Supplement to:

Pulmonary artery enlargement and cystic fibrosis pulmonary exacerbations: a cohort study

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Supplementary Methods:

Patient Population of the Validation Cohort. We built our validation cohort using de-identified patient data collected by the Prince Charles Hospital (TPCH) Adult Cystic Fibrosis Centre, Brisbane) for patients who were evaluated and had CT imaging performed between 2002 and 2014 as outlined in **Figure 1B**. During this time, the center provided care for 456 patients ranging in age from 15 to 70 years. The number of patients in the cohort in 2002 was 180 and increased to 282 in 2014, consistent with the growth of the vast majority of adult CF centers globally. All patients attending the adult CF center for the validation cohort are routinely registered into a long-term local CF database, and all microbiology data is housed in this database. We utilized the database identifiers to search the local radiology database for CT chest scan performed between 2007 and 2014. When more than one scan was performed the first scan was included in this analysis. The clinical and microbiological data from the 197 (of 456) patients who had a CT scan were then extracted from the Australian CF Data Registry (ACFDR). Prior to 2007 annual summary data was entered in the ACFDR and from 1/1/2007 encounter-based data entry of all clinical encounters has been entered. Clinical data of interest based on the endpoints for the Derivation population were sought from the ACFDR. As there is no formal definition of pulmonary exacerbation we utilized a surrogate of hospital admissions for respiratory indications, as also reported by Rowe et al and other retrospective studies of this sort. All patients in the validation cohort had a formal CF diagnosis based on the CF Foundation diagnosis guidelines[1], although these legacy values of sweat chloride performed in childhood were not always available in the database and were too remote to feasibly obtain.

Genotype and CFTR function. In the derivation cohort, all subjects had documentation of the presence of a nonsense mutation in at least one allele of the CFTR gene. Each CFTR mutation in trans was queried through the Clinical and Functional Translation of CFTR database (www.cftr2.org) and each genetic mutation was dichotomized into partially functional and non-functional status. In the validation cohort, genotypes were not limited to nonsense mutations and were classified similarly. Sweat chloride concentration was measured by pilocarpine iontophoresis as previously reported. Sweat chloride was available for all subjects in the derivation cohort, but only in 29 (15%) of the validation cohort of adult subjects. The allelic frequencies of CFTR mutations for both cohorts are shown in **Table S1**.

Spirometry. In the derivation cohort, each subject underwent pre- and post- bronchodilator spirometry using a study-specific spirometer before and after inhalation of two puffs of albuterol with a spacer according to the American Thoracic Society (ATS) criteria[2]. Validation Reference values obtained from the National Health and Nutrition Examination Survey (NHANES) III data were used in both cohorts[3]. All subjects in the derivation cohort had spirometry conducted at the time of CT scan. In the validation cohort, spirometry data was extracted from the TPCH registry with preference given to spirometry performed at the time closest to the date of CT scan. If only one spirometry was available, that value was recorded. The median (IQR) time between spirometry and CT was 2 (15) days.

Supplemental References:

1. Rosenstein BJ, Cutting GR. The diagnosis of cystic fibrosis: a consensus statement. Cystic Fibrosis Foundation Consensus Panel. *J Pediatr* 1998; **132**(4): 589-95.
2. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. *Eur Respir J* 2005; **26**(2): 319-38.
3. Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general U.S. population. *Am J Respir Crit Care Med* 1999; **159**(1): 179-87.

Table S1. Gene mutation frequency in the derivation and validation cohorts

A) Derivation cohort.

Mutation	Percent of Cohort with the Indicated Mutation	Mutation	Percent of Cohort with the Indicated Mutation
DeltaF508	33.7	**p.A455E**	0.4
p.W1282X	19.6	**p.G1244E**	0.4
p.G542X	16.8	**p.G550X**	0.4
p.R553X	4.2	**p.G551D**	0.4
p.R1162X	3.5	**p.G745X**	0.4
p.E60X	2.1	**p.G85E**	0.4
c.3717+12191C>T	1.4	**p.L1065P**	0.4
p.D1152H	0.7	**p.L88X(T>G)**	0.4
p.H1079P	0.7	**p.Q220X**	0.4
p.N1303K	0.7	**p.Q414X**	0.4
p.Q552X	0.7	**p.Q493X**	0.4
p.R347P	0.7	**p.R117H**	0.4
p.R709X	0.7	**p.R334W**	0.4
p.R792X	0.7	**p.R560T**	0.4
p.W57X	0.7	**p.R810X**	0.4
p.Y1092X(C>A)	0.7	**p.S1196X**	0.4
c.1248insT	0.4	**p.S341P**	0.4
c.1585-1G>A	0.4	**p.S549R(A>C)**	0.4
c.1679+1634A>G	0.4	**p.W1089X**	0.4
c.2051_2052delAAinsG	0.4	**p.W1310X**	0.4
c.2583delT	0.4	**p.W401X (TGA)**	0.4
c.2657+5G>A	0.4	**p.W846X(TAG)**	0.4
c.3849+10kbC>T	0.4	**p.W846X(TGA)**	0.4
c.489+1G>T	0.4	**p.W882X**	0.4
c.490-2A>C	0.4	**p.Y913X**	0.4
del ex17a-18	0.4		

B) Validation cohort.

Mutation	Percent of Cohort with the Indicated Mutation	Mutation	Percent of Cohort with the Indicated Mutation
F508del	73.0	**c.1408A>G (p.Met470val)**	0.3
G551D	6.7	**c.1687 T>A**	0.3
621 +1G>T	1.8	**c.1766+1G>A**	0.3
G542X	1.5	**c.2184delA**	0.3
N1303k	1.2	**E60X**	0.3
W1282X	1.2	**G85E**	0.3
R1162X	0.9	**I507del**	0.3
Unknown	0.9	**L1335P**	0.3
1585 1G>A	0.6	**p.C524**	0.3
1717-1	0.6	**p.I1027T**	0.3
A455E	0.6	**p.Leu1399fs**	0.3
c.3718.2477C>T	0.6	**p.Met470val**	0.3
D1152H	0.6	**p.Q493**	0.3
p.Ser341fs	0.6	**p.Val1108Hisfs*47**	0.3
Q493X	0.6	**P67L**	0.3
R117H	0.6	**Q1291H**	0.3
1154 ins TC	0.3	**R334W**	0.3
1717-1G-A	0.3	**R347P**	0.3
2052delA	0.3	**R560T**	0.3
2184delA	0.3	**S489X**	0.3
2657 + 5G>A	0.3	**T1053I**	0.3
3849+10Kb	0.3	**T12461**	0.3

Table S2. Baseline characteristics of all subjects in the derivation cohort with CT data available.

	All (n=140)	PA:A ≤1 (n=73)	PA:A >1 (n=67)
Age, years	28±8	29±9	26±6†
White race	136 (97%)	72 (99%)	64 (96%)
Male gender	72 (52%)	33 (45%)	39 (58%)
BMI, kg/m^2	21.7±2.8	21.9±2.6	21.2±2.3
***Pseudomonas* positive status**	62 (44%)	32 (44%)	30 (45%)
Sweat chloride, mmol/L	97.9±15.6	95.2±16.4	102.3±10.3†
FEV_1% predicted	58±14	60±14	56±13†
FVC% predicted	75±13	78±13	72±12†
FEV_1/FVC	0.65±0.10	0.65±0.11	0.65±0.09
PA, cm	2.51±0.32	2.45±0.34	2.57±0.29†
A, cm	2.54±0.37	2.70±0.36	2.37±0.29†
PA:A ratio	0.99±0.11	0.91±0.06	1.09±0.08†

Data are expressed as mean±SD or number (%). Abbreviations: FEV_1 = forced expiratory volume in 1 second, FVC = forced vital capacity, PA = pulmonary artery diameter, A = ascending aortic diameter. †P<0.05 for PA:A>1 group versus PA:A≤1 group within the same cohort.

Table S3. Factors associated with acute pulmonary exacerbations during follow-up in patients who did not have index CT scans performed as part of a hospitalization in the validation cohort.

≥1 Acute pulmonary exacerbation at 2-years of follow-up				
	Univariate		Multivariate[a]	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Age, year	1.01 (0.97-1.05)	0.75		
Male gender	1.14 (0.51-2.59)	0.75		
BMI, kg/m^2	0.95 (0.85-1.07)	0.43		
Positive *Pseudomonas* status	2.99 (0.86-10.3)	0.08	1.59 (0.32-7.82)	0.57
PA:A>1	3.17 (1.28-7.83)	0.01	4.59 (1.56-13.5)	0.006
FEV_1% predicted	1.00 (0.98-1.02)	0.99		
FEV1/FVC	0.15 (0.02-1.52)	0.11	0.17 (0.01-3.22)	0.24
Exacerbation in the previous year	19.4 (4.20-89.2)	<0.001	22.2 (4.47-110)	<0.001

[a]Multivariate logistic regression model includes: FEV_1/FVC, positive Pseudomonas status, PA:A>1, prior exacerbation (R^2=0.29, P<0.001). In this subgroup, 47 (51%) patients had ≥1 exacerbation (median 1, IQR 2) at 2-years of follow-up, including 22 (69%) in the PA:A>1 and 25 (41%) in the PA:A A≤1 group (P=0.01).

Figure S1.

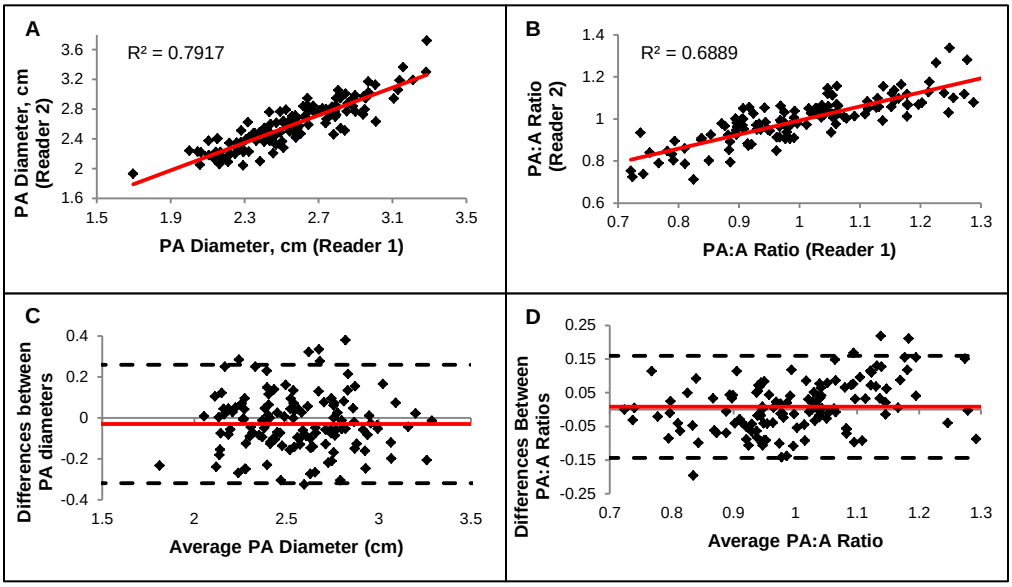

Figure S1. Agreement between different readers for measuring the pulmonary artery (PA) and the Pulmonary artery to Aorta (PA:A) ratio. A-B: The correlation between two independent blinded readers for measurement of the PA diameter (A) and PA:A ratio (B) are shown. C-D: Bland-Altman analysis indicates the mean difference between PA diameter (C) and PA:A ratio (D) were not different between the two readers and were independent of vessel diameter.

Figure S2.

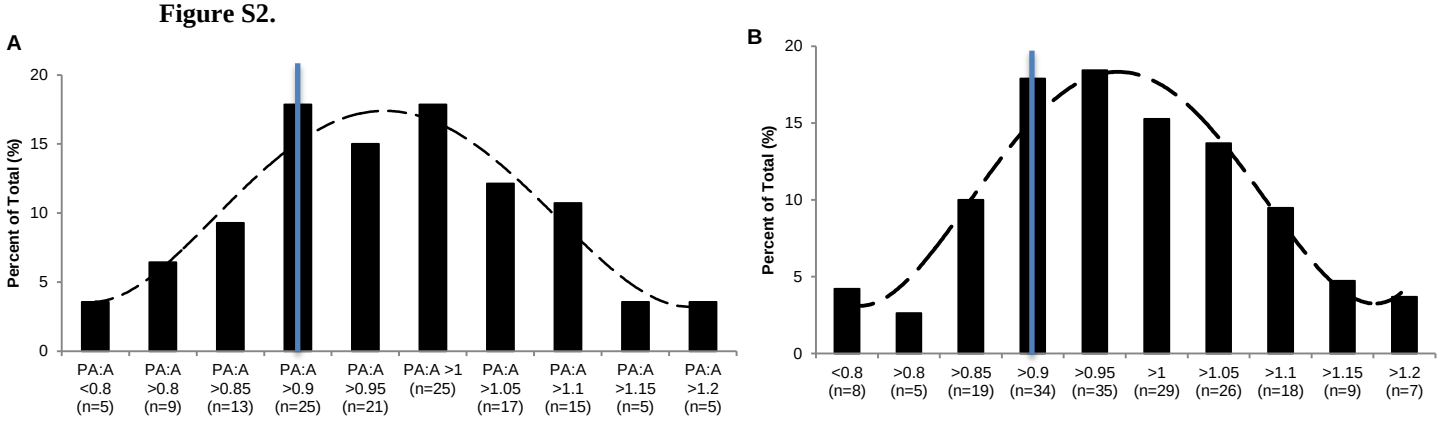

Figure S2. Histogram showing the distribution of the PA:A ratio in the adult CF cohort. (A) Of the 140 subjects with baseline CT scans available for analysis, 80.7% (n=113/140) have a PA:A >0.9 and 47.9% (n=67/140) have a PA:A >1. (B) We observed a similar distribution in a validation cohort, with PA:A>0.9 present in 83.2% (n=158/190) and PA:A>1 present in 46.8% (n=89/190) subjects. The blue line at PA:A >0.9 indicates the 90th percentile cutoff for the normal value of the PA:A ratio in healthy subjects.

Figure S3.

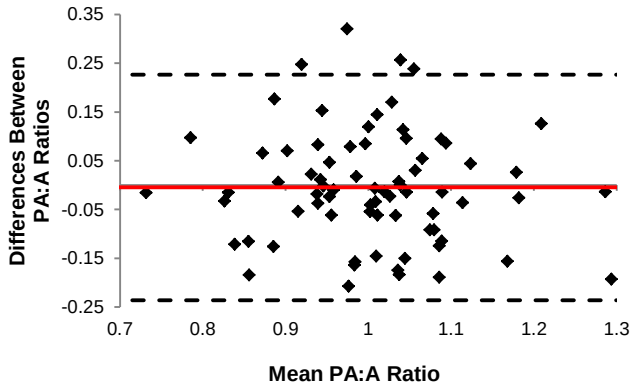

Figure S3. Bland-Altman analysis depicting the relationship between baseline and follow-up PA:A ratios. There was a mean difference of -0.005 in the PA:A ratio between baseline and follow-up CT images.

Figure S4.

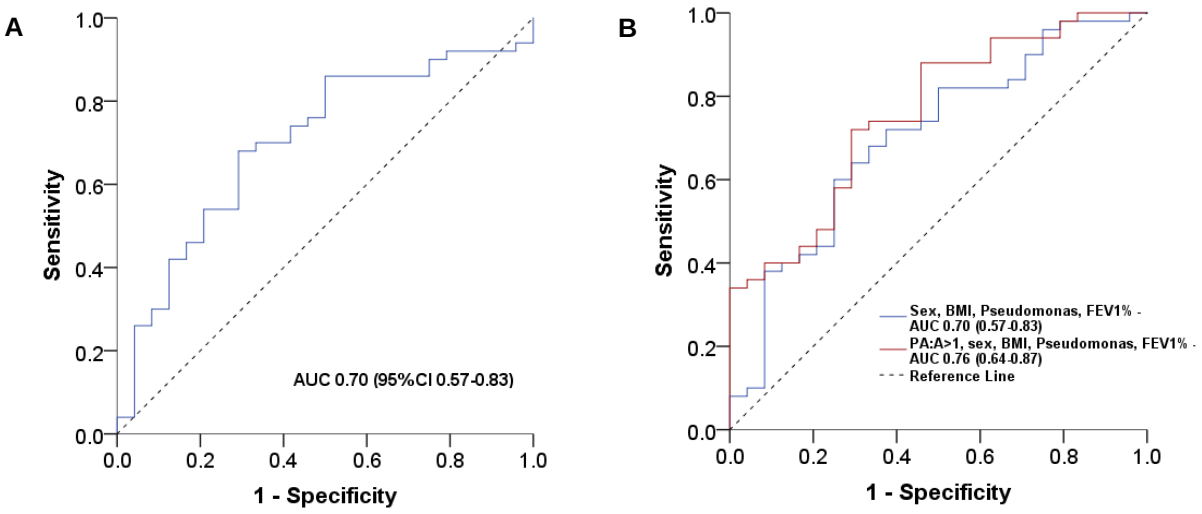

Figure S4. Performance of the PA:A>1. ROC curves for detection of exacerbation at 1-year in the derivation cohort by A) the PA:A and by B) the multivariable model. ROC = receiver operating characteristic, AUC = area under the curve.