# INTERALVEOLAR PORES INCREASE IN AGING AND SEVERE AIRWAY OBSTRUCTION

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*To the Editor:*

*Introduction*

Interalveolar pores are thought to equalize pressure between adjacent alveoli through collateral ventilation, but their extent, both in aging and lung disease, remains unclear. We recently demonstrated an association between aging and small airway loss (2), but changes in collateral ventilation might form another hallmark of aging. This may especially be true in pathological conditions with severe airway obstruction, to prevent retro-obstructive alveolar collapse. Bronchiolitis obliterans syndrome (BOS) after lung transplantation represents a typical airway-centered disease, with irreversible obstructive pulmonary function deficit due to obliterative bronchiolitis (3). We therefore provide a detailed assessment of interalveolar communications in a cohort of aging lungs and correlate these findings with terminal bronchiole counts and other physiological and structural parameters related to aging, and assess interalveolar communications in BOS explant lungs. We hypothesize that aging and BOS are associated with increased interalveolar communications.

*Methods*

We included 20 unused donor lungs from never-smoking donors, previously used to demonstrate decreased small airway counts in aging (2), and 10 BOS-explant lungs (*Table 1*). One explant lung was processed as previously described (2), and two initially extracted tissue cores from matched locations from each lung (upper-lower lobe, total n=60) were included and processed for scanning electron microscopy (Philips XL30). Twenty representative images (350x magnification) were obtained per location (n=40/lung); pores were analyzed using FIJI-software. Terminal bronchiole counts, Global Lung Function Initiative (GLI)-predicted Tiffeneau-index, computed tomography (CT)-calculated lung volume, mean-linear-intercept,

and surface density results were obtained from the previously generated dataset (2). All data was normally distributed (Shapiro-Wilk) and presented as mean (standard deviation). P-value <0.05 was considered significant. The relation between the overall mean number/size of interalveolar pores (i.e. of n=40 analyzed images/lung) with age was analyzed using univariate linear regression with Pearson correlation. The mean number/size of interalveolar pores in upper vs lower lobe samples (i.e. of n=20 analyzed images/location) was compared using paired t-test. BOS and donor lungs were compared using multivariate linear regression analysis, adjusted for age and donor sex. Ethics Committee approval was obtained (S52174, S59648, S61653). BOS patients provided written informed consent; donor lungs were exempt from written informed consent following Belgian legislation.

*Results*

We found a significant association between interalveolar pore numbers and aging (R=0.93, 95%CI [0.82, 0.97], p<0.0001) (*Figure 1A, representative examples shown in C, E, G, H*). Overall mean pore size was 11.86 µm (SD 1.78), without correlation with age (R=0.010 [-0.36, 0.52], p=0.68). Sub-analysis revealed significantly larger pores in upper versus lower lobe derived samples (mean size 13.08 µm (SD 2.71) versus 10.64 µm (SD 1.92), p=0.0020), without difference between pore numbers (mean number of pores/field = 15.76 (SD 9.66) versus 15.25 (SD 9.28), p=0.43). An inverse association was found between interalveolar pore numbers and terminal bronchiole counts/mL lung tissue (R=-0.58 [-0.81, -0.19], p=0.0072) (*Figure 1B*). Moreover, interalveolar pore numbers inversely correlated with GLI-predicted Tiffeneau-index (R=-0.85, [-0.94, -0.66], p<0.0001). There was no association between interalveolar pore numbers and CT-calculated lung volume (R=0.015, [-0.43, 0.45], p=0.95), mean-linear-intercept (R=0.39, [-0.058, 0.71], p=0.085) or surface density (R=-0.37, [-0.70, 0.086], p=0.11).

In BOS explant lungs, there was no correlation between total graft age (donor age + time between transplantation and graft loss) and interalveolar pore numbers (R=0.33, 95%CI [-0.38, 0.79], p=0.35) (*Figure 1A, D, F*). Mean pore size was 11.87 µm (SD 1.88) (R=0.37, [-0.34, 0.81], p=0.29). Sub-analysis of upper versus lower lobes revealed no difference in pore numbers (mean number of pores/field = 33.17 (SD 10.83) versus 36.84 (SD13.20), p=0.23) or size (mean size 12.48 µm (SD 2.18) versus 11.26 µm (SD 2.51), p=0.11). No association was found between Tiffeneau-index and interalveolar pore numbers (R=0.077, 95%CI [-0.77, 0.43], p=0.44). Multivariate analysis, adjusted for age and donor sex, revealed more interalveolar pores in BOS versus the aging cohort (p<0.0001, estimate = 24.78 mean numbers of pores/field (SD 2.93)), but similar pore sizes (p=0.88). In addition, donor sex was not significantly associated with pore numbers or size (p=0.26 and p=0.88, respectively).

*Discussion*

We found a significant increase in interalveolar pore numbers with aging, while pore sizes showed no age-dependent association. To our knowledge, this report forms the largest cohort of human explant lungs investigating number and size of interalveolar communications. Our findings are consistent with physiological measurements in healthy subjects which reported lower resistance to collateral ventilation (Rcoll) in older individuals, indicating age-dependent increased collateral ventilation (4). Animal studies on aging demonstrated conflicting results. Martin et al. reported no increase in number/size of interalveolar pores after the first year of life in dogs (5), whereas Rcoll decreased in older sheep (6). Interestingly, we found an inverse correlation between interalveolar pore numbers and terminal bronchiole numbers. We previously reported ±50% reduction in terminal bronchioles from 30 to 80 years (2). While the clinical significance and sequence remains uncertain, small airway loss may lead to increased

airway resistance and lower Rcoll. Increased numbers of interalveolar pores, through turbulent alveolar airflow, may directly add to age-dependent FEV1-decline (7). Further mechanistical studies are necessary to assess the underlying pathophysiology. Mechanical factors, such as chronic altered interalveolar pressure differences with lower Rcoll may cause increased alveolar pore numbers. In addition, alveolar pores are associated with type II pneumocytes (8), and biological processes involved in cellular aging (e.g. altered surfactant production, telomere shortening, cellular senescence) may also cause an increase in alveolar pores.

We further report an approximately threefold increase in interalveolar pore numbers in BOS compared to aging. To our knowledge, no data is available about collateral ventilation in BOS. As >75% of small airways can become segmentally obstructed in end-stage BOS with a preserved number and diameter of terminal bronchioles (9), collateral ventilation might form an important mechanism to prevent retro-obstructive alveolar collapse and maintain gas exchange. Future (patho)physiological studies should address the clinical relevance of collateral ventilation in BOS. In addition, we did not assess broncho-alveolar and inter-bronchiolar connections, as their complex architecture hampers visualization.

In conclusion, we report a significant increase in interalveolar pore numbers with aging, while pore sizes were not correlated with aging. In addition, interalveolar pores are markedly increased in BOS.

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## Figure Legends

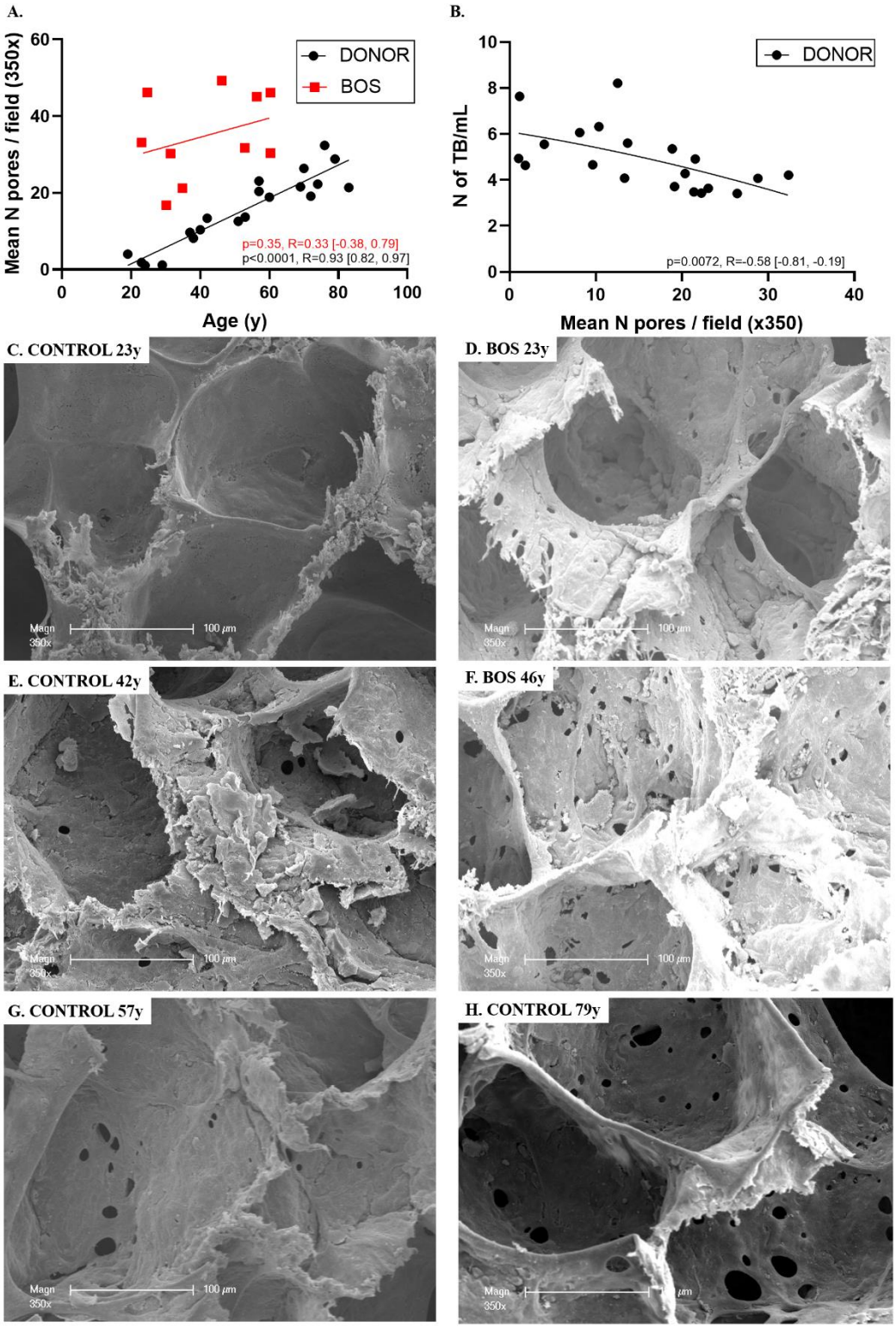

**Figure 1.** A. Association between age and mean number of interalveolar pores per field. A significant positive association between donor age and mean number of pores was found in non-transplanted donor lungs (Pearson R=0.93, 95%CI [0.82-0.97], p<0.0001), while there was no association between total graft age (= donor age + time between transplantation and graft loss) and pore numbers in bronchiolitis obliterans syndrome (R=0.33, 95%CI [-0.38, 0.79], p=0.35). B. There was a significant negative correlation between the number of terminal bronchioles per mL of lung tissue and mean number of pores per field (R=-0.58, 95%CI [-0.81, -0.19], p=0.0072). C. Representative scanning electron microscopy (SEM) image of 23 years old donor lung. D. Representative SEM image of BOS explant lung of 23 years total graft age. E. Representative SEM image of 42 years old donor lung. F. Representative SEM image of BOS explant lung of 46 years total graft age. G and H. Representative SEM images of a 57 years old and 79 years old donor lung, respectively.

| **Table 1.** Patient characteristics | | 181 |
|---|---|---|
| | **Aging cohort** | **BOS** |
| **Patients, N** | 20 | 10 |
| **Donor characteristics** | | |
| Age (y) | 52.7 (20.2) | 36.9 (16.0) |
| Male, N (%) | 15 (75) | 4 (40) |
| Weight (kg) | 75 (12) | 68 (12) |
| Height (cm) | 173 (8) | 171 (9) |
| **Cause of death donor, N (%)** | | |
| Cerebral ischemia | 11 (55) | 4 (40) |
| Cardiac arrest | 3 (15) | |
| Cranio-cerebral trauma | 2 (10) | 5 (50) |
| Suicide | 2 (10) | 1 (10) |
| Extra-pulmonary tumor | 1 (5) | |
| Hemodynamic collapse | 1 (5) | |
| **Recipient characteristics** | | |
| Age (y) | | 50.4 (13.5) |
| Male, N (%) | | 5 (50) |
| Weight (kg) | | 61.3 (18.8) |
| Height (cm) | | 168 (12) |
| Time to BOS (y) | | 3.11 (2.23) |
| Time LTx to graft loss (y) | | 5.15 (2.75) |
| Time BOS to graft loss (y) | | 2.12 (1.78) |
| Total graft age (y) | | 42.1 (14.8) |
| **Pulmonary function*** | | |
| FEV1 (l) | 3.44 (0.83) | 0.61 (0.21) |
| FEV1% | | 19.5 (4.9) |
| FVC (l) | 4.32 (0.95) | 1.79 (0.62) |
| FVC% | | 48.7 (12.8) |
| FEV1/FVC | 0.79 (0.036) | 0.34 (0.056) |

182 **Table 1.** Data are presented as number (%) or mean (standard deviation). (*) for the aging
183 cohort, FEV1 and FVC values are Global Lung Function Initiative (GLI)-predicted values; for BOS
184 patients, the last available pulmonary function test before graft loss was included. BOS:
185 bronchiolitis obliterans syndrome; CVA: cerebrovascular accident; FEV1: forced expiratory
186 volume in one second; FVC: forced vital capacity.