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Original article

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LUNG FUNCTION TRAJECTORY AND BIOMARKERS

IN THE TASMANIAN LONGITUDINAL HEALTH STUDY

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Authors' contributions

Study concept and design: SCD, EHW, MJA, RF, AA. Acquisition of data: SCD, EHW, PST, CL. JLP. Analysis and interpretation of data: DSB, SCD, RF, AA. Drafting of the manuscript: DSB, RF, AA, SCD, CL, Critical revision of the manuscript for important intellectual content: all authors. Statistical analysis: DSB, RF, AA SCD. Obtained funding: SCD, EHW, MJA, RF, AA.

Take home message: In the general population, two circulating biomarkers (CRP and CC16) are associated with different lung function trajectories leading to COPD in adulthood.

ABSTRACT

Background and objective. Different lung function trajectories through life can lead to chronic obstructive pulmonary disease (COPD) in adulthood. This study investigates if circulating levels of biomarkers can differentiate those with accelerated (AD) from normal decline (ND) trajectories.

Methods. The Tasmanian Longitudinal Health Study (TAHS) is a general population study that measured spirometry and followed up participants from ages 7 to 53 years. Based on their FEV_1 trajectories from age 7 to 53 years, this analysis included those with COPD at age 53 years (60 with AD and 94 with ND) and controls (C, n=720) defined as never smokers with an average FEV_1 trajectory. Circulating levels of selected biomarkers determined at 53 and 45 years of age were compared between trajectories.

Results. Results showed that CC16 levels (an anti-inflammatory protein) were lower and CRP (a pro-inflammatory marker) higher in the AD than in the ND trajectory. Higher CC16 levels were associated with a decreased risk of belonging to the AD trajectory (OR=0.79 [0.63-0.98] per unit increase) relative to ND trajectory. Higher CRP levels were associated with an increased risk of belonging to the AD trajectory (OR=1.07 [1.00, 1.13] per unit increase). Levels of CC16 (AUC=0.69 [95%CI: 0.56-0.81], p=0.002), CRP (AUC=0.63 [0.53-0.72], p=0.01) and the combination of both (AUC=0.72 [0.60-0.83], p<0.001) were able to discriminate between the AD and ND trajectories. Other quantified biomarkers (IL4, IL5, IL6, IL10 and TNFα) were not significantly different between AD, ND and C.

Conclusions. Circulating levels of CRP and CC16 measured in late adulthood identify different lung function trajectories (AD *vs* ND) leading to COPD at age 53 years.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) has been traditionally considered a self-inflicted disease caused by tobacco smoking and characterized by an accelerated age-related decline (AD) of the forced expiratory volume in the 1st second (FEV_1) [1]. Research over the past few years, however, has shown that only about half of adult COPD patients have followed this AD trajectory, whereas the other half never achieved normal peak lung function in early adulthood and develop COPD following a normal rate of FEV_1 decline (ND) [2-4]. Importantly, these latter patients suffer a higher prevalence and an earlier incidence of cardio-metabolic comorbidities and premature death [5], so identifying what trajectory a given COPD patient has followed when seen in the clinic for the first time in their fifties or sixties may have implications for prognosis and management [6, 7]. In real life, however, spirometry is rarely measured in childhood, adolescence or early adulthood [4], so circulating biomarkers associated with different life-long lung function trajectories leading to COPD may be potentially useful for the appropriate stratification of adult COPD patients.

The Tasmanian Longitudinal Health Study (TAHS) is a general population study that began in 1968 when 8,583 Tasmanian children born in 1961 (7 years of age) were enrolled [8, 9]. Spirometry (and clinical assessment) were repeated at 13, 18, 45, 50 and 53 years [8, 9]. Previous analysis of the TAHS cohort have identified six distinct FEV_1 trajectories from age 7 to 53 years (Figure 1) [3]. The prevalence of COPD at age 53 years increased exponentially between them (Figure 1). Accordingly, TAHS offers a unique opportunity to explore the hypothesis that some circulating biomarkers may be differentially associated with the AD and ND trajectories leading to adult COPD. To test this hypothesis, we contrasted the serum levels of a panel of biomarkers determined in adulthood (45 and 53 years), including club cell secretory protein (CC16), C-reactive protein [CRP], interleukin [IL]4, IL5, IL6, IL10 and tumour necrosis factor [TNF]α, in individuals with COPD at age 53 years who had followed a life-long AD *vs.* ND trajectory in TAHS.

METHODS

Study Design, Participants and Ethics

The design and methods of TAHS have been summarized above and reported in detail elsewhere [8, 9]. In 1968, 8,583 students (98.8% of all school students aged 7 year and born in Tasmania) were recruited. The children underwent a clinical examination including spirometry and parents completed a questionnaire. Follow-up studies were conducted at 13, 18, 45, 50 and 53 years with spirometry measured. In 2002, when the participants were 45 years old, we traced 7,562 (88.1%) of the original 1968 cohort and 5,729 (78.4%) of those traced completed a postal survey. A subgroup of these respondents, enriched for cases of asthma or cough reported in childhood or adulthood, were invited to participate in another clinical study, which included spirometry and collection of blood sample (n=1,405). In the most recent TAHS follow-up in 2012, all those from the original cohort who were alive (53 years) and had up-to-date contact details, were invited to attend a clinical visit, where pre and post-BD spirometry was measured again and a new peripheral venous sample were obtained and processed [8]. The TAHS study was approved by Ethics Committees of all participating institutions, and all participants signed their written informed consent.

Biomarker quantification

The serum levels of CRP and CC16 were quantified by ELISA in blood samples obtained at both 45 and 53 years of age; and levels of IL4, IL5, IL6, IL10 and TNFα were quantified for only blood samples at 45 years as published elsewhere [10-12].

Lung function trajectories over time and COPD at 53 years.

In our previous analysis, we applied group-based trajectory modelling technique to model FEV1 trajectories from 7 to 53 years [3]. We identified six distinct trajectories of them three were

associated with an increased risk of COPD (defined as post-BD FEV1/FVC<0.7) at 53 years (Figure 1). Among these three "disadvantaged" trajectories, one (namely "early below average, accelerated decline") had accelerated lung function decline while other two (namely "below average" and "persistently low") had lower lung function from childhood and normal lung function decline in adulthood.

Data analysis

For the current analysis, we included 154 COPD cases at 53 years who had followed the three "disadvantaged" FEV1 trajectories (Figure 1). These COPD cases were classified into two COPD groups: (1) COPD cases included in the "early below average, accelerated decline" FEV1 trajectory (n=60), labelled here as Accelerated Decline (AD); and (2) COPD cases included in the "below average" or "persistently low" FEV1 trajectories, labelled here as Normal Decline (ND) (n=94). These two COPD groups (AD and ND) were primary comparison groups in this analysis. We additionally included a control group who were never smoker participants in the "average" FEV1 trajectory who had normal lung function at age 53 (n=702), labelled here as controls (C).

Results are summarized as n (%), mean ± SD or median [interquartile range -IQR], as appropriate. Groups were compared using t , Kruskal-Wallis and chi-squared tests where appropriate. Levels of biomarkers were then associated with lung function trajectories using regression models, adjusted for age, sex, socio-economic status, current asthma, smoking status, cumulative smoking exposure (packyears) and childhood asthma and pneumonia. Receiver Operating Characteristic (ROC) analysis was used to assess the ability of biomarkers to discriminate among trajectories as indicated by the Area Under the Curve (AUC) and 95% confidence intervals [CI]. All analyses were performed with STATA V.15.1 (Stata Corporation 2019, College Station, Texas, USA).

RESULTS

Participan characteristics

Table 1 presents the main characteristics of C, AD and ND participants at age 53 years. Main observations were that: the proportion of females was higher in C, intermediate in AD and lowest in the ND; BMI was higher in AD than in ND; the prevalences of current smoking and parental smoking were similar in AD and in ND but cumulative smoking exposure was higher in AD; both AD and ND had more childhood pneumonia/pleurisy, and were diagnosed with asthma in childhood and adulthood more frequently than C; and finally, by definition, spirometry was normal in C, whereas airflow limitation at the age of 53 was generally more severe in AD than in ND.

Table 2 presents the biomarker levels determined in the three groups at 45 or 53 years of age. Compared with C, AD participants had higher CRP and lower CC16 levels, both at 45 and 53 years of age. They also showed reduced concentrations of the Th2-related cytokines IL4 and IL5, whilst IL6, IL10 and TNFα levels were similar to C. By contrast, ND participants showed a biomarker pattern that resembled that of C, with normal CRP levels and mildly reduced CC16 concentrations, normal IL4, IL5, IL6, IL10 values, although they had slightly increased TNFα concentrations (Table 2, Figure S1). Finally, CC16 levels were significantly lower in the AD than in the ND trajectories, both at 45 and 53 years, and CRP levels higher in the AD than in the ND trajectory at 53 years. The remaining measured biomarkers were not different between AD and ND (Table 2). In the sensitivity analysis when smokers with normal lung function were added to the control group (C), we observed similar findings (Table S1). Between 45 and 53 year, CC16 increased by 0.59 (95%CI:0.38-0.81, SD=2.9) unit. CRP reduced by 0.77(95%CI:0.43, 1.1; SD=4.6) unit.

Regression analysis adjusted for age, sex, current asthma, smoking, packyears and childhood factors, including childhood asthma and pneumonia, showed that higher CC16 levels at 53 years (OR=0.79 [95%CI: 0.63, 0.98] per one unit increase in CC16) and 45 years (OR=0.69 [95%CI: 0.52, 0.91] per one unit increase in CC16 level) were associated with a decreased risk of belonging to the AD trajectory relatively to ND as the reference (Table 3). Higher levels of CRP at 53 years were associated with an increased risk of AD (OR=1.07 [95%CI: 1.00, 1.13] per one unit increase in CRP level) relatively to ND as the reference. Although BMI has been associated with increased CRP levels, additional adjustment for BMI did not appreciably change our findings. ROC analysis showed that the combined consideration of CC16 and CRP levels had an AUC of 0.72 [95%CI: 0.60-0.83, $p<0.001$] to discriminate the AD from the ND trajectories (Figure 2). The Youden method identified a cut-off point for CC16 is 5.11, which has a corresponding sensitivity of 60% and specificity of 71%. For CRP, the cut-off point is 4.72 with a sensitivity of 55% and specificity of 70%.

DISCUSSION

In this study, we investigated biomarkers associated with different lung function trajectories leading to COPD in middle age. The main and novel observations of this analysis are that the AD trajectory was associated with significantly higher CRP and lower CC16 circulating levels than the ND one; and the combined assessment of these two biomarkers can effectively discriminate between the two trajectories among adults with COPD.

It is now well accepted that different lung function trajectories can lead to COPD in adulthood [2-4, 13, 14] albeit their relationship with circulating biomarkers is unclear. Guerra *et al* [15] have recently shown using several cohorts with different, limited age ranges that low concentrations of CC16 in serum are associated with reduced lung function in childhood, accelerated lung function

decline in adulthood, and development of moderate airflow limitation in adults population [15]. In a subsequent publication, they showed that CC16 levels increased from birth to childhood to 32 years of age, likely related to increasing body/lung size with age, that there was intrasubject tracking of CC16 levels across all ages, and that several environmental (maternal age at delivery, maternal smoking, and parental education) and genetic (sex and the single nucleotide polymorphism rs3741240) were associated to CC16 levels [16]. Our results confirm that CC16 is an important biomarker in this context, although two important differences with these previous studies are worth noting. First, Guerra *et al* combined different cohorts followed during varying periods of time [15, 16] whereas our cohort studied the same participants followed from infancy (7 years) to late adulthood (53 years) [8, 9]. And, second, whereas Guerra *et al* explored the predictive value of CC16 determined early in life, we used the reverse approach and explored the potential usefulness of quantifying CC16 (and other biomarkers) in late adulthood to estimate the trajectory that the individual with chronic airflow limitation in adulthood has already followed, a scenario which resembles much more current clinical practice. Finally, it is also important to acknowledge that, in patients with moderate to severe COPD recruited into the ECLIPSE study, Vestbo *et al* [17] showed that CC16, CRP and fibrinogen were significantly associated with the FEV1 value determined at recruitment (63 years of age), and that the values of CC16 ($p=0.04$) and CRP ($p=0.07$) were related to the annual rate of FEV1 change during three years follow-up, with no relationship observed for other biomarkers investigated (IL6, IL8, surfactant protein D and TNFα) neither with basal FEV1 or change over time.

We investigated the ability of CC16 and CRP to differentiate the two COPD groups. CC16 was found to have better predictive ability. Although the combination of CRP and CC16 improved the predictive ability compared to CC16 alone, this improvement is relatively small and not statistically significant.

Our observation that the AD trajectory leading to COPD was associated with higher CRP and lower CC16 levels supports that an excessive inflammatory response may be an important endotype underlying this trajectory [18], whereas the fact that the ND trajectory was associated with a much more "normal" biomarker pattern suggests that inflammation is not the main endotype underlying this trajectory and that poor lung development mechanisms are likely to play a more relevant pathogenic role here [19]. If so, it is possible that individuals in the AD trajectory (identified by low CC16 and high CRP levels) may benefit most from treatment with anti-inflammatory drugs, whereas those in the ND (and no evidence of abnormal inflammatory response) are unlikely to do so. This hypothesis may be explored in previous RCTs, if blood samples are available for analysis, or considered in the design of future ones, thus helping to delineate more clearly any potential therapeutic effect or to repurpose existing drugs [20].

Our study has both some strengths and limitations. The fact that participants in TAHS have been followed since childhood to late adulthood, allows for lifetime trajectories that capture both lung growth and decline, which is a clear strength. Lack of replication is a limitation. Unfortunately, the uniqueness of TAHS makes it impossible to validate our observations in other cohorts. We acknowledge that our selection of biomarkers based on available data at the time the study was set up is a limitation, and exploration of other candidates using unbiased proteomics is warranted [21-26] and will occur with a future planned follow up. As we only had data on biomarkers at 45 and 53 years, having such data over time from childhood would have provided more information on the longitudinal association with lung function trajectories leading to COPD. Although different spirometers were used in different follow-ups in TAHS, we used standardised measures (z-scores) to develop lung function trajectories, which reduces the impact of this limitation. As TAHS spans six decades, lost to follow-up is not unexpected. However, those participated at 53 years had similar baseline characteristics to those lost to follow-up, suggesting that the attrition is unlikely to explain our findings. We were able to control for smoking in this study, however, stratified analyses for smoking were not possible due to small sample sizes. Finally, while we have assed serum levels of

the biomarkers, further studies measuring CC16 levels in the sputum and EBC are required to provide a direct measure of the airway pathology.

In conclusion this study shows that two circulating biomarkers (CC16 and CRP) are differentially associated with the AD and ND trajectories leading to COPD in adulthood and that their combined assessment can significantly discriminate both trajectories with potential implications both for clinical practice and future research.

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Table 1. Main characteristics of C, AD and ND at age 53 years.

	Controls (C)		**Accelerated Decline (AD)**		**Normal decline (ND)**		**P value**		
Demographics	**n**	**mean ± SD or %**	**n**	**mean ± SD or %**	**n**	**mean ± SD or %**	**C vs. AD**	**C vs. ND**	**AD vs. ND**
Age at last follow up, years	702	52.6 ± 0.7	60	53.2 ± 0.7	94	52.8 ± 0.8	**<0.001**	0.41	**0.04**
Females, %	702	55	60	45	94	35	0.15	**<0.001**	0.22
Height, cm	702	169.3 ± 9.1	60	169.5 ±9.1	94	172.1 ± 10.1	0.99	**0.019**	0.24
Body mass index, kg/m^2	702	28.0 ± 5.2	60	29.9 ±7.2	94	26.5 ± 4.6	**0.028**	**0.026**	**<0.001**
SES at 53 years, %	702		60		94				
1 (highest)		41.4		25		24.2	**0.013**	**0.002**	0.90
2		16.2		13.3		14.3			
3		29.6		40		32.9			
4		5.7		11.7		7.7			
5		7.1		10		20.9			
Smoking exposure									
Pack-years	702	0	60	21.9 ± 19.5	94	16.7 ± 16.8	**<0.001**	**<0.001**	**<0.001**
Smoking status, %	702		60		94				
Never		100		10.0		29.8	-	-	**0.004**
Current		0		41.7		46.8			
Past		0		48.3		23.4			
Maternal smoking, %	702	28.8	60	49.1	94	44.4	**0.001**	**0.003**	0.57
Paternal smoking, %	702	49.7	60	64.9	94	65.2	**0.028**	**0.006**	0.90
Family history									
Maternal asthma, %	702	9	60	14	94	12.2	0.21	0.32	0.70

Paternal asthma, %	702	10	60	10.7	94	12.3	0.86	0.49	0.76
Previous personal									
Low birth weight(<2.5kg)	450	5.8	39	7.7	62	6.4	0.60	0.80	0.80
Small gestational age (<37	450	15	39	12.8	62	18	0.70	0.59	0.50
Childhood pneumonia, %	702	10.5	60	22	94	18.1	**0.008**	**0.030**	0.55
Childhood asthma, %	702	14.7	60	41.7	94	24.5	**<0.001**	**0.015**	**0.025**
Asthma status, %	702		60		94		**<0.001**	**<0.001**	0.10
Never		84.7		38.3		57.4			
Past		8.7		13.3		7.5			
Current		6.6		48.3		35.1			
Childhood hay fever, %	702	11.5	60	26.3	94	17	**0.001**	0.13	0.17
Childhood eczema, %	702	9.3	60	25	94	12.9	**<0.001**	0.28	0.055
Childhood food allergy, %	702	6.3	60	11.8	94	10.6	0.10	0.12	0.81
Lung function at 53 yrs.									
Post-BD FEV_1/FVC, %	702	81.6 ± 3.9	60	63.6 ± 5.9	94	65.7 ± 4.2	**<0.001**	**<0.001**	**0.005**
Post-BD FEV_1, % predicted	702	109.7 ± 8.8	60	72.4 ± 8.7	94	86.1 ± 8.6	**<0.001**	**<0.001**	**<0.001**
Post-BD FVC, % predicted	702	106.7 ± 9.6	60	90.5 ± 10.6	94	103.6 ± 10.2	**<0.001**	**0.015**	**<0.001**

SES: Socio-economic status

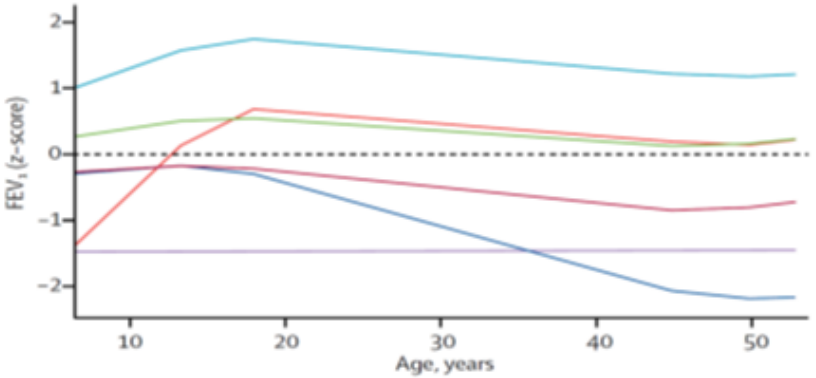
Table 2. Biomarker values (median [IQR]) in C, AD and ND participants.

	Controls (C) (N=702)		**Accelerated decline (AD) (N=60)**		**Normal decline (ND) (N=94)**		**P-value**		
	n	**Median [IQR]**	**n**	**Median [IQR]**	**n**	**Median [IQR]**	**C vs. AD**	**C vs. ND**	**AD vs. ND**
CRP									
45 years	232	2.3 [1.1-5.2]	37	3.2 [1.6-8.6]	45	2.6 [1.8-6.0]	**0.04**	0.17	0.36
53 years	648	2.1 [1.0-4.4]	55	5.1 [1.9, 10.2]	84	2.6 [1.3-5.9]	**<0.001**	0.14	**0.01**
CC16									
45 years	217	6.7 [5.1-9.0]	33	3.9 [2.3-5.9]	43	5.6 [4.2-7.6]	**<0.001**	**0.02**	**0.003**
53 years	214	7.7 [5.5-9.8]	34	3.9 [2.6-5.5]	42	5.4 [3.7-8.6]	**<0.001**	**<0.001**	**0.005**
IL4 (45 years)	212	194 [17-555]	37	15.3 [0.3-265]	40	37 [6-412]	**0.003**	0.08	0.21
IL5 (45 years)	212	0.65 [0.14-1.8]	37	0.27 [0.1-.0]	40	0.44 [0.15-1.1]	**0.02**	0.38	0.11
IL6 (45 years)	212	21 [6.6-5.3]	37	9.3 [4.0-49]	40	17.4 [3.9-73]	0.19	0.78	0.49
IL10 (45 years)	212	4.4 [0.3-11]	37	4.1 [0.6-9.1]	40	8.7 [1.7-30]	0.78	0.07	0.12
TNFα (45 years)	212	6.1 [4.4-8.8]	37	7.2 [4.6-9.0]	40	7.7 [5.4-9.5]	0.53	**0.05**	0.39

Table 3. Adjusted association between CC16 and CRP levels and the two COPD groups

	COPD with accelerated lung function decline (AD) OR (95%CI) ł
CC16 levels at 53 years, per unit increase	0.79 (0.63, 0.98)*
CC16 levels at 45 years, per unit increase	0.69 (0.52, 0.91)**
CRP levels at 53 years, per unit increase	1.07 (1.00, 1.13)*
CRP levels at 45 years, per unit increase	1.08 (0.96, 1.21)

ł Compared to COPD with early low-normal lung function decline (ND) as the "non-disease"/reference group; OR=odds ratio per unit increase in each biomarker.
Adjusted for age, sex, current asthma, smoking, packyears, inhaled corticosteroid use and childhood factors, including childhood asthma and pneumonia.
*p<0.05; **p<0.001



Trajectory[4]	Prevalence[4]	% participants with FEV1/FVC<0.7 at 53 yrs.
Persistently high	12%	0.2%
Early low, accelerated growth, normal decline	8%	0.5%
Average	39%	2.2%
Below average	32%	9.1%
Persistently low	6%	17.6%
Early below average, accelerated decline	4%	62.5%

Figure 1. Six lung function trajectories from age 7 to 53 years identified in TAHS [3]. Table shows the prevalence of each of these six trajectories as well as the proportion of participants in each trajectory with evidence of COPD at the age of 53 years. For further explanations, see text Modified from [3].

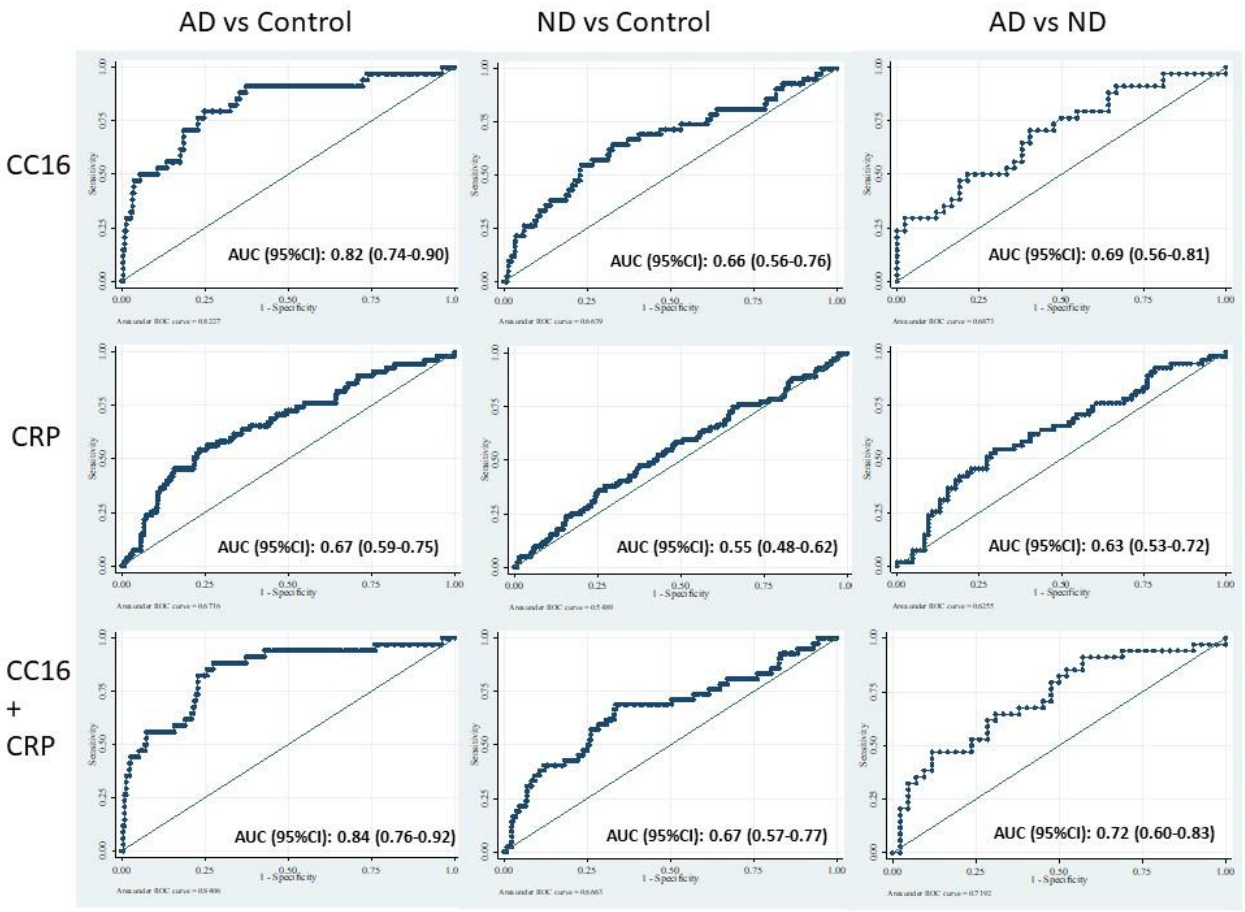


Figure 2. ROC curves (and corresponding AUC values) of CC16 (top row), CRP (middle row) and the combination of both biomarkers (bottom row) in the three-potential comparison in TAHS. CRP and CC16 were measured at 53 years in this figure. AD: accelerated decline group. ND: early low-normal decline group. For further explanations, see text.

ONLINE SUPPLEMENT

LUNG FUNCTION TRAJECTORY AND BIOMARKERS IN THE TASMANIAN LONGITUDINAL HEALTH STUDY

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Lung function tests

Pre- and post-BD spirometry were performed according to international standards (1). Spirometry z-scores were derived from the Global Lung Initiative (GLI) reference equations (2).

Table S1. Biomarker values (median [IQR]) in C, AD and ND participants (C included both smokers and non-smokers).

	Controls (C) (N=1398)		**Accelerated decline (AD) (N=60)**		**Normal decline (ND) (N=94)**		**P-value**		
	n	**Median [IQR]**	**n**	**Median [IQR]**	**n**	**Median [IQR]**	**C vs. AD**	**C vs. ND**	**AD vs. ND**
CRP									
45 years	437	2.4 [1.1-5.1]	37	3.2 [1.6-8.6]	45	2.6 [1.8-6.0]	**0.05**	0.23	0.36
53 years	1286	2.3 [1.1-4.8]	55	5.1 [1.9, 10.2]	84	2.6 [1.3-5.9]	**<0.001**	0.27	**0.01**
CC16									
45 years	413	6.8 [4.9-8.9]	33	3.9 [2.3-5.9]	43	5.6 [4.2-7.6]	**<0.001**	**0.02**	**0.003**
53 years	410	7.5 [5.3-9.7]	34	3.9 [2.6-5.5]	42	5.4 [3.7-8.6]	**<0.001**	**0.002**	**0.005**

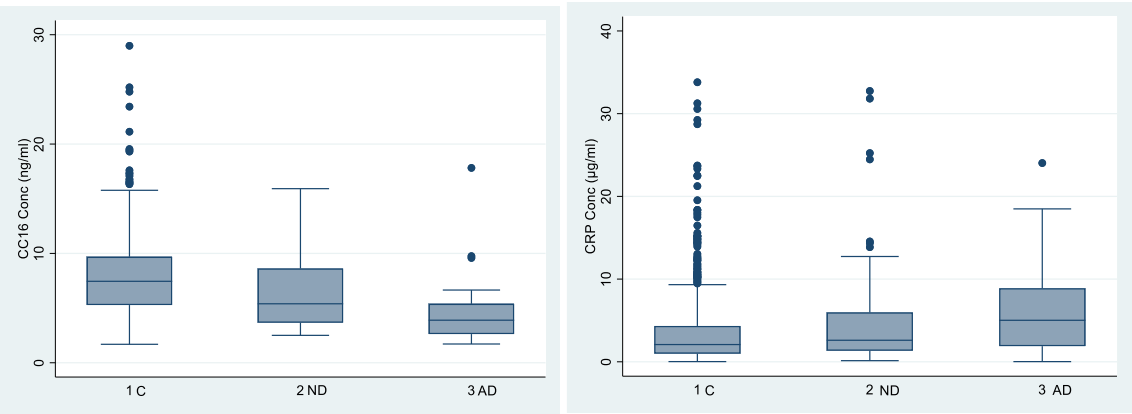

Figure S1. CC16 and CRP levels at 53 years across three groups. AD=accelerated decline. ND=Early low-normal decline; C=controls

Reference

1. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. *Eur Respir J* 2005; 26: 319-38.

2. Quanjer PH, Stanojevic S, Cole TJ, et al. Multi-ethnic reference values for spirometry for the 3–95-yr age range: the global lung function 2012 equations. *Eur Respir J* 2012; 40: 1324-43.