytokine storm mediated by a SARS-CoV-2 infection in patients with lung injury, and its more severe form; acute respiratory distress syndrome (ARDS) suggests that pro-inflammatory cytokines, chemokines or its intermediaries may play a vital role in the assessment of the severity of SARS-CoV-2 infection [9]. It has also been observed that severely ill SARS-CoV-2 patients that require ICU admission release large amounts of pro-inflammatory cytokines [10].

Cardiovascular or pulmonary diseases are associated with hyperactivity of ACE, and the absence of the protective function of ACE2. Thus, making the lungs and heart vulnerable to the damaging effects of ACE/Ang-II. ACE2 binds with a high affinity to Ang-II and cleaves it to produce a potent heptapeptide: Ang (1–7). The Ang (1–7) is a biologically active metabolite that counterbalances the effects of Ang-II by exerting vasodilatation, and anti-inflammatory effects via activation of Mas receptor [11].

Angiotensin profile has become a major target for pharmacological therapies [12]. The ACE inhibitors (ACEIs) and/ Angiotensin II receptor blockers (ARBs) are first-line therapies for the treatment of cardiovascular diseases [13]. The clinical utility of ACEIs is attributed to the inhibition of Ang-II formation, leading to diminished NAD(P)H oxidase expression and O^2- formation by vascular endothelium [14]. It also indirectly stimulates the accumulation of bradykinin which exhibits vasoprotective function [15]. Additionally, corticosteroid drugs have been suggested to be potent anti-inflammatory drugs that could be used as a treatment for cytokine storm mediated by SARS-CoV-2 infection [16].

The catalytic activity of ACE elicits pro-inflammatory cytokines such as interleukin–6 (IL-6) and IL-8 triggered by Ang-II [17]. A similar pathophysiology has also been confirmed to contribute to the severity of SARS-CoV-2 infection [10]. Under this condition, clinical assessment of ACE/Angiotensin profile could potentially improve the diagnosis of the SARS-CoV-2 infection, predominantly for severely ill patients, and further provide evidence-based prescription of ARBs and corticosteroid drugs.

Measurement of ACE kinetics and/ Angiotensin profile

The ACE activity has clinical significance for the diagnosis and treatment monitoring of hypertension, and sarcoidosis. Clinical chemistry laboratories mainly use spectrophotometric assays based on Furyl-acryloyl-phenylalanyl-glycyl-glycine (FAPGG) as a substrate for the measurement of ACE [18]. The cleavage of FAPGG by endogenous ACE generates furylacryloylphenylalanine (FAP) and glycylglycine [18]. The kinetics of this reaction is quantified by recording a decrease in absorbance at 340 nm.

The fluorescence resonance energy transfer (FRET) substrate such as *o*-aminobenzoic acid-FRK(Dnp)P-OH is also used for assessing human ACE status [19]. This method provides real-time kinetic readings of enzyme activity utilizing wavelengths of 320 nm (excitation) and 420 nm (emission) [20].

Despite its wide application, the drawback of spectrophotometric and spectroscopic assays is the lack of specificity as it measures the proteolytic products of renin-Angiotensin systems (RAS) by quantifying change in colour or fluorescence [21].

The recent development of proteolytic assays for monitoring cardiovascular pathology that are based on liquid or gas chromatography/mass spectrometry (LC/MS or GC/MS) offer rapid and sensitive alternatives compared to predating techniques[22]. These new proteolytic assays also provide a comprehensive analysis of biologically active ACE/Ang-II and ACE2/Ang-(1–7) [22].

Selection of an ACE assay with corrected serum deletion (D)/insertion (I) genotype reference values has been shown to improve clinical sensitivity of ACE reporting since there is strong evidence confirming serum ACE levels are mainly dependant on genetic influence [23]. Serum ACE was found to be influenced by a deletion D/I polymorphism defined by the presence or absence of a 287-bp DNA fragment in the intron 16 of the ACE gene [24]. The D/D genotype is linked to high serum ACE levels, whilst in I/I ACE genotype is generally low [25].

The distribution of ACE D/I polymorphism accounts for a 28% variation in serum ACE levels of Caucasian origin [26]. However, a statistically powered Nigerian cohort study did not find the clinical significance of ACE D/I polymorphism, the varying serum ACE was attributable to other 13 single-nucleotide polymorphisms (SNPs) across the entire ACE gene. [27] The interpretation of serum ACE values without reference to ACE D/I polymorphism may lead to an incorrect diagnosis. In addition, there was a study conducted by Biller et al. [23] that reported on genotype-corrected reference values for serum ACE of Caucasians for two test kits from: Buhlmann Laboratories AG and Trinity Biotech. Therefore, this suggests that genotyping is an essential step that aids the clinical significance of serum ACE reporting.

Conclusion

The status of ACE may provide a comprehensive clinical picture for SARS-CoV-2 patients. Based on the utility of these assays, Angiotensin profile could be assessed at the early stages of infection, as a prognostic biomarker, and for monitoring the therapeutic activity of various treatment interventions. A systematic review on RAS assays may also reveal the most sensitive assay that could be used to assess the Angiotensin profile on SARS-CoV-2 patients.

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.

CRediT authorship contribution statement

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