Mathematical Modeling of Impacts of Patient Differences on Renin-Angiotensin System and Applications to COVID-19 Lung Fibrosis Outcomes

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Abstract

Patient-specific premorbidity, age, and sex are significant heterogeneous factors that influence the severe manifestation of lung diseases, including COVID-19 fibrosis. The renin-angiotensin system (RAS) plays a prominent role in regulating the effects of these factors. Recent evidence suggests patient-specific alteration of RAS homeostasis with premorbidity and the expression level of angiotensin converting enzyme 2 (ACE2) during COVID-19. However, conflicting evidence suggests decreases, increases, or no changes in RAS after SARS-CoV-2 infection. In addition, detailed mechanisms connecting the patient-specific conditions before infection to infection-induced RAS alteration are still unknown. Here, a mathematical model is developed to quantify the systemic contribution of heterogeneous factors of RAS during COVID-19. Three submodels are connected—an ABM COVID-19 in-host lung tissue model, a RAS model, and a fibrosis model to investigate the effects of patient-group-specific factors in the systemic alteration of RAS and collagen deposition in the lung. The model results indicate cell death due to inflammatory response as a major contributor to the reduction of ACE and ACE2. In contrast, there are no significant changes in ACE2 dynamics due to viral-bound internalization of ACE2. Reduction of ACE and ACE2 reduces the homeostasis of RAS, including angiotensin II (ANGII), in the lung tissue. At the same time, the decrease in ACE2 increases systemic ANGII and results in severe lung injury and fibrosis. The model explains possible mechanisms for conflicting evidence of RAS alterations in previously published studies, and simulated results are consistent with reported RAS peptide values for SARS-CoV-2-negative and SARS-CoV-2-positive patients. We observed decreased RAS peptides for all patient groups with aging in both sexes. In contrast, large variations in the magnitude of reduction were observed between male and female patients in the older and middle-aged groups. We also predicted that feedback of ANGII•AT1R to renin could restore ANGI homeostasis but fails to restore homeostasis values of RAS peptides downstream of ANGI. In addition, the results show that ACE2 variations with age and sex significantly alter RAS peptides and lead to collagen deposition with slight variations depending on age and sex. This model may find further applications in patient-specific calibrations of tissue models for acute and chronic lung diseases to develop personalized treatments.

Keywords: SARS-CoV-2, Renin-Angiotensin System, TGF-β, pulmonary fibrosis

1. Introduction

The renin-angiotensin system (RAS) has gained significant attention as alterations in RAS peptides and enzymes directly correlate with patient-specific premorbidity, age, and sex differences in response to COVID-19 lung diseases (Kutz et al., 2021; Liu et al., 2021; Martins et al., 2021; Pagliaro and Penna, 2020). RAS is important in maintaining physiological processes, including blood pressure and vascular permeability (Kutz et al., 2021). The cascade of RAS's local and systemic regulatory processes begins with renin from the kidney and angiotensinogen (AGT) from the liver, which enter into the systemic process through blood and migrate to the lung. The renin activity on AGT causes its conversion to angiotensin I (ANGI). ANGI uses the angiotensin converting enzyme (ACE) from the cell surface of lung tissue to convert to angiotensin II (ANGII). ANGII binds with angiotensin converting enzyme 2 (ACE2) from the cell surface to form the ACE2•ANGII complex, which converts to angiotensin 1–7 (ANG1–7). ANGII also binds with angiotensin type 1 receptor (AT1R) and forms the ANGII•AT1R complex, which regulates the feedback

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signaling to the systemic renin production rate. In the subsequent reactions, ANGII binds with angiotensin type 2 receptor (AT2R) and forms the ANGII•AT2R complex, ANGII converts to angiotensin IV (ANGIV) by enzymatic reaction, and ANG1–7 binds with MAS receptor to form the MAS•ANG1–7 complex. The expressions of ACE2 (Pagliaro and Penna, 2020) and homeostasis values of RAS peptides (Hilliard et al., 2013) are patient-dependent.

Kutz et al. (2021) observed reductions in all RAS peptides and renin activity without significant differences in ACE and ACE2 activity in the serum of COVID-19 patients compared with matched controls. Contrarily, Liu et al. (2021) reported increased ANGII in critically ill SARS-CoV-2 positive patients compared to non-critically ill SARS-CoV-2 positive patients. Liu et al. (2020) observed increased plasma ANGII levels in SARS-CoV-2-infected patients compared to healthy individuals. The up-regulation in ACE2 expression has also been observed in COVID-19 patients (Zhang et al., 2020). Contrary to all, Kintscher et al. (2020) did not observe any significant differences in plasma RAS peptides and ACE2 activity between control and COVID-19 patients. To explain the conflicting data on RAS peptide alteration, Kutz et al. (2021) highlighted the differences between their study and that of Liu et al. (2021), particularly the selection of the baseline patient groups. The baseline patient group in Kutz et al. (2021) was SARS-CoV-2-negative, whereas Liu et al. (2021) selected SARS-CoV-2-positive patients with normal ANGII range. In addition to these contradictions and limitations of scarce patient data, detailed mechanisms suitable for explaining the conflicting observations in different patient groups still need to be determined.

Patient-specific premorbidity, age, and sex differences can account for response variations between patient groups. Pendergrass et al. (2008) observed variations in RAS with sex differences for hypertensive rats. Xudong et al. (2006) reported dramatically reduced ACE2 expression with aging and higher ACE2 content in old female rats than in males. In a recent mathematical study, Bastolla (2021) showed a direct correlation between the severity of COVID-19 fatality depending on age and sex across three countries and experimental data for the expression of ACE2 from Xudong et al. (2006). Miesbach (2020) reported that male patients were more affected by severe manifestations than female patients and depended on the activation of RAS and pathological roles of ANGII.

Downregulation of ACE2 inhibits the protective activity of downstream RAS peptides, resulting in inflammation and fibrosis (Yim et al., 2021; Wigén et al., 2020). RAS regulates fibrosis by directly and indirectly activating and inhibiting latent TGF-β (Murphy et al., 2015). ANGII•AT1R complex in RAS activates latent TGF-β directly or via Smad and ERK/p38/MAPK signaling pathways, whereas ANGII•AT2R complex inhibits TGF-β production. Via *in vitro* experiments, ANGII•AT1R was reported to induce procollagen synthesis from human fetal lung fibroblasts directly through mitogenesis and indirectly through TGF-β (Marshall et al., 2004). ANGII-induced TGF-β production was also observed in vascular smooth muscle cells (Gibbons et al., 1992) and glomerular mesangial cells (Kagami et al., 1994). In our earlier mathematical modeling work (Islam et al., 2023), we also identified TGF-β as a critical contributor to COVID-19 fibrosis. TGF-β activates and recruits macrophages and fibroblasts, and fibroblasts are responsible for excess collagen deposition and fibrosis (Islam et al., 2023; Ceresa et al., 2018; Jin et al., 2011).

Bleomycin-mouse models are widely used to evaluate the effect of RAS peptides and enzymes in lung fibrosis (Mecozzi et al., 2020). Li et al. (2008) observed increased ANGII and collagen for ACE2 knockout mice in bleomycin-induced lung fibrosis models. Rey-Parra et al. (2012) also ran similar experiments for ACE2 knockout male and female mice and observed increased collagen in both males and females with significantly increased collagen in males compared to females (Rey-Parra et al., 2012); they also highlighted that a higher AT2R/AT1R ratio in female mice could be a possible explanation for the sex differences in collagen deposition. The bleomycin-induced lung fibrosis mouse model of Kuba et al. (2005) showed increased ANGII in the presence of SARS-CoV spike proteins. Another study from the same lab reported that ACE2 knockout mice also increased ANGII (Imai et al., 2005). The mouse model of Wang et al. (2015) showed exogenous ACE2 attenuates bleomycin-induced lung injury by attenuating TGF-β production and collagen deposition.

Multiple computational and mathematical models have been developed to integrate and quantify the effect of premorbidity, age, and sex differences in RAS and RAS-mediated fibrosis. Leete et al. (2018) developed a mathematical model to investigate the impact of sex differences in RAS, and they identified ANGII•AT1R feedback mechanism as a significant modulator of sex differences. Pucci et al. (2020) developed a mathematical model to investigate the pathogenic mechanisms of SARS-CoV-2 and RAS and applied their model for *in silico* testing of potential modulators to restore functionality. Sadria and Layton (2020) expanded a RAS model with membrane-bound ACE2, shedding of ACE2, and internalized ACE2; connected that to a damage response model; and used the model to investigate the effects of drugs targeting ACE and AT1R. The mathematical modeling framework of Voutouri et al. (2021) connected SARS-CoV-2 infection, RAS, innate and adaptive immune cells, and coagulation cascade to investigate the heterogeneity in treatment response and clinical outcome as a function of patient comorbidities, age, and immune response; the model predicted increases in ANGII in severe COVID-19 patients. Barbiero and Lió (2020) developed a computational patient model integrating cardiovascular, RAS, and diabetic processes. They analyzed the effects of age, diabetes, and renal impairment during SARS-CoV-2 infection in a functional context. Our lab has also previously developed mathematical models of RAS for normal and impaired renal functions (Ford Versypt

et al., 2017) and glucose dependency of RAS in diabetic kidney disease (Pilvankar et al., 2018, 2019).

Here, we developed a mathematical model that contributes to understanding patient-specific premorbidity, age, and sex differences on RAS during SARS-CoV-2 infection. We connected a RAS model to our earlier agent-based model (ABM) framework for COVID-19 tissue response in the lung and fibrosis (Islam et al., 2023) to investigate and quantify the local and systemic effects of the patient-specific premorbidity, age, and sex differences on RAS in the progression of fibrosis due to SARS-CoV-2 infection.

2. Methods

2.1. COVID-19 RAS fibrosis model

The COVID-19 RAS fibrosis model (Fig. 1) consists of three submodels: an ABM COVID-19 in-host lung tissue model to quantify tissue scale dynamics of ACE2 receptor trafficking and inflammatory immune response due to SARS-CoV-2 infection, a RAS model to account for patient-specific local and systemic changes of RAS peptides and enzymes, and a fibrosis model to quantify the effects of immune modulation by dysregulated RAS peptides and systemic contributions to lung fibrosis. We refer to the COVID-19 RAS fibrosis model as the "overall model". Each of the submodels is detailed in turn.

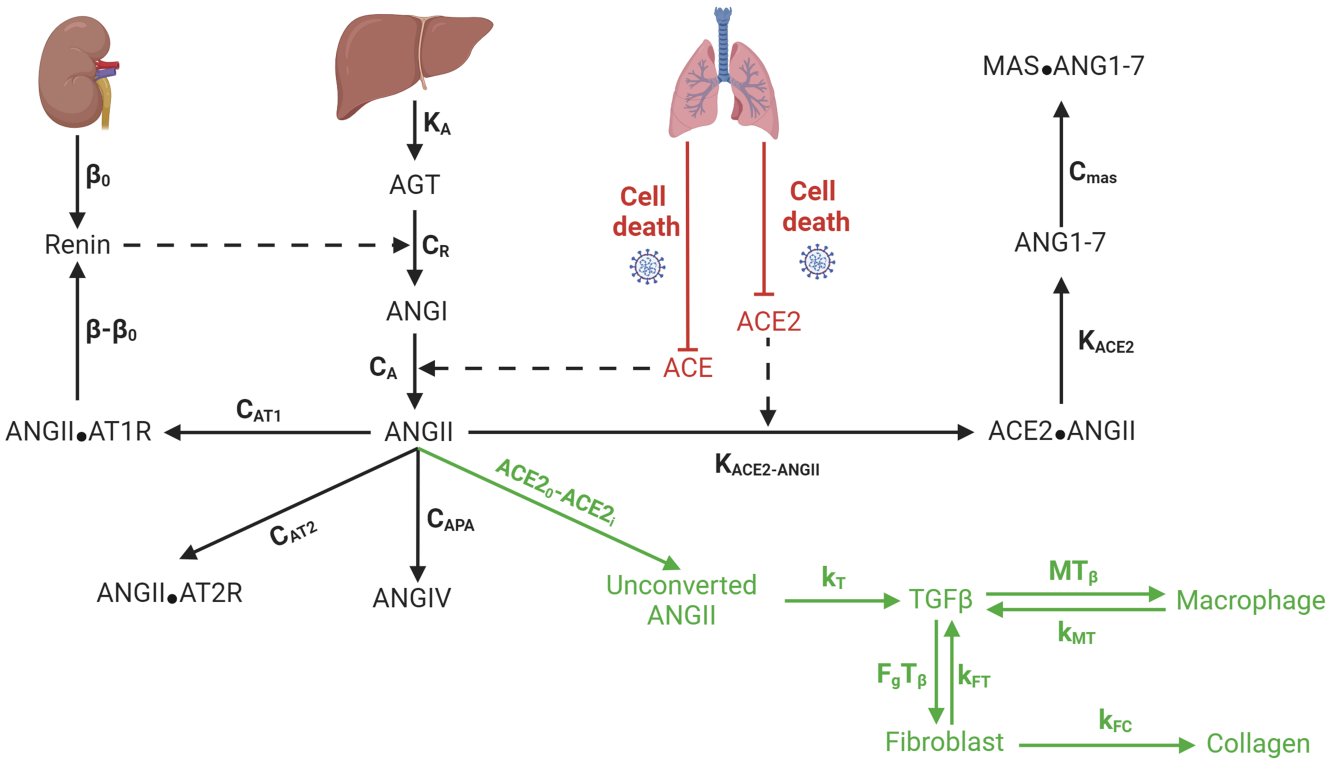

Fig. 1: Schematic diagram of COVID-19 RAS Fibrosis model. Renin is produced from the kidney and angiotensinogen (AGT) from the liver. The renin activity on AGT causes its conversion to angiotensin I (ANGI). ANGI uses the angiotensin converting enzyme (ACE) from the cell surface of lung tissue to convert to angiotensin II (ANGII). ANGII binds with angiotensin converting enzyme 2 (ACE2) from the cell surface to form the ACE2•ANGII complex, which converts to angiotensin 1–7 (ANG1–7). ANGII also binds with angiotensin type 1 receptor (AT1R) and forms the ANGII•AT1R complex, which regulates the feedback signaling to the systemic renin production rate. In the subsequent reactions, ANGII binds with angiotensin type 2 receptor (AT2R) and forms the ANGII•AT2R complex, ANGII converts to angiotensin IV (ANGIV) by enzymatic reaction, and ANG1–7 binds with MAS receptor to form the MAS•ANG1–7 complex. During infection, the death of epithelial cells downregulates both ACE and ACE2, which increases unconverted ANGII. Unconverted ANGII induces TGF-β production and activates the fibrosis cascade.

2.1.1. COVID-19 tissue model

In our earlier work, we developed a SARS-CoV-2 tissue simulator (Islam et al., 2023), which we call the "COVID-19 tissue model". We use the model to determine the ACE and ACE2 receptor profiles. Here, we briefly recap the most salient features of the model.

The COVID-19 tissue model is developed in an open-source multiscale ABM framework PhysiCell (Ghaffarizadeh et al., 2018) and simulates a tissue section of 800 μm × 800 μm × 20 μm, representing a monolayer of stationary epithelial cells on an alveolar surface of lung tissue. Initially, 2793 epithelial cells, 50 resident macrophages, 28 dendritic cells, and 57 fibroblasts are present in the simulated tissue. Using a uniform random distribution, we infect the simulated tissue by placing SARS-CoV-2 viral particles in the extracellular space. Viral particles diffuse through tissue and bind with unoccupied or unbound external ACE2 receptors (ueACE2) on the epithelial cell surfaces to form bound external ACE2 receptors (beACE2). These bound external ACE2 receptors internalize via endocytosis to form bound internal ACE2 receptors (biACE2), release the virions to form unbound internal ACE2 receptors (uiACE2), and recycle back to the cell surface.

The released virions from biACE2 replicate through intracellular viral replication kinetics, export back to the extracellular domain by exocytosis, and diffuse in the tissue. Our model considers the interactions between virion and ueACE2 as discrete events, whereas diffusion of the virus in tissue is continuous. Details of the intracellular virus model for replication kinetics, viral response, receptor trafficking, and rules for discrete to continuous transition of the virus are described in much greater detail in our earlier manuscript (Islam et al., 2023; Getz et al., 2020). Viral infection activates the innate and adaptive immune response and recruits immune cells from the lymph nodes. Further immune and lymph node model details are available elsewhere (Islam et al., 2023; Getz et al., 2020, 2021; Jenner et al., 2021). Viral infection and immune response activate latent TGF-β and shift the phenotype of pro-inflammatory M1 macrophages to anti-inflammatory M2 macrophages to produce TGF-β in the later phase of infection, which leads to fibroblast-mediated collagen deposition and fibrosis. Details of the effects of TGF-β sources in fibroblast-mediated collagen deposition at damaged sites of SARS-CoV-2 infected tissue are available in our earlier work (Islam et al., 2023). Here, we build upon that work to consider the effects of ACE2 receptor dynamics on the RAS network and lung fibrosis.

We assume the same initial number and dynamics of ACE and ACE2 receptors. This assumption is based on the experimental observation of similar intensity of ACE and ACE2 protein expressions in lung tissue in the control mice experiments of Roca-Ho et al. (2017). Losses of ACE and ACE2 occur due to epithelial cell death and binding between virus and ACE2 during SARS-CoV-2 infection. We use the dynamics of ueACE2 from the COVID-19 tissue model to account for the changes in ACE and ACE2, that is

$$ueACE2(t) = ACE(t) = ACE2(t) \tag{1}$$

2.1.2. RAS model

The rate of change of renin is

$$\frac{d[R]}{dt} = \beta - \frac{\ln 2}{h_R}[R] \tag{2}$$

$$\beta = \begin{cases} \beta_0, & \text{if no feedback} \\ \beta_0 + \left(\frac{[ANGII \cdot AT1R]_0}{[ANGII \cdot AT1R]}\right)^{\delta} - 1, & \text{else if } [ANGII \cdot AT1R] \geq [ANGII \cdot AT1R]_L \\ \beta_0 + \left(\frac{[ANGII \cdot AT1R]_0}{[ANGII \cdot AT1R]_L}\right)^{\delta} - 1, & \text{otherwise} \end{cases} \tag{3}$$

where $[R]$ is renin concentration, β is the production rate, and h_R is the half-life of renin. The term β has two contributions: a constant source of renin from the kidney, β_0, and feedback of ANGII•AT1R to the production of renin, given by Eq. 3. The feedback signaling depends on the initial concentration of ANGII•AT1R ($[ANGII \cdot AT1R]_0$), changes in ANGII•AT1R after infection ($[ANGII \cdot AT1R]$), and the lower value of ANGII•AT1R ($[ANGII \cdot AT1R]_L$). We estimated $[ANGII \cdot AT1R]_L$ based on the experimental study of Kutz et al. (2021). The strength of the feedback signaling is defined by a dimensionless number, δ.

The rate of change of AGT is

$$\frac{d[AGT]}{dt} = k_A - c_R \frac{ACE(t)}{ACE(t_i)}[R] - \frac{\ln 2}{h_A}[AGT] \tag{4}$$

where $[AGT]$ is concentration of AGT, k_A is a constant source of AGT from the liver, c_R relates renin concentration to its activity on AGT, $ACE(t)$ is the ACE receptor profile determined from the COVID-19 tissue model and described in Section 2.1.1, $ACE(t_i)$ is the initial value ACE receptor, and h_A is the half-life of AGT. Here, we consider plasma renin activity (c_R) based on earlier models (Hallow et al., 2014; Pucci et al., 2020). Kutz et al. (2021) reported 58.5% reduction in plasma renin activity in COVID-19 patients. We also assume that changes in c_R after SARS-CoV-2 infection depend on the profile of the ACE receptor in the COVID-19 tissue model to account for the changes in plasma renin activity.

The rate of change of ANGI is

$$\frac{d[ANGI]}{dt} = c_R \frac{ACE(t)}{ACE(t_i)}[R] - c_A[ANGI]ACE(t) - \frac{\ln 2}{h_{A1}}[ANGI] \tag{5}$$

where $[ANGI]$ is the concentration of ANGI, c_A is the rate constant for ACE catalyzed conversion of ANGI to ANGII, and h_{A1} is the half-life of ANGI.

The rate of change of ANGII is

$$\begin{aligned}\frac{d[ANGII]}{dt} &= c_A[ANGI]ACE(t) - c_{APA}[ANGII] - c_{AT1}[ANGII] - c_{AT2}[ANGII] \\ &\quad - k_{ACE2\cdot ANGII}[ANGII]ACE2(t) - \frac{\ln 2}{h_{A2}}[ANGII]\end{aligned} \tag{6}$$

where $[ANGII]$ is concentration of ANGII, c_{APA} is the rate constant for APA catalyzed conversion of ANGII to ANGIV, c_{AT1} and c_{AT12} are the binding rate constants for formation of ANGII•AT1R and ANGII•AT2R, respectively, $k_{ACE2\cdot ANGII}$ is the binding rate constant for formation of ACE2•ANGII, $ACE2(t)$ is the ACE2 receptor profile determined from the COVID-19 tissue model and described in Section 2.1.1, and h_{A2} is the half-life of ANGII.

The rates of change of ACE2•ANGII and ANG1–7 are

$$\frac{d[ACE2 \cdot ANGII]}{dt} = k_{ACE2\cdot ANGII}[ANGII]ACE2(t) - k_{ACE2}[ACE2 \cdot ANGII] \tag{7}$$

and

$$\frac{d[ANG1\text{–}7]}{dt} = k_{ACE2}[ACE2 \cdot ANGII] - c_{mas}[ANG1\text{–}7] - \frac{\ln 2}{h_{A17}}[ANG1\text{–}7] \tag{8}$$

where $[ACE2 \cdot ANGII]$ and $[ANG1\text{–}7]$ are concentrations of ACE2•ANGII and ANG1–7, respectively, k_{ACE2} is the rate constant for the conversion rate of ACE2•ANGII to ANG1–7, c_{mas} is the binding rate constant for the formation of MAS•ANG1–7, and h_{A17} is the half-life of ANG1–7. Here, we considered the reduction in ACE2 from the lung tissue model (Section 2.1.1) and assumed no natural degradation of ACE2•ANGII.

The rate of change of ANGIV is

$$\frac{d[ANGIV]}{dt} = c_{APA}[ANGII] - \frac{\ln 2}{h_{A4}}[ANGIV] \tag{9}$$

where $[ANGIV]$ is concentration of ANGIV and h_{A4} is the half-life of ANGIV.

ANGII binds with AT1R and AT2R receptors, and ANG1–7 binds with MAS receptors. The rates of change of these complexes are

$$\frac{d[ANGII \cdot AT1R]}{dt} = c_{AT1}[ANGII] - \frac{\ln 2}{h_{AT1}}[ANGII \cdot AT1R], \tag{10}$$

$$\frac{d[ANGII \cdot AT2R]}{dt} = c_{AT2}[ANGII] - \frac{\ln 2}{h_{AT2}}[ANGII \cdot AT2R], \tag{11}$$

and

$$\frac{d[MAS \cdot ANG1\text{–}7]}{dt} = c_{mas}[ANG1\text{–}7] - \frac{\ln 2}{h_{mas}}[MAS \cdot ANG1\text{–}7] \tag{12}$$

where $[ANGII \cdot AT1R]$, $[ANGII \cdot AT2R]$, and $[MAS \cdot ANG1\text{–}7]$ are concentrations and h_{AT1}, h_{AT2}, and h_{mas} are the half lives of ANGII•AT1R, ANGII•AT2R, and MAS•ANG1–7, respectively.

The parameters $[R_0]$, β, k_A, c_A, $k_{ACE2\cdot ANGII}$, k_{ACE2}, c_{APA}, c_{AT1}, c_{AT2}, and $[MAS \cdot ANG1\text{–}7_0]$ in Eqs. 2–12 are patient-specific and require homeostasis values of RAS in calibration. A list of fixed parameters for the overall model is available in Table 1, and mean homeostasis RAS peptide concentrations for normotensive and hypertensive patients are listed in Table 2. We further calibrate the RAS model with the initial number of ACE and ACE2 receptors of virtual lung tissue in COVID-19 tissue model. We calculate the patient-specific parameters for normotensive and hypertensive patients using the homeostasis values of RAS peptides and the initial number of ACE and ACE2 receptors from virtual lung tissue. We further assume $c_{mas} = c_{AT2}$ and solve Eqs. S1–S11 in the Supplementary material to calculate the patient-specific parameters (Table 2).

Table 1: List of fixed parameters for the overall model.

Symbol	Definition	Value	Units	Source
c_R	Renin activity on AGT	2×10^1	min^{-1}	(Pucci et al., 2020)
h_R	Half-life of renin	1.2×10^1	min	(Pucci et al., 2020; Hallow et al., 2014)
h_A	Half-life of angiotensinogen	6×10^2	min	(Pucci et al., 2020; Hallow et al., 2014)
h_{A1}	Half-life of angiotensin I	5×10^{-1}	min	(Pucci et al., 2020; Hallow et al., 2014)
h_{A2}	Half-life of angiotensin II	5×10^{-1}	min	(Pucci et al., 2020; Hallow et al., 2014)
h_{A17}	Half-life of angiotensin 1–7	5×10^{-1}	min	(Pucci et al., 2020; Hallow et al., 2014)
h_{A4}	Half-life of angiotensin IV	5×10^{-1}	min	Estimated
h_{AT1}	Half-life of angiotensin II & receptor 1 complex	1.2×10^1	min	(Pucci et al., 2020; Hallow et al., 2014)
h_{AT2}	Half-life of angiotensin II & receptor 2 complex	1.2×10^1	min	(Pucci et al., 2020; Hallow et al., 2014)
h_{mas}	Half-life of angiotensin 1–7 & MAS complex	1.20×10^1	min	(Pucci et al., 2020)
δ	Strength of feedback to Renin	8×10^{-1} [0, 1]	Unitless	(Leete et al., 2018; Pucci et al., 2020)
$[ANGII \cdot AT1R]_L$	Lower value of ANGII·AT1R	3	fmol mL^{-1}	(Kutz et al., 2021)
DS	TGF-β activation rate from a damaged site	2×10^{-9}	ng min^{-1}	Estimated in (Islam et al., 2023)
MS	TGF-β secretion rate from an M2 macrophage	2×10^{-9}	ng min^{-1}	Estimated in (Islam et al., 2023)
k_T	ANGII-induced TGF-β production rate	5.0×10^{-4}	$\text{ng mL min}^{-1}\ \text{fmol}^{-1}$	Estimated
k_{MT}	Macrophage TGF-β production rate	4.86×10^{-5}	min^{-1}	(Ceresa et al., 2018; Jin et al., 2011)
k_{FT}	Fibroblast TGF-β production rate	2.78×10^{-6}	min^{-1}	(Ceresa et al., 2018; Jin et al., 2011)
d_T	TGF-β degradation rate	1.04×10^{-2}	min^{-1}	(Ceresa et al., 2018)
d_M	Macrophage apoptosis rate	4.17×10^{-4}	min^{-1}	(Ceresa et al., 2018; Jin et al., 2011)
d_F	Fibroblast apoptosis rate	8.30×10^{-5}	min^{-1}	(Ceresa et al., 2018; Jin et al., 2011)
k_{FC}	Fibroblast collagen production rate	2.52×10^{-7}	$\mu\text{g min}^{-1}$	Estimated from (Hao et al., 2015)
$V_{T\beta}$	Rate parameter for TGF-β-dependent collagen deposition	9.42×10^{-1}	Unitless	Estimated from (Roberts et al., 1986; Ignotz and Massague, 1986) data in (Islam et al., 2023)
$k_{T\beta}$	Saturation parameter for TGF-β-dependent collagen deposition	1.74×10^{-1}	Unitless	Estimated from (Roberts et al., 1986; Ignotz and Massague, 1986) data in (Islam et al., 2023)
$M(T_\beta)$	TGF-β-dependent macrophage recruitment rate	interpolated	cell min^{-1}	(Wahl et al., 1987)
$F_g(T_\beta)$	TGF-β-dependent fibroblast recruitment rate	interpolated	cell min^{-1}	(Wahl et al., 1987)

Table 2: Homeostasis concentrations of species and parameter values in RAS network for uninfected normotensive and hypertensive patients.

Symbol	Definition	Hypertensive	Normotensive	Unit	Source
$[AGT]_0$	angiotensinogen	6×10^5	6×10^5	fmol mL^{-1}	(Pucci et al., 2020; Katsurada et al., 2007)
$[ANGI]_0$	angiotensin I	110	70	fmol mL^{-1}	(Pucci et al., 2020; Chappell, 2016; Pendergrass et al., 2008)
$[ANGII]_0$	angiotensin II	156	28	fmol mL^{-1}	(Pucci et al., 2020; Chappell, 2016; Pendergrass et al., 2008)
$[ANG1\text{–}7]_0$	angiotensin 1–7	92	36	fmol mL^{-1}	(Pucci et al., 2020; Chappell, 2016; Pendergrass et al., 2008; Sullivan et al., 2015)
$ACE2_0$	angiotensin converting enzyme 2	200–2000	200–2000	cell^{-1}	COVID-19 tissue model
$[ACE2 \cdot ANGII]_0$	angiotensin II & ACE2 receptor complex	2.1×10^4	2.1×10^4	fmol mL^{-1}	(Voutouri et al., 2021)
$[ANGIV_0]$	angiotensin IV	1	1	fmol mL^{-1}	(Pucci et al., 2020; Nussberger et al., 1986)
$[ANGII \cdot AT1R]_0$	angiotensin II & type 1 receptor complex	85	15	fmol mL^{-1}	(Pucci et al., 2020; Leete et al., 2018)
$[ANGII \cdot AT2R]_0$	angiotensin II & type 2 receptor complex	27	5	fmol mL^{-1}	(Pucci et al., 2020; Leete et al., 2018)
$[R]_0$	Renin	2.53×10^1	9.43×10^0	fmol mL^{-1}	calculated
β_0	Renin production rate	1.46×10^0	5.45×10^{-1}	$\text{fmol mL}^{-1}\ \text{min}^{-1}$	calculated
k_A	angiotensinogen production rate	1.2×10^3	8.82×10^2	$\text{fmol mL}^{-1}\ \text{min}^{-1}$	calculated
c_A	ANGI to ANGII conversion rate	1.15×10^{-6}	4.69×10^{-7}	$\#\text{ACE}^{-1}\ \text{min}^{-1}$	calculated
$k_{ACE2 \cdot ANGII}$	ANGII & ACE2 receptor binding rate	2.95×10^{-7}	6.43×10^{-7}	$\#\text{ACE2}^{-1}\ \text{min}^{-1}$	calculated
k_{ACE2}	ANGII–ACE2 to ANG1–7 conversion rate	6.12×10^{-3}	2.39×10^{-3}	min^{-1}	calculated
c_{APA}	ANGII to ANGIV conversion rate	8.89×10^{-3}	4.95×10^{-2}	min^{-1}	calculated
c_{AT1}	ANGII & type 1 receptor binding rate	3.15×10^{-2}	3.09×10^{-2}	min^{-1}	calculated
c_{AT2}	ANGII & type 2 receptor binding rate	1×10^{-2}	1.03×10^{-2}	min^{-1}	calculated
$[MAS \cdot ANG1\text{–}7]_0$	angiotensin 1–7 & MAS receptor complex	1.6×10^1	6.43×10^0	fmol mL^{-1}	calculated

2.1.3. Fibrosis model

The reduction of ACE2 reduces ANGII at tissue scale. Meanwhile, a surplus of ANGII will remain unconverted at a systemic level and increase the systemic concentration of ANGII. With less ACE2 in the lung, systemic ANGII is converted to ACE2·ANGII at a slower rate, providing some surplus of ANGII not converted along this pathway towards ANG1–7. We term the additional ANGII due to loss of ACE2 as "unconverted ANGII". We calculate the unconverted ANGII as

$$[U_{ANGII}](t) = k_{ACE2 \cdot ANGII}\big(ACE2(t_i)[ANGII]_0 - ACE2(t)[ANGII]\big) \tag{13}$$

where $[U_{ANGII}]$ is the concentration of unconverted ANGII, $ACE2(t_i)$ is the number of available ACE2 before time of infection ($t = t_i$), $ACE2(t)$ is the number of available ACE2 at time $t > t_i$ after infection.

We assume that the unconverted ANGII modulates the direct and indirect activation of TGF-β, and we consider first-order reaction kinetics for ANGII-induced TGF-β production rate. Eq. 14 shows the dynamics of TGF-β:

$$\frac{d[T_\beta]}{dt} = k_T[U_{ANGII}] + k_{MT}[M] + k_{FT}[F] - h_T[T_\beta] \tag{14}$$

where $[T_\beta]$ is TGF-β concentration, k_T is ANGII-induced TGF-β production rate, k_{MT} is TGF-β production rate from macrophages, k_{FT} is TGF-β production rate from fibroblasts, and h_T is degradation rate of TGF-β.

TGF-β-dependent macrophage recruitment rate ($M(T_\beta)$) and fibroblast recruitment rate ($F_g(T_\beta)$) are estimated from the experimental observations of Wahl et al. (1987). Linear interpolation is used for the missing ranges of data. Fig. S1 in the Supplementary material shows the dynamics of $M(T_\beta)$ and $F_g(T_\beta)$ with TGF-β. The population balances for macrophages and fibroblasts are

$$\frac{d[M]}{dt} = M(T_\beta) - d_M[M] \tag{15}$$

and

$$\frac{d[F]}{dt} = F_g(T_\beta) - d_F[F] \tag{16}$$

where $[M]$ and $[F]$ are the populations and d_M and d_F are the apoptosis rates of macrophages and fibroblasts, respectively.

The TGF-β-dependent collagen deposition rate from fibroblasts is

$$\frac{d[C]}{dt} = k_{FC}\frac{V_{T\beta}[T_\beta]}{k_{T\beta} + [T_\beta]}[C] \tag{17}$$

where $[C]$ is collagen concentration, k_{FC} is the collagen production rate from fibroblasts, and $V_{T\beta}$ and $k_{T\beta}$ are corresponding rate and saturation parameters defining the TGF-β dependency on collagen deposition (Islam et al., 2023).

In the fibrosis model, we use the same parameters as our earlier work (Islam et al., 2023) except parameter values for TGF-β activation rate from a damaged site (DS) and TGF-β secretion rate from an M2 macrophage (MS) are fixed (Table 1), and the initial number of ACE2 receptors per cell is varied ($ueACE2_0$, Table 3) to represent patient groups. The initial values of the COVID-19 tissue model and fibrosis model variables (Table 3) are all relative to changes from the baseline of an uninfected patient rather than absolute numbers and concentrations. The code repository for our earlier fibrosis model is available in a repository at `https://github.com/ashleefv/covid19fibrosis` (Islam and Ford Versypt, 2023). The code repository for the COVID-19 RAS fibrosis model is available in a repository at `https://github.com/ashleefv/covid19fibrosisRAS` (Islam and Ford Versypt, 2024).

Table 3: Initial conditions for COVID-19 tissue and fibrosis submodels.

Symbol	Definition	Initial condition	Unit
$ueACE2_0$	Unoccupied external ACE2 receptors	200-2000	#
$beACE2_0$	Virus-bound external ACE2 receptors	0	#
$biACE2_0$	Virus-bound internal ACE2 receptors	0	#
$ueACE2_0$	Unoccupied internal ACE2 receptors	0	#
$T_{\beta 0}$	Initial concentration of TGF-β	0	ng mL^{-1}
M_0	Initial number of macrophages	0	cells
F_0	Initial number of fibroblasts	0	cells
C_0	Initial amount of collagen	0	μg

2.2. Sensitivity analysis

We use dynamic local sensitivity analysis for parameters to quantify the input parameters that significantly affect the output variables. Eq. 18 shows the local sensitivity index for $[ANGII]$ with respect to the one-at-a-time variation of each model parameter (P_i), which is approximated by a difference equation given a small perturbation ΔP_i to the model parameter (Ford Versypt et al., 2017):

$$\frac{\partial[ANGII]}{\partial P_i} = \lim_{\Delta P_i \to 0^+} \frac{[ANGII](P_i + \Delta P_i, P_{n\neq i}) - [ANGII](P_i, P_{n\neq i})}{\Delta P_i} \tag{18}$$

where $[ANGII](\mathbf{P})$ are the model predictions evaluated for parameter set $\mathbf{P}$. We normalize the local sensitivity index to eliminate the effect of units. Nominal input values (I_s) and output values (O_s) are calculated before varying the parameters. The input values are multiplied by a multiplier (m_S) to change one input at a time. We calculate the change of new input value (I_n) as: $\Delta I(t) = I_n(t) - I_s(t)$ where $I_n(t) = m_S I_s(t)$. The model for normalized sensitivity index, ($S_{i,t}$), for the predicted new output value (O_n) is shown in Eq. 19.

$$
\begin{aligned}
S_{i,t} &= \frac{\Delta O(t)}{O_s(t)} \frac{I_s(t)}{\Delta I(t)} \\
&= \frac{O_n(t) - O_s(t)}{O_s(t)} \frac{1}{m_S - 1}
\end{aligned}
\tag{19}
$$

where i denotes parameters or variables, and t denotes time. We applied additional rules to avoid division by zero, as described in Eq. 20.

$$
S_{i,t} = \begin{cases} \frac{O_n(t)-O_s(t)}{O_s(t)} \frac{1}{m_S-1}, & \text{if } O_s(t) \neq 0 \\ \frac{O_n(t)}{m_S-1}, & \text{otherwise} \end{cases}
\tag{20}
$$

2.3. Data extraction

Experimental data from the literature used in this paper were extracted from graphs in the sources (referenced in the text) using the web-based program WebPlotDigitizer (Rohatgi, 2022).

3. Results and Discussion

The overall model has three phases. In the first phase, we infected the virtual lung tissue considering different initial numbers of ACE2 per cell ($ueACE2_0$) using the COVID-19 tissue model. The model was used to evaluate the dynamics of ACE2 receptors after SARS-CoV-2 infection. In the second phase, we calibrated the RAS model with patient-specific homeostasis peptide concentration from Table 2 and an initial number of ACE2 receptors from the COVID-19 tissue model. The model was used to simulate the dynamics of RAS peptides before infection. In the third phase, the predicted ueACE2 dynamics from the COVID-19 tissue model were used as input for ACE and ACE2 dynamics in the RAS model. Here, we quantified the effects of patient-group-specific premorbidity by varying initial RAS peptide concentration and the impact of age and sex by varying initial numbers of ACE2. Finally, we extended the model to investigate the effect of dysregulation in RAS peptides on COVID-19 lung fibrosis outcomes.

3.1. Dynamics of ACE2 and tissue damage after SARS-CoV-2 infection depends on initial number of ACE2

In our earlier COVID-19 tissue model, we considered a constant initial ueACE2 ($ueACE2_0 = 1000$). Here, we varied initial ueACE2 in each cell from 200–2000 with an interval of 200 to account for age and sex differences. Fig. 2 and Figs. S2 and S3 in the Supplementary material showed the dynamics of the cell population, ACE2 receptors, and tissue damage with variations in initial ueACE2. We observed that the magnitude of reduction of ueACE2 increases with lower initial ueACE2 (Fig. 2B, 2F, and 2J). We also observed ACE2 reach a new steady state around six days as the removal of the virions from the system occurred around six days. We did not observe any significant variations in ACE2 dynamics due to viral binding and internalization of ACE2 (beACE2, biACE2, and uiACE2) as the recycling process moved a portion of internalized ACE2 back to the surface and ample availability of surface ACE2 receptors in neighboring uninfected cells (Fig. 2C, 2G, and 2K). However, we observed a direct correlation between the dynamics of total number of epithelial cells and ueACE2. We normalized the dynamics of total cells and ueACE2 with their initial value for comparison (Fig. 2D, 2H, and 2L). The findings suggest that the dysregulation of ACE2 in the lung tissue will mainly occur due to the loss of major sources of ACE2 by the death of epithelial cells during infection. We also observed complete destruction of tissue for $ueACE2_0$ 200 and 400, increased cell survivability for ranges between 600–1000, and steady behavior for $ueACE2_0$ of 1200 and above (Fig. S3 in the Supplementary material). Since recent studies showed a direct correlation between ACE2 expressions and COVID-19 fatality where male patients were more affected compared to female patients (Bastolla, 2021; Miesbach, 2020), we selected the age and sex of our virtual patient cohort based on the tissue damage dynamics. The lower ranges of $ueACE2_0$ represent older adults (200–800), intermediate ranges represent middle-aged (800–1200), and upper ranges of $ueACE2_0$ represent young patients (1200–2000). The variations of $ueACE2_0$ within a specific age group account for sex differences. ACE2 expression in the female rat lung was higher than in male rats (Bastolla, 2021; Xudong et al., 2006). So, we selected that within a specific age group, lower $ueACE2_0$ represents male patients, and higher $ueACE2_0$ represents female patients.

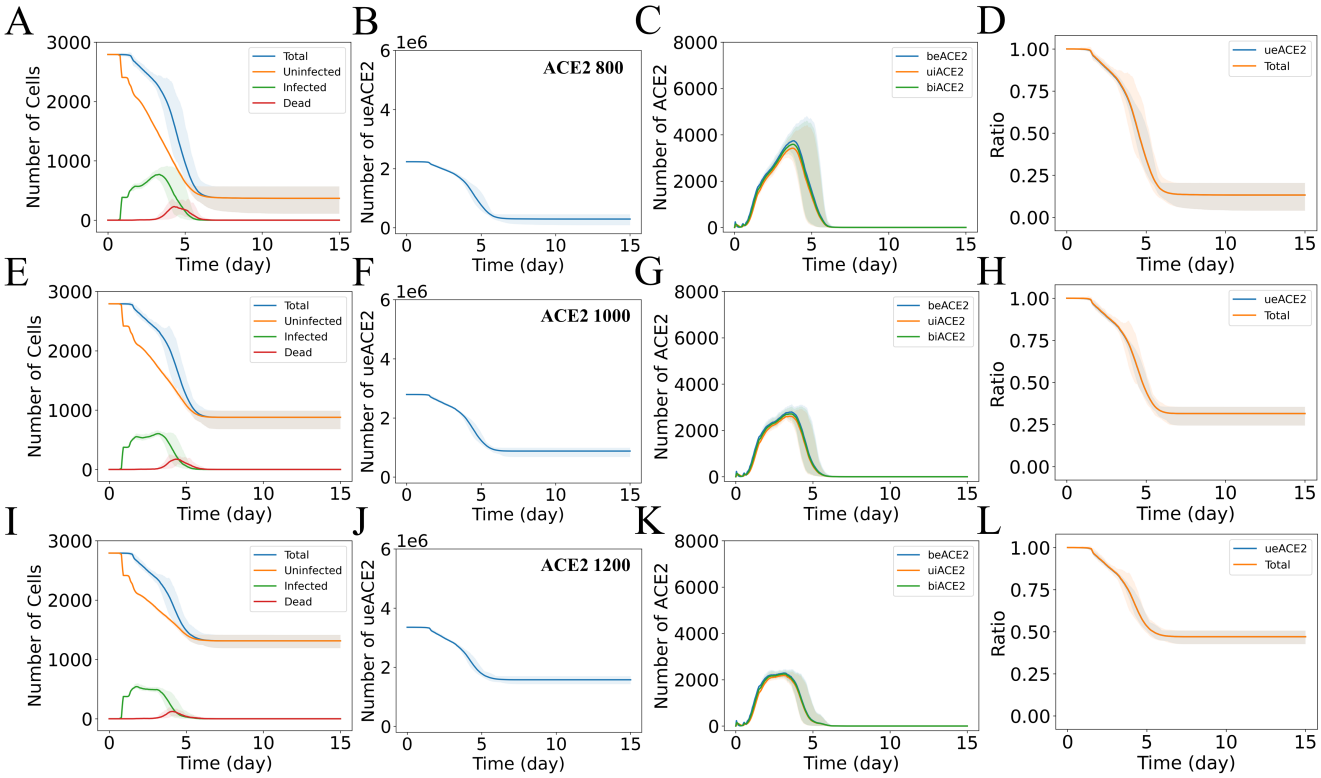

Fig. 2: Epithelial cells and ACE2 response to the different initial values of ACE2. Dynamics of (A, E, I) total, uninfected, infected, and dead cells, (B, F, J) unbound external ACE2 (ueACE2) (C, G, K) bound external ACE2 (beACE2), bound internal ACE2 (biACE2), unbound internal ACE2 (uiACE2), and (D, H, L) normalized comparison between the dynamics of ueACE2 and total cells. Each row represents a fixed initial ueACE2 number ranging from 800–1200, top to bottom. The solid lines represent the mean, and a shaded area represents the 5th and 95th percentile of 15 iterations.

3.2. Dynamics of RAS peptides with variations in ACE2

As described earlier, in the second phase, we calibrated the overall model with $ueACE2_0$ from the COVID-19 tissue model at the uninfected condition. We ran the simulation for one day to calibrate the parameters of RAS for different patient groups. Here, we considered hypertensive patients with no feedback of ANGII•AT1R to the production of renin. The third phase started after one day of preloading with the onset of infection, where we account for the variations in ACE2 dynamics from the COVID-19 tissue model. We simulated the third phase for ten days post infection as ACE2 reached a new steady state around six days after infection and investigated the variations in RAS peptides. Figs. 3 and S4 in the Supplementary material show the dynamics of RAS peptides. We observed decreased ANGI and downstream peptides for all patient groups with aging in both sex groups. The magnitude of the reduction is highest for older patients ($ueACE2_0$=200–800), intermediate for middle-aged ($ueACE2_0$=800–1200), and lower for younger patients ($ueACE2_0$=1200–2000). We also observed large variations in the magnitude of reduction between male and female patients in the older and middle-aged groups. In contrast, the variations due to sex were relatively slight for the young patient group.

3.3. Model predictions for experimentally observed decrease of RAS

Kutz et al. (2021) studied patient data to investigate RAS peptide alteration between SARS-CoV-2-positive and SARS-CoV-2-negative patients with respiratory infections. The study design included older adults with premorbid conditions, such as hypertension, diabetes, and obesity, for both SARS-CoV-2-positive and SARS-CoV-2-negative patients. They observed a lower equilibrium serum RAS peptides and plasma renin activity in SARS-CoV-2-positive patients compared to SARS-CoV-2-negative patients. They also reported the ranges of variations in ANGI (3–595 pmol L^{-1}), ANGII (10–1687 pmol L^{-1}), and ANG1-7 (3–166 pmol L^{-1}) in SARS-CoV-2-negative patients and the ranges of variations in ANGI (2.7–188 pmol L^{-1}), ANGII (2.1–357 pmol L^{-1}), and ANG1-7 (3–7.3 pmol L^{-1}) in SARS-CoV-2-positive patients. However, the time points for the data taken were not reported.

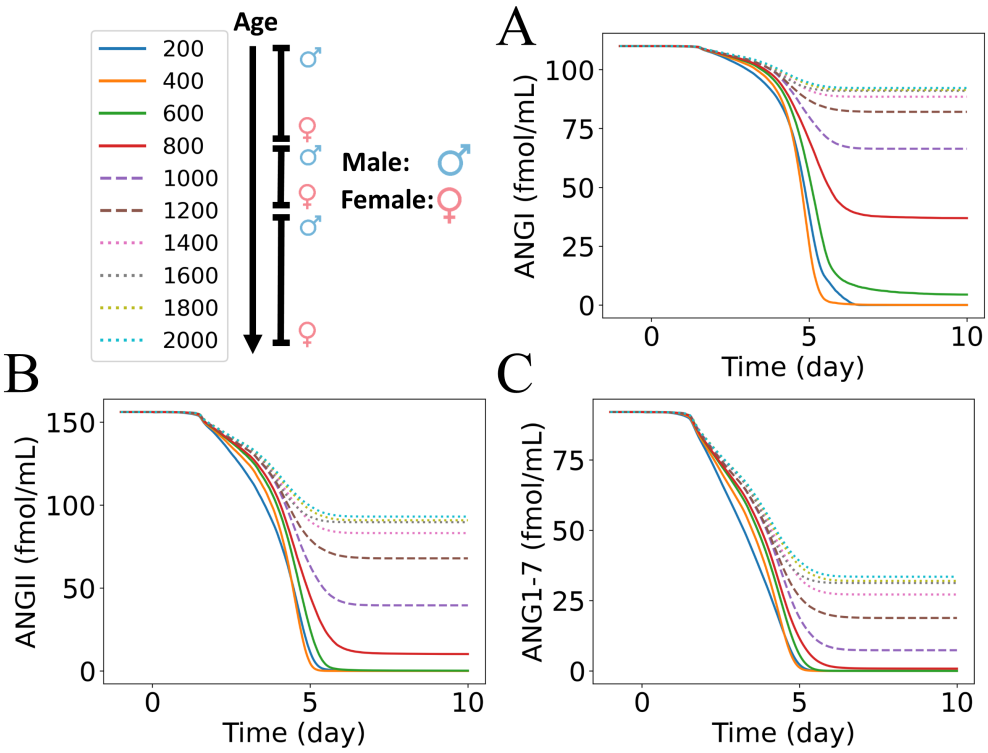

Fig. 3: Dynamics of RAS peptides with initial unbound external ACE2. Dynamics of A) ANGI, B) ANGII, and C) ANG1-7 for initial unbound external ACE2 ranges from 200–2000.

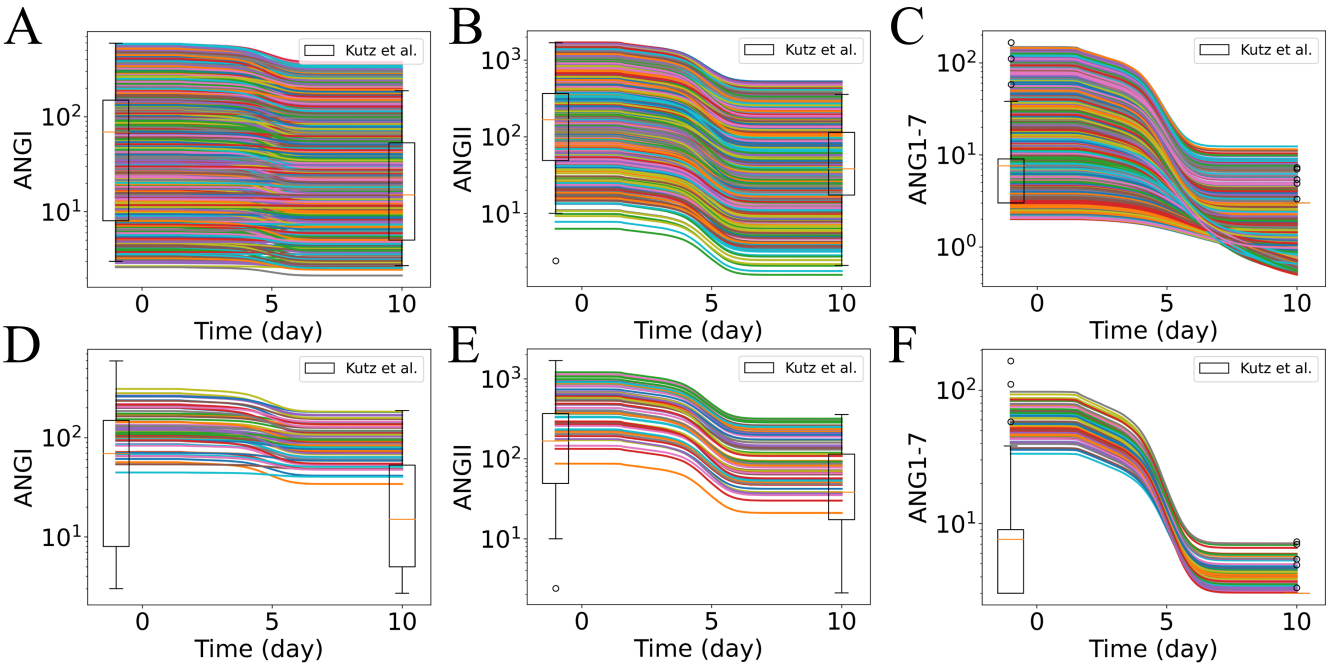

Fig. 4: effect of initial values of ANGI, ANGII, and ANG1-7 in the dynamics of RAS peptides. (A, B, C) Initial values of ANGI, ANGII, and ANG1-7 are sampled within the experimentally observed ranges of SARS-CoV-2-negative patients reported in Kutz et al. (2021). (D, E, F) A subset of top figures showing similar reduction within the experimentally observed ranges of SARS-CoV-2-positive patients reported in Kutz et al. (2021).

Here, we investigated the effects of variations in the initial values of RAS peptides. We sampled the initial values of ANGI, ANGII, and ANG1-7 using log uniform distribution within the ranges of experimentally observed values of SARS-CoV-2-negative patients. Also, we account for the relative differences in the initial values of ANGI, ANGII, and ANG1-7 using the bounds in Eq. 21.

$$[ANGII]_0 \geq \gamma_1[ANGI]_0 \cap [ANGII]_0 \geq \gamma_2[ANG1-7]_0 \cap [ANGI]_0 \geq \gamma_3[ANG1-7]_0 \tag{21}$$

where γ_1 is the ratio of ANGII to ANGI, γ_2 is the ratio of ANGII to ANG1-7, and γ_3 is the ratio of ANGI to ANG1-7. We selected $\gamma_1 = 1.4$, $\gamma_2 = 1.7$, and $\gamma_3 = 1.2$ using the homeostasis RAS peptides concentration for hypertensive patients listed in Table 2. The concentration of downstream RAS peptides ANGIV, ANGII·AT1R, ANGII·AT2R were also scaled based on ANGII value for hypertensive patients listed in Table 2 and new ANGII value sampled from the distribution as they derived from ANGII. For a sampled ANGII value $[ANGII]_{0,s}$ and ANGII value for hypertensive patients listed in Table 2 $[ANGII]_0$, we calculated the scaling factor as $\left(1 + \frac{[ANGII]_{0,s}-[ANGII]_0}{[ANGII]_0}\right)$, and multiplied $[ANGIV]_0$, $[ANGII \cdot AT1R]_0$, and $[ANGII \cdot AT2R]_0$ with the scaling factor. Fig. 4A-C showed the dynamics of ANGI, ANGII, and ANG1-7 for 1000 set of initial values of ANGI ranges from 2–600 fmol mL^{-1}, ANGII ranges from 2–1700 fmol mL^{-1}, and ANG1-7 ranges from 2–150 fmol mL^{-1} satisfying the bounds in Eq. 21. Fig. 4D-F are the subset of Fig. 4A-C where the reduction in ANGI, ANGII, and ANG1-7 are in similar ranges of experimentally observed values of SARS-CoV-2-positive patients at ten days post infection. Our simulated result showed the dynamics of 65 patients in Fig. 4D-F that are consistent with experimentally reported values of SARS-CoV-2-negative and SARS-CoV-2-positive patients.

3.4. Effect of feedback from ANGII·AT1R to renin

Next, we investigated the effect of feedback from ANGII·AT1R to renin for hypertensive and normotensive patients. Neubauer et al. (2018) provided contradictory evidence for direct feedback of ANGII·AT1R to renin in the mice kidney and suggested indirect effects (e.g., blood pressure) as the required concentrations of ANGII to induce renin secretion from renin cells was not achieved in *in vivo* settings. But Leete et al. (2018) suggested the feedback strength as the major difference between male and female RAS. Here, we investigated the response with and without feedback but did not vary the strength of the feedback signaling (δ). We considered four patient groups: **group 1** hypertensive patients with no feedback from ANGII·AT1R to renin, **group 2** normotensive patients with no feedback from ANGII·AT1R to renin, **group 3** hypertensive patients with feedback from ANGII·AT1R to renin, and **group 4** normotensive patients with feedback from ANGII·AT1R to renin. Fig. S5 in the Supplementary material shows the dynamics of RAS peptides, and Figs. 5 and S6 in the Supplementary material show the dose-response of ueACE2 to RAS peptides at 10 days post infection. We observed a similar magnitude of reduction between normotensive and hypertensive patients without feedback (group 1 and group 2 in Fig. 5) and with feedback (group 3 and group 4 in Fig. 5). But we observed that feedback of ANGII·AT1R to renin was able to restore ANGI concentration to homeostasis level for both hypertensive (group 1 and group 3 in Fig. 5) and normotensive (group 2 and group 4 in Fig. 5) middle-aged and younger patients. Although the feedback signaling increase the ANGII concentration slightly compared to the cases without feedback signaling, we did not observe any variations in RAS peptides downstream of ANGII. So, feedback of ANGII·AT1R to renin could restore ANGI homeostasis but fails to restore homeostasis values of RAS peptides downstream of ANGI.

3.5. Sensitivity analysis

The dynamic local sensitivity analysis for parameters was conducted to quantify the sensitivity of model output ANGII with input parameters. The analyses were performed for the parameters: β, k_A, c_A, c_R, $k_{ACE2 \cdot ANGII}$, k_{ACE2}, c_{APA}, c_{AT1}, and c_{AT2}. We ran the sensitivity analyses using $m_S = [0.1, 0.3, 0.5, 0.7, 0.9, 1.1, 1.5, 2, 5, 10]$ as input. Fig. 6 shows the dynamic local sensitivity for parameters for hypertensive patients with no feedback from ANGII·AT1R to renin. The sensitivity analysis of ANGII shows parameters β, c_A, and $k_{ACE2 \cdot ANGII}$ will change the steady state of ANGII before infection. Parameters c_A and $k_{ACE2 \cdot ANGII}$ modulate ANGII response positively and negatively, respectively, before infection and positively after infection.

3.6. Dynamics of fibrosis model with patient differences in RAS network

Multiple studies identified a reduction in ACE2 and an increase in ANGII as regulators of excess collagen deposition and fibrosis (Li et al., 2008; Rey-Parra et al., 2012; Kuba et al., 2005; Imai et al., 2005; Wang et al., 2015). Here, we hypothesize that the reduction of ACE2 in the lung tissue will contribute to a surplus of ANGII (unconverted ANGII) at a systemic level and increase the systemic concentration of ANGII. In a recent study, Liu et al. (2021) collected plasma samples and demographic data from 55 SARS-CoV-2-positive patients. They reported increased plasma ANGII above the normal range (25-129 pg mL^{-1}), mainly in critically ill patients. The measured range

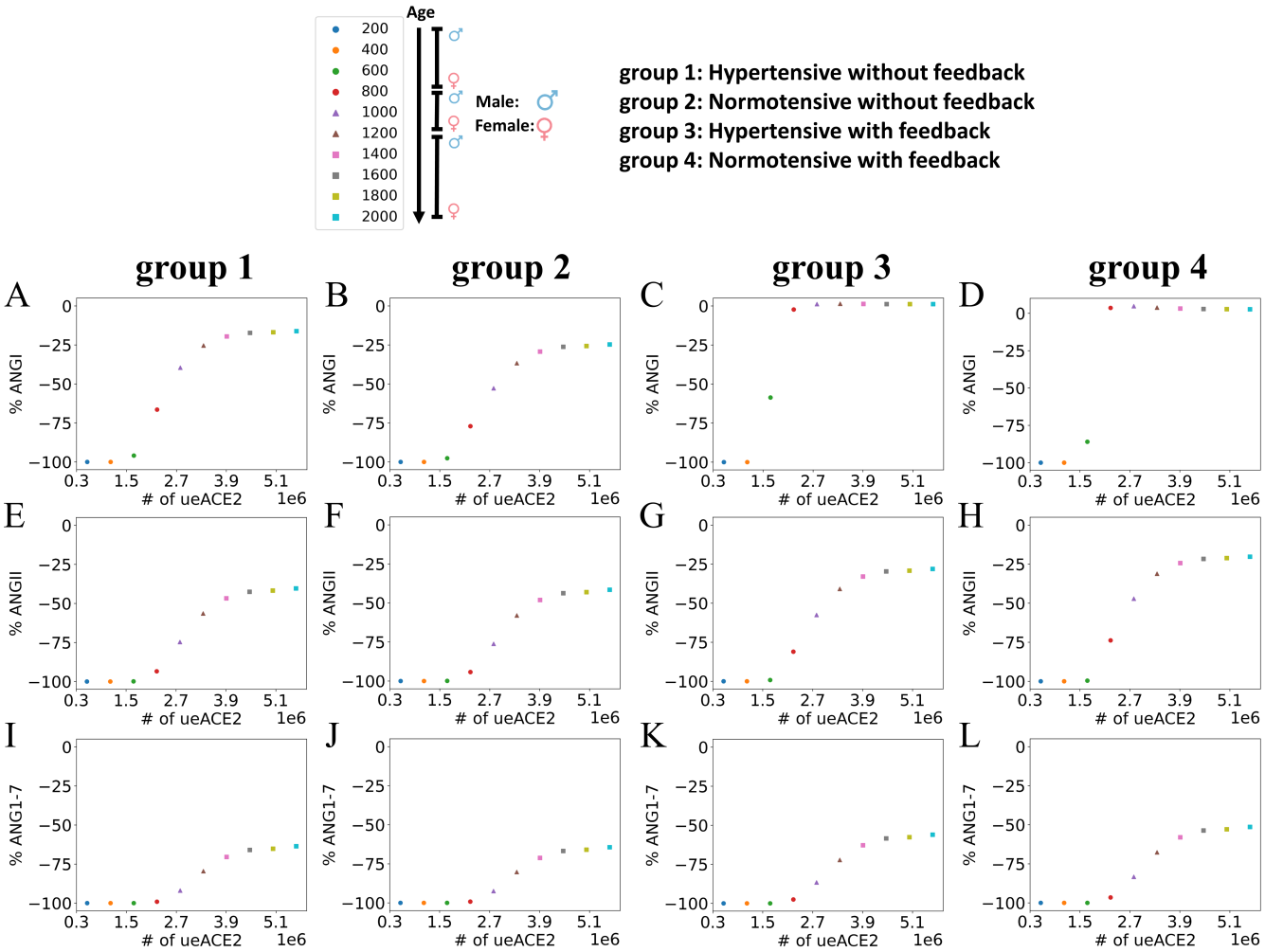

Fig. 5: Dose-response of unbound external ACE2 ($ueACE2_0$) to RAS peptides at day 10 after SARS-CoV-2 infection. **group 1:** hypertensive patients with no feedback from ANGII·AT1R to renin, **group 2:** normotensive patients with no feedback from ANGII·AT1R to renin, **group 3:** hypertensive patients with feedback from ANGII·AT1R to renin, and **group 4:** normotensive patients with feedback from ANGII·AT1R to renin.

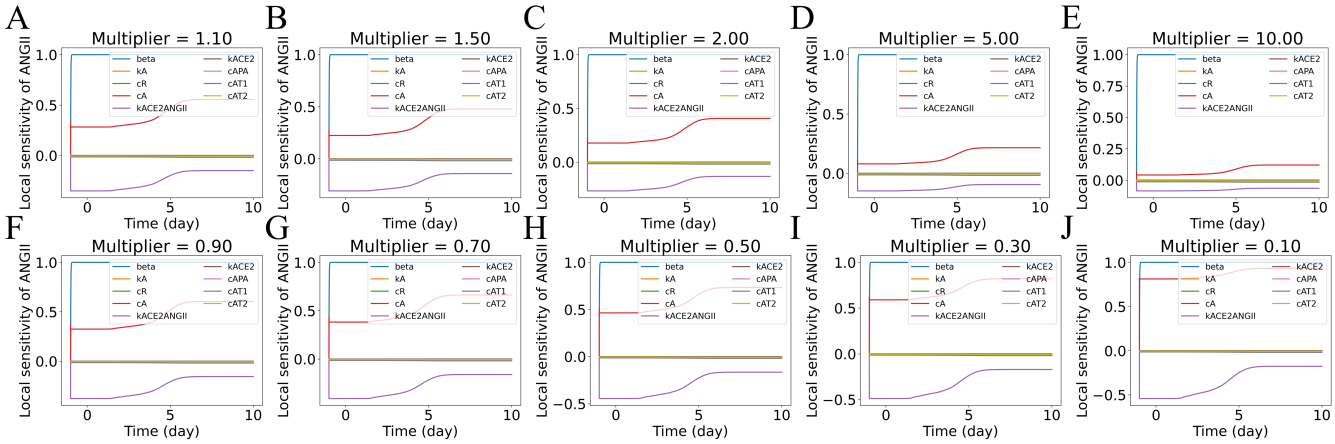

Fig. 6: Dynamic local sensitivity analysis of parameters for ANGII for hypertensive patients with no feedback from ANGII·AT1R to renin. The parameters are multiplied by a multiplier (m_S) A) 1.1, B) 1.5, C) 2, D) 5, E) 10, F) 0.9, G) 0.7, H) 0.5, I) 0.3, and J) 0.1.

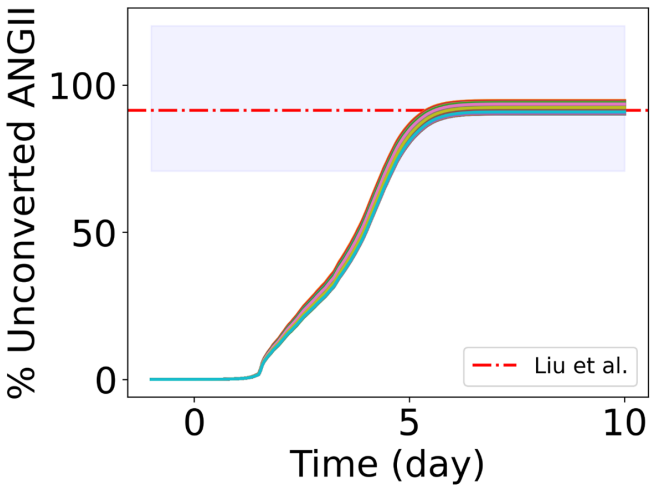

Fig. 7: Dynamics of unconverted ANGII for 1000 virtual patients. The dashed line and shaded area represent experimentally reported increase in ANGII in critically ill patients (Liu et al., 2021) from the mean ANGII of the normal range.

of ANGII in the critically ill patients group was 149.7 (137.8, 165.1) pg mL^{-1}. So, from the mean of the normal range of ANGII (77 pg mL^{-1}), ANGII in the critically ill patients group increased by 94.4%(79%, 114.4%). Here, we assumed that the increase in ANGII in critically ill patients from the mean value of ANGII of normal patients is due to the dysregulation in ACE2. Unconverted ANGII in our model represents the difference in ANGII between baseline ACE2 ($ACE2(t_i)$) and reduced ACE2 dynamics considering cell death ($ACE2(t > t_i)$. Figure 7 shows the percentage unconverted ANGII increase from baseline with initial RAS peptide concentration variations for 1000 virtual patients sampled in Section 3.3. We predicted a similar increase in unconverted ANGII at $ueACE2_0 = 1000$ as the experimental observations of Liu et al. (2021).

Then, we considered a simplified first-order reaction kinetics to account for ANGII-induced TGF-β production from unconverted ANGII. The *in vitro* experiments of Lai et al. (2003) showed increased TGF-β concentration in human mesangial cells (279-630 pg mL^{-1}) with increasing ANGII ranges (0-10^{-7} M). Singh et al. (1999) also observed changes in TGF-β concentration from 0.827-1.65 ng mL^{-1} with changes of ANGII from the control condition to 10^{-8} M in their in vitro experiments on human mesangial cells. The changes in ANGII in our model occur at a much slower range compared to these experiments. However, similar experiments in lung cells can be used to parameterize k_T. We varied k_T between (1-10)$\times$10^{-4} for hypertensive patients with no feedback from ANGII•AT1R to renin and observed linear changes in immune cells and collagen dynamics with variations in k_T (Fig. S7 in the Supplementary material). Our goal was to keep TGF-β concentration within the range of experimental observations (0–10 ng/mL) where the TGF-β-dependent functions for fibroblasts were defined (Jin et al., 2011; Ceresa et al., 2018; Islam et al., 2023). We selected $k_T = 5 \times 10^{-4}$ to simulate the dynamics of immune cells and collagen for different age and sex groups. Fig. 8 shows the dynamics of fibrosis model species: $unconvertedANGII$, TGF-β, immune cells, and collagen, and Fig. S8 in the Supplementary material shows the dose-response of $ueACE2_0$ to immune model species at ten days post infection for four patient groups described in the Section 3.4. Our simulated result showed an increased macrophage and fibroblast population and increased collagen deposition. However, we observed slight variations with aging in both sexes.

Our earlier COVID-19 tissue fibrosis model (Islam et al., 2023) identified M2 macrophage as the key regulator for fibrosis. In this model, we only used experimental data of TGF-β-dependent macrophage recruitment for up to 1 ng mL^{-1} and assumed a constant response from 1-10 ng mL^{-1} (Fig. S1 in the Supplementary material). The additional experimental studies on TGF-β-dependent macrophage recruitment for TGF-β ranges from 1-10 ng mL^{-1} may yield the effect of patient differences in the current model. Also, we observed variations in collagen area fraction depending on age and sex in our COVID-19 tissue model. Fig. S9 in the Supplementary material shows variations in the collagen area fraction depending on age and sex, and the variations increase from day 10 to day 15. Currently, we are running the COVID-19 tissue model and RAS model separately. However, an integrated model with the additional systemic influx of immune cells due to dysregulation in RAS may simulate the experimentally observed patient differences in lung fibrosis outcomes.

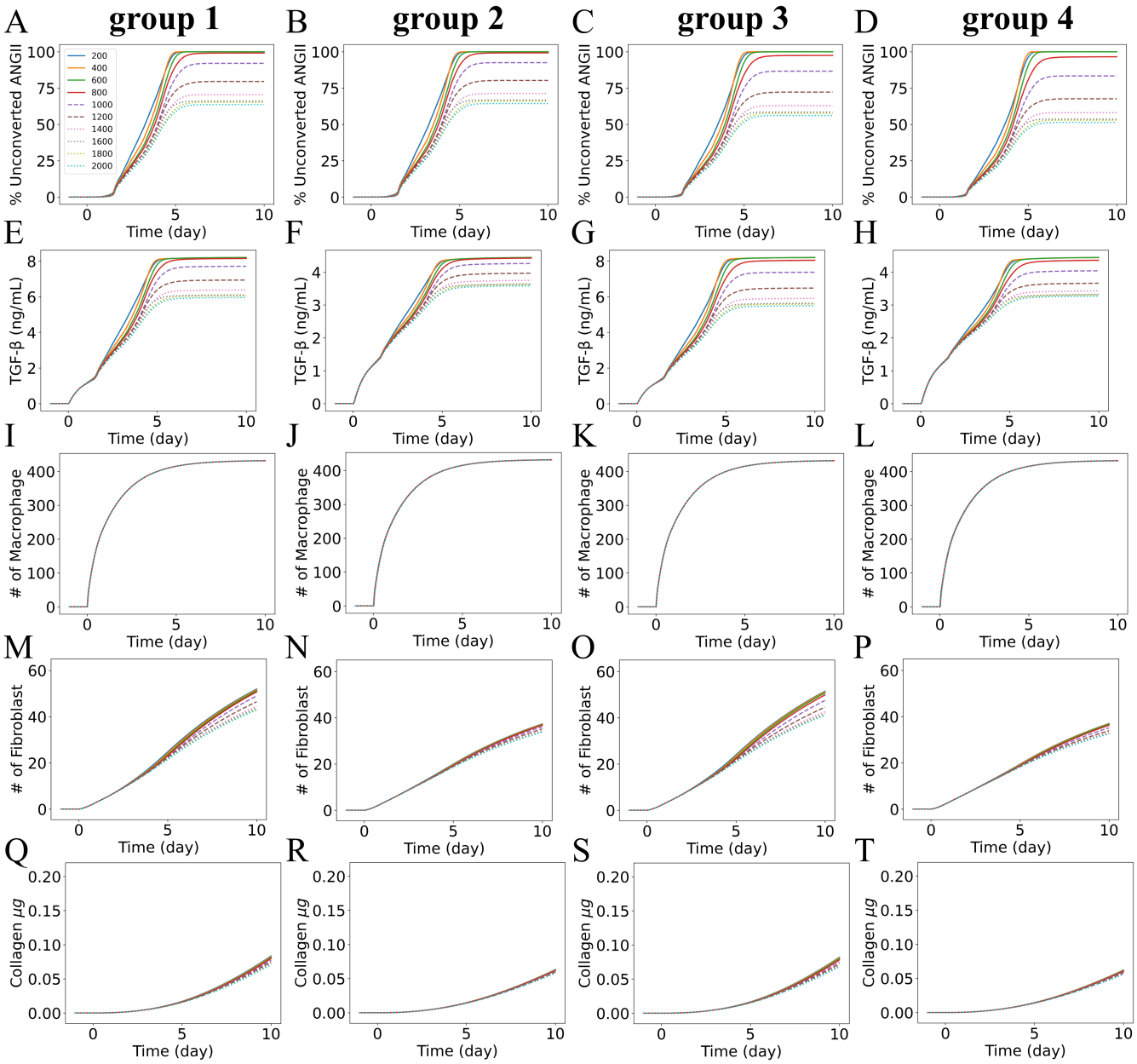

Fig. 8: Dynamics of unconverted ANGII, TGF-β, immune cells, and collagen. **group 1:** hypertensive patients with no feedback from ANGII·AT1R to renin, **group 2:** normotensive patients with no feedback from ANGII·AT1R to renin, **group 3:** hypertensive patients with feedback from ANGII·AT1R to renin, and **group 4:** normotensive patients with feedback from ANGII·AT1R to renin.

4. Conclusions

Heterogeneity in the severity of COVID-19 disease depends on the patient-specific premorbid condition, age, and sex differences. We developed an integrated mathematical model to investigate and quantify the effect of these heterogeneous factors on RAS during COVID-19. We identified cell death as a major reduction source of ACE2 and did not observe any significant difference due to viral-bound ACE2. We observed increased disease severity with aging and significant variations between male and female patients in the older and middle-aged groups. Our results predicted the mechanisms for conflicting RAS alterations in two previous experimental studies. We also predicted systemic immune recruitment and collagen deposition due to RAS alteration during COVID-19. Our model may find application in calibrating patent-specific local and systemic regulations of RAS with bleomycin-induced tissue fibrosis model and autopsy and biopsy tissue samples.

5. Acknowledgments

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6. Supplementary material

Supplementary material associated with this article can be found in the online version.

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