

Airspace Dimension Assessment with Nanoparticles (AiDA) in Comparison to Established Pulmonary Function Tests

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Background: Airspace Dimensions Assessment with nanoparticles (AiDA) is a new method for non-invasive measurement of pulmonary distal airspaces. The aim of this study was to compare AiDA measurements with other pulmonary function variables to better understand the potential of AiDA in a clinical context.

Methods: AiDA measurements and pulmonary function tests were performed in 695 subjects as part of the Swedish CARDIOpulmonary bioImage Study. The measurement protocol included spirometry, measurement of diffusing capacity of carbon monoxide, oscillometry and pulmonary computed tomography. AiDA indices were compared to all other pulmonary examination measurements using multivariate statistical analysis.

Results: Our results show that AiDA measurements were significantly correlated with other pulmonary function examination indices, although covariance was low. We found that AiDA variables explained variance in the data that other lung function variables only influenced to a minor extent.

Conclusion: We conclude that the AiDA method provides information about the lung that is inaccessible with more conventional lung function techniques.

Keywords: aerosol nanoparticles, respiratory diagnostics with nanoparticles, distal airspaces, COPD diagnostics, airspace dimension assessment with nanoparticles

Plain Language Summary

There is a need for methods to measure changes deep down in the lung to detect lung disease at an early stage. However, the techniques available for these types of measurements are either too expensive, inaccessible or require ionizing radiation. In this study, we explore the potential of a new method called airspace dimension assessment with nanoparticles (AiDA) for the measurement of airspace dimensions in the deep lung. With AiDA, subjects inhale a small number of aerosolized nanoparticles and the number of particles that are deposited deep down in the lungs is measured with high precision. With knowledge of how inhaled nanoparticles behave in the lung, we derive the size of airspaces in the deep lung.

Our study shows that the AiDA measurements stand out when compared to more conventional pulmonary function measurements. Our results are important since they underline that the AiDA method can become an important tool for the detection of lung diseases in the deep lung.

Introduction

Chronic respiratory diseases are among the leading causes of death worldwide, affecting more than 500 million people.¹ There are no cures for chronic respiratory diseases, but pharmacological and physical treatment can help relieve symptoms, slow down the progression^{2,3} and decrease the risk of premature death.⁴ It is important to identify early signs of disease, which can facilitate measures to prevent the disease from progressing.

There is a discrepancy between the clinically evident form of chronic respiratory diseases and their early pathological manifestations in the lung distal to the terminal bronchioles. It is hard to detect abnormalities and changes in the most distal lung, and therefore the first signs of disease can be missed or misinterpreted. Diseases such as chronic obstructive pulmonary disease (COPD) and asthma entail damage to the distal lung. Tissue damage can modify distal airspace sizes, lower the surface area available for gas exchange, and reduce lung function. When the disease progresses slowly, with mild or absent initial symptoms, diagnosis is often delayed.^{5,6} Therefore, early detection of pathological changes to distal airspaces is key for early diagnosis of chronic respiratory diseases.

There are several techniques available for the assessment of lung disease, but non-invasive and cost-effective methods sensitive to the distal airspaces are lacking. The primary diagnostic tool used for the assessment of the distal lung is dynamic spirometry. The GOLD criterion to detect airflow obstruction is to study the ratio between forced expiratory volume in one second (FEV₁) and vital capacity (VC). Pulmonary function tests (PFTs) also include measurement of diffusing capacity of carbon monoxide (D_{LCO}). While both spirometry and D_{LCO} are accessible, spirometry primarily assess airflows in the upper airways and often fails to detect early changes in the distal lung.⁷ D_{LCO} has been found to correlate with both microscopic and macroscopic measures of the distal lung,^{8,9} but is sensitive to factors such as hemoglobin and carbon monoxide saturation of the blood, as well as recent smoking.¹⁰ Pulmonary computed tomography (CT) offers detailed information about anatomical structures, with for instance quantitative analysis of lung volume and lung density. However, CT requires ionizing radiation and is expensive, and evaluation of the generated images is interpretation- and software-dependent.¹¹ Oscillometry (also known as forced oscillation technique, FOT),¹² measures respiratory mechanics, ie pulmonary Resistance (R_{rs}) and Reactance (X_{rs}),¹³ by the application of oscillating pressure signals. While R_{rs} is presumably related to airway calibre, X_{rs} is related to elastance and inertance, reflecting lung heterogeneity. An association between X_{rs} and FEV₁ in COPD patients has been found,¹⁴ and self-reported chronic respiratory diseases have been associated with high pulmonary R_{rs} and low pulmonary X_{rs}.¹⁵ While the data indicate that oscillometry can be sensitive to airflow obstruction, it cannot discriminate between obstructive and restrictive lung disorders.¹⁶ Magnetic Resonance Imaging (MRI) can be used for detection of changes in the distal lung,^{17,18} but the technique is far too advanced and expensive to be employed in standard lung function testing. Therefore, cost-effective diagnosis techniques sensitive to early and small signs of damage or changes to the distal airspaces are needed.

Airspace Dimensions Assessment with nanoparticles (AiDA) is a novel method developed for quantification of distal airspaces by measurement of the deposition of inhaled nanoparticles.^{19,20} The method relies on the fact that inhaled nanoparticles reach the distal airspaces, where some of them deposit by diffusion, while the rest are exhaled. With AiDA the recovery of particles in exhaled air is measured with high accuracy.²¹ Analysis of AiDA data yields two variables: (1) a root-mean-square (rms) measure of the dimension of a collection of airspaces, referred to as the effective airspace radius, r_{AiDA} , and (2) the extrapolated recovery at zero-second breath-hold, R_0 . While r_{AiDA} reflects distal airspace dimensions, R_0 is presumably related to particle losses during the dynamic process of breathing^{19,21,22} and lung heterogeneity.²³

We have previously found that r_{AiDA} agrees with distal airspace dimensions, and R_0 correlates with lung heterogeneity, as quantified by MRI with inhaled hyperpolarized gas.²³ We have shown that AiDA measurements can detect early emphysema as evaluated with pulmonary CT,^{24,25} and that AiDA data correlate with lung density as measured with standard pulmonary MRI.²⁶ Based on these observations, we believe that AiDA has the potential to improve diagnosis of disease in the distal lung. AiDA is a cost-effective, non-invasive, and easy-to-use method, without radiation, that can be used stand-alone or combined with other pulmonary examination techniques.

The aim of this study is to explore the AiDA variables, r_{AiDA} and R_0 , by comparing them to other pulmonary function measures. The measurements include spirometry, D_{LCO}, CT and oscillometry measurements for a large group of subjects.

This is the first study that aims to understand the relationship between R_0 and other standard lung function variables. Measurements were performed in a population-based group from the Swedish CARDioPulmonary bioImage Study (SCAPIS). Parts of the AiDA data were published²⁴ when we investigated the potential of AiDA as a biomarker for emphysema from the difference between r_{AiDA} values in CT-justified emphysematous and non-emphysematous subjects. In the present study, we elaborate further and more broadly on the potential of AiDA in a clinical setting based on multivariate statistical analyses between AiDA variables and standardized lung function measurements. Here, we also introduce and discuss results on the AiDA extrapolated recovery at zero-second breath-hold index, R_0 , which have never been published before.

Materials and Methods

Study Population

The study was performed with a subset of participants in the multicenter population-based national CARDioPulmonary bioImage Study (SCAPIS),²⁷ which is a collaboration between six Swedish universities (Gothenburg, Linköping, Malmö/Lund, Stockholm, Umeå and Uppsala). SCAPIS is initiated to identify risk factors of cardiovascular diseases and COPD, with enrolment of men and women aged 50–64 years from the Swedish population register. Enrolment took place by random selection. No exclusion criteria were applied except for the inability to understand written and spoken Swedish for informed consent. Of the 6251 participants recruited in Malmö, 744 participants were examined with AiDA. All participants answered a questionnaire (including questions about life-style, such as smoking habits) and took part in extensive physical examinations, lung function testing and imaging.²⁷ Measurements were performed from 2014 to 2016. All participants gave written and informed consent. SCAPIS was approved by the Regional Ethical Review Board in Umeå (Dnr 2010–228-31M), Lund (2016/1031) and Linköping (2018/478-31) and complied with the Declaration of Helsinki.

AiDA Measurements

The AiDA setup used for particle deposition measurements has been described in detail elsewhere.²¹ Figure 1A shows a schematic illustration of the setup used to measure inhaled and exhaled aerosol particle concentrations. The nanoparticle aerosol was generated by aerosolizing 50 nm polystyrene latex nanospheres (PSL) using an electrospray aerosol

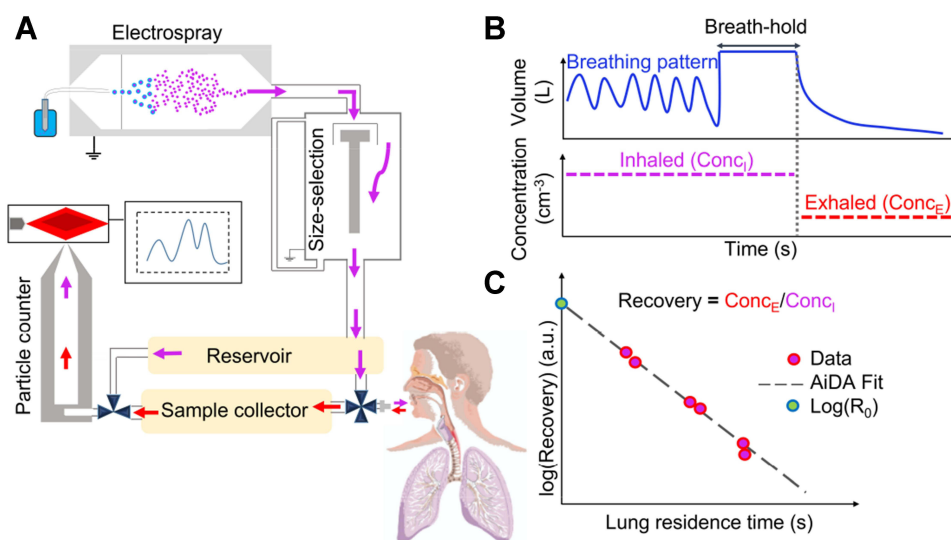


Figure 1 Schematic diagram of the AiDA setup, example output data and nanoparticle recovery as a function of lung residence time. **(A)** Nanoparticles were electrospray-generated, size-selected and transferred to the reservoir from which the particle concentration was measured. The inhaled and exhaled nanoparticle concentrations were quantified with the same CPC. **(B)** Diagrams of a recorded breathing pattern and measured particle concentrations. The breathing-pattern and the inhaled and exhaled particle concentrations were analyzed to determine particle residence time in the lungs and the fraction of recovered particles after exhalation at each residence time. **(C)** A linear-least squares line was fitted to the recovery as a function of lung residence time, from which r_{AiDA} and R_0 were determined.

generator (EAG) (Model 3480, TSI Inc., Shoreview, MN). A monodisperse aerosol was obtained by size-selecting the 50 nm particles with a differential mobility analyzer (DMA, Model 3071, TSI GmbH, Aachen, Germany). The aerosol was diluted with particle free air in a flexible antistatic reservoir. The inhalation system included a computer-controlled four-way valve, which directed the flow of aerosol to the mouthpiece and the sampling of exhaled aerosol into the sample collector, consisting of a 1-m-long metal tube with about 35 mm inner diameter. Inhaled and exhaled nanoparticle number concentrations were determined by a condensation particle counter (CPC), and particle losses in the system were characterized.

Precise measurement of monodispersed nanoparticle recovery from a predetermined lung depth of 1300 mL was measured for three different breath-hold times: 5, 7 and 10 seconds. The inhaled nanoparticle concentrations ranged between 1000 and 6000 cm⁻³, with an average inhaled concentration of 4600 cm⁻³. Nanoparticle residence time in the lungs was established from the breathing pattern and the fraction of recovered particles was determined from the inhaled and exhaled particle concentrations (see Figure 1B). The recovery of particles from the 1300 mL lung depth ranged between 0.7% and 65%, with a median value of 15% for all participants, depending on breath-hold. A linear least-squares regression line was fitted to the recovery as a function of residence time (see Figure 1C). R_0 was estimated from extrapolation of the fit to zero-second residence time (green marker in Figure 1C) and r_{AiDA} was established from the nanoparticle diffusion coefficient (D) and the half-life time ($t_{1/2}$) of the inhaled nanoparticles (available from the slope of the fit), according to

$$r_{AiDA} = 2.89 \sqrt{Dt_{1/2}},$$

as previously described.²² AiDA measurements depended, to some extent, on the skills of the testing personnel, just like standard PFT, CT evaluation and oscillometry measurements. To account for this, two measurements were performed for each breath-hold time.

For the 744 participants, measurements for 728 (346 M, 382 F) passed data quality checks such that the participant managed to do at least four AiDA breathing maneuvers, with no instrumental errors (eg leakages, incorrect measurement and quantification) detected and the inhaled particle concentration was stable ($\pm 10\%$), and high enough (>1000 cm⁻³). For measurements passing data quality checks, data for 695 (328 M, 367 F) participants had a coefficient of determination value (r^2) for the AiDA fit (Figure 1C) higher than 0.8, which was considered a good enough fit.

Pulmonary Function Testing

All pulmonary function measurements were performed in accordance with the American Thoracic Society (ATS) and European Respiratory Society (ERS) standards.^{28,29} Spirometry (Jaeger MasterScreen PFT, CareFusion, Hoechberg, Germany) was performed 15 minutes after bronchodilation using salbutamol with subjects in sitting position and wearing a nose clip. FEV₁, slow vital capacity (SVC) and forced vital capacity (FVC) were obtained. D_{LCO} was measured using a single-breath carbon monoxide diffusion test (Jaeger MasterScreen PFT). Oscillometry measurements were performed with an impulse oscillometry system (Jaeger MasterScreen IOS) according to Oostveen et al,¹⁶ with the exception that only one measurement was performed, which has been shown to be enough in studies with a large number of participants.³⁰ Resistance at 5 Hz (R_5) and 20 Hz (R_{20}), reactance at 5 Hz (X_5), the area of reactance (AX) and the resonant frequency (f_{res}) were measured. The difference in resistance for 5 and 20 Hz was calculated (R_5-R_{20}).

CT Examination

Pulmonary CT was performed using a protocol with low radiation dose³¹ and interpreted by one of the four radiologists. The measurements were obtained according to the European Respiratory Society (ERS) standards.²⁸ CT-derived total lung capacity by volumetric CT (VOL) was calculated, along with quantification of the 15th percentile density (PD₁₅), defined as the value (in Hounsfield Units) below which 15% of voxels with the lowest density are distributed. The percentage of low attenuation volume (LAV), corresponding to a density measurement of the lung parenchyma was determined.

Statistical Analysis

AiDA variables were compared to spirometry values VC, FEV₁, and their ratio (FEV₁/VC), D_{LCO}, IOS variables R₅, R₅-R₂₀, X₅, AX, f_{res} and CT variables Vol, LAV and PD₁₅. The relationship between AiDA variables and all other measures were explored with correlation analysis, linear regression with adjustment to age and height, and principal components analysis. A P-value <0.05 was considered significant. Calculations were performed using MATLAB Version 2020a (The MathWorks, Inc., Natick, Massachusetts, United States) and Python 3.6.12 (Python Software Foundation).

Results

AiDA measurements, anthropometric data and lung function examination results are shown in Table 1, for the study population of 695 participants that passed all data quality checks. The distal airspace radius, r_{AiDA} , and zero-seconds recovery, R_0 , were within limits of previously measured values.^{23,24,26} About half of all the participants, 359 participants (52%), reported pack-years (1 pack-years = 20 cigarettes smoked per day for one year), above zero. A low number of the participants, 87 individuals (12.5%) had FEV₁/VC <0.7, which is the GOLD criteria for airway obstruction and COPD. Within the study population, 56 individuals (8%) had mild-to-moderate emphysema as visually detected with CT, which was further investigated in another study.²⁴ Table A1 in the Supplement presents subject demographics and lung examination results for non-smokers (Packyears = 0), smokers (Packyears >0), men and women, respectively. These data show that some indices vary significantly between groups, for instance r_{AiDA} is

Table 1 Subject Demographics, Anthropometric Data, AiDA Measurements and Other Lung Function Examination Results. Mean, Median, Standard Deviations and Inter Quartile Range (the Difference Between Quartile 3 and Quartile 1), for the Study Population are Presented

Characteristics	Mean (SD)	Median (IQR)	Min	Max
Anthropometry				
Age (years)	57.4 (4.5)	57.3 (8.0)	50	65
Height (cm)	172 (10)	171 (15)	146	199
Weight (kg)	81 (16)	79 (22)	43	147
Waist (cm)	96 (14)	96 (19)	64	142
M/F	328/367	-	-	-
Pack-years (a.u.)	10 (13)	6 (17)	0	86
AiDA				
r_{AiDA} (μm)	288 (41)	282 (48)	200	582
R_0 (a.u.)	0.50 (0.21)	0.49 (0.30)	0.05	1
Pulmonary function tests				
FEV ₁ (L)	3.15 (0.76)	3.05 (1.11)	1.00	5.35
VC (L)	4.00 (0.94)	3.91 (1.42)	2.00	7.32
D _{LCO} (mmol L ⁻¹ s ⁻¹)	8.12 (1.66)	8.00 (2.17)	2.19	14.30
FEV ₁ /VC	0.78 (0.08)	0.79 (0.09)	0.28	0.94
Forced oscillation technique				
R ₅ (kPa L ⁻¹ s)	0.34 (0.09)	0.33 (0.12)	0.15	0.73
R ₅ -R ₂₀ (kPa L ⁻¹ s)	0.040 (0.045)	0.040 (0.040)	-0.36	0.35
X ₅ (kPa L ⁻¹ s)	-0.09 (0.044)	-0.09 (0.05)	-0.42	0.06
AX (kPa L ⁻¹)	0.27 (0.29)	0.18 (0.22)	-0.02	3.42
f_{res} (Hz)	10.85 (3.18)	9.86 (4.16)	5.94	24.84
Computed tomography				
Vol (L)	5.42 (1.28)	5.27 (1.74)	0.79	10.11
LAV (%)	5.85 (4.12)	5.21 (5.1)	0.10	25.40
PD ₁₅ (HU)	-919 (22)	-922 (26)	-969	-722

Abbreviations: IQR, inter-quartile range; FEV₁, forced expiratory flow in one second; VC, vital capacity; D_{LCO}, diffusing capacity for carbon monoxide; R₅, resistance at 5 Hz; R₂₀, resistance at 20 Hz; X₅, reactance at 5 Hz; AX, area of reactance; f_{res} , resonance frequency; Vol, CT-derived lung volume; LAV, low attenuation volume; PD₁₅, 15th percentile density point.

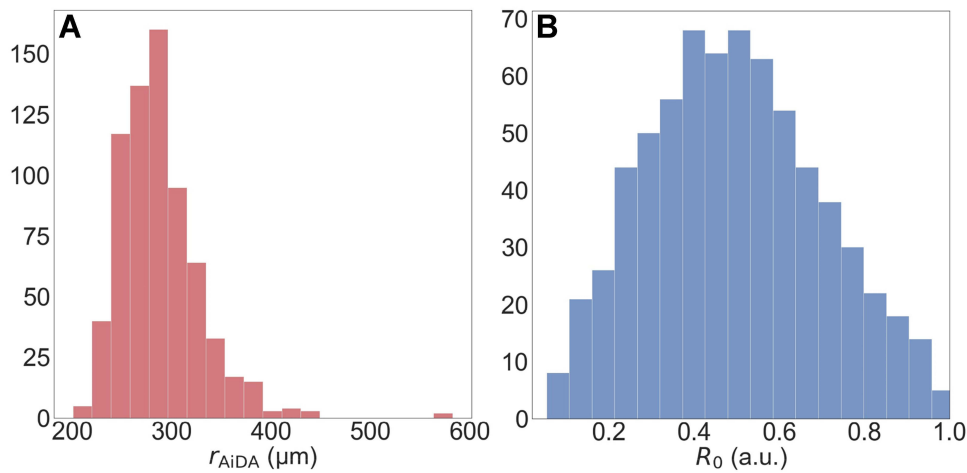


Figure 2 The r_{AiDA} and R_0 distributions for the 695 participants. **(A)** The r_{AiDA} distribution is skewed, largely due to the outliers at $r_{AiDA} > 400$. Note that one participant had an r_{AiDA} value close to 600 μm . **(B)** The R_0 distribution approached a normal distribution with a mean recovery of 50% at an imaginary zero seconds breath-hold.

slightly higher for men than for women ($P < 0.01$) and higher in smokers than non-smokers ($P < 0.05$). However, [Table A1](#) shows that the study population was rather homogeneous. [Figure 2](#) displays the r_{AiDA} and R_0 distributions for the whole study population. According to the Kolmogorov–Smirnov normality test, both the r_{AiDA} and R_0 distributions deviated from normal distributions. However, the R_0 distribution approached a normal distribution in [Figure 2](#), which was also seen when studying the norm–norm plot produced for R_0 , and the small difference between its mean and median values.

[Table 2](#) displays Spearman’s rank correlation coefficients for AiDA variables, the anthropometric data, age, and pack-years. The airspace radius, r_{AiDA} , was significantly positively associated with age, height, and pack-years, while the zero-second recovery index, R_0 , was associated with weight, height and pack-years, but correlation coefficients were low.

Table 2 Spearman’s Rank Coefficient (ρ) with Associated P-values, for AiDA Variables r_{AiDA} and R_0 , with Age, Weight, Height, Waist and Pack-Years (Left). Simple (Unadjusted) Linear Regression of r_{AiDA} and R_0 on Age, Weight, Height, Waist and Pack-Years (Right). For the Regression, β -Coefficients, Standard Errors (SE), 95% Confidence Intervals (CI) and P-values are Given. The Level of Significance is Indicated with Asterisk According to $P < 0.0001$ (****), $0.0001 \leq P < 0.001$ (***), $0.001 \leq P < 0.01$ (**) and $P < 0.05$ (*)

	Spearman Correlation		Linear Regression		
r_{AiDA} (μm)					
	ρ	P-value	β -Coefficient	SE	P-value
Age (years)	0.13	**	0.93	0.350	**
Weight (kg)	0.00	0.93	−0.04	0.095	0.64
Height (cm)	0.17	****	0.46	0.163	**
Waist (cm)	0.00	0.91	−0.02	0.117	0.86
Pack-years (a.u.)	0.13	**	0.53	0.116	****
R_0 (a.u.)					
	ρ	P	β -Coefficient	SE	P-value
Age (years)	0.01	0.86	0.0012	0.002	0.52
Weight (kg)	0.09	*	0.0009	0.000	0.05
Height (cm)	0.19	****	0.0044	0.001	****
Waist (cm)	0.03	0.52	0.0005	0.001	0.42
Pack-years (a.u.)	−0.17	****	−0.0023	0.001	****

Table 3 demonstrates correlations between AiDA variables, r_{AiDA} and R_0 , and all other lung function indices by Spearman's rank coefficient (ρ) and linear regression models with associated P-values. The linear regression has been adjusted for age and height. The AiDA variables were significantly correlated with several lung function measurements, but all correlations were associated with low correlation coefficients, ie lower than |0.3|. The overall strongest associations were seen between r_{AiDA} and the CT LAV measure ($\rho = 0.22$, $P < 0.0001$) and between R_0 and FEV_1 ($\rho = 0.3$, $P < 0.0001$). AiDA variables were found to be significantly ($P < 0.01$) dependent on sex, with men prone to have slightly higher r_{AiDA} and R_0 compared to women (see Table A1 in the supplemental material), as assessed with Student's t -test. However, the differences based on sex were numerically minimal (mean r_{AiDA} of $290 \pm 39 \mu\text{m}$, mean R_0 of 0.52 ± 0.19 for men, and mean r_{AiDA} of $284 \pm 42 \mu\text{m}$, R_0 of 0.47 ± 0.21 for women), and were therefore not adjusted for in the linear regression analyses performed.

Table 3 Spearman's Rank Coefficient (ρ) with Associated P-values, Between AiDA Variables r_{AiDA} and R_0 , and Lung Function Measurements (Left), with No Adjustment for Confounders. Coefficients, Standard Errors (SE), 95% Confidence Intervals (CI) and P-value for the Multiple Regression Model of r_{AiDA} and R_0 on VC, FEV_1/VC , D_{LCO} , R_5 , $R_5\text{-}R_{20}$, X_5 , AX, f_{res} , VOL, LAV and PD_{15} , Adjusted for Age and Height (Right). The Level of Significance is Indicated with Asterisk According to $P < 0.0001$ (***), $0.0001 \leq P < 0.001$ (**), $0.001 \leq P < 0.01$ (*) and $P < 0.05$ (*)

	Spearman Correlation		Linear Regression (Age- and Height-Adjusted)		
r_{AiDA} (μm)					
	ρ	P-value	β -Coefficient	SE	P-value
R_0 (a.u)	-0.009	0.81	-21.2	7.6	0.05
FEV ₁ (L)	0.11	*	-1.9	3.2	0.54
VC (L)	0.19	****	8.8	2.9	**
FEV ₁ /VC (a.u)	-0.15	****	-125.0	21.8	****
D _{LCO} (mmol L ⁻¹ s ⁻¹)	-0.04	0.34	-6.5	1.4	****
R ₅ (kPa L ⁻¹ s)	-0.09	*	3.8	19.2	0.85
R ₅ -R ₂₀ (kPa L ⁻¹ s)	-0.05	0.23	26.5	34.1	0.44
X ₅ (kPa L ⁻¹ s)	0.17	****	18.4	40.5	0.65
AX (kPa L ⁻¹)	-0.12	**	10.0	5.4	0.06
f_{res} (Hz)	-0.09	*	0.3	0.5	0.51
VOL (L)	0.2	****	0.0	0.01	**
LAV (%)	0.22	****	2.2	0.4	****
PD ₁₅ (HU)	-0.2	****	-0.2	0.1	**
R_0 (a.u)					
	ρ	P-value	β -Coefficient	SE	P-value
r_{AiDA} (μm)	-0.009	0.81	0.0005	0.0	0.05
FEV ₁ (L)	0.3	****	0.10	0.016	****
VC (L)	0.29	****	0.08	0.014	****
FEV ₁ /VC (a.u)	0.05	0.215	0.23	0.111	0.04
D _{LCO} (mmol L ⁻¹ s ⁻¹)	0.17	****	0.006	0.006	0.34
R ₅ (kPa L ⁻¹ s)	-0.2	****	-0.208	0.096	0.03
R ₅ -R ₂₀ (kPa L ⁻¹ s)	-0.21	****	-0.669	0.169	****
X ₅ (kPa L ⁻¹ s)	0.29	****	1.090	0.198	****
AX (kPa L ⁻¹)	-0.29	****	-0.121	0.027	****
f_{res} (Hz)	-0.26	****	-0.012	0.002	****
VOL (L)	0.26	****	3.226 10 ⁻⁵	8.12 10 ⁻⁶	****
LAV (%)	0.25	****	0.010	0.002	****
PD ₁₅ (HU)	-0.25	****	-0.002	0.000	****

Abbreviations: FEV_1 , forced expiratory flow in one second; VC, vital capacity; D_{LCO} , diffusing capacity for carbon monoxide; R_5 , resistance at 5 Hz; R_{20} , resistance at 20 Hz; X_5 , reactance at 5 Hz; AX, area of reactance; f_{res} , resonance frequency; Vol, CT-derived lung volume; LAV, low attenuation volume; PD_{15} , 15th percentile density point.

Principal components analysis (PCA) of the lung function data, including r_{AiDA} and R_0 variables, yielded the first five PCs to explain 83% of the variance in the data. The explained variance per PC is illustrated in Figure 3. Each PC is constructed as a linear combination of the original variables in the data, to maximize the variance explained for each PC. The first five PCs explained 39%, 20%, 10%, 8% and 6%, of the full data variance, respectively. The principal component coefficient loadings are shown as a heat map in Figure 4. The loadings indicate how much each lung function variable contributes (on a scale from -1 to 1) to each PC. Here, a more saturated color indicates a higher contribution to the respective PC. The heat map shows that PC1 was constructed mainly from spirometry measures (FEV_1 , VC), D_{LCO} , IOS variables (R_5 , R_{5-20} , X_5 , AX , f_{res}) and lung volume measured with CT (VOL). PC2 was constructed mainly from FEV_1/VC , R_5 - R_{20} , X_5 , AX , f_{res} , and CT indices VOL, LAV and PD_{15} . PC3 was mainly

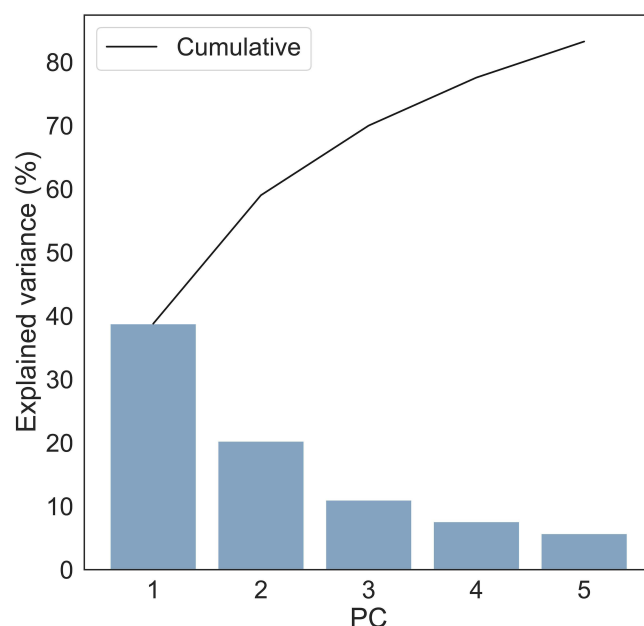


Figure 3 Explained variance per PC. The black line marks the cumulative explained variance while the bars show the explained variance per PC. Together the first five PCs explained 83% of the variance in the data, with each PC explaining 39%, 20%, 10%, 8% and 6% respectively, in descending order.

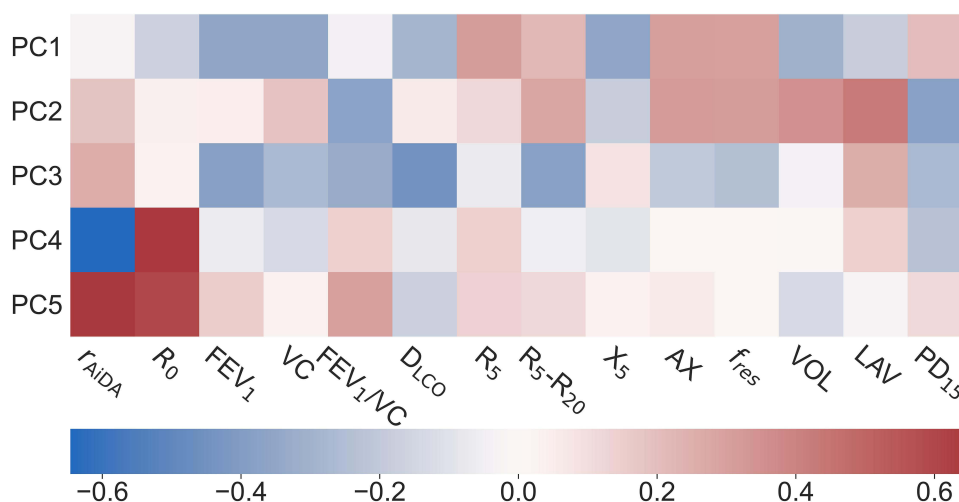


Figure 4 Heat map on the principal component factor loading for the first five PCs. The heatmap shows that the AiDA variables are not important components of the first two PCs, r_{AiDA} is important for PC3, and r_{AiDA} and R_0 are the dominating contributors to PC4 and PC5, with relatively low contributions from all other lung function variables. The variance in the data explained by PC4 and PC5 (14% of the variance in the data) is mainly explained by the AiDA variables.

constructed from FEV₁, D_{LCO} and R₅-R₂₀. It can be noted that the contributions from AiDA to the first three PCs were negligible, which indicates that the AiDA variables did not contribute much to the variance explained by these first three PCs. The main contributors to PC4 and PC5 were the AiDA variables (indicated by the darker colors in the heat map in Figure 4). All other variables contributed negligibly to PC4 and PC5.

Discussion

This study displays the lack of fundamentally strong relationships between AiDA and established lung function examinations in a generally healthy study population. The results show that the lung properties measured with AiDA, the effective airspace radius, r_{AiDA} , and the extrapolated recovery at zero-second breath-hold, R_0 , cannot be assessed with conventional pulmonary function tests.

The radius measured with AiDA, r_{AiDA} , is a root mean square (rms) estimate of the diffusional distance that inhaled nanoparticles traverse to reach the airspace walls. The r_{AiDA} values here measured ranged from 200 to 582 μm with a mean value of 288 μm . These estimated airspace dimensions correspond well with cast models predicting the inner diameter of distal airspaces to be between 270 and 500 μm , depending on airway generation, and an alveolar sac diameter of about 250 μm , independent of generation.^{32,33} These measures also agree with Weibel's lung model that has a diameter of 410 μm for the 23rd generation of the respiratory system, where the alveoli are located.³²

The association between r_{AiDA} and the other lung function measures shown in Table 3 were in line with expectations. A negative association was found for r_{AiDA} with FEV₁/VC. A low FEV₁/VC ratio is the GOLD criteria for diagnosis of COPD, which often includes some degree of emphysema. Since emphysema enlarges distal airspace, r_{AiDA} increases with decreasing FEV₁/VC. The strongest associations observed for r_{AiDA} were found for the CT indices. The effective airspace radius, r_{AiDA} , was found to be positively correlated with the lung volume measured with CT (VOL). A positive association was also found for r_{AiDA} and LAV. A high LAV means a larger volume with less dense lung tissue and hence larger airspaces and thus a higher r_{AiDA} . The association between LAV and r_{AiDA} indicates that AiDA is at least partly related to the region of the lung with low attenuation volume. A negative association between r_{AiDA} and PD₁₅ was found, which means that a higher 15th percentile density is associated with a lower r_{AiDA} value. This is logical, as a higher density means more lung tissue and smaller airspaces. According to Spearman's rank coefficient r_{AiDA} was associated with R₅, X₅, AX and f_{res} , but when assessing these relationships with linear regression with adjustment for age and height none of the relationships were significant.

The physiological information contained in R_0 still remains to be further investigated. R_0 presumably reflects a nanoparticle–lung interaction effect from the breathing phase of the AiDA measurements. Conceptually, R_0 corresponds to a measure of the recovery of aerosol particles from the deep lung at zero-seconds breath-hold. As such, R_0 is attributed to estimate the particle losses in the small conducting airways (<2 mm). Hence, R_0 is presumably related to small airways, but not to the most distal airspaces.

The relationships between R_0 and IOS indices show that R_0 is related to airway calibre and lung heterogeneity. According to the theory of oscillometry the resistance, R_{rs} reflect airway calibre, where narrower and longer airways have higher resistance due to greater frictional pressure loss in them. The reactance, X_{rs}, is related to stiffness of the entire respiratory system and to lung heterogeneity, governed by the product of resistance and compliance.¹³ When heterogeneity increases, the stiffness and hence X_{rs} decreases. The results underline the relationship already found between R_0 and lung heterogeneity, as assessed with MRI with hyperpolarized gas.²³

It cannot be assumed that all lung physiological variables correlate linearly with one another. With the analysis performed, it was our intention to show the general associations between variables. With Spearman's rank coefficient we studied the degree of association without assuming linearity, and linearity was explored, over all ranges for all variables, using linear regression. It is plausible that some of the variables were only partly linearly correlated, but this was not visible in the linear regression analysis here performed.

Since the r_{AiDA} distribution was skewed, as seen in Figure 2, we also log-transformed r_{AiDA} in the correlation and regression analysis for r_{AiDA} compared with other lung physiology indices. This resulted in similar associations as presented in Tables 2 and 3. However, the significance of the relationship of r_{AiDA} with PD₁₅ increased when r_{AiDA} was log-transformed.

The study population was randomly selected from the Swedish population register and most subjects were healthy, with only a few subjects with mild-to-moderate lung disease. We studied subjects with mild-to-moderate emphysema as diagnosed with CT in another publication.²⁴ The narrow age range (50–64 years) limits conclusions about the relationship between AiDA variables and possible age-dependencies. The effective airspace radius, r_{AiDA} , was very weakly associated with age ($P < 0.01$), height ($P < 0.01$) and pack-years ($P < 0.01$), both according to Spearman's rank coefficient and the linear regressions. For a variable that describes the size of distal airspaces these associations are expected. Airspace size is age-dependent,³⁴ lung volume depends on height, and pack-years are related to alterations of both bronchi and peripheral airspaces. However, for all correlations found, coefficients were low. R_0 was somewhat associated with height ($P < 0.0001$) and pack-years ($P < 0.0001$), which underlines the potential association between R_0 and lung properties.

In line with the results of the correlation analysis, the PCA showed that the AiDA variables did not explain the same variance in the data as the other lung function measurements. These results indicate that the AiDA method stands out among the lung function measurement techniques and that AiDA probes qualities of the deep lung that are not measurable by other means.

In a previous study,²³ we showed that r_{AiDA} quantified distal airspace dimensions and that r_{AiDA} corresponded well with distal lung dimensions assessed with hyperpolarized gas MRI. In the same study, we also observed that R_0 corresponded to the lung heterogeneity measured with that same technique. Since no close correspondence with other lung function parameters was found in the current study, we conclude that the method so far most closely related to AiDA is the more advanced hyperpolarized gas MRI method. Hyperpolarized gas MRI is, however, primarily available in research and only exists in few places around the world.

The measured AiDA variables are in accordance with those found in previous AiDA studies.^{23,24,26} Closely related methods include the technique referred to as Aerosol-Derived Airway Morphometry (ADAM).^{35,36} While certain versions of the ADAM method resemble AiDA, the main difference between the two techniques is that ADAM uses larger particles, typically 0.8–1 μm in diameter and that the ADAM method requires a controlled low breathing flow (typically about 250 mL/s). Therefore, in contrast to AiDA, where the particle deposition is dominated by diffusional deposition, for ADAM most particles presumably deposit by sedimentation. Due to the different physics of diffusion and sedimentation, the relationship between peripheral airspace dimensions and measured particle deposition differs. ADAM gives an effective airway diameter (EAD), which is related to r_{AiDA} , although deviating as it is a geometrical mean value. ADAM measurement of EAD presumably reflects larger airways, while r_{AiDA} is associated with more distal airspaces. Studies with ADAM have not reported on a measure corresponding to R_0 .³⁶

The data here presented indicate that AiDA has the potential to evaluate the occurrence of distal airspace abnormalities by measurement of r_{AiDA} . Since AiDA is non-invasive it could potentially be used with the purpose of facilitating early detection of distal airspace abnormalities, such as early emphysema.

The AiDA indices, r_{AiDA} and R_0 , provide data that complement more traditional lung function measurements, such as spirometry, lung volumes, specific conductance, and diffusing capacity. Therefore, AiDA could become an adjunct to spirometry, for detection of abnormalities in the distal lung which may be undetected by spirometry. AiDA allows the estimation of distal airspace dimensions with high accuracy and reproducibility^{19,21} and AiDA is important since more traditional lung function tests are not sensitive to detect early changes in distal airspaces. Early detection of abnormalities in the distal lung can prevent the occurrence of obstructive ventilator impairment if medical treatment, smoke cessation or other lifestyle changes can be implemented. While one measurement per breath-hold is enough to derive the recovery, since AiDA is still under development, two measurements are made for each breath-hold. The AiDA measurement maneuver is easy to perform and very similar to that of D_{LCO} measurements. AiDA is less exhausting for patients than spirometry but could still be challenging for someone with severe lung disease. Personnel working in pulmonary healthcare can be trained to perform these types of measurements within a few days.

One strength of this study is that the study group is population based. As such, the representative sample is largely healthy, and only has a low prevalence of serious lung diseases. On the downside, the population-based participants make it hard to draw conclusions on the potential of AiDA to identify and detect lung disease in this study, since specific AiDA values cannot clearly be paired with specific states of disease. In addition to AiDA, the study included an extensive

protocol of measurements, with spirometry, D_{LCO} , IOS and CT. However, in addition to the dynamic spirometry, it would have been ideal to also measure static lung volumes.

Due to the novelty of the AiDA method, we had several instrumental problems at the beginning of the study. The proportion of measurements not fulfilling the technical criteria, 49 out of 744 participants, can be explained by several factors. These include insufficient data quality checks at the time of measurements, the difficulty for some participants to perform the breathing maneuver and technical difficulties with the instrument. Technical errors that generated faulty measurement data include insufficient particle concentrations generated with the EAG, leakage through the nanoparticle reservoir and air entering the system through the mouthpiece.

For measurements of the distal airspaces, AiDA requires hydrophobic nanoparticles (diameters <100 nm). Diffusion is faster for smaller particles. Therefore, for particles with diameters <50 nm, a large fraction of the particles deposit in the upper airways. To avoid too large particle losses in the upper airways, 50 nm particles were chosen. The particles need to be hydrophobic, so they do not grow by condensation due to the high relative humidity close to 99.5% in the lungs.³⁷ Once a nanoparticle reaches an airspace wall, it deposits without bouncing.

The dose of nanoparticles delivered to the lung with AiDA is low. Most people are routinely exposed to much higher average nanoparticle concentrations in urban settings.^{38,39} With AiDA, subjects inhale a low predefined controlled aerosol nanoparticle concentration for a short time. Before and after the inhalation of nanoparticles, subjects breathe particle-free clean air to minimize the aerosol concentration in the lungs. Therefore, the total cumulative dose of aerosol nanoparticles delivered to lung tissue is lower with AiDA than for exposure for the same duration in a typical indoor office environment.

The polystyrene latex (PSL) nanoparticles used with AiDA are well characterized. PSL particles have been used extensively to study translocation, clearance, and ventilation in both human and animal lungs.^{40–45} These, and similar studies with other types of particles,^{46,47} show that the translocation of nanoparticles to other organs is very limited for particles in the range 40–100 nm. In-vitro studies with human lung cells exposed to PSL particles showed indistinguishable effects from controls.⁴⁸ Based on the low concentrations used in AiDA, we appraise the risks associated with exposure during AiDA measurements to be negligible.

Future research is needed to understand the diagnostic accuracy, sensitivity, and specificity of AiDA. AiDA measurements in groups with varying ages and varying lung function, including studies with patients who have been diagnosed with pulmonary diseases, are needed to further study the potential of AiDA as a diagnostic tool.

Conclusions

In this work, the AiDA method has been measured in a large national cohort. AiDA variables have been evaluated against other lung function metrics including spirometry, D_{LCO} , pulmonary CT and IOS. The primary aim of this study was to investigate the relationship between AiDA measures, r_{AiDA} and R_0 , and other pulmonary function tests. This study indicates that AiDA measurements are weakly associated with lung function variables derived from spirometry, D_{LCO} , IOS and pulmonary CT, but also with age and height. In conclusion, the study shows that, in contrast to conventional lung function techniques, AiDA assesses properties that are not accessible by conventional lung function measurements.

Abbreviations

AiDA, Airspace Dimension Assessment with nanoparticles; r_{AiDA} , effective airspace radius; R_0 , zero-second breath-hold; SCAPIS, Swedish CardioPulmonary bioImage Study; COPD, chronic obstructive pulmonary disease; FEV_1 , forced expiratory volume in one second; VC, vital capacity; PFTs, pulmonary function tests; D_{LCO} , diffusing capacity of carbon monoxide; CT, computed tomography; LAV, low attenuation volumes; R_{rs} , respiratory resistance; X_{rs} , respiratory reactance; MRI, Magnetic resonance imaging; AX, area of reactance; f_{res} , resonant frequency; PD_{15} , 15th percentile density.

Data Sharing Statement

The data generated and/or analyzed in this study can be made available to researchers by the corresponding author upon request but will be subject to a review of secrecy and require an approved ethical application.

Ethics Approval and Informed Consent

The study was approved by the regional ethical review board in Umeå (Dnr 2010-228-31M), Lund (2016/1031), and Linköping (2018/478-31). All participants gave written and informed consent. The consent was obtained prior to the study commencement.

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Author Contributions

All authors made significant contributions to the work reported in the form of either conception, study design and/or data acquisition. All authors have substantially revised and critically reviewed the article, have agreed on the journal to which the article is submitted and have reviewed and agreed on all versions of the article before submission. All authors agree to be accountable for all aspects of the work.

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Disclosure

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