Expression of ACE2, the SARS-CoV-2 receptor, in lung tissue of patients with type 2 diabetes

Sara RA Wijnant, MD^{1,2,3,4*}, Merel Jacobs, MSc^{1*}, Hannelore P Van Eeckhoutte, MSc¹,

Bruno Lapauw, Prof⁵, Guy F Joos, Prof¹, Ken R Bracke, Prof^{1,**}, Guy G Brusselle, Prof^{1,2,4,**}

¹Laboratory for Translational Research in Obstructive Pulmonary Diseases, Department of Respiratory Medicine, Ghent University Hospital, Ghent, Belgium.

²Department of Epidemiology, Erasmus Medical Center, Rotterdam, The Netherlands

³Department of Bioanalysis, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium.

⁴Department of Respiratory Diseases, Erasmus Medical Center, Rotterdam, The Netherlands.

⁵Department of Endocrinology, Ghent University Hospital, Ghent, Belgium.

*These authors contributed equally

**These authors supervised the work equally

Short running title: ACE2 in lungs of diabetes patients

Corresponding author: Dr. Sara Wijnant, Department of Respiratory Medicine, Ghent University Hospital, C. Heymanslaan 10, 9000 Ghent, Belgium, +32(0)4/72467895,

sara.wijnant@UGent.be

ONLINE SUPPLEMENT



Supplementary Figure 1. Flowchart of participants

Flowchart showing the selection of subjects for both RT-PCR and immunohistochemical analyses in our large biobank. *3 subjects with diabetes were not included due to the retrospective nature of assigning a diabetes diagnosis.

Supplementary Figure 2. ACE2 immunohistochemistry reveals positive signal in both bronchial and alveolar epithelium



Representative images of ACE2 immunohistochemical staining in A) positive staining in alveolar epithelium, B) negative isotype control staining in alveolar epithelium, C) positive staining in bronchial epithelium, and D) negative isotype control staining in bronchial epithelium, at 400x magnification. Small inlays are representative of a larger image from the same area at a 200x magnification.

Supplementary Figure 3. Pulmonary ACE2 protein expression according to diabetes and smoking status



Bar plots depict median values of ACE2 expression in patients with (right dark gray bar) and without diabetes (left light gray bar) and stratified by smoking status. Error bars represent 25th and 75th percentiles. P values <0.1 are shown.

Supplementary Figure 4. Forest plots of pulmonary ACE2 protein expression and mRNA expression of ACE2 and TMPRSS2



Forest plots depict regression coefficients from linear regression analyses with determinants diabetes, pre-operative blood glucose values, ACE-inhibitor use or ARB use, and with outcomes A) ACE2+ alveolar cell count, B) ACE2+ alveolar tissue percentage C) ACE2+ bronchial cell count or D) ACE2 mRNA expression. Model 1 is adjusted for age, sex, BMI and current smoking. Model 2 is adjusted for age, sex, BMI, current smoking, COPD, hypertension, inhalation corticosteroid use, oral corticosteroid use and atherosclerotic vascular disease. ACE = angiotensin converting enzyme, ARB = angiotensin receptor blocker, BMI = body mass index, COPD = chronic obstructive pulmonary disease. P values for regression coefficients for diabetes are A) 0.004, 0.012 and 0.040, B) 0.002, 0.002 and 0.009, C) <0.001, <0.001 and <0.001, D) 0.945, 0.741 and 0.622. P values for regression coefficients for glucose are A) 0.028, 0.040 and 0.071, B) 0.019, 0.013 and 0.072, C) 0.098, 0.195 and 0.285, D) 0.677, 0.807 and 0.444. P values for regression coefficients for ACEinhibitors are A) 0.066, 0.157 and 0.349, B) 0.433, 0.521 and 0.841, C) 0.070, 0.195 and 0.602, D) 0.251, 0.446 and 0.240. P values for regression coefficients for ARBs are A) 0.612, 0.587 and 0.818, B) 0.806, 0.559 and 0.990, C) 0.366, 0.456 and 0.833, D) 0.041, 0.042 and 0.079.

Supplementary Figure 5. Pulmonary ACE2 protein expression according to HbA1c in patients with diabetes



Scatter plot of ACE2 protein expression according to HbA1c. P values <0.1 are shown. Bar plots depict median values of ACE2 expression in diabetes patients with HbA1c values smaller than 8% or greater than or equal to 8%. Error bars represent 25th and 75th percentiles. RAAS-I = renin-angiotensin-aldosterone-sytem-inhibitors, ACE = angiotensin converting enzyme, ARB = angiotensin receptor-blocker.

Supplementary Table 1. Characteristics of subjects enrolled in the ACE2 mRNA expression analysis by RT-PCR (n=106)

	No type 2 diabetes	Type 2 diabetes	P value
Number	91	15	
Age (years)	62.68 (9.46)	67.00 (7.48)	0.096
Male sex (%)	54 (59.3)	13 (86.7)	0.081
BMI	24.73 (4.62)	31.04 (6.28)	< 0.001
Smoking			
Current (%)	43 (47.3)	7 (46.7)	0.745
Pack-years	30.00 (0.00-43.00)	34.00 (17.00-50.50)	0.269
Laboratory			
Glucose (mg/L)	1.21 (0.31)	1.67 (0.54)	< 0.001
Serum creatinine (mg/dL)	0.85 (0.23)	0.94 (0.13)	0.136
eGFR (ml/min/1.73 m ²))	76.39 (20.15)	64.13 (11.61)	0.024
White blood cell count $(x10^9/L)$	9.31 (3.31)	10.33 (3.75)	0.282
CRP (mg/dL)	0.60 (0.20-3.00)	2.80 (1.70-4.80)	0.022
Comorbidities			
Hypertension (%)	52 (57.1)	14 (93.3)	0.017
COPD (%)	33 (36.3)	9 (60.0)	0.145
Kidney disease (%)	4 (4.5)	1 (6.7)	1.000
Atherosclerotic vascular disease (%)	16 (17.8)	5 (33.3)	0.296
Medication			
Metformin* (%)	0 (0.0)	11 (73.3)	< 0.001
Insulin* (%)	0 (0.0)	2 (13.3)	0.013
Sulfaniluree* (%)	0 (0.0)	3 (20.0)	0.001
Diuretics (%)	14 (15.4)	5 (33.3)	0.188
ACE-inhibitors (%)	11 (12.1)	6 (40.0)	0.019
ARBs (%)	15 (16.5)	1 (6.7)	0.552
Statins (%)	16 (17.6)	8 (53.3)	0.006
Inhalation corticosteroids (%)	21 (23.1)	6 (40.0)	0.283
Oral corticosteroids (%)	8 (8.8)	3 (20.0)	0.389

BMI (body mass index); eGFR (estimated glomerular filtration rate); CRP (C-reactive protein); COPD (chronic obstructive pulmonary disease); ACE-inhibitor (angiotensin converting enzyme inhibitor); ARBs (angiotensin receptor blockers); *All antidiabetic medication use is listed in the table, and none of the subjects used glinides, glitazones, GLP-1-analogues, DDP-4-inhibitors or SGLT2-inhibitors.