Supplementary Material

Emphysema- and airways-dominant COPD phenotypes defined by standardised quantitative CT

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CT imaging protocols

Non-contrast CT imaging was performed (15 minutes after inhalation of salbutamol 400 micrograms) in a caudo-cranial direction, in order to reduce artefacts secondary to diaphragmatic motion. Scanning was undertaken with subjects in the supine position and with both arms fully abducted, thereby positioned outside the field of view. A foam box (LD15, Styrotech Ltd, West Bromwich, UK) housing three electron density rods (LN300, LN450, 'solid water') from an RMI467 electron density CT phantom (Gammex – RMI Ltd, Nottingham, UK) was secured over the mid-point on the sternum using a *Velcro* belt (Figure S1). Subjects were requested to take three breaths to maximum inspiration prior to breath-holding at full inspiration in order to image the whole lung within a single breath hold. The scanning field of view (FOV) used for the emphysema imaging protocol was selected to ensure inclusion of the whole lung and at least 4 cm of air ventral to the foam box, thereby allowing measurement of air density for internal image calibration. The airways disease protocol employed limited imaging (1, 2) of the region between the aortic arch and 2 cm caudal to the carina. The scanner-specific parameters and image reconstructions are shown in table S1.

CT image analysis

Emphysema: All imaging series were analysed using a semi-automated software program (Pulmo-CMS; Medis Medical Imaging, Leiden, Netherlands) by a single operator (DS) Analysis consisted of 3 parts: internal image calibration utilising measurements of air and blood density as previously described (3, 4); semi-automated, threshold-based lung segmentation; derivation of lung densitometric indices from the voxel frequency distribution histogram, as previously described (5).

Airways disease: Airway cross-sectional geometry of the right upper lobe apical bronchus (RB1) was determined with a semi-automated software program (Emphylx-J V 1.00.01 (6)) by a single operator (SG) utilising the full width at half maximum (FWHM) method (7). Following manual placement of a seed point in the airway lumen of RB1, 64-128 radial trajectories were cast across the airway wall and the boundaries of the airway wall were defined by the mid-point of the profile of CT numbers across each radial trajectory. Lumen area (LA) and wall area (WA) were measured as described previously (1, 8).

Preclinical phantom study of inter-scanner variability in CT lung densitometry and validation of proposed standardisation methodology

Interscanner variability was assessed by imaging an anthropomorphic lung phantom (KCARE, Kings College Hospital, London, UK), containing fixed whole dog lungs in a GE Lightspeed VCT 64 scanner using a reference imaging series protocol (protocol 1, table S1) and five other scanners using the closest equivalent scanner settings. In addition, other scanning protocols were employed in each scanner in order to generate a total of twenty-five imaging protocols with a wide range of parameters (Table S2). The pitch was 1.375:1 for all imaging performed in the GE scanners and 1.5:1 in the Siemens scanners. Whole lung densitometry (Perc15) of the dog lung was assessed for each imaging series using Pulmo-CMS and variability assessed as mean and standard deviation.

A method for standardisation of densitometric indices was explored using density measurements obtained from a number of 'standards', including a novel Warwick densitometry phantom (WDP; see figure S2), that were imaged synchronously with the dog lung phantom. The WDP was constructed of a milled housing of 'solid water' (Gammex – RMI Ltd, Nottingham, UK) that contained a series of synthetic lung cores designed to mimic

the structure and heterogeneous density of lung tissue. Nine cores were made to provide a range of density measurements comparable to lungs with a range of emphysema severity. The lung cores were modelled on data from volumetric lung CT imaging of patients with a range of emphysema severity. These images were imported into a software package (Mimics, Materialise, Belgium) and used to construct a laser-sintered (EOS P380i - EOS GmbH, Munich, Germany) polyamide-12 polymer (PA2200 - EOS GmbH, Munich, Germany) 'skeleton' of high-density structures, equivalent to airway walls and blood vessels. The open volumes of the cores were subsequently filled with stiff polyurethane (PU) expanding foam (DRF002, Tiranti Ltd, Thatcham, UK) of different densities, generated by using pure PU foam and PU foam mixed with 5% or 10 % acetone (C3H6O) prior to casting.

Three electron density rods (Two rods with density values equivalent to lung tissue (LN300, LN450), and one rod with equivalent density to water ('solid water')) from an RMI467 electron density CT phantom (Gammex – RMI Ltd, Nottingham, UK) were secured by a *Velcro* strap to the sternal region of the dog lung phantom for all imaging series. Density measurements of these rods and the WDP 'solid water' housing were obtained using the region of interest (ROI) facility of Pulmo-CMS. In addition, density measurements of air ventral to the phantom sternal region were obtained using the ROI sampling facility of Pulmo-CMS and used for internal air calibration, as previously described (3, 4).

Regression equations were calculated from measurements of the densitometry standards for each of the scanning protocols in relation to the reference protocol (protocol 1, table S2). Each regression equation was used to adjust the density measurements of the lung phantom to standardize towards the lung densitometry value obtained using the reference protocol on the

reference scanner. The efficacy of the standardisation procedures was assessed by comparison of the mean and standard deviation.

Preclinical phantom study of inter-scanner variability in CT airway measurement and validation of proposed standardisation methodology

Two airway phantom models were used to assess inter-scanner variability and to explore standardisation methodology:

A) CTP674© Phantom, The Phantom Laboratory (http://www.phantomlab.us/)

The CTP674© Phantom was used as a surrogate 'patient' to assess the interscanner variability in CT airway measurements and the efficacy of the standardisation methodology. This phantom consists of an outer ring (Catphan® Uniformity Material Ring) that simulates soft tissue attenuation with an electron density just above water, and a central oval insert that simulates lung attenuation. The central oval insert contains 6 tubes (CTP666, 1-6) that simulate airways of different dimensions, air holes of varying dimensions, an acrylic reference material and an insert for a small bottle filled with sterile water (9).

B) Leicester Airway Phantom (LAP)

Measurements for standardisation were obtained from the LAP, which consists of a polystyrene block housing nine plastic tubes of varying dimensions that simulate airways of different dimensions (1, 2). In the current study, the three tubes with smallest dimensions were excluded as they were not measurable with the FWHM method on images reconstructed using the EvA airway scanning protocol.

CT imaging of both the LAP and CTP674© phantoms was performed at each centre using the EvA airway scanner-specific protocols as shown in table S1, with a FOV of 350mm for image reconstruction. All LAP and CTP674© phantom imaging series were analysed with a semi-automated software program (Emphylx-J V 1.00.01 (6)) by a single operator (SG). Morphometry of LAP tubes and CTP674© phantom tubes was performed using the full-width at half-maximum (FWHM) method (14) to derive lumen area (LA) and wall area (WA). Percentage WA (% WA) was derived from LA and WA [%WA=WA/(WA+LA) x 100].

Interscanner variability was assessed from CT airway morphometry (LA, WA and %WA) of the CTP674© phantom tubes (Table S3). Linear regression equations were derived using LAP tubes measurements obtained from the CT scanner at each EvA centre and the reference scanner (Table S3). Standardisation of airway morphometry of the CTP674© phantom at each EvA centre was achieved by regression towards the airway measurements of the CTP674© phantom obtained at the reference centre. Standardised percentage WA (% WA) was derived from standardised LA and WA [%WA:WA/(WA+LA) x 100]. The efficacy of the standardisation method was assessed by percentage error reduction in %WA measurements of the CTP674© phantom following standardisation (Table S3).

Standardisation of Clinical CT Lung Densitometry

Imaging of the WDP was performed at all centres using the emphysema imaging protocols in table S1. Regression equations were calculated for all patient imaging series by combining data derived from centre-specific densitometry of the WDP cores, 'solid water' (Gammex – RMI Ltd, Nottingham, UK) measurements from the WDP housing, ROI measurements of air density derived from WDP imaging series and patient-specific densitometry using measurements from the 3 EDR imaged in each patient series. Internal air and blood calibration was also performed as previously described (3, 4). Adjustment of whole lung

Perc15 values for each patient was performed by linear regression in order to standardize measurements to the densitometry reference scanner (centre 3). To reduce the confounding effects of variation in inspiratory level, the total lung capacity (TLC) derived from body plethysmography was used to adjust densitometry using the 'sponge-model' approach (10).

Standardisation of clinical CT airway dimensions

Linear regression equations (Table S3) were derived from morphometry of the LAP as described above. LA and WA values of the RB1 for each patient were adjusted by linear regression using equations derived from the LAP phantom in order to standardize measurements to the airway reference scanner (centre 2). Standardised percentage WA (% WA) was derived from standardised LA and WA [%WA:WA/(WA+LA) x 100].

Results

Preclinical phantom study to validate CT lung densitometry standardisation

Figure S3 shows the difference between Perc15 values of the dog lung phantom using protocols 2-25 (table S2), and the value obtained from the reference imaging series (protocol 1, table S1) for unstandardised and standardised data. A mean difference of 26.2 HU (SD 13.4) was observed in the unstandardised data. Standardisation reduced the mean difference to 4.1 HU (SD 2.3 HU), representing an error reduction of 84 %.

Preclinical phantom study to validate CT airway morphometry standardisation

Mean tube measurements of the CTP674© phantom imaged at each EvA centre, and the regression equations used for standardisation of the tube measurements, are shown in table S3.

A mean (SD) difference of 2.9 (1.9) was observed in the %WA dimensions prior to standardisation (see figure E4). Mean differences (SD) in %WA measurement across all EvA centres following standardisation was 0.6 (0.5), p=0.002. The mean (SD) %WA of the CTP674© phantom tubes were significantly lower on the Siemens-derived imaging compared to the GE-derived imaging [62.0 (11.6) versus 66.4 (12.0), p=0.007].

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Table S1. 'Emphysema' and 'airway' disease clinical imaging protocols.

Scanner model	**GE Brightspeed 16**	**GE Lightspeed VCT 64**	**Siemens Sensation 16**	**Siemens Emotion 16**	**Sensation 64 and Definition 64**
Scan type	Helical	VCT Helical	Spiral	Spiral	Spiral
Scan field of view	50 cm	50 cm	50 cm	50 cm	50 cm
Matrix	512 x 512	512 x 512	512 x 512	512 x 512	512 x 512
Rotation time (s)	0.5	0.5	0.5	0.5	0.5
Detector Rows	16	64	16	16	64
Detector width	0.625 mm	0.625 mm	0.75 mm	0.6 mm	0.6 mm
Emphysema protocol					
Pitch	1.375	1.375	1.5	1.5	1.5
kVp	120	120	120	110	120
mAs	40	40	40	40	40
Algorithm	Soft	Soft	B30f	B30f	B30f
Thickness (mm)	5	5	5	5	5
Interval (mm)	2.5	2.5	2.5	2.5	2.5
Airway protocol					
Pitch	0.983	0.983	1.1	1.1	1.1
kVp	120	120	120	110	120
mAs	50	50	50	50	50
Algorithm	BONE	BONE	B70f	B70f	B70f
Thickness (mm)	0.625	0.625	0.75	0.75	0.75
Interval (mm)	0.5	0.5	0.5	0.5	0.5

GE: General Electric; kVp: tube voltage; mAs: milliampere second (tube current).

Table S2. Imaging protocols used in the preclinical study for validation of densitometry standardisation. (* indicates the protocol used in each scanner that was closest to the reference imaging series protocol.)

Scanner Model	Protocol number	kV	mAs	Slice thickness (mm)	Slice interval (mm)	FOV (mm)	Reconstruction Filter
GE Lightspeed VCT 64	1*	120	40	5.0	2.5	360	Soft
GE Lightspeed 16	2*	120	40	5.0	2.5	360	Soft
	3	120	20	2.5	1.25	360	Soft
	4	120	20	2.5	1.25	360	Standard
	5	120	20	5.0	2.5	360	Soft
	6	120	40	2.5	1.25	360	Soft
	7	120	40	5.0	2.5	500	Standard
	8	120	80	5.0	2.5	360	Soft
	9	120	80	2.5	1.25	450	Soft
	10	120	80	5.0	2.5	450	Soft
Siemens Sensation 16	11*	120	40	5.0	2.5	360	b30f
	12	120	80	3.0	1.5	360	b30f
	13	120	80	1.5	1.5	360	b30f
	14	120	80	3.0	3.0	360	b30f
	15	120	80	5.0	5.0	360	b41f
GE Lightspeed 8	16*	120	40	5.0	2.5	360	Soft
	17	120	40	2.5	1.25	360	Soft
	18	120	40	1.25	1.25	360	Soft
GE Lightspeed 16	19*	120	40	5.0	2.5	360	Soft
	20	120	40	5.0	2.5	360	Soft
	21	120	40	2.5	1.25	360	Soft

FOV: field of view; GE: General Electric; kVp: tube voltage; mAs: milliampere (tube current).

Table S3: Mean airway measurements of the CTP674© phantom imaged at each EvA centre.

Centre and Scanner Type	Mean (SD) airway measurement CTP674© phantom		Significant difference in airway measurements compared to reference scanner (p value)	Regression equations for standardisation (LA and WA)	Error reduction in %WA following standardisation (%)
1 GE Brightspeed 16	LA	15.4 (10.0)	0.03	(LA*1.0724)-0.0831	
	WA	23.5 (10.1)	0.01	(WA*0.914)+0.4753	
	%WA	65.5 (13.2)	0.005		69
2 * Siemens Sensation 16	LA	16.4 (10.8)	na	na	
	WA	21.5 (9.0)	na	na	
	%WA	61.7 (12.2)	na		Na
3 GE Lightspeed VCT 64	LA	14.8 (9.7)	0.02	(LA*1.0602)+0.3644	
	WA	24.5 (10.6)	0.008	(WA*0.9259)-0.4496	
	%WA	67.3 (12.8)	<0.001		77
4 Siemens Emotion 16	LA	15.8 (10.1)	0.07	(LA*1.0027)+0.2722	
	WA	22.5 (9.5)	0.003	(WA*0.9816)-0.4388	
	%WA	63.5 (11.8)	0.001		89
5 Siemens Sensation 64	LA	16.6 (10.6)	0.55	(LA*0.9429)+0.229	
	WA	20.8 (8.9)	0.008	(WA*1.0128)-0/0892	
	%WA	60.8 (12.6)	0.03		99
6 GE Lightspeed VCT 64	LA	15.0 (9.9)	0.01	(LA*1.052)+0.4666	
	WA	24.2 (10.3)	0.005	(WA*0.9298)-0.3498	
	%WA	66.7 (13.1)	<0.001		84
7 Siemens Sensation 16	LA	16.2 (10.4)	0.4	(LA*0.9873+0.063	
	WA	21.6 (9.2)	0.3	(WA*0.9985)-0.2161	
	%WA	62.0 (12.0)	0.2		41
8 Siemens Emotion 16	LA	15.8 (10.3)	0.02	(LA*1.0457)-0.1652	
	WA	22.8 (9.5)	0.002	(WA*0.9782)-0.3254	
	%WA	64.2 (13.1)	0.004		52
9	LA	16.8 (10.8)	0.2	(LA*0.9837)-0.2005	
Siemens Definition 64	WA	20.5 (8.8)	0.02	(WA*1.008)+0.1707	
	%WA	60.1 (12.6)	0.1		81
10 GE Lightspeed VCT 64	LA	15.2 (10.0)	0.008	(LA*1.0572)+0.3667	
	WA	24.3 (10.5)	0.007	(WA*0.9297)-0.2666	
	%WA	66.2 (12.1)	<0.001		89

LA: lumen area; na: not applicable; SD: standard deviation; WA: wall area. *Airway reference scanner.

Table S4: Reasons for failure to undertake image analysis

Reasons for image analysis failure	**N**
Scanning protocol violation	40
Data incompatible with software	26
Airway not measurable / collapsed	5
Excessive movement artefact	1
No TLC measurement	10
No phantom standardisation data	11
Total	**93**

Table S5. Comparison of characteristics of patients with chronic obstructive pulmonary disease with and without assessable CT scans and according to CT scanner manufacturer with which imaging was performed (SD in brackets).

	Unassessable CT scans n=93	**General Electric n=172**	**Siemens n=269**	**P value assessable versus unassessable CT scans**	**P value GE versus Siemens**
Age, years	65 (7)	66 (7)	64 (10)	0.52	0.15
Sex, % male	67	66	70	0.77	0.47
BMI, kg.m^{-2}	27.5 (5.2)	27.9 (6.6)	27.7 (5.8)	0.20	0.32
Smoking exposure, pack years	37 (17)	40 (29)	38 (27)	0.12	0.64
FEV_1 % predicted	74 (21)	71 (26)	71 (26)	0.11	0.97
FEV_1/FVC, %	57 (11)	58 (14)	56 (17)	0.08	0.11
DLco/VA % predicted	93 (52)	72 (30)	84 (40)	0.18	<0.001 *
TLC, L	7.1 (1.5)	6.9 (1.7)	7,3 (2,1)	0.70	0.11
6MWD, m	448 (98)	467 (128)	469 (125)	0.32	0.68
BODE score	1.95 (1.64)	1 (2)	1 (2)	0.35	0.57

6MWD: six-minute walk distance; HU: Hounsfield units; mMRC: modified Medical Research Council dyspnoea scale; Perc15: 15th percentile point; % WA: % wall area.

Table S6. Patient distribution according to center

Center	E-dominant	A-dominant	Mixed disease	Mild disease
1 Munich GE	24	8	6	14
2 Leicester Sie	7	4	4	17
3 Coventry GE	13	1	1	17
4 Manchester Sie	13	14	3	28
5 Freiburg Sie	9	16	2	40
6 Ferrara Ge	15	23	7	16
7 Warsaw Sie	7	7	2	50
8 Budapest Sie	19	0	0	7
9 Marburg Sie	9	0	3	8
10 Hannover GE	8	6	6	7

	airway	both	emphysema	mild	no data	
01Muenchen	8	6	24	14	3	GE
03Coventry	1	1	13	17	4	GE
06Ferrara	23	7	15	16	5	GE
10Hannover	6	6	8	7	7	GE
	38	**20**	**60**	**54**	**19**	
02Leicester	4	4	7	17	10	Siemens
04Manchester	14	3	13	28	25	Siemens
05Freiburg	16	2	9	40	16	Siemens
07Warszawa	7	2	7	50	7	Siemens
08Budapest	0	0	19	7	6	Siemens
09Marburg	0	3	9	8	10	Siemens
	41	**14**	**64**	**150**	**74**	

Table S7. Patient distribution according to scanner manufacturer.

Scanner Manufacturer	E-dominant	A-dominant	Mixed disease	Mild disease
GE	60	38	20	54
Siemens	64	41	14	150

Table S8. Eex versus Aex clinical characteristics

	Eex **62**	**Aex** **40**	**P value**
Age (years)	67 (9)	67 (8)	0.92
Gender (% male)	63	73	0.39
Smoking history pack years	42 (19)	46 (20)	0.36
FEV_1/FVC lower limit of normal (LLN[%])	98	65	< 0.001 *
BODE	2 (3)	2 (2)	0.17
BMI (kg/m^2)	25.3 (6.5)	30.5 (7.7)	< 0.001 *
6MWD (m)	447 (79)	405 (177)	0.094
Dyspnea score	1 (1)	1 (2)	0.45
Spirometry			
FEV_1 L	1.6 (0.6)	2.0 (0.7)	< 0.001 *
FEV_1 % predicted	62 (18)	68 (19)	0.004
FEV_1/FVC (%)	47.1 (15.2)	62.7 (10.1)	< 0.001 *
BD reversibility L	0.13 (0.17)	0.18 (0.17)	0.29
Lung mechanics			
Raw (kPa x sec /L)	0.34 (0.21)	0.37 (0.19)	0.28
sRaw (kPa x sec)	1.7 (1.3)	1.5 (1.3)	0.21
Rin (kPa x sec /L)	0.2 (0.13)	0.3 (0.10)	0.073
Rex (kPa x sec /L)	0.5 (0.4)	0.4 (0.4)	0.75
Lung volumes			
TLC L	8.1 (2.4)	6.4 (1.4)	< 0.001 *
TLC % predicted	132 (20)	105 (17)	< 0.001 *
RV L	4.2 (1.4)	3.2 (0.7)	< 0.001 *
RV % predicted	181 (53)	136 (34)	< 0.001 *
RV/TLC (% predicted)	1.32 (0.25)	1.29 (0.32)	0.086
Gas exchange			
pO2 (mmHg)	66.1 (11.8)	74.5 (10.1)	< 0.001 *
pCO2 (mmHg)	36.7 (3.9)	39.7 (3.2)	< 0.001 *
A-a gradient	37.8 (13.0)	27.5 (10.8)	< 0.001 *
TLCO/Va % predicted	60 (21)	94 (27)	< 0.001 *
Blood parameters			
Leukocytes (10^9 cells/L)	7.7 (2.4)	6.4 (1.6)	< 0.001 *
Lymphocytes (10^9 cells/L)	1.8 (0.7)	1.9 (0.6)	0.74
Neutrophils (10^9 cells/L)	4.8 (2.4)	3.8 (1.7)	0.002
Eosinophils (10^9 cells/L)	0.16 (0.20)	0.18 (0.18)	0.80
Hb (mg/dL)#	14.7 (1.2)	13.9 (1.4)	0.0012*
Hk (%)	44 (4)	41 (3)	0.0013 *
erythropoetin (mIU/mL)	10.1 (9.2)	11.8 (5,0)	0.39
IgE (kU/L)	29 (92)	68 (173)	0.081
CRP (mg/dL)	0.4 (0.37)	0.4 (0.25)	0.187
AAT (mg/dL)	138 (36)	130 (26)	0.057
CT parameters			
WA/BSA (mm^2 / m^2)	17.9 (7.1)	13.8 (6.4)	< 0.001 *
LA/BSA (mm^2 / m^2)	11.9 (7.6)	4.3 (2.6)	< 0.001 *
Percentage WA (%)	58.6 (7.1)	74.8 (4.4)	< 0.001 *
Lung density (Perc15 HU)	-942 (12.2)	-897 (23.5)	< 0.001 *
Emphysema distribution	3.5 (45.2)	-0.7 (25.3)	0.35

given is median and IQR; # = mean +/-SD

Table S9 Selected parameters in mild cases below and above lower limit normal for FEV1/FVC

	Controls n=280	**Cases n=441**	**Mild below LLN n= 138**	**Mild above LLN n=65**	**p value* above LLN vs controls**
Raw (kPa x sec /L)	0.18 (0.11)	0.3 (0.21)	0.28 (0.20)	0.22 (0.45)	0.002
TLC (L)	6.5 (1.8)	7.1 (2.0)	6.8 (1.8)	6.7 (1.8)	0.882
TLCO/Va (% predicted)	100 (21)	79 (33)	80 (30)	99 (34)	0.286

given is median and IQR *Mann-Whitney-Wilcoxon

The cases, which are below LLN and have COPD also by this criterion show more pronounced changes in LFTs and have values matching the average of the COPD cases, which are defined based on the 0.7 FEV1/FVC ratio cut-off criterion (see Table S9). Among the cases above lower limit normal, which have COPD according to the 0.7 FEV1/FVC ratio cut-off but not according to LLN, the LFTs are similar to controls for TLC and TLCO/Va, but the Raw value is still significantly increased compared to controls, indicating that this group exhibits airflow limitations when looking at resistance.

Figures

Figure S1. Axial clinical CT image showing the positioning of 3 electron density rods over the sternal region.

Figure S2. Warwick densitometry phantom. A. Photograph of the Warwick densitometry phantom, housing the nine fabricated lung cores. **B.** Cross-sectional CT image following densitometric analysis (Pulmo-CMS) showing varying extent of low density areas (below -930 HU, highlighted in pink) equivalent to a range of emphysema severity.

Figure S3. Influence of standardisation method on interscanner variability in 15th percentile point (Perc15) measurements. Scanner variability is expressed as the absolute difference in mean (and standard deviation) Perc15 values of the dog lung phantom between each of the 24 image series compared to the reference image series (protocol 1, table E3).

Figure S4. Influence of standardisation method on interscanner variability in %Wall area (%WA) measurements. Scanner variability is expressed as the absolute difference in mean %WA values of the CTP674© phantom between each of the 9 image series acquired from each EvA centre compared to the airway reference image series.

Figure S1

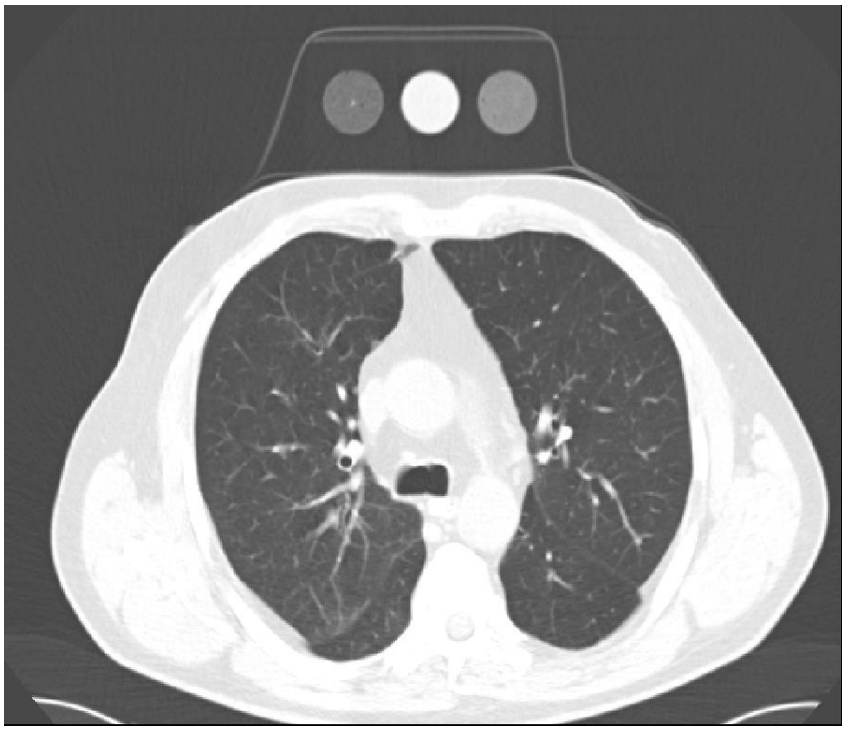


Figure S2

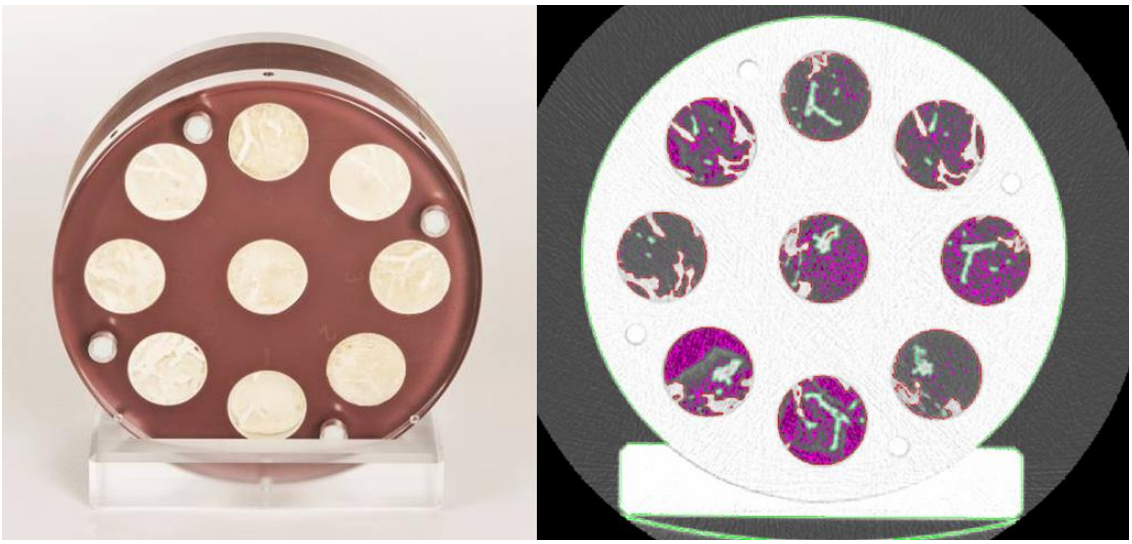


Figure S3

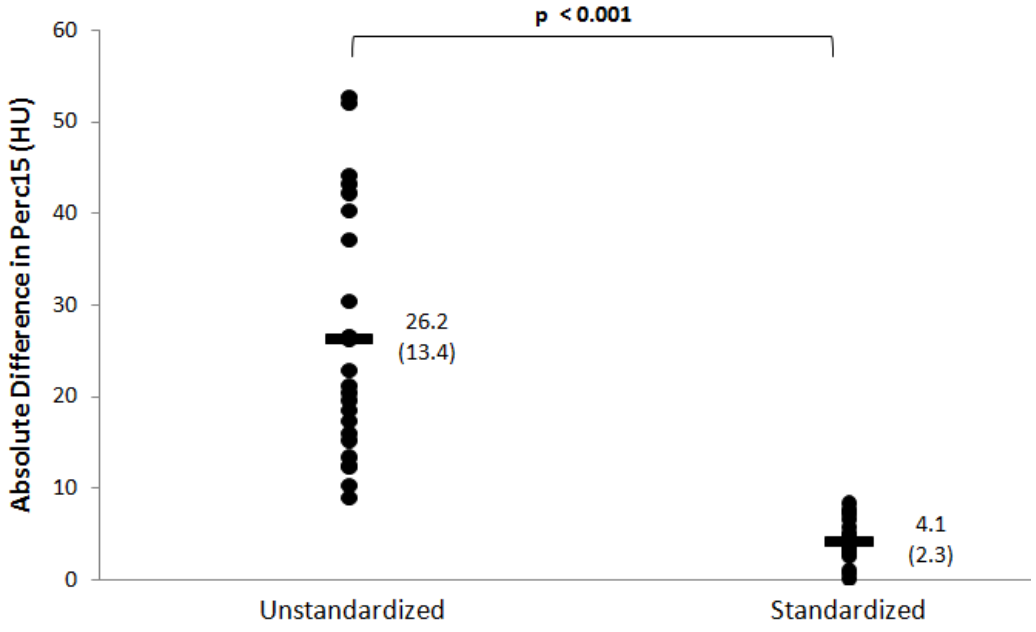
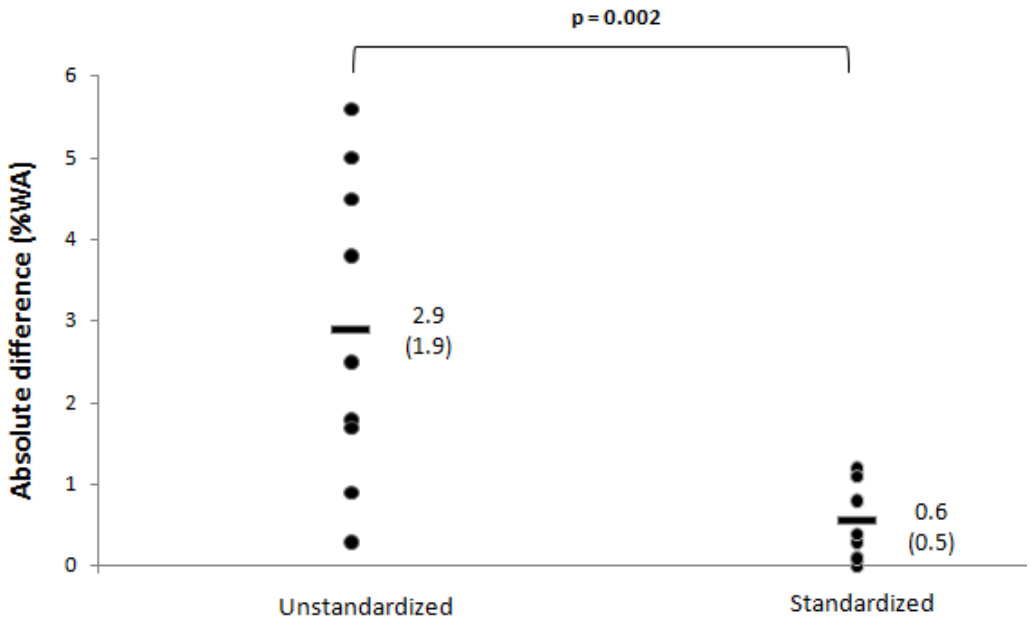

p < 0.001
Absolute Difference in Perc15 (HU)
60
50
40
30
20
10
0
26.2
(13.4)
4.1
(2.3)
Unstandardized
Standardized

Figure S4


p = 0.002
Absolute difference (%WA)
6
5
4
3
2
1
0
2.9
(1.9)
0.6
(0.5)
Unstandardized
Standardized