Renin-Angiotensin System Blockade Influences ACE2 in Human Type II Pneumocytes

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Research Article

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Abstract

Rationale—Angiotensin converting enzyme (ACE) 2 and the transmembrane protease serine 2 (TMPRSS2) are key for cellular entry of the type 2 coronavirus that causes severe acute respiratory syndrome (SARS-CoV2), the etiological agent of coronavirus-19 disease (COVID-19). There has been a growing concern that renin-angiotensin system (RAS) blockade with ACE inhibitors (ACEIs) or type 1 angiotensin (Ang II) receptor blockers (ARBs) increases ACE2 expression and then elevate patient susceptibility to SARS-CoV-2. However, evidence about RAS blockade and ACE2 in human lung are lacking.

Objective– To investigate RAS blockade on ACE2 and TMPRSS2 in type II pneumocytes of human lung parenchymal of untreated and ACEI/ARB-treated hypertensive subjects.

Methods and Results– ACE2 and TMPRSS2 protein expression were measured by immunohistochemistry. We found that smoking and RAS blockade influence on the percentage of human ACE2-expressing type II pneumocytes (p= 0.026). Smokers subjects under RAS blockade treatment exhibited higher percentage of ACE2-expressing type II pneumocytes than normotensive ones. Within the ACEI/ARB-treated group, the percentage of ACE2-expressing type II pneumocytes was higher in smokers than never smokers. A significant association between ACE2 immunostaining intensity and smoking on subjects over 60 years old was found (p= 0.05): older smokers exhibited higher ACE2 protein levels compared to youngers. The percentage of TMPRSS2-expressing type II pneumocytes was greater in men than women (p= 0.026) and in subjects under 60 years old (p= 0.040) and trend to be higher in ACEI/ARB-treated subjects than normotensives (p= 0.060). A significant association between TMPRSS2 immunostaining intensity with smoking and age or with RAS blockade and age or with RAS blockade and smoking was observed. Older or smokers subjects under ACEI/ARB treatment exhibited higher TMPRSS2 protein levels than youngers or never smokers.

Conclusions—ACE2 and TMPRSS2 are influenced by smoking and ACEI/ARB treatment. These findings help explain the increased susceptibility to COVID-19 in subjects with treated cardiovascular-related pathologies.

Introduction

The renin-angiotensin system (RAS) is composed of two axis with opposing functions. The depressor and protective axis, represented by angiotensin (Ang) (1-7) and its Mas receptor counterbalancing the classic pressor axis represented by Ang II and the angiotensin type 1 receptor (AT1R). Overexpression of the pressor axis Ang II/AT1R promotes cardiovascular, renal, pulmonary and brain organ damage due to its oxidative stress, proinflammatory, fibrotic and hypertrophic effects among others.[1] Angiotensin converting enzyme 2 (ACE2) is a key element of the protective axis of the RAS. ACE2 catalyzes Ang II degradation into Ang-(1-7), a component of the RAS that exerts hypotensive, anti-oxidant, anti-fibrotic, anti-inflammatory and protective effects opposite to those displayed by Ang II.[2,3] ACE2 is widely expressed in the human body with strong expression in the gastrointestinal tract, heart and kidney.[4,5] In

addition to its enzymatic function counterbalancing the pressor arm of the RAS, ACE2 acts as a receptor for the type 2 coronavirus that causes severe acute respiratory syndrome (SARS-CoV2), the etiological agent of coronavirus-19 disease (COVID-19).[6,7] The spike protein on SARS-CoV-2 binds ACE2 followed by priming by the transmembrane protease serine 2 (TMPRSS2) allowing viral fusion and cellular entry.[7]

Through single-cell ACE2 RNA sequencing, organs identified at risk are lung, heart, esophagus, kidney, bladder, and ileum, and located specific cell types (i.e., type II alveolar cells, myocardial cells, proximal tubule cells of the kidney, ileum and esophagus epithelial cells, and bladder urothelial cells) which are vulnerable to SARS-CoV2 infection.[6] Type II alveolar cells (pneumocytes) are key cells to maintain lung homeostasis.[8] Type II pneumocytes are cuboidal secreting cells responsible for secretory functions of the peripheral lung, thus reducing the surface tension of the alveoli and preventing them from collapsing.[8] ACE2 is mainly expressed in type II pneumocytes[9–12] and protects against acute lung injury in several animal models of acute respiratory distress syndrome (ARDS).[13] The pressor arm of the RAS is implicated in the pathogenesis of acute lung injury, thus, the protective role of ACE2 results from Ang II downregulation and Ang-(1-7) upregulation.[13–16]

Antihypertensive drugs targeting the pressor axis of the RAS such as ACE inhibitors (ACEIs) or type 1 Ang II receptor blockers (ARBs) are extensively used worldwide to treat many cardiovascular disorders. Evidence in animals show that ACEIs and ARBs increase ACE2 expression.[17–20] Regarding human tissues, hypertension and RAS blockers are not associated with ACE2 expression in the kidney[21] but ACE2 expression is slightly reduced in human upper (nasal) respiratory tract of patients taking ACEI compared to matched controls.[10] In contrast, human intestine luminal ACE2 expression is increased by ACEI.[22] Thus, evidence in animals gave rise to the concern that antihypertensive treatment with ACEIs or ARBs may be harmful because hypertensive subjects under those treatments would be more susceptible to be infected by the virus. In contrast, other authors postulate that these antihypertensive treatments would be beneficial because Ang II levels decrease, thus decreasing the harmful effect of overexpression of the pressor arm of the RAS, while the levels of the protective component of the RAS, Ang-(1-7), increase. Although clinical studies have shown that these antihypertensive treatment did not increase the percentage of deaths or those hospitalized with COVID-19[23–26], up to date there is no experimental evidence showing whether ACEIs or ARBs induce changes in ACE2 in human type II pneumocytes. In this study we investigate Ang II and Ang-(1-7) levels in lung parenchymal and ACE2 and TMPRSS2 protein expression in type II pneumocytes from subjects treated with ACEIARB compared to control untreated subjects.

Methods

Please see the Major Resources Table in the Supplemental Materials.

Data Availability

The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Human lung samples

The present is an observational retrospective study with insensitive information, thus informed consent was not required. The procedures followed were in accordance with institutional guidelines. The Ethics and Clinical Research Committee of Hospital Provincial del Tórax “Dr. A. Cetrángolo” and Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires (PV-2020-00851131) have approved the present study.

Lung parenchymal samples from untreated subjects (control) (n=42) and subjects treated with the ACEIs enalapril (n=22) or perindopril (n= 1), or the ARBs valsartan (n= 9), losartan (n= 7), telmisartan (n= 1) or candersartan (n= 1) (total ARB-treated subjects= 18) who underwent resection (segmentectomy or lobectomy) were obtained from the unified archives of the Pathology Service from Hospital Municipal de Vte. López “Prof. B. Houssay and Hospital Provincial del Tórax “Dr. A. Cetrángolo”. Those were resection samples obtained from subjects undergoing surgery for various reasons (cancer, interstitial lung diseases, bullas, bronchiectasis, infections and others). Tissues were only used if characterized as non-diseased.

Ang II and Ang-(1-7) levels

Ang II and Ang-(1-7) endogenous levels were determined in 84 samples (42 from control subjects and 41 from ACE/ARB-treated subjects). Angs levels were measured by radioimmunoassay as previously described.[27–29] Briefly, samples were dewaxed, rehydrated and homogenized in acid ethanol (0.1 mol/L HCl/80% ethanol) containing 0.44 mmol/L o-phenanthroline, 1 mmol/L Na+para-chloromercuribenzoate, 0.12 mmol/L pepstatin A and 25 mmol/L EDTA. Homogenates were centrifuged at 20000 xg for 30 min at 4ºC. Proteins in the supernatant were quantified. The supernatant was subsequently lyophilized and Angs extraction and recovery was performed as previously described.[28,29] Each sample was corrected for each recovery. Angs levels were quantified by radioimmunoassay using Angs labelled in our laboratory as previously described.[30] Radioimmunoassay for Ang-(1-7) has been previously validated.[31] Intra-assay and inter-assay variability were 13.7 ± 2.3% and 12.4 ± 3.1%, respectively. Results were expressed as pg/g tissue.

ACE2 Immunohistochemistry

Immunohistochemistry staining was performed on a Discovery Ultra VENTANA systems (Roche) automated Stainer. Fifty seven samples were analyzed (20 from untreated subjects and 37 from ACE/ARB-treated subjects). Samples were exposed to a rabbit polyclonal anti-ACE2 antibody (Abcam cat. ab15348, lot GR3333640-5 dilution 1:500) or to a monoclonal recombinant anti-TMPRSS2 antibody (Abcam cat. ab92323, lot GR3344246-11, dilution 1:1500) together with a mouse monoclonal antibody TTF-1 (Cell Marque clone 8G7G3/1, lot 077115 dilution 1:500) to label type II pneumocytes. Antibody binding to ACE2 or TMPRSS2 and type II pneumocytes was visualized by incubation with the ultraView

Universal Alkaline Phosphatase Red Detection Kit (Roche cat. 0785269814001) and the ultraView Universal DAB Detection Kit (Roche cat. SAP 5269806001), respectively. Images were taken on a Olympus CX31 microscope. Two independent qualified pathologists specialized in human lung analyzed ACE2 or TMPRSS2 staining in type II pneumocyte in a blinded fashion following principles for reproducible tissue scores. Percentage of ACE2- or TMPRSS2-expressing type II pneumocytes was measured in representative lung fields sufficient to count no fewer than 450 pneumonocytes. ACE2 intensity was classified from 1 to 3 (1+, very weak; 2+, moderate and 3+, strong).

Statistical Analysis

All analyses were conducted using the IBM SPSS Statistics 19 software. Kolmogorov-Smirnov test was applied to verify normal distribution of the variables. Homogeneity between groups was verified by the Levene test. The general linear model was applied to detect differences. The stratified Fisher's Exact Test was used to investigate association between the variables. A p value <0.05 was considered statistically significant.

Results

Baseline Characteristics of the Total Study Population

We analyzed lung samples obtained from white subjects, untreated (n=42) and treated with ACEI/ARB (n= 41). Mean systolic and diastolic pressure of those subjects were 120$\pm$5.4 and 67.3$\pm$2.0 mmHg in the untreated group and 124.4$\pm$5 and 73.3$\pm$4.4 mmHg in the ACEI/ARB treated group, respectively. The mean age of untreated subjects was 51$\pm$17 and of ACEI/ARB-treated subjects was 63$\pm$9 years old. The male-to-female ratio was 42/41. Characteristics of the population investigated are presented under table 1.

Lung Ang II levels are Influenced by Age, Sex, Smoking and ACEI/ARB Treatment

ACE2 has high catalytic efficiency for Ang II. In order to investigate ACE2 activity as the ratio between Ang-(1-7) and Ang II, we measured Ang II and Ang-(1-7) levels by radioimmunoassay. Figure 1 shows Ang II levels in pg/g tissue in lung parenchyma of untreated and ACEI/ARB-treated subjects. We found that age, sex, smoking and ACEI/ARB treatment influence on Ang II levels ($p<0.05$). Within the untreated population, subjects under 60 years old, smokers or never smokers, showed no difference in Ang II levels irrespective of their sex. Conversely, Ang II levels were higher in smoker and former smoker men older than 60 years old compared to smoker and former smoker women of the same age. In addition, Ang II levels were higher in older smokers men compared to younger smoker and never smoker women. Unfortunately, our study lacks samples from never smoker subjects older than 60 years old to make a comparison between older never smoker and smokers. Regarding Ang-(1-7) levels, we observed a trend ($p= 0.08$) to have reduced Ang-(1-7) levels in untreated older and smoker subjects, female and male compared to younger smoker subjects, that is, age and smoking showed a tendency to induce a decrease in Ang-(1-7) levels in untreated subjects (Figure 2). Altogether, our results showed that untreated older smoker or former smoker men displayed higher Ang II levels compared to women, while Ang-(1-7) levels were not

changed by sex at the same age. In contrast, younger smoker women showed slightly higher Ang II levels compared to men.

Age and smoking also influence Ang II levels in those subjects under RAS blockade (p= 0.001). We found that Ang II levels were reduced in those subjects treated with ACEI/ARB, never smoker or smoker, under 60 years old compared to subjects older than 60 years old, independently of their sex (Figure 1). RAS blockade treatment showed a tendency to increase Ang-(1-7) levels in subjects under 60 years old of any sex, smoker or never smoker compared to older subjects (p= 0.08). Instead, antihypertensive treatment did not change Ang-(1-7) levels in subjects over 60 years old (Figure 2).

Because ACE2 catalyzes the conversion of Ang II into Ang-(1-7), we then evaluated Ang-(1-7)/Ang II ratio as an indirect measurement of ACE2 activity. We observed no difference in Ang-(1-7)/Ang II ratio between the investigated groups, suggesting no change in ACE2 activity (Figure 3S).

ACE2-expressing type II pneumocytes are Influenced by Age, Smoking and ACEI/ARB Treatment

Because type II pneumocytes are key cells to maintain lung homeostasis[8] and that ACE2 is mainly expressed in these cells[9,10,12,32], we measured the percentage of ACE2-expressing type II pneumocytes by immunohistochemistry in lung parenchymal from untreated and subjects under ACEI/ARB treatment. If we consider only untreated and ACEI/ARB-treated subjects, we found no difference in the percentage of type II pneumocytes that expresses ACE2 (Figure 3A), being ACE2 present in 61.8 ± 7.5 % of type II pneumocytes from untreated subjects and in 62.4 ± 8.6 % of type II pneumocytes from ACEI/ARB-treated subjects. We also did not find significant differences if we only compare never smokers, former smokers or smokers (Figure 3B) or male and female (Figure 3C). However, we observed that subjects over 60 years old exhibited lower percentage of ACE2-expressing type II pneumocytes (p= 0.012) (Figure 3D). When we compared all the variables at the same time, that is, smoking, sex, age and antihypertensive treatment, we found a significant interaction between ACEI/ARB treatment and smoking on the percentage of ACE2-expressing type II pneumocytes (p= 0.026). This means that the percentage of ACE2-expressing type II pneumocytes depends on whether the subject is a smoker, former smoker or never smoker, under or not RAS blockade treatment. Regarding smokers subjects, those under RAS blocking treatment exhibited higher percentage of ACE2-expressing type II pneumocytes than untreated ones. Within the group of subjects under ACEI/ARB treatment, the percentage of ACE2-expressing type II pneumocytes was higher in smokers compared to never smokers (Figure 4). Altogether, these data suggest that smoking and RAS blocking treatment enhances the percentage of ACE2-expressing type II pneumocytes.

ACE2 Protein Levels are Influenced by Age and Smoking

We evaluated ACE2 immunostaining intensity, indicative of ACE2 protein levels. We found a significant association between ACE2 immunostaining intensity and smoking on subjects equal or older than 60 years old (p= 0.05). The greater percentage of subjects that exhibited higher ACE2 protein levels was that of older smokers and former smokers (Figure 5B).

We next evaluated whether RAS blockade and age are associated with ACE2 protein immunostaining intensity. Although the p value of that association was lower than 0.1, this fact shows a tendency to older subjects treated with ACEI/ARB to display higher ACE2 protein expression levels (Figure 5C). Thus, age and RAS blockade trends to enhance ACE2 protein expression levels.

TMPRSS2-expressing type II pneumocytes are Influenced by Age and Sex

Since TMPRSS2 is needed for SARS-CoV2 entry[7], we next determined the percentage of TMPRSS2-expressing type II pneumocytes. We found a significant difference by age (p= 0.04) and sex (p= 0.026) but not by smoking in the percentage of TMPRSS2-expressing type II pneumocytes (Figure 6). Although we did not observe a significant difference between TMPRSS2-expressing cells in untreated vs ACEI/ARB-treated subjects, the p value of 0.06 indicated a trend of hypertensive treated subjects to have higher percentage of TMPRSS2-expressing cells compared to those untreated (Figure 6).

TMPRSS2 Protein Levels are Influenced by Age, Smoking and RAS blockade

We then evaluated the association between TMPRSS2 immunostaining intensity (indicative of protein levels) with age, sex, smoking and ACEI/ARB treatment. We found a significant association with age and smoking (p= 0.001) being the higher TMPRSS2 protein levels in older smoker and former subjects (Figure 7A). Regarding ACEI/ARB treatment, we observed a significant association in TMPRSS2 protein levels with age (p= 0.001) (Figure 7B) and smoking (p= 0.039) (Figure 7C). Thus, the greater percentage of ACEI/ARB-treated subjects that exhibited higher TMPRSS2 protein levels was that of olders and smokers.

Discussion

We report novel findings in this translational research study: 1) age, sex, smoking and RAS blockade influence lung Ang II levels, 2) age, smoking and ACEI/ARB treatment influence the percentage of human ACE2-expressing type II pneumocytes, 3) age and smoking enhance ACE2 protein expression levels, 4) RAS blockade shows a tendency to enhance ACE2 protein levels in older subjects, 5) age and sex influence the percentage of human TMPRSS2-expressing type II pneumocytes, and 6) RAS blockade enhances TMPRSS2 protein levels in older and smoker subjects. Figure 8 resumes the most relevant conclusions of our study. The effect of RAS blockade on human lung Ang II levels, ACE2 and TMPRSS2 has never been previously reported to our knowledge.

We found that Ang II levels were higher in smoker men older than 60 years old compared to smoker and former smoker women of the same age or younger subjects, but they were reduced under ACEI/ARB treatment. Conversely, a trend to have their Ang-(1-7) levels reduced were observed in untreated older and smoker subjects. Altogether these data suggest that smoking and aging induces RAS disbalance. In accord, chronic cigarette smoking and aging are associated with an upregulation of the pressor arm and downregulation of the compensatory ACE2/Ang-(1-7)/MasR axis of the RAS.[33–35] In a chronic cigarette smoke-induced pulmonary arterial hypertension rat model, lung Ang II levels and ACE expression were augmented while ACE2 expression was diminished.[36,37] The ARB losartan reduced lung Ang II levels,

restored ACE2 expression and ameliorated the deleterious effects induced by cigarette smoking in this model[36,37] suggesting an AT1R-dependent mechanism. Accordingly, here we report that Ang II levels were augmented in lung from older smoker men but they were reduced in subjects under RAS blockade. Furthermore, we found that the percentage of ACE2-expressing type II pneumocytes were lower in older subjects compared to youngers, reinforcing the concept of a RAS disbalance with aging. [34,35] Accordingly, aging caused a reduction in ACE2 expression in various organs of mice, being the lowest in the lungs.[38,39]

We observed that smoking and RAS blockade influence on the percentage of human lung ACE2-expressing type II pneumocytes: the percentage of ACE2-expressing type II pneumocytes was similar between untreated never smokers or smokers but increased in smokers under RAS blockade. Furthermore, we also found that the greater percentage of subjects that exhibited higher ACE2 protein levels was that of older smokers and former smokers. Accordingly, some reports showed that current smokers and never smokers have closely similar bronchial epithelial cell mRNA levels of ACE2[40] or no change in ACE2 protein levels in both bronchial and alveolar epithelial cells[41] and in nasal ciliary cells.[10] In contrast, others have reported higher ACE2 expression in smokers compared to never-smokers.[42–44] ACE2 RNA expression in the human lung at single-cell resolution showed that ACE2 expression was higher in the small airway epithelia of smokers than never smokers and that male smokers exhibited higher ACE2 RNA levels than female smokers or never smokers of either sex.[45] In contrast, Smith et al. showed that lung ACE2 levels do not vary by age or sex, but smokers exhibit upregulated ACE2.[44] Purkayastha and colleagues, through modeling the direct effects of cigarette smoke on SARS-CoV-2 infection, showed that acute cigarette smoke exposure increases the number of infected and apoptotic cells, prevents the normal airway basal stem cell repair response, suggesting that smoke increases COVID-19 severity.[46] However, it has been shown that smoking was not associated with increased mortality in COVID-19 pneumonia[47,48], but cumulative smoking exposure, as measured by pack/years, was associated with a higher risk of hospitalization and mortality from COVID-19 in a dose-dependent manner.[49] In contrast, cigarette smoke-pretreatment potently abrogates SARS-CoV-2 replication in an vitro model[41]. An ongoing clinical trial focused on nicotine and smoking in COVID-19 subjects listed on ClinicalTrials.gov (NCT04429815) should address that concept. Nevertheless, this is the first report showing that smoking and RAS blockade treatment enhance ACE2 positive type II penumocytes.

TMPRSS2 is a key protein in SARS-CoV2 entry.[7] We observed that TMPRSS2-expressing type II pneumocytes decreased with age. In accord, recent data showed a weak decreasing age-related trend in TMPRSS2 expression in human lung.[50] However, increased TMPRSS2 expression with aging was reported in mice and humans. [51] The difference between this study and ours may be due to the age range and the type cell investigated (type I vs type II pneumocytes). We also observed that the percentage of TMPRSS2-expressing type II pneumocytes was higher in men than women. In contrast, no statistically significant differences have been identified in TMPRSS2 expression in lung tissues by stratifying for gender.[50] Again, the difference may be due to the type of sample under investigation.

There has been a growing concern that the use of ACEI and/or ARBs blockers by subjects

with cardiovascular-related pathologies could increase the expression of ACE2 and then elevate patient susceptibility to SARS-CoV-2 infection. An observational multicenter cohort study with patients from USA and Spain showed no clinically significant increased risk of hospital admission with COVID-19 associated with ACEI or ARB.[26] Through a transcriptomic analysis of over 700 lung samples of patients with comorbidities associated with severe COVID-19 it was found that ACE2 was highly expressed in these patients, compared to control individuals, although hypertensive taking RAS inhibitors were not evaluated.[52] In a small sample size of patients taking ACEI, upper respiratory tract ciliary ACE2 expression was slightly decreased compared to matched controls.[10] In contrast, we found that smoking and RAS blockade influence on the percentage of ACE2-expressing type II pneumocytes, being higher in smokers under ACEI/ARB treatment. Differences between our study and that from Lee et al.[10] may be due to the area investigated (alveolar vs upper respiratory tract), the type of cell (type II pneumocytes vs ciliary cells) and the number of samples under study. Furthermore, those authors used upper respiratory tract and ciliary cells, an area that lacks type II pneumocytes.[53] Pneumocytes together with alveolar macrophages are essential to maintain lung homeostasis.[7] We also found a trend for subjects older than 60 years under ACEI/ARB treatment to exhibit higher ACE2 expression in type II pneumocytes compared to youngers. The increased ACE2 expression in type II pneumocytes would be protective due to Ang II downregulation. In fact, older ACEI/ARB-treated subjects exhibited lower Ang II levels (present results). But at the same time, the increased percentage of ACE2-expressing type II pneumocytes in older smokers under RAS blocking treatment would facilitate SARS-CoV2 infection, thus increasing patient susceptibility to uncontrolled ARDS development because of an unbalance between the pressor and depressor axis of the RAS due to ACE2 sequestration by the virus.

Regarding TMPRSS2 protein levels in type II pneumocytes of ACEI/ARB-treated subjects we found that the higher percentage of people with higher protein levels is that one of older than 60 years old and smokers, thus RAS blockade together with aging and smoking influence on TMPRSS2 protein expression. In contrast, it has been shown that RAS inhibition did not affect mRNA abundance of ACE2 or TMPRSS2 in male C57BL/6J mice administered ACEI or ARB.[54] Differences with our results may be due to differences in species (rat vs human) and the sample investigated (whole lung vs type II pneumocytes). In addition, those mice were not exposed to smoke. Evidence shows that smoking increases TMPRSS2[52,55], which is in accordance with our results.

Our study is limited by a retrospective design, which is vulnerable to uncontrolled biases. We were unable to assess the impact of time since smoking cessation in the former smokers subjects population. We also do not consider the amount of cigarettes that each subject usually consumes in the smokers subjects population, the pathology by which the subject underwent lung surgery and the doses of ACEIs or ARBs taken by each subject. Another limitation was the lack of lung samples from uncontrolled hypertensive subjects and from never smoker older subjects.

In conclusion, we found that older smoker men displayed the highest Ang II levels in their lungs while they were reduced under RAS blockade. Smokers under ACEI/ARB treatment displayed higher percentage of

ACE2-expressing type II pneumocytes, which may increase their susceptibility to be infected by SARS-CoV2. Thus, due to ACE2 sequestration by the virus there will be a disbalance in the RAS with the consequent ARDS development. The fact that olders and smokers under RAS inhibition treatment displayed greater ACE2 and TMPRSS2 protein levels may help explain the increased susceptibility to COVID-19 in subjects with treated cardiovascular-related pathologies.

Abbreviations And Acronyms

ACE2 angiotensin converting enzyme 2

ACEI angiotensin converting enzyme inhibitor

Ang angiotensin

ARB type 1 angiotensin II receptor blocker

ARDS acute respiratory distress syndrome

AT1R angiotensin type 1 receptor

COVID-19 coronavirus-19 disease

HT ACEI/ARB-treated hypertensive

RAS renin-angiotensin system

SARS-CoV2 type 2 coronavirus that causes severe acute respiratory syndrome

TMPRSS2 transmembrane protease serine 2

Declarations

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Disclosures

None.

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Table

Table 1. Baseline Characteristics of the Investigated Population

	Normotensive subjects	Hipertensive subjects treated with ACEI/ARB
Number of subjects, n (%)	42 (100)	41 (100)
Age (year), □ ± SD	51 ± 17	63 ± 9
< 60 years old, n (%)	28 (67)	12 (29)
≥ 60 years old, n (%)	14 (33)	29 (71)
Treated with ACEI, n (%)	0 (0)	23 (55)
Treated with ARB, n (%)	0 (0)	18 (44)
Female, n (%)	19 (45)	22 (54)
Male, n (%)	23 (55)	19 (46)
Never smoker, n (%)	16 (38)	9 (22)
Smoker, n (%)	18 (43)	17 (41)
Former smoker, n (%)	8 (19)	15 (37)
Chronic obstructive pulmonary disease, n (%)	1 (2)	9 (22)
Type 2 diabetes, n (%)	1 (2)	6 (15)
Cardiovascular disease, n (%)	0 (0)	5 (12)
Chronic kidney disease, n (%)	0 (0)	0 (0)
Lung cancer, n (%)	6 (14)	12 (29)

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin type 1 receptor blocker

Figures

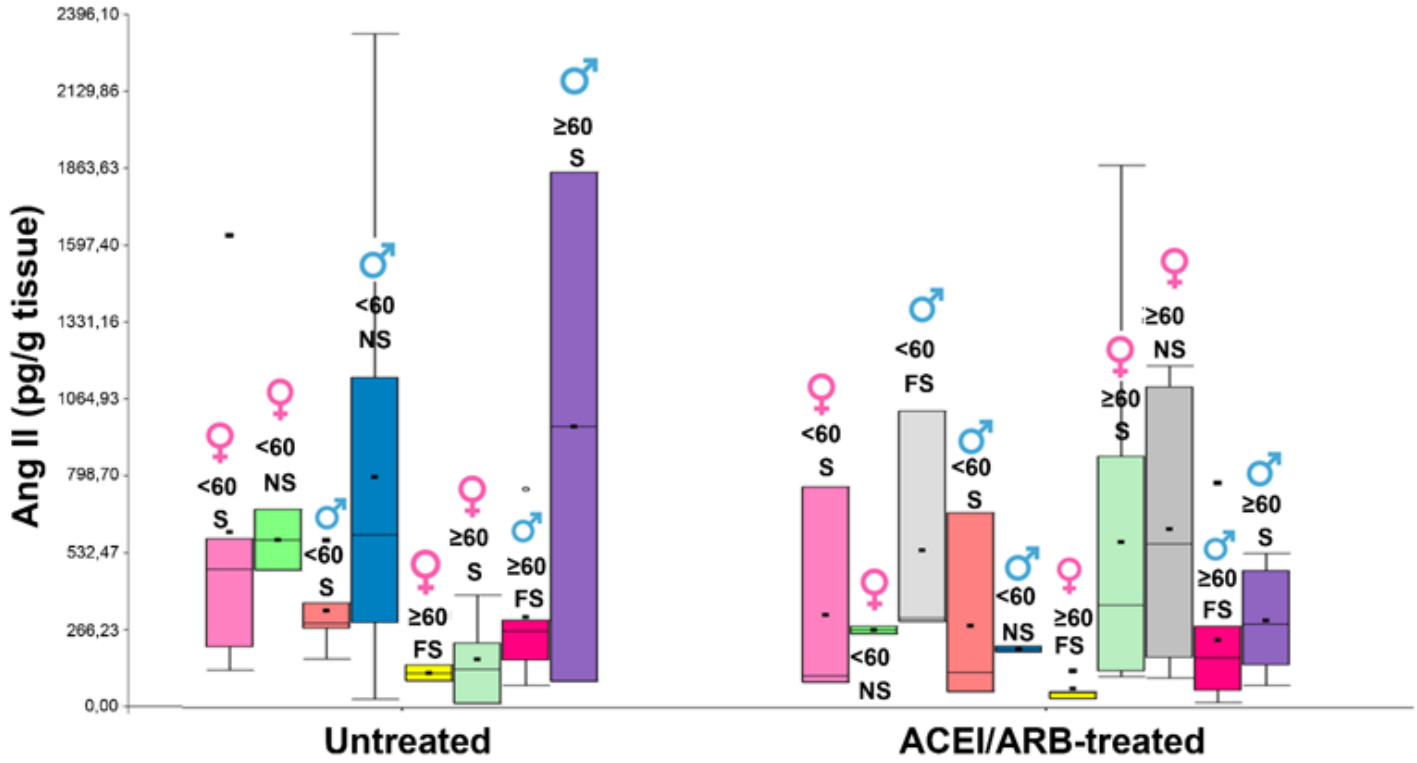

Figure 1

Ang II levels are Influenced by Age, Sex and Smoking. Ang II levels expressed as pg/g tissue in lung parenchyma of untreated or ACEI/ARB-treated women (♀) or men (♂), smokers (S), never smokers (NS) or former smokers (FS) under 60 years old (<60) or equal to or over 60 years old (≥ 60). Because the number of samples of untreated females never smokers ≥60 years old and of males former smokers <60 years was very low, they were obviated to be represented in the graph but they were included in the statistical analysis. The bottom and top of the box plots represent the 25th and 75th percentiles, respectively. The bands within the box show the median value, the dot is the media and the whiskers extending from both ends of the boxes are minimum and maximum values. Statistical analysis (general linear model) showed a significant interaction between age and smoking ($p = 0.001$) and age and sex ($p = 0.013$) on Ang II levels. Data distribution of such interaction is presented in Figure 1S.

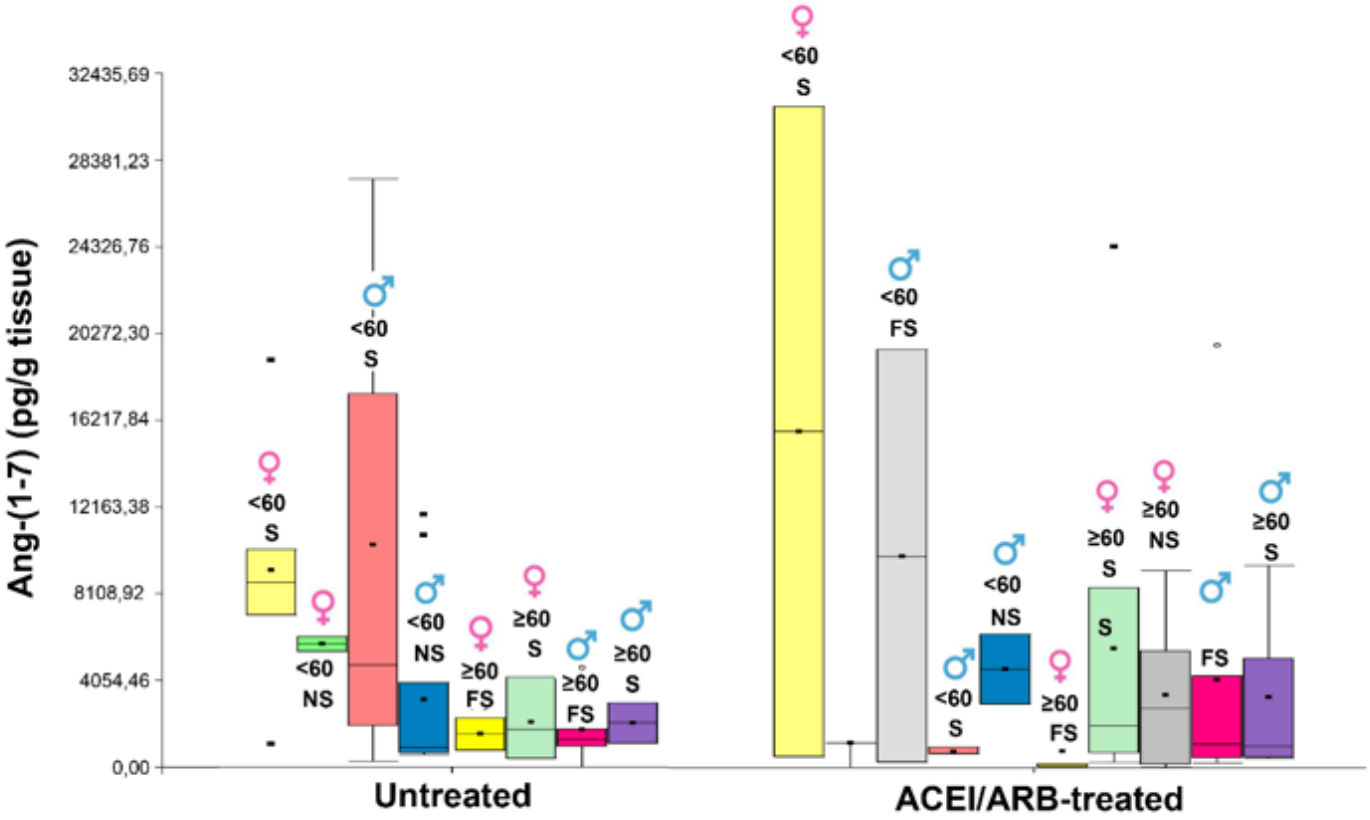

Figure 2

Ang-(1-7) Levels Showed a Trend to Be Influenced by Age and Smoking. Ang-(1-7) levels expressed as pg/g tissue in lung parenchyma of untreated or ACEI/ARB-treated women (♀) or men (♂), smokers (S), never smokers (NS) or former smokers (FS) under 60 years old (<60) or equal to or over 60 years old (≥ 60). Because of the low n in untreated female never smokers ≥ 60 years old and in males former smokers <60 years old they were obviated to be represented in the graph but they were included in the statistical analysis. The bottom and top of the box plots represent the 25th and 75th percentiles, respectively. The bands within the box show the median value, the dot is the media and the whiskers extending from both ends of the boxes are minimum and maximum values. Data distribution is presented in Figure 2S. Statistical analysis (general linear model) showed an interaction between age and smoking (p= 0.086) and age and ACEI/ARB treatment (p = 0.08) on Ang-(1-7) levels.

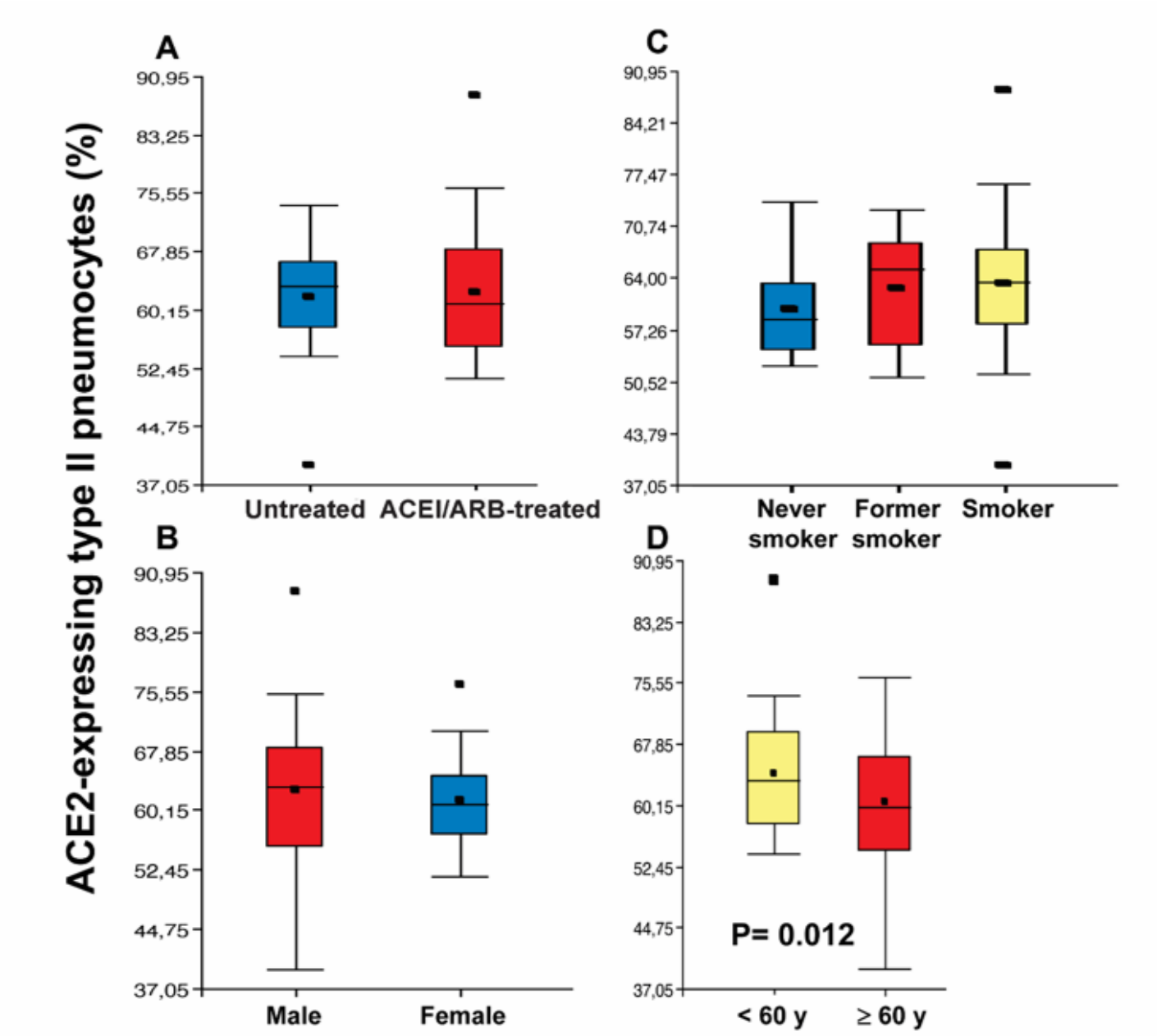

Figure 3

ACE2-Expressing type II Pneumocytes are Influenced by Age. Percentage of ACE2-expressing type II pneumocytes of lung parenchyma of untreated and ACEI/ARB-treated subjects (A), never smokers, former smokers or smokers (B), male or female (C) or from subjects under 60 years old (<60 y) or equal or over 60 years old (≥ 60 y) (D). Statistical analysis (general linear model) showed a significant difference by age (p= 0.012). The bottom and top of the box plots represent the 25th and 75th percentiles, respectively. The bands within the box show the median value, the dot is the media and the whiskers extending from both ends of the boxes are minimum and maximum values. Black points outside the box are outliers. Data distribution is presented in Figure 4S. The general linear model was used for statistical analysis.

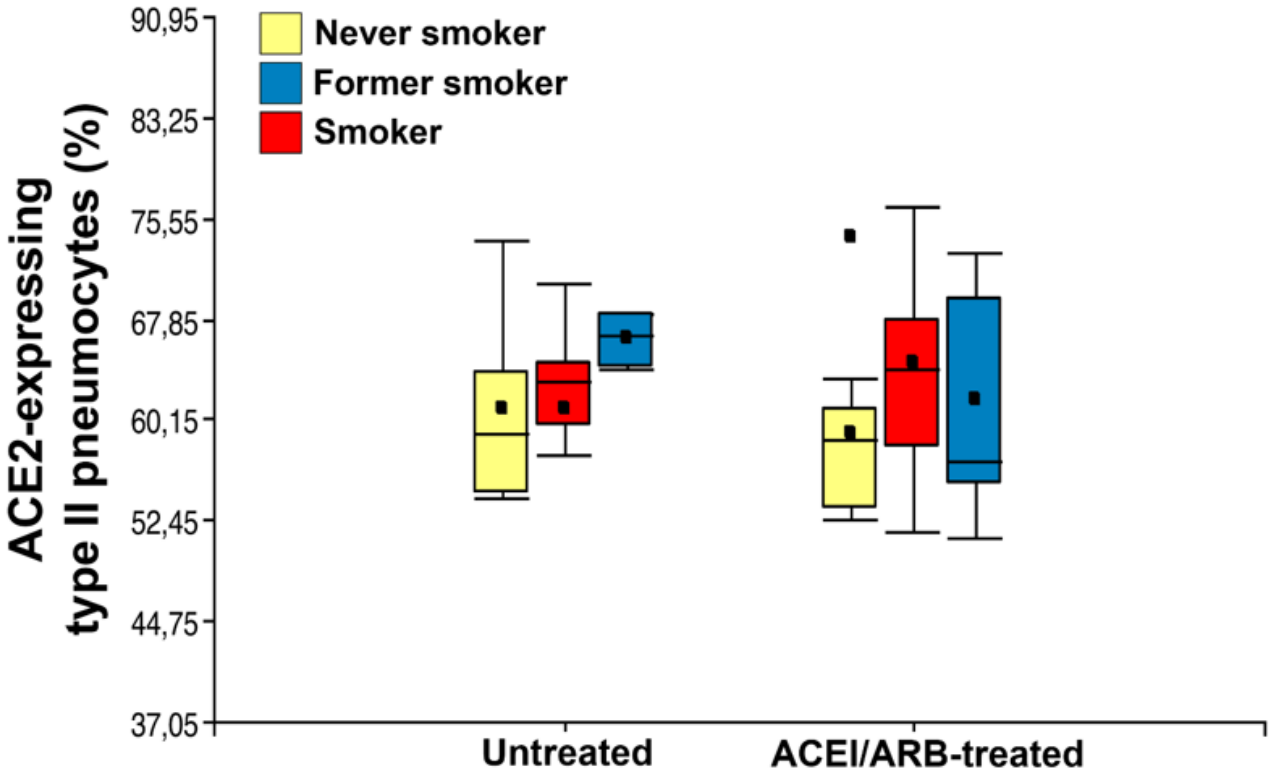

Figure 4

ACE2-Expressing type II Pneumocytes are Influenced by Smoking and ACEI/ARB Treatment. Percentage of ACE2-expressing type II pneumocytes of lung parenchyma of untreated and ACEI/ARB-treated never smokers, former smokers or smokers. Statistical analysis (generali linear model) showed a significant interaction between ACEI/ARB treatment and smoking ($p = 0.026$) on the percentage of ACE2-expressing type II pneumocytes. The bottom and top of the box plots represent the 25th and 75th percentiles, respectively. The bands within the box show the median value, the dot is the media and the whiskers extending from both ends of the boxes are minimum and maximum values. Black points represent outliers. Data distribution is presented in Figure 5S.

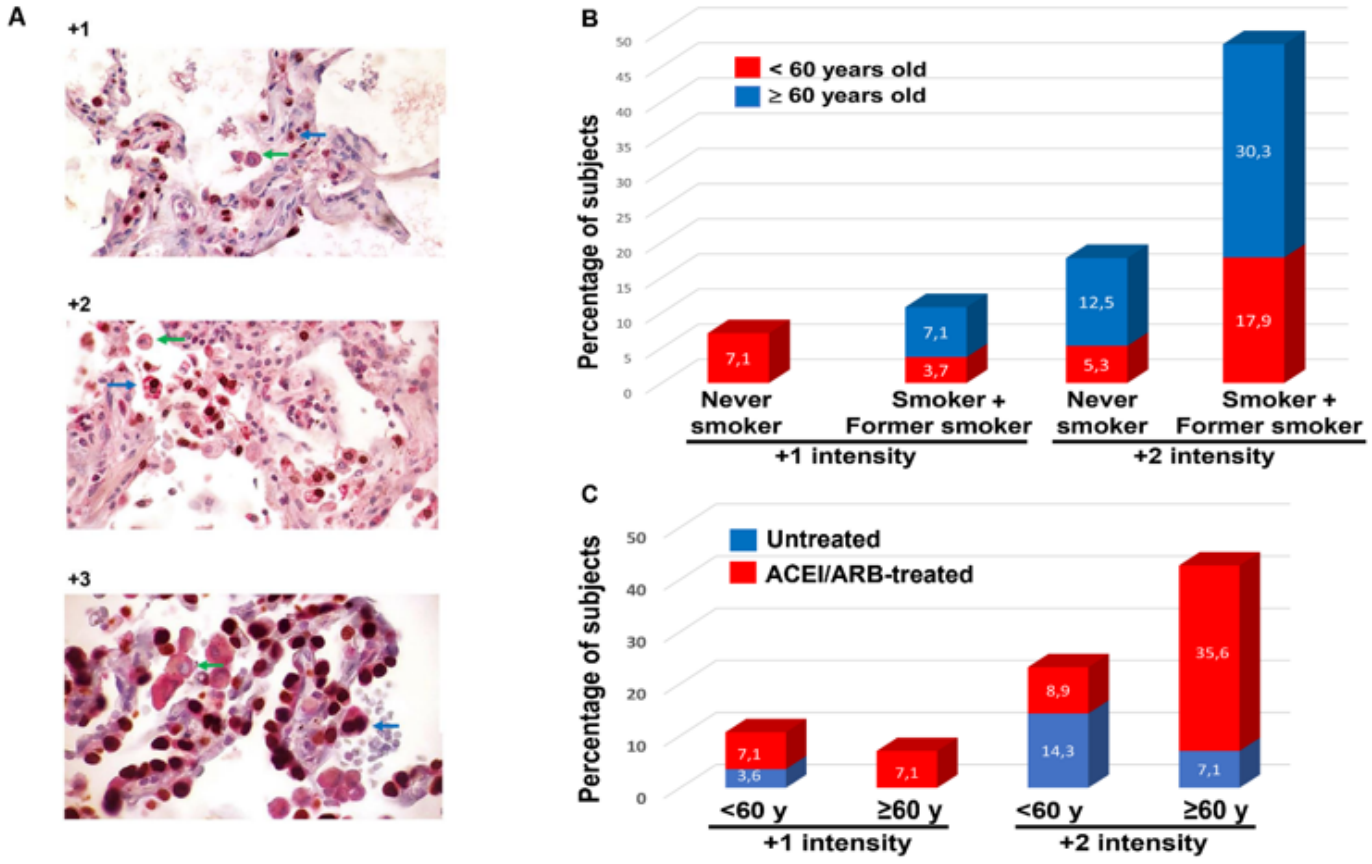

Figure 5

ACE2 Protein Expression is Greater in Older Smokers. Percentage of subjects with ACE2 immunostaining intensity (+1 or +2) in type II pneumocytes of lung parenchyma from never smokers and smokers + former smokers subjects under 60 years old (<60 y) or equal or over 60 years old (≥60 y) (B) or from untreated and ACEI/ARB-treated subjects under 60 years old (<60 y) or equal or over 60 years old (≥60 y) (C). Representative images (40x) of surgically resected lung tissue stained for ACE2 protein (red) and type II pneumocytes (brown) counterstained with hematoxylin (blue) (A). Positive ACE2 staining in type II pneumocytes and in alveolar macrophages is indicated by blue and green arrows, respectively. ACE2 intensity was classified from +1 to +3. ACE2 intensity corresponding to the score +3 was not presented because of the low number of samples with that classification, but they were included when statistical analysis was performed. Percentage of subjects with ACE2 intensity of +1 or +2 in each group is displayed inside the box. The stratified Fisher's Exact Test shows an association between ACE2 intensity, smoking and subjects equal or older than 60 years old (p= 0.05) and between ACE2 intensity, age and ACEI/ARB treated (p = 0.09). Although this last p value is greater than 0.05 but less than 0.1, this result shows a trend that these factors are associated.

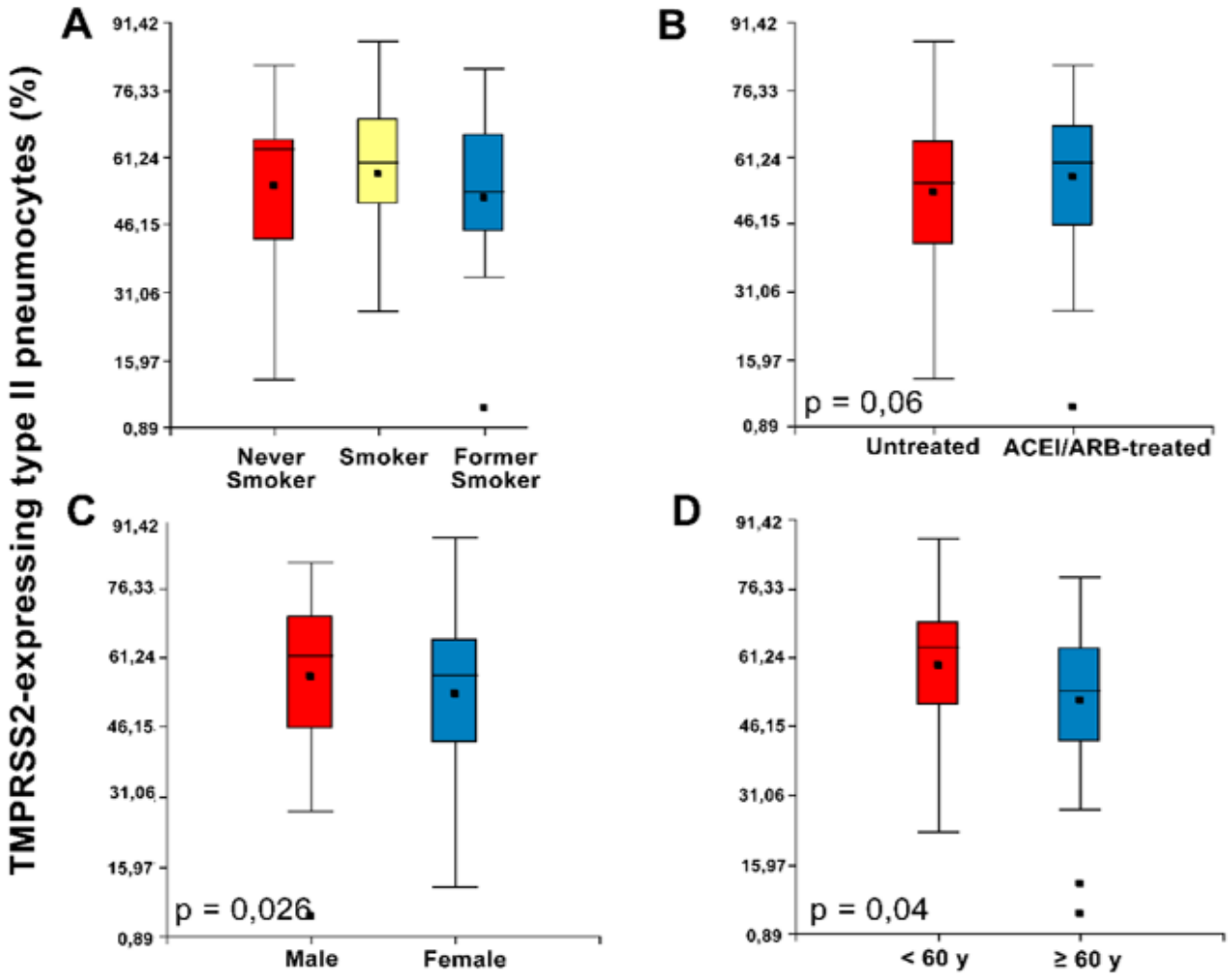

Figure 6

TMPRSS2-Expressing type II Pneumocytes are Influenced by Age and Sex. Percentage of TMPRSS2-expressing type II pneumocytes of lung parenchyma of never smokers, former smokers or smokers (A), untreated and ACEI/ARB-treated subjects (B), male or female (C) or from subjects under 60 years old (<60 y) or equal or over 60 years old (≥ 60 y) (D). The bottom and top of the box plots represent the 25th and 75th percentiles, respectively. The bands within the box show the median value, the dot is the media and the whiskers extending from both ends of the boxes are minimum and maximum values. Black points outside the box are outliers. Data distribution is presented in Figure 6S. The general linear model was used for statistical analysis.

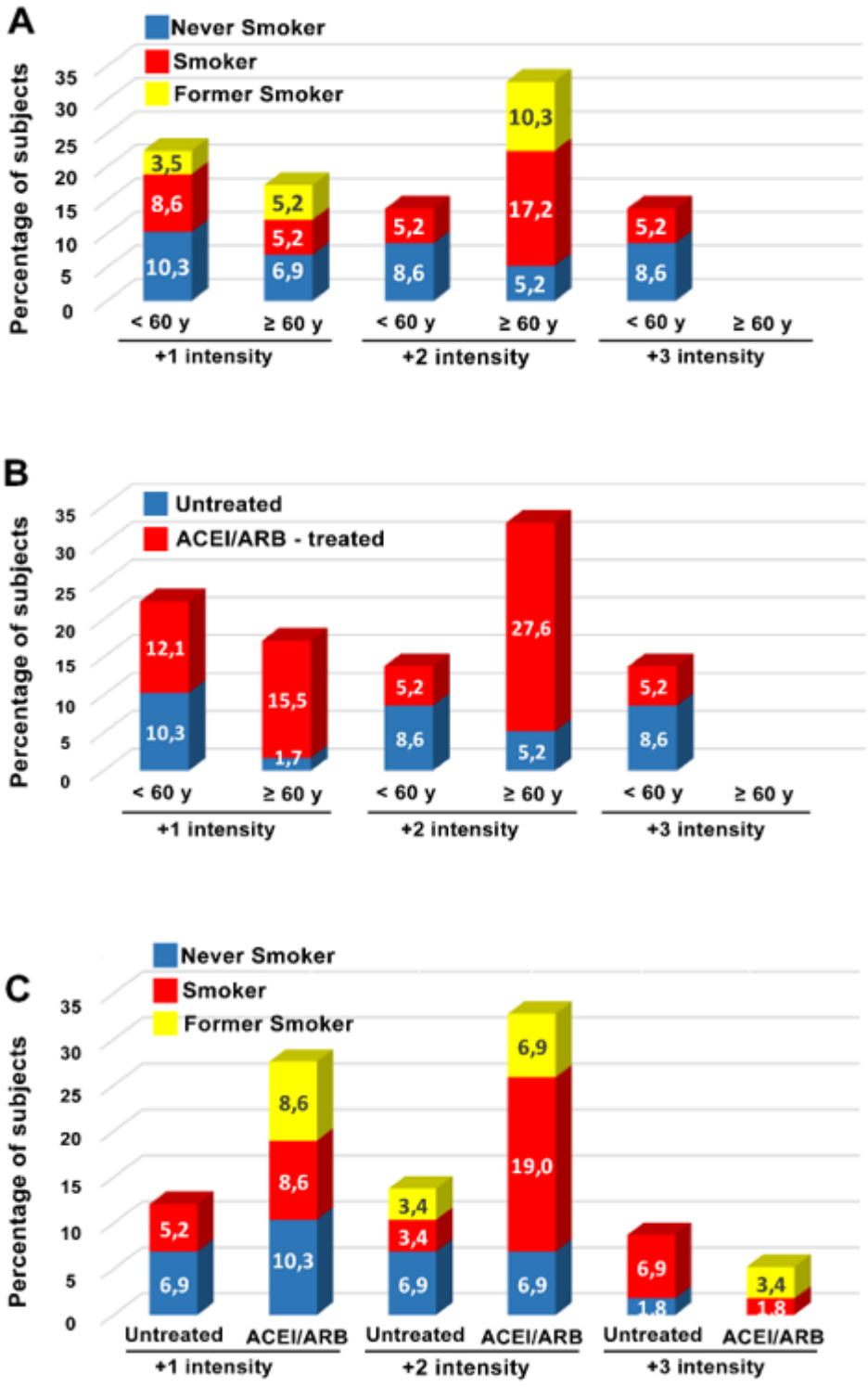

Figure 7

TMPRSS2 Protein Levels are Greater in Older Smokers and in Olders or Smokers under RAS Blockade Treatment. Percentage of subjects with TMPRSS2 immunostaining intensity (+1, +2 or +3) in type II pneumocytes of lung parenchyma from never smokers, smokers or former smokers subjects under 60 years old (<60 y) or equal or over 60 years old (≥60 y) (A) or from untreated and ACEI/ARB-treated subjects under 60 years old (<60 y) or equal or over 60 years old (≥60 y) (B) or from untreated and

ACEI/ARB-treated (ACEI/ARB) subjects never smokers, smokers or former smokers (C). TMPRSS2 intensity was classified from +1 to +3. Representative images (40x) of surgically resected lung tissue stained for TMPRSS2 protein are presented in Figure 7S. The percentage of subjects with TMPRSS2 intensity of +1, +2 or +3 in each group is displayed inside the box. The stratified Fisher's Exact Test was applied to verify association.

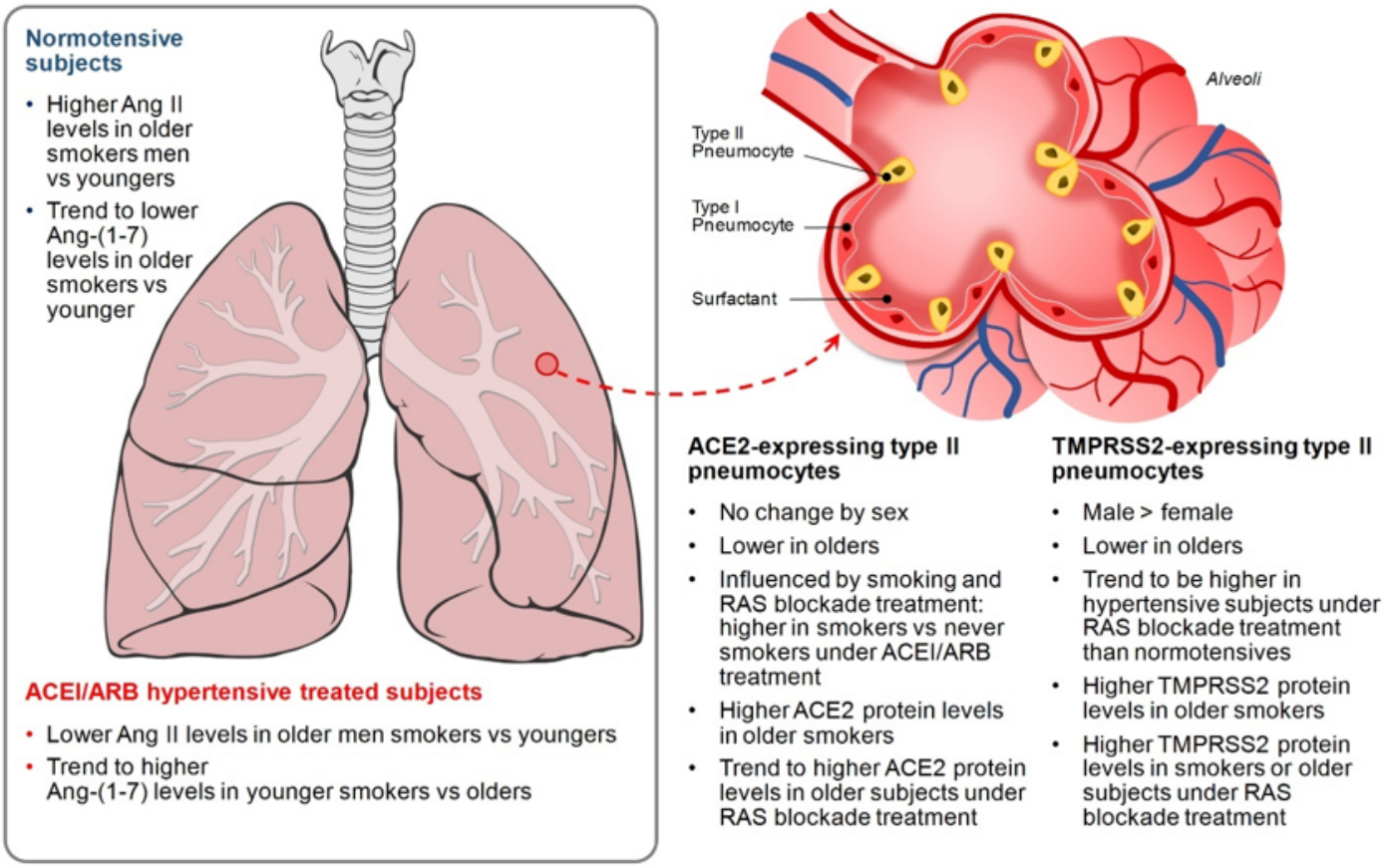

Figure 8

Scheme that resumes the conclusions raised in the present work