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Using Hyperpolarized Xenon-129 MRI to Quantify Early-Stage Lung Disease in Smokers

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Abstract

Rationale and Objectives: Hyperpolarized xenon-129 magnetic resonance (MR) provides sensitive tools that may detect early stages of lung disease in smokers before it has progressed to chronic obstructive pulmonary disease (COPD) apparent to conventional spirometric measures. We hypothesized that the functional alveolar wall thickness as assessed by hyperpolarized xenon-129 MR spectroscopy would be elevated in clinically healthy smokers before xenon MR diffusion measurements would indicate emphysematous tissue destruction.

Materials and Methods: Using hyperpolarized xenon-129 MR we measured the functional septal wall thickness and apparent diffusion coefficient of the gas phase in 16 subjects with smoking-related COPD, 9 clinically healthy current or former smokers, and 10 healthy never smokers. All subjects were age-matched and characterized by conventional pulmonary function tests. 11 data sets from younger healthy never smokers were added to determine the age dependence of the septal wall thickness measurements.

Results: In healthy never smokers the septal wall thickness increased by 0.04 μm per year of age. The healthy smoker cohort exhibited normal pulmonary function test measures that did not significantly differ from the never-smoker cohort. The age-corrected septal wall thickness correlated well with diffusion capacity for carbon monoxide ($R^2 = 0.56$) and showed a highly significant difference between healthy subjects and COPD patients (8.8 μm versus 12.3 μm ; $p < 0.001$), but was the only measure that actually discriminated healthy subjects from healthy smokers (8.8 μm versus 10.6 μm ; $p < 0.006$).

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Conclusion: Functional alveolar wall thickness assessed by hyperpolarized xenon-129 MR allows discrimination between healthy subjects and healthy smokers and could become a powerful new measure of early-stage lung disease.

Keywords

Hyperpolarized MRI; lung function; smoking; chronic obstructive pulmonary disease

INTRODUCTION

Despite preserved pulmonary function, many former and current smokers experience physical function impairments, reduced respiratory-specific quality of life, and an increased number of exacerbation events (1–3). Studies based on computed tomography (CT), thin-section CT, and high-resolution CT (HRCT), have demonstrated radiologically-detectable pulmonary abnormalities including ground-glass opacities, parenchymal micronodules, abnormal bronchial wall thickening, and emphysematous changes in a large fraction of non-symptomatic current or former smokers in statistically significant excess to the levels found in healthy never-smokers (4, 5). Further, using magnetic resonance imaging (MRI) Alamidi et al (6) showed a negative correlation between pack years and the relaxation time of the lung parenchyma possibly due to pathological decreases in microvascular blood flow in mild chronic obstructive pulmonary disease (COPD) (7). Nevertheless, the clinical relevance of these findings has not been established and it remains infeasible to identify those individuals who will develop progressive COPD (8).

Hyperpolarized gas MRI has provided additional insights in the ventilation, microstructure and function of the lungs that are difficult to obtain with existing conventional lung imaging modalities. By filling the pulmonary airspaces with hyperpolarized helium-3 gas several groups have been able to identify statistically significant differences in ventilation patterns (9, 10), fractional ventilation (11), lung morphology (12–14), and pulmonary oxygen tension (15, 16) between normal control subjects, healthy smokers, and COPD patients. However, hyperpolarized helium-3 MRI provides only indirect measures of lung function due to its very low tissue solubility.

Hyperpolarized xenon-129 (HXe) much more readily dissolves in the lung parenchyma (17) and, importantly, experiences a shift in resonance frequency of approximately 200 ppm in the process (18). Several HXe MRI studies have already demonstrated the utility of using snap shots of the static distribution of xenon dissolved in lung tissue, the so-called dissolved-phase magnetization, for characterizing lung disease (19–28). In addition, it is possible to obtain dynamic xenon gasexchange information by selectively destroying the dissolved-phase magnetization with radiofrequency pulses and subsequently observe pulmonary gas exchange as a regrowth of the dissolved-phase signal. This approach is commonly termed chemical shift saturation recovery (CSSR) MR spectroscopy (29–33) and it is already becoming a powerful tool to directly quantify lung function (23, 34–39). In particular, by fitting the CSSR measurement data to gas uptake models (31, 40–42) an apparent alveolar wall thickness can be computed. The purpose of this work was to assess whether the thickness of the alveolar wall as obtained by HXe MR spectroscopy permits a discrimination

between emphysematous COPD patients, non-symptomatic healthy smokers and age-matched normal subjects.

MATERIALS AND METHODS

Subjects

Study groups were composed of 16 subjects (9 female) with smoking-related COPD (> 10 pack years, $FEV_1/FVC < 70$), 10 (6 female) age-matched healthy, never smokers, and 9 (8 female) age-matched, clinically healthy current or former smokers (> 10 pack years, $FEV_1/FVC > 70$ and $FEV_1 > 80\%$ predicted). HXe spectroscopy data from 11 (8 female) healthy never smokers (19 – 54 years of age) who were not part of these study cohorts were combined with the results from the age-matched study cohort to estimate the age dependence of the septal wall thickness measurements.

Physiologic Measurements

Ahead of the MR experiments, lung function - including total lung capacity (TLC), residual volume (RV), functional residual capacity (FRC), diffusion capacity for carbon monoxide (DLCO) and 6-minute walk test (6MWT) - was assessed in the University of Virginia pulmonary function lab for the subjects in the three study cohorts.

In all subjects spirometry, including forced vital capacity (FVC), forced expiratory flow over the middle half of the FVC (FEF 25–75%), and forced expiratory volume in 1 second (FEV1), was also performed immediately before and after the imaging session using a hand-held device (Koko; PDS Ferraris, Louisville, CO). Throughout the imaging session the subject's heart rate and oxygen saturation level were monitored (3150 MRI Patient Monitor; Invivo Research Inc., Orlando, FL), and the subject was assessed for central nervous system side effects of the inhaled xenon. All studies were supervised by a physician.

Gas Polarization and Administration

Enriched xenon gas (87% xenon-129) was polarized using a prototype commercial system (XeBox-E10; Xemed, LLC, Durham, NH) that provided gas polarizations of 30–40%. Immediately before MR data acquisition, 0.5 L of HXe gas was dispensed into a 600-ml Tedlar bag (Jensen Inert Products, Coral Springs, FL). Starting from residual lung volume, the subjects inhaled the HXe gas from the bag. An assistant removed the emptied bag from the subjects' mouth while they began the inhalation of room air and then held their breath at total lung capacity.

HXe MRI Data Acquisition

The HXe MRI data were acquired using apparent diffusion coefficient imaging (24), which detects changes in the size of the alveolar airspaces and CSSR (23) spectroscopy as previously described. MR acquisitions were performed using a 1.5 T commercial whole-body MR scanner (Avanto; Siemens Medical Solutions, Malvern PA) equipped with the multinuclear imaging option, permitting operation at the xenon-129 resonant frequency of 17.6 MHz. A flexible, circularly-polarized, vest-shaped chest radio-frequency coil (Clinical

MR Solutions, Brookfield, WI) was used for the experiments. The subject was positioned supine on the scanner table with the coil around their chest.

Data Analysis

All post-processing and data analysis was performed using MatLab (Mathworks, Natick, MA, USA). The areas under the gas-phase peak and the dissolved-phase peaks were integrated and the ratio between the tissue-plasma peak areas and the gas-phase peak area were calculated for normalization purposes. This ratio as a function of the delay time between dissolved-phase signal destruction and spectral data sampling captures the temporal dynamics of the accumulating xenon magnetization in the lung tissue. Over the years, various theoretical gas-diffusion and gas transport models that take pulmonary perfusion into account have been developed to map these dynamics to parameters of the underlying physiology (30, 31, 40, 41, 43). In particular, the rate with which the xenon magnetization saturates the alveolar septal wall is dependent on a combination of the thickness of the septal wall, xenon solubility in lung parenchyma, and the xenon diffusion constant in tissue. Unfortunately, it is highly impractical to measure the diffusion constant directly in vivo, so it is usually estimated at a fixed value of $3.3 \times 10^{-6} \text{ cm}^2/\text{s}$ (43). In this work, the experimental data was fit to the theoretical model proposed by Patz et al (40) and the mean septal wall thickness for the entire lung was extracted (23).

Statistical Analysis

One-way ANOVA was utilized to analyze lung function and HXe MRI derived measures of lung function. Traditional Q-Q plots were constructed to test whether the quantiles of ANOVA standardized residuals were in alignment with the quantiles of the Standard Normal distribution. We found no cases of misalignment between the quantiles of ANOVA standardized residuals and the quantile of the Standard Normal distribution that warranted either a data transformation of the response variable (e.g. log-transformation), or the use of a non-parametric analytical approach. Linear regression was utilized to examine the relationship between DLCO Dsb and septal wall thickness. Logistic regression was utilized to separate AMN and HS individuals based on traditional and HXe derived lung function parameters; and conventional receiver operator characteristics (i.e. area under ROC curve (AUC), sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV)), were utilized to assess the utility of the traditional and HXe lung function parameters to discriminate between AMN versus HS individuals. $P < 0.05$ was considered an indication for statistical significance. The software packages SAS version 9.4 (SAS Institute Inc., Cary, NC), and the R version 3.1.2 (R Foundation for Statistical Computing, R Development Core Team, Vienna, Austria) were used to conduct the statistical analyses and the graphics were produced via the Spotfire Splus version 8.2 package (TIBCO Inc., Palo Alto, CA).

Descriptive Data Summaries

Continuous scaled variables were summarized either by the mean and standard deviation of the distribution, or by the mean and 95% confidence interval, while categorical data were summarized by frequencies and percentages.

Analysis of Lung Function

Lung function test measurements for vital capacity (VC), FRC, RV, FVC, FEV1 predicted [%], FEV1/FVC predicted [%], DLCO Dsb, and 6MWT were compared between the 3 groups of individuals (i.e. AMN, HS, and COPD) by way of One-way ANOVA. An F-test was utilized to test the global null hypothesis that there is no association between the mean of the lung function measurement distribution and study-group. Hypothesis tests related to the pairwise between-group comparisons of the means of lung function measurement distributions were conducted via the Welch 2-sample test (44); i.e. a parametric test that is akin to the 2-sample Student's t-test, but does not have the underlying assumption of between-group measurement variability homogeneity. A Bonferroni corrected $p = 0.05$ criterion was established *a priori* as the null hypothesis rejection rule for this set of pairwise between-group comparisons.

First Order Derivation of Age-Corrected Septal Wall Thickness

The linear relationship between age and septal wall thickness was determined via ordinary least-squares regression (OLSR) for a group of 21 normal health individuals. The estimate for the slope of this relationship was then utilized in the framework of linear-interpolation to derive first order approximations for age-corrected septal wall thickness for the 3 study-groups of individuals (AMN, HS, and COPD); where the age corrected septal wall thickness for any particular individual represents the first order approximation for the individual's septal wall thicknesses at age 63 years. The expression that was utilized to derive the age corrected first order approximation for septal wall thickness is: AC-63 Septal Wall Thickness (μm) = Septal Wall Thickness (μm) -0.0371 ($\mu\text{m}/\text{year}$) $\times$ [True Age (years) -63 years], where "AC-63 Septal Wall Thickness (μm)" is the individual's age corrected septal wall thickness corrected to 63 years of age, "Septal Wall Thickness" is the individual's HXe MRI derived septal wall thickness, " 0.0371 " is the OLSR parameter estimate for the slope of the relationship between septal wall thickness (μm) and chronological age (years), "True Age" is the individual's true age in years, and "63 years" is the reference age of the correction. 63 years of age was selected as the reference age of the correction because 63 years was the average age of the individuals in the 3 study-groups.

Analysis of HXe MRI Derived Lung Function Measurements

The distributions of the HXe MRI derived apparent diffusion constants (ADC), as well as the distributions of the HXe MRI derived septal wall thicknesses and the age corrected (AC-63) septal wall thicknesses were compared between the 3 study-groups of individuals (AMN, HS, and COPD) by way of One-way ANOVA. An F-test was utilized to test the global null hypothesis that there is no association between the mean of the lung function measurement distribution and study-group. Hypothesis tests related to the pairwise between-group comparisons of the means of lung function measurement distributions were conducted via the 2sample Welch test and a Bonferroni corrected $p = 0.05$ criterion was established *a priori* as the null hypothesis decision rule for this set of pairwise between-group comparisons.

Analysis of DLCO Dsb and Septal Wall Thickness

The relationship between DLCO Dsb and septal wall thickness was determined for the entire group of individuals (i.e. AMN, HS, and COPD combined) and for the AMN and HS subset of individuals by way of OLSR. Septal Wall Thickness was considered the independent variable in the OLSR analyses, while DLCO was considered the dependent variable. DLCO versus septal wall thickness association was tested by way of an F-test, and a $p = 0.05$ rejection rule was the criterion utilized for rejecting the null hypothesis that there is no association between DLCO and septal wall thickness.

Lung Function-based Separation of AMN and HS Individuals

Logistic regression and receiver operator characteristic (ROC) analyses were conducted to determine if the AMN and HS individuals could be separated into their respective groups based on lung function. Traditional lung function measures (VC, FRC, RV, TLC, FVC, FEV1 pred [%], FEV1/FVC pred [%] and DLCO Dsb) and the HXe MRI lung function measures (Septal Wall Thickness, AC-63 Septal Wall Thickness, and ADC) were evaluated individually in univariate fashion.

A logistic regression analysis was conducted per lung function measure to determine if there is an association between the lung function measure study-groups. In the analyses the response variable was assigned the value 1 if the individual was a member of the HS study-group and the response variable was assigned 0 if the individual was a member of the AMN study group. The test of association was based on the Wald chi-square test and a $p = 0.05$ decision rule was utilized as the null hypothesis rejection criterion. Strength of association was quantified by comparing between the 3rd and 1st quartiles of the lung function measure empirical distribution, the odds for HS group membership.

Pre lung function measure, common diagnostic receiver operator characteristics (ROC) were utilized to determine how accurately the AMN and HS individuals could be assigned into their respective groups based on the lung function measure. In the ROC analyses individuals in the HS study-group were considered “True Positives”, while individuals in the AMN study-group were considered “True Negatives”. Measure of classification accuracy included: area under ROC curve (AUC), sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV). These measures of classification accuracy were estimated at the threshold value of the lung function measure which minimized the sum of the false positive and false negative error rates. Confidence interval construction for the diagnostic receiver operator characteristics was based on the bootstrap methods of Carpenter and Bithell (45).

Experiments were performed under a physician’s investigational new drug application for MR imaging with HXe using a protocol approved by our Institutional Review Board. Written informed consent was obtained from each subject after the nature of the procedure had been fully explained.

RESULTS

Demographics and Conventional Lung Function Measurements

The subjects in the normal, smokers, and COPD study-groups were age-matched with mean ages of 60.8 ± 10.3 years, 59.7 ± 8.4 years, and 65.8 ± 8.3 years, respectively. The age range for the additional 8 female and 3 male normal subjects that were used to determine the age-dependence of the septal wall thickness was 19 – 54 years with a mean of 28.1 ± 11.8 years.

The empirical distributions of lung function measures are displayed in Figure 1. By inclusion criteria, all spirometric functions for the AMN and HS study-groups were in the normal range with no statistically significant differences in FEF 25–75%, FRC, RV, TLC, FVC, FEV1 %pred, and FEV1/FVC %pred. Similarly, no statistically significant differences in DLCO and 6MWT were observed between the two groups. However, the values for FEF 25–75%, FEV1 %pred, FEV1/FVC %pred, and DLCO in the COPD cohort showed a statistically significant difference from those in the AMN and HS groups. No significant differences were observed for the FRC, TLC, and FVC measurements while there were statistically significant differences between AMN and COPD subjects for RV and 6MWT but not between HS and COPD subjects.

Age Dependence of Septal Wall Thickness

The septal wall thickness measured by MR spectroscopy for all healthy subjects, comprised of the AMN cohort as well as the additional 11 younger subjects, as a function of age is plotted in Figure 2. A linear regression line was fitted to the data ($R^2 = 0.64$) and revealed an increase in septal wall thickness of approximately $0.04 \mu\text{m}$ per year. Although the number of data points is too small to be conclusive, the graph hints that the thickening process might accelerate beyond 60 years of age.

HXe MRI Lung Function Measurements

The observed slope of the regression line in Figure 2 was used to perform a first-order age correction of the measured septal wall thickness in all subjects in the 3 study cohorts to the mean age of all subjects of 63 years. The empirical distributions for the measured septal wall thickness, the age-corrected (AC-63) septal wall thickness, and the mean apparent diffusion constant in the alveolar airspaces for the 3 study groups are shown in Figure 3 while the between study-group comparisons of these measures are presented in Table 1. The mean difference in septal wall thickness due to the age correction (i.e. age-corrected – non-age-corrected) was $0.07 \mu\text{m}$ (95% CI: $[-0.36, 0.21 \mu\text{m}]$, $p=0.578$) for AMN, while $0.11 \mu\text{m}$ (95% CI: $[-0.37, 0.13 \mu\text{m}]$, $p=0.324$) for HS, and $-0.11 \mu\text{m}$ (95% CI: $[-0.06, 0.28 \mu\text{m}]$, $p=0.186$) for COPD. Subject-to-subject variability in the septal wall thicknesses was not impacted by the age correction ($p=0.501$, $p=0.923$, and $p=0.963$, for AMN, HS and COPD, respectively). The mean AC-63 wall thickness was $8.8 \pm 0.7 \mu\text{m}$, $10.6 \pm 1.3 \mu\text{m}$ and $12.3 \pm 2.3 \mu\text{m}$ and the mean apparent diffusion constant was $0.042 \pm 0.004 \text{ cm}^2/\text{s}$, $0.043 \pm 0.004 \text{ cm}^2/\text{s}$, and $0.059 \pm 0.012 \text{ cm}^2/\text{s}$ in AMN, HS and COPD, respectively. The differences in AC-63 septal wall thickness for the AMN – HS ($p < 0.006$), the AMN – COPD ($p < 0.001$), and the HS – COPD ($p < 0.03$) pairs as well as the apparent diffusion constants for the AMN – COPD ($p < 0.001$) and HS – COPD ($p < 0.001$) pairs were statistically significant.

Figure 4 depicts scatterplots of the xenon gas-phase diffusion coefficient versus the measured (Figure 4A) and the age-corrected (Figure 4B) septal wall thickness. In particular, the age-corrected plot indicates that the three study-groups are clustered in different plot quadrants separated by fairly sharp transition zones surrounding the dashed lines at a diffusion constant of approximately $0.046 \text{ cm}^2/\text{s}$ and a septal wall thickness of approximately $9.5 \text{ }\mu\text{m}$: AMN in the lower left quadrant; HS in the lower right quadrant; COPD subjects in the upper right quadrant. The upper left quadrant is empty.

Comparison of Septal Wall Thickness and DLCO

Conceptually, the septal wall thickness measurements using CSSR MR spectroscopy are rather similar to the DLCO measurement as both assess lung function through the quantification of a gas uptake process. Indeed, as illustrated in Figure 5A, these measurements are highly related ($R^2 = 0.56$, $p < 0.001$). However, a closer inspection of the data reveals that this association is mainly driven by the COPD subjects. The association within the two healthy study groups (i.e. AMN and HS) is actually much less evident ($R^2 = 0.27$, $p = 0.023$) and, critically, there is no statistically significant difference in DLCO between HS and AMN subjects while there is in the septal wall thickness (Table 1). This same set of findings hold true for the age-corrected (63years) septal wall thickness measurement and the DLCO measurement in which these two sets of measurement are highly related when all three study groups are considered ($R^2 = 0.56$, $p < 0.001$), but less so when only the two healthy study groups are considered ($R^2 = 0.25$, $p = 0.031$).

Lung function based separation of AMN and HS individuals

The results from the logistic regression analyses and from the receiver operator characteristic analyses that were carried out to determine if AMN and HS individuals can be separated into their respective groups based on traditional and HXe MRI measures of lung function are summarized in Table 2.

With regard to the logistic regression analyses, two measures of lung function were found to discriminate HS from AMN. One measure was HXe MRI septal wall thickness and the other was HXe MRI age-corrected septal wall thickness. A septal wall thickness of $10.4 \text{ }\mu\text{m}$ (3rd quartile of the empirical distribution) versus a septal wall thickness of $8.5 \text{ }\mu\text{m}$ (1st quartile of the empirical distribution), increased the odds for HS membership by a factor of 15.38 (95% CI: [1.35, 174.24], $p = 0.027$), while an age-corrected (63 years) septal wall thickness of $10.2 \text{ }\mu\text{m}$ (3rd quartile of the empirical distribution) versus an age-corrected septal wall thickness of $8.6 \text{ }\mu\text{m}$ (1st quartile of the empirical distribution), increased the odds for HS membership by a factor of 30.34 (95% CI: [1.47, 629.33], $p = 0.027$).

With regard to the ROC analyses, at the thresholds of the lung function measures that minimize the misclassification error rate, the ROC AUC ranged from 0.52 (95% CI: [0.50, 0.81]) for RV to 0.90 (95% CI: [0.75, 1.00]) for age-corrected (63-year) septal wall thickness. Note that an AUC of 0.5 indicates that the classification is no better than a classification scheme based on the flip of a fair coin, while an AUC of 1.0 indicates that the classification is perfect.

For the two HXe MRI lung-function measures (Septal Wall Thickness and AC-63 Septal Wall Thickness) that were identified by logistic regression to be predictors of HS versus AMN studygroup membership, the ROC estimates for classification sensitivity, specificity, PPV and NPV ranged from 80.0% to 88.9% for both lung function measures. Note that the estimates for PPV and NPV in Table 2 are prevalence dependent and these estimates assume that the prevalence for HS is the same as the observed prevalence of 0.473.

DISCUSSION

Smoking is a factor crucial to the development of pulmonary emphysema; however, there are no current prognostic tools to determine which smokers will develop emphysema or have progression. Nevertheless, in COPD patients the strongest predictor for frequent exacerbations seems to be a history of previous exacerbations (46). Hence, the identification of the distinct COPD phenotype that is at the root of such exacerbation susceptibility might prove to be very valuable especially if it can be verified at the earliest disease stages. Evidence for clinical and radiologic disease manifested as pathological disease patterns in CT and proton MRI scans, as well as abnormalities in lung function as assessed by hyperpolarized gas MRI even in smokers with normal spirometry has been reported in recent years. Still, it remains unclear which of the observed abnormalities should be considered most predictive for rapid disease progression.

In our study of three age-matched cohorts of normal control subjects, healthy smokers and COPD patients we quantified the functional alveolar septal wall thickness and the alveolar gasphase apparent diffusion coefficient using HXe MRI. By including additional MRI data from previous studies performed in younger healthy subjects we identified an increase in the functional septal wall thickness of approximately 0.04 μm per year of age in line with histological (47) and CSSR-based (37) measurements in the literature. The age-related thickening was subsequently used to correct the measured functional wall thickness values for all subjects in the three study cohorts to the mean subject age of 63. We compared these findings to conventional pulmonary function metrics. The inclusion criteria for the healthy smoker cohort required normal spirometry results during the initial subject screening. As expected from the study inclusion criteria, the spirometric lung function tests showed no statistically significant differences between the healthy smokers and the age-matched normals. In addition, for these two cohorts there was also no statistically significant difference in the DLCO and 6MWT parameters, which had not been part of the inclusion criteria.

The functional septal wall thickness extracted from CSSR MR spectroscopy was obtained by monitoring the rise of signal from xenon dissolved in the lung parenchyma as a function of time. Hence, the numerical value thereby obtained reflects the underlying properties of the lung tissue, such as the xenon diffusion constant in lung parenchyma or the xenon tissue solubility, that facilitate or obstruct the diffusion-driven xenon gas transport into and within the lung tissue. As a result, the spectroscopic measurements will differ from histological wall thickness measurements that only take geometric dimensions into account and not how rapidly certain distances within the tissue can be traversed by the lung gases. While the latter is likely to yield better correlations with anatomical CT imaging we would argue that the

functional alveolar wall thickness derived by the former might be of greater interest for the assessment of the actual pulmonary gas exchange processes.

For the analysis of the MRI measurements we plotted the apparent gas diffusion coefficient over the functional septal wall thickness. In such a graph the healthy subjects appear tightly clustered in the lower left quadrant with a septal wall thickness $< \sim 9.5 \mu\text{m}$ and an alveolar xenon gas diffusion coefficient of $< \sim 0.046 \text{ cm}^2/\text{s}$. In other words, healthy never-smokers have thin septal walls and small alveolar sizes apparent in the geometric restrictions imposed on the free gas diffusion. An age-correction of the septal wall thickness to the mean subject age of 63 further tightened the standard deviation of the wall thickness distribution from 11% to 7% in healthy subjects. Only two subjects in the never-smoker cohort exhibited an age-corrected septal walls thickness above $9.5 \mu\text{m}$. One of these two was also the only never smoker with a gas diffusion coefficient above $0.046 \text{ cm}^2/\text{s}$. It is probably no coincidence that at 79 years of age these two subjects were also by far the two oldest members of the cohort (all other non-smokers were younger than 65 years). As mentioned above, the aging lung is characterized by a thickening of the alveolar walls and an overall enlargement of the size of the airspaces (47, 48). The lower right-hand quadrant of the diffusion – wall thickness plot is almost entirely populated by subjects of the current and former smoker cohort. This observation can be interpreted as the alveolar size being normal within the cohort, i.e. a process of emphysematous lung tissue destruction has not begun at a sufficiently large scale to be apparent in the global gas-phase diffusion measurement. In only two members of the cohort a diffusion coefficient above $0.046 \text{ cm}^2/\text{s}$ was found. However, except for one subject, all members of the current and former smoker cohort had a functional septal wall thickness exceeding $9.5 \mu\text{m}$. In the absence of a relaxation of the wall tension due to tissue destruction, one possible explanation is a swelling of the septal walls from inflammatory processes or a permanent remodeling involving the synthesis of additional connective tissue (49). Of equal importance for the sensitivity of this measurement is the very narrow zone of overlap in the septal wall thickness of never smokers and current or former smokers. This strong discriminatory characteristic of the age-corrected septal wall thickness is further confirmed by the ROC analysis for the two cohorts, which yielded an AUC of 0.9.

Lastly, the subjects in the smoking-induced COPD cohort appear concentrated in the upper right quadrant with an age-corrected septal wall thickness above $9.5 \mu\text{m}$ as well as an alveolar diffusion coefficient above $0.046 \text{ cm}^2/\text{s}$. Also, while only one healthy smoker and none of the healthy control subjects had a wall thickness of more than $11.5 \mu\text{m}$, 56% of the COPD cohort exhibited thicker septal walls with a maximum measured value of $16.7 \mu\text{m}$. This finding implies that the observed wall thickening is not just an acute response to cigarette smoke, but worsens further with the onset of emphysematous changes. Only 2 out of 16 COPD subjects exhibited a diffusion coefficient below $0.046 \text{ cm}^2/\text{s}$ and only one had a wall thickness below $9.5 \mu\text{m}$, possibly indicative of simultaneous parenchymal inflammation and emphysematous lung tissue destruction in almost the entire cohort, in agreement with morphometric findings by Vlahovic et al (49).

Another notable result of our study was that we did not identify any subjects with an elevated gas-phase diffusion coefficient but a septal wall thickness comparable to the one found in healthy subjects. In other words, the upper left-hand quadrant of the diffusion –

wall thickness plot is empty. The most plausible explanation for this observation is that we detected no emphysematous lung tissue destruction without simultaneous increases in septal wall thickness most likely related to inflammation (50).

The measurement of the functional septal wall thickness by CSSR MR spectroscopy adds another dimension to the quantification of lung function parameters that could extend hyperpolarized gas MRI to the characterization of pre-clinical stages of lung disease as can be found, for instance, in clinically healthy smokers. The capability of septal wall thickness measurements to delineate smokers from age-matched healthy control subjects is particularly impressive considering that the smokers cohort in our study is a considerably healthier subpopulation of smokers than the one evaluated in the Regan study (2) since in our smokers cohort not only spirometry was normal but also 6 MWT and DLCO. It has already been demonstrated in the literature that the onset of emphysematous lung tissue destruction is associated with a loss of peripheral airways (51) and a decrease of regional perfusion (52). Additional studies will have to show whether a thickening of the functional septal wall precedes these other observed changes, occurs as a result of these changes, or is reflective of a different disease phenotype altogether.

Another advantage of functional septal wall thickness measurements, in addition to their sensitivity, is that the wall thickness has been shown to be fairly insensitive to the inflation level of the lung (23). Thus, although all subjects were instructed to hold their breath at TLC, the measured wall thickness value, unlike for spirometric measures, is not highly effort dependent. Further, the theoretical model describing the xenon gas uptake by the lung parenchyma to which the measurement data was fitted takes the effect of gas transport due to perfusion into account. Hence, a more rapid xenon accumulation as the result of reduced perfusion (52) will not introduce an unaccounted bias to the wall thickness quantification. Although the functional septal wall thickness evaluated in our study was a global metric for the whole lung, efforts are under way to incorporate at least coarse spatial information into the wall thickness measurement (36, 53, 54). In particular for strongly heterogeneous disease patterns such additional information would further improve the sensitivity of the technique by reducing partial volume effects.

As mentioned previously, the functional septal wall thickness quantified in this study is related to, but not the same as, the structural septal wall thickness measured by histology. While it is quite possible that the observed increase in functional septal wall thickness in both healthy smokers and COPD patients is at least in part associated with a structural wall thickening, other explanations also need to be taken into consideration. Because in CSSR measurements the functional wall thickness is inextricably tied to the structural wall thickness, the xenon solubility, and the xenon diffusion constant in lung tissue, pathological remodeling of the septal wall might affect the latter two parameters as well, thereby reducing the correlation between structural and functional wall thickness. Further, although oxygen and xenon diffusion from the alveolar volume to hemoglobin inside red blood cells is conceptually very similar, these two gases differ considerably in their physical and chemical properties. Hence, the implied reduction in xenon gas transfer due to an increased functional septal wall thickness might not necessarily affect the oxygen gas transfer in a proportional manner. Additional investigations will be required for validation.

In conclusion, our cross-sectional study suggests great potential of functional septal wall thickness measurements for detecting early stages of lung disease but future studies will have to show whether this technique can be used to more sensitively classify the risk for developing emphysematous lung tissue destruction. It might be of particular interest to perform longitudinal studies to investigate if healthy smokers with thicker septal walls are more likely to develop emphysema in the future.

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Figure 1.

Empirical distributions of conventional lung function measures. If any of the letters above the boxplots match between groups they did not differ at the $p < 0.05$ level after Bonferroni correction. For subjects to be included in the study, all spirometric functions (FEF 25–75%, FRC, RV, TLC, FVC, FEV₁ %pred, and FEV₁/FVC %pred) for the AMN and HS study-groups had to be in the normal range. No statistically significant differences in DLCO and 6MWT were observed between the AMN and HS groups, either. Statistically significant differences for FEF 25–75%, FEV₁ %pred, FEV₁/FVC %pred, and DLCO in the COPD cohort relative to those values in the AMN and HS groups were observed.

Figure 2.

Function alveolar septal wall thickness as assessed by CSSR spectroscopy in healthy never smokers plotted as a function of age. The septal wall thickness shows a good linear correlation with the age of subject and increases by approximately 0.04 μm per year of age.

Figure 3.

Empirical distribution of global measures of septal wall thickness, age-corrected septal wall thickness and apparent gas-phase diffusion coefficient for the three study cohorts. Correcting the measured septal wall thickness for the age of the subjects reduces the variability in the AMN cohort and further increases the statistical significance of the difference between the AMN and HS cohorts. The COPD cohort is characterized by a large increase in gas-phase diffusion relative to the AMN and HS cohorts, most likely due to emphysematous lung tissue destruction. If any of the letters above the boxplots match between groups they did not differ at the $p < 0.05$ level after Bonferroni correction.

Figure 4.

Plot of the apparent gas-phase diffusion constant as a function of the measured (A) and age-corrected (B) septal wall thickness. By subdividing the graph at a diffusion coefficient of 0.046 cm²/s and a septal wall thickness of 9.5 μ m the three study cohorts appear clustered in the lower left (AMN), lower right (HS) and upper right (COPD) quadrants. The fourth quadrant (upper left) is empty.

Figure 5.

Plot of DLCO Dsb as a function of the measured (A) and age-corrected (B) septal wall thickness. Although there appears to exist a reasonable strong negative correlation between the septal wall thickness and DLCO ($R^2 = 0.56$) this correlation is mainly driven by the COPD subjects. If only the HS and AMN cohorts are considered the correlation disappears and the septal wall thickness alone is discriminative for the two cohorts.

Table 1.

Between study-group comparisons of Septal Wall Thickness, AC-63 Septal Wall Thickness and Apparent Diffusion Coefficient (ADC).

HXe MRI Derived Parameter	AMN mean [95% CI]	HS mean [95% CI]	COPD mean [95% CI]	P-value Ho: All Means Equal
Septal Wall Thickness [μm]	8.7 [8.0, 9.4] ^{a (a)}	10.5 [9.4, 11.6] ^{b (b)}	12.4 [11.2, 13.7] ^{c (b)}	<0.001
AC-63 Septal Wall Thickness [μm]	8.8 [8.3, 9.3] ^{a (a)}	10.6 [9.5, 11.7] ^{b (b)}	12.3 [11.1, 13.6] ^{c (b)}	<0.001
Apparent Diffusing Coefficient (cm ² /s)	0.04 [0.04, 0.05] ^{a (a)}	0.04 [0.04, 0.05] ^{a (a)}	0.06 [0.05, 0.07] ^{b (b)}	<0.001

For the analysis of variances (ANOVA) in which the null hypothesis that all study-group means are equal is rejected at the 0.05 significance level, pairwise tests were conducted. Study-group means with same superscript outside of parenthesis signify the means did not differ at the two-sided $p < 0.05$ significant level, while study-group means with the same superscript inside of parenthesis signify that the mean did not differ at the Bonferroni multiple comparison corrected two-sided $p < 0.05$ significant level.

Table 2.

Logistic regression odds ratios and receiver operator characteristics (ROC) for predicting AMN and HS individuals based on lung function characteristics.

Logistic Regression Odds Ratio Summary				Receiver Operator Characteristics (ROC)					
Predictor	Predictor Ratio 3 rd Quartile : 1 st	Odds Ratio [95% CI]	Pvalue	ROC AUC [95% CI]	Predictor Threshold	Sensitivity [95% CI]	Specificity [95% CI]	PPV [95% CI]	NPV [95% CI]
VC [L]	4.2 : 3.2	0.29 [0.07, 1.25]	0.097	0.76 [0.52, 1.00]	3.85	88.9 [55.6, 100]	70.0 [40.0, 100]	72.7 [58.3, 100]	87.5 [66.7, 100]
FRC [L]	3.8: 2.6	0.78 [0.22, 2.87]	0.725	0.58 [0.50, 0.87]	2.95	66.7 [22.2, 100]	70.0 [40.0, 100]	66.7 [50.0, 100]	70.0 [55.6, 100]
RV [L]	2.4: 1.7	1.49 [0.60, 2.69]	0.384	0.52 [0.50, 0.81]	1.99	66.7 [11.1, 100]	60.0 [40.0, 100]	60.0 [50.0, 100]	66.7 [55.6, 88.9]
TLC [L]	6.9: 5.0	0.60 [0.14, 2.51]	0.481	0.63 [0.50, 0.92]	6.12	77.8 [33.3, 100]	60.0 [40.0, 100]	63.6 [53.9, 100]	75.0 [58.3, 100]
FVC [L]	3.9: 3.0	0.36 [0.09, 1.38]	0.137	0.74 [0.50, 0.99]	3.19	77.8 [55.6, 100]	80.0 [40.0, 100]	77.8 [57.1, 100]	80.0 [66.7, 100]
FEV1 pred [%]	2.9: 2.2	0.42 [0.12, 1.46]	0.172	0.71 [0.50, 0.96]	2.66	77.8 [33.3, 100]	60.0 [30.0, 100]	63.6 [53.9, 100]	75.0 [60.0, 100]
FEV1/FVC pred [%]	96: 91	0.99 [0.36, 2.69]	0.978	0.57 [0.50, 0.84]	97.00	33.3 [11.1, 100]	90.0 [30.0, 100]	75.0 [50.0, 100]	60.0 [55.6, 100]
DLCO Dsb [ml/min/mm Hg]	24.3: 18.5	0.75 [0.25, 2.25]	0.607	0.56 [0.50, 0.83]	21.19	77.7 [22.2, 100]	50.0 [20.0, 100]	58.3 [50.0, 100]	71.5 [55.6, 100]
6MWT [steps]	486: 400	1.13 [0.32, 4.04]	0.084	0.77 [0.55, 0.99]	486	100 [33.3, 100]	50.0 [40.0, 100]	64.3 [60.0, 100]	100 [62.5, 100]
Septal Wall Thickness [µm]	10.4: 8.5	15.38 [1.35, 174.24]	0.027	0.86 [0.69, 1.00]	9.25	88.9 [55.6, 88.9]	80.0 [50.0, 80.0]	80.0 [64.3, 81.8]	88.9 [71.4, 90.9]
AC-63 Septal Wall Thickness [µm]	10.2: 8.6	30.34 [1.47, 629.33]	0.027	0.90 [0.75, 1.00]	9.53	88.9 [55.6, 88.9]	80.0 [69.7, 90.0]	80.0 [69.2, 90.0]	88.9 [71.4, 90.0]
ADC [cm ² /s]	0.045:0.039	1.13 [0.32, 4.04]	0.848	0.59 [0.50, 0.87]	0.04	77.8 [22.2, 100]	50.0 [30.0, 100]	58.3 [50.0, 100]	71.4 [55.6, 100]

[†]The predictor variable threshold for classifying individuals as AMD (predictor variable < threshold) or HS (predictor variable > cut point) was determined by identifying the threshold value that minimized the false classification error rate.