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CIRCULATING BIOMARKERS IN YOUNG INDIVIDUALS WITH LOW PEAK FEV_1

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Take home message: Biomarkers associated with COPD and multimorbidity in older patients, but not aging hallmarks, are also associated to low peak FEV_1 in young individuals.

It is now well established that there is a range of lung function trajectories throughout the life course (1, 2). Specifically, 4-12% of young adults in the general population never achieve normal peak lung function, as determined by the volume of gas expired in the 1st second of a forced spirometry (FEV_1) (3). These individuals are at higher risk of developing COPD in adulthood (4), suffer a higher prevalence and a decade earlier incidence of cardiovascular and metabolic disorders, and die prematurely (3, 5). The biological mechanisms underlying these observations are unknown.

Older patients with chronic obstructive pulmonary disease (COPD) often present abnormal levels of circulatory inflammatory markers (IL-6, IL-8, CCL19) (6), pneumo-proteins (CC16, SP-D, sRAGE, CCL18) (6) and ageing hallmarks (telomere attrition and mitochondrial damage) (7). Whether or not these biological abnormalities also occur in young individuals with low peak lung function has not been investigated before. To explore this, we studied 300 individuals aged 25-35 years from the Lifelines Cohort Study (8) with FEV_1 < (n=147) or ≥ (n=153) than their lower limit of normal (LLN) for their age (according to the GLI equations). Demographic and clinical factors had been recorded as described elsewhere (8). Because groups were balanced by sex and smoking exposure, their potential effect could not be investigated here. The serum levels of IL-8, IL-6, sRAGE, SP-D, CCL2, CCL19, Pentraxin-3, TSLP, CC16, CCL18, BNDF, Leptin, vWFA-2, Collagen I 1, all previously associated with COPD (6), were quantified using the Luminex MAGPIX® platform (R&D systems). Because IL-6 and TSLP concentration were below the detection level of the assay in more than 80% of samples, they were excluded from analysis. For the included biomarkers, determinations below the detection limit were imputed with a 1/4 of that value. The maximum

number of imputed samples was n=11 (out of 300 measured, i.e. 3.7%) for both IL-8 and Pentraxin-3. Telomere length and the ratio between mitochondrial to nuclear DNA (12S rRNA/RNAseP)), two well-stablished aging hallmarks (7), were measured by quantitative PCR (qPCR) in whole blood DNA. Differences between groups were compared using the Mann-Whitney U test. A step-wise multivariate logistic regression model that included clinical factors associated with low peak FEV_1 (table 1) (3, 9-11)) and the biomarkers measured here was used to identify variables independently associated to FEV_1 <LLN.

Table 1 compares selected clinical characteristics and biomarkers levels in participants with FEV_1 ≥ or <LLN. A diagnosis of asthma was similarly prevalent in both groups but ever wheezing and eosinophil counts were higher in participants with FEV1<LLN, so we cannot exclude asthma underdiagnoses. Triglycerides were higher in participants with FEV_1 <LLN, who also showed a tendency towards shorter pregnancy duration and breathing problems. The serum level of most measured biomarkers was similar in both groups except for lower CC16, and higher CCL19 and leptin levels in individuals with FEV_1<LLN. Telomere length and the mitochondrial/nuclear DNA ratio were similar in both groups.

Multivariate logistic regression showed that triglycerides, HbA1c, ever wheezing, CC16, CCL2, sRAGE, CCL19 and Leptin were independently related to low lung function in this young population (Figure 1). In a sensitivity analysis in the population where information on eosinophils was available (n=291) we found that they did not have a significant effect (OR 1.18, 95% C.I. 0.90-1.55, p= 0.222), while the other variables preserved the direction of effect and significance.

To explore if the biomarkers associated here to low peak lung function were also related to early lung function decline, we calculated FEV_1 changes during 5 years follow-up in 70 of the 300 individuals in whom this information was available. In this admittedly small population, we observed that FEV_1 changed a median of -1.85 ml/yr. (IQR 63.8 ml/yr.) in those with baseline FEV_1 < LLN (n=32) and -18.7 ml/yr. (IQR 58.8 ml/yr.) in those with baseline FEV_1 > LLN (n= 38; p=0.02), suggesting that low peak lung function is not associated with early lung function decline in the studied population.

CC16 is a homodimer protein with anti-inflammatory properties secreted mostly by non-ciliated bronchiolar club cells (10). Previous studies have reported lower circulating CC16 levels in relation to low lung function in childhood, smoking, increased airway inflammation, airflow limitation in the general population, accelerated FEV_1 decline and asthma (10, 12, 13). In line with these reports, we observed that systemic CC16 levels were lower in individuals with FEV_1<LLN, supporting that CC16 is a biomarker of abnormal lung development (14). This can be the consequence of early life respiratory infections (11), but this information was not available in this cohort. CCL19 is a chemokine involved in cell trafficking that activates dendritic and B cells to produce pro-inflammatory cytokines (15). Increased levels of CCL19 have been described in smoking mice models of COPD, in the airway smooth muscle of patients with asthma (16) and in severe COPD related to B cell responses (17). Our observations here suggest that activation of dendritic and B cells in young individuals can drive an inflammatory response. Interestingly the levels of CCL2, a monocyte homing cytokine, and those of surfactant protein D, also an innate

immune response protein (6), were also associated with reduced FEV_1 levels in the multivariate analysis. This further supports a role of inflammation in this young population that goes beyond smoking according to our study design.

We observed that low peak FEV_1 in early adulthood was associated with biomarkers of extra-pulmonary organ dysfunction, such as of the metabolic system (HbA1c) and leptin. This supports that low lung function in early adulthood is a marker of poor development of the lungs and also other organ systems which may contribute to multimorbidity later in life (3)(4).

We did not find differences in telomere length and mitochondrial DNA copy number between groups suggesting that abnormal ageing does not play a significant role in young adults with low peak lung function. This is at variance with what has been reported in both old and young severe COPD patients (7) albeit it may be too early in the disease course to observe these abnormalities.

In conclusion, we showed that low peak lung function in early adulthood is associated with some circulating biomarkers (CC16, CCL19, CCL2, SP-D and RAGE) previously associated with airflow limitation in older patients with COPD as well as with markers of systemic organ dysfunction (HbA1c and Leptin) but not with abnormal ageing. These observations are partly in line with the 'Dutch hypothesis'(18), since some of these individuals present asthma features and are likely to develop COPD later in life, particularly if exposed to noxious stimuli. Also, these observations pinpoint towards still poorly known mechanisms linking abnormal lung and other systemic organs

development. Understanding them better may open novel opportunities for prevention and early intervention with the long-term aim of promoting healthier ageing.

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Declaration of interests

The authors declared no conflict of interests.

Data sharing statement

Lifelines data can be requested following the procedures described in: https://www.lifelines.nl/researcher/how-to-apply

REFERENCES

1. Agusti A, Faner R. Lung function trajectories in health and disease. *Lancet Respir Med* 2019; 7: 358-364.
2. Bui DS, Lodge CJ, Burgess JA, Lowe AJ, Perret J, Bui MQ, Bowatte G, Gurrin L, Johns DP, Thompson BR, Hamilton GS, Frith PA, James AL, Thomas PS, Jarvis D, Svanes C, Russell M, Morrison SC, Feather I, Allen KJ, Wood-Baker R, Hopper J, Giles GG, Abramson MJ, Walters EH, Matheson MC, Dharmage SC. Childhood predictors of lung function trajectories and future COPD risk: a prospective cohort study from the first to the sixth decade of life. *Lancet Respir Med* 2018; 6: 535-544.
3. Agusti A, Noell G, Brugada J, Faner R. Lung function in early adulthood and health in later life: a transgenerational cohort analysis. *Lancet Respir Med* 2017; 5: 935-945.
4. Lange P, Celli B, Agusti A, Boje Jensen G, Divo M, Faner R, Guerra S, Marott JL, Martinez FD, Martinez-Camblor P, Meek P, Owen CA, Petersen H, Pinto-Plata V, Schnohr P, Sood A, Soriano JB, Tesfaigzi Y, Vestbo J. Lung-Function Trajectories Leading to Chronic Obstructive Pulmonary Disease. *N Engl J Med* 2015; 373: 111-122.
5. Vasquez MM, Zhou M, Hu C, Martinez FD, Guerra S. Low Lung Function in Young Adult Life Is Associated with Early Mortality. *Am J Respir Crit Care Med* 2017; 195: 1399-1401.
6. Faner R, Tal-Singer R, Riley JH, Celli B, Vestbo J, MacNee W, Bakke P, Calverley PM, Coxson H, Crim C, Edwards LD, Locantore N, Lomas DA, Miller BE, Rennard SI, Wouters EF, Yates JC, Silverman EK, Agusti A. Lessons from ECLIPSE: a review of COPD biomarkers. *Thorax* 2014; 69: 666-672.
7. Casas-Recasens S, Mendoza N, Lopez-Giraldo A, Garcia T, Cosio BG, Pascual-Guardia S, Acosta-Castro A, Borras-Santos A, Gea J, Garrabou G, Agusti A, Faner R. Telomere Length but Not Mitochondrial DNA Copy Number Is Altered in Both Young and Old COPD. *Front Med (Lausanne)* 2021; 8: 761767.
8. Scholtens S, Smidt N, Swertz MA, Bakker SJ, Dotinga A, Vonk JM, van Dijk F, van Zon SK, Wijmenga C, Wolffenbuttel BH, Stolk RP. Cohort Profile: LifeLines, a three-generation cohort study and biobank. *Int J Epidemiol* 2015; 44: 1172-1180.
9. Okyere DO, Bui DS, Washko GR, Lodge CJ, Lowe AJ, Cassim R, Perret JL, Abramson MJ, Walters EH, Waidyatillake NT, Dharmage SC. Predictors of lung function trajectories in population-based studies: A systematic review. *Respirology* 2021.
10. Guerra S, Halonen M, Vasquez MM, Spangenberg A, Stern DA, Morgan WJ, Wright AL, Lavi I, Tares L, Carsin AE, Dobano C, Barreiro E, Zock JP, Martinez-Moratalla J, Urrutia I, Sunyer J, Keidel D, Imboden M, Probst-Hensch N, Hallberg J, Melen E, Wickman M, Bousquet J, Belgrave DC, Simpson A, Custovic A, Anto JM, Martinez FD. Relation between circulating CC16 concentrations, lung function, and development of chronic obstructive pulmonary disease across the lifespan: a prospective study. *Lancet Respir Med* 2015; 3: 613-620.
11. Berry CE, Billheimer D, Jenkins IC, Lu ZJ, Stern DA, Gerald LB, Carr TF, Guerra S, Morgan WJ, Wright AL, Martinez FD. A Distinct Low Lung Function Trajectory from Childhood to the Fourth Decade of Life. *Am J Respir Crit Care Med* 2016; 194: 607-612.
12. Zhai J, Insel M, Addison KJ, Stern DA, Pederson W, Dy A, Rojas-Quintero J, Owen CA, Sherrill DL, Morgan W, Wright AL, Halonen M, Martinez FD, Kraft M, Guerra S, Ledford JG. Club Cell Secretory Protein Deficiency Leads to Altered Lung Function. *Am J Respir Crit Care Med* 2019; 199: 302-312.
13. Johnson MDL, Younis US, Menghani SV, Addison KJ, Whalen M, Pilon AL, Cress AE, Polverino F, Romanoski CE, Kraft M, Martinez FD, Guerra S, Ledford JG. CC16 Binding to alpha4beta1 Integrin Protects against Mycoplasma pneumoniae Infection. *Am J Respir Crit Care Med* 2021; 203: 1410-1418.

14. Bui DS, Agusti A, Walters H, Lodge C, Perret JL, Lowe A, Bowatte G, Cassim R, Hamilton GS, Frith P, James A, Thomas PS, Jarvis D, Abramson MJ, Faner R, Dharmage SC. Lung function trajectory and biomarkers in the Tasmanian Longitudinal Health Study. *ERJ Open Res* 2021; 7.
15. Ebert LM, Schaerli P, Moser B. Chemokine-mediated control of T cell traffic in lymphoid and peripheral tissues. *Mol Immunol* 2005; 42: 799-809.
16. Kaur D, Saunders R, Berger P, Siddiqui S, Woodman L, Wardlaw A, Bradding P, Brightling CE. Airway smooth muscle and mast cell-derived CC chemokine ligand 19 mediate airway smooth muscle migration in asthma. *Am J Respir Crit Care Med* 2006; 174: 1179-1188.
17. Faner R, Cruz T, Casserras T, Lopez-Giraldo A, Noell G, Coca I, Tal-Singer R, Miller B, Rodriguez-Roisin R, Spira A, Kalko SG, Agusti A. Network Analysis of Lung Transcriptomics Reveals a Distinct B Cell Signature in Emphysema. *Am J Respir Crit Care Med* 2016.
18. Jindal SK. Dutch hypothesis: revisited? *Chest* 2004; 126: 329-331.

Table 1. Selected characteristics, and biomarkers, in young individuals (25-35 yrs) with $FEV_1 \geq$ or <LLN. ¥ indicates that the variable was included in the step-wise selection for logistic regression (Figure 1).

	FEV_1 <LLN		$FEV_1 \geq$LLN		P-value
	N	**Mean ± SD or number (%)**	**N**	**Mean ± SD or number (%)**	
Demographics and exposures					
Sex Female¥	147	88 (59.86%)	153	88 (57.52%)	0.725
Age, yrs¥	147	29.37 ± 3.22	153	29.84 ± 2.97	0.149
Body Mass Index kg/m2¥	147	25.15±4.46	153	24.65±3.43	0.68
Smoking status > or < 5 py¥					
Ever smoker with > 5 packs-year	147	0 (0%)	153	0 (0%)	1.00
Ever smoker with <= 5 packs-year		98 (66.67%)		102 (66.67%)	
Never smoker		49 (33.33%)		51 (33.33%)	
Tobacco exposure¥					
Second hand, exposed and more hours than the average (40 min)	141	18 (12.77%)	149	18 (12.08%)	0.977
Second hand, Exposed but less hours than the average (40 min)		12 (8.51%)		12 (8.05%)	
No exposed		111 (78.72%)		119 (79.87%)	
Age of starting smoking and smoking status > or < 5 py¥					
Ever smoker with <= 5 packs-year and age of starting above average (15.8 yrs)	146	57 (39.04%)	152	60 (39.47%)	1.00
Ever smoker with <= 5 packs-year and age of starting below average (15.8 yrs)		40 (27.4%)		41 (26.97%)	
Never smoker		49 (33.56%)		51 (33.55%)	

Early life events					
Pregnancy duration (weeks) ¥	144	33.82±13.32	152	34.36±13.41	0.079
Mother ever smoked regularly during your childhood? ¥	146	65 (44.52%)	153	59 (38.56%)	0.348
Education level¥					
Low Education	147	8 (5.44%)	152	3 (1.97%)	0.331
Medium Education		72 (48.98%)		71 (46.71%)	
High Education		66 (44.9%)		77 (50.66%)	
Respiratory diagnoses and symptoms					
Ever asthma diagnosed by doctor¥	138	13 (9.42%)	145	16 (11.03%)	0.699
Asthma onset ¥					
Asthma and onset above the average (9.7 yrs)	136	<10	143	<10	0.723
Asthma and onset below the average (9.7 yrs)		<10		<10	
No asthma		125 (91.91%)		129 (90.21%)	
Have you ever suffered from wheezing? ¥	147	38 (25.85%)	153	21 (13.73%)	**0.009**
Do you at times have breathing problems? ¥	145	39 (26.9%)	152	28 (18.42%)	0.096
Do you usually cough in winter during daytime or at night? ¥	147	18 (12.24%)	153	21 (13.73%)	0.734
Respiratory medicines¥	147	21 (14.29%)	152	19 (12.5%)	0.735
Allergies					
Known allergies¥	147	65 (44.22%)	153	71 (46.41%)	0.914
Nasal allergy (including hay fever) ¥	147	42 (28.57%)	153	42 (27.45%)	0.898
Spirometry					
FEV1 % ref.	147	74.71±4.96	153	101.33±8.13	**0**
FVC % ref.	147	83.61±8.76	153	103.64±8.56	**0**
FEV1/FVC (L)	147	0.75±0.07	153	0.81±0.06	**0**

Analytics					
Leukocytes (10E9/L) ¥	147	6.15±1.6	153	5.87±1.51	0.099
Lymphocytes (10E9/L)	141	2.12±0.61	150	2.04±0.55	0.249
Neutrophil Granulocytes (10E9/L)	141	3.24±1.03	150	3.16±1.12	0.351
Monocytes (10E9/L)	141	0.46±0.12	150	0.45±0.14	0.217
Eosinophil Granulocytes (10E9/L)	141	0.19±0.12	150	0.16±0.12	**0.035**
Triglycerides (mmol/L) ¥	147	1.11±0.56	152	0.96±0.51	**0.002**
Creatinine (umol/L)	147	71.29±11.44	152	73.63±10.76	**0.046**
HbA1c (%)¥	147	5.41±0.35	153	5.35±0.28	0.224
Hematocrit (v/v) ¥	147	0.42±0.03	153	0.42±0.04	0.534
HDL Cholesterol (mmol/L) ¥	147	1.43±0.35	152	1.48±0.32	**0.047**
LDL Cholesterol (mmol/L) ¥	147	2.81±0.76	152	2.75±0.84	0.345
Cardiovascular					
Heart rate ¥	147	68.16±12.91	153	66.28±9.95	0.309
Systolic Blood Pressure in mm Hg ¥	147	120.64±12.9	153	120.3±10.41	0.964
Diastolic Blood Pressure in mm Hg ¥	147	69.86±8.13	153	69.71±6.31	0.714
Non-respiratory health problems					
Diabetes mellitus ¥	146	<10	153	0 (0%)	0.238
Heart valve problems ¥	147	<10	153	<10	1.000
Rheumatoid arthritis (joint inflammation) ¥	147	<10	153	<10	1.000
Hypertension ¥	147	<10	153	<10	0.614
Biomarkers					
BNDF (pg/mL) ¥	147	26.56 ± 17.91	153	27.62 ± 21.81	0.521
CCL18 (pg/mL) ¥	147	49.33 ± 44.07	153	45.92 ± 38.34	0.766
Collagen-1α (pg/mL) ¥	147	10.86 ± 16.99	153	12.1 ± 20.99	0.157
CCL19 (pg/mL) ¥	147	0.05 ± 0.05	153	0.04 ±0.04	**0.045**
CCL2 (pg/mL) ¥	147	0.27 ± 0.3	153	0.23 ± 0.3	0.219

IL-8 (pg/mL) ¥	147	0.59 ± 1.26	153	0.90 ± 1.93	0.223
Leptin (pg/mL) ¥	147	17.41 ± 32.4	153	10.09 ± 20.95	**0.01**
Pentraxin 3 (pg/mL) ¥	147	0.62 ± 1.38	153	0.74 ± 1.32	0.528
sRAGE (pg/mL) ¥	147	2.53 ± 1.72	153	2.79 ± 1.49	0.246
SP-D (pg/mL) ¥	147	10.76 ± 8.25	153	10.70 ± 8.77	0.609
vWFA-2 (pg/mL) ¥	147	0.03 ± 0.05	153	0.03 ± 0.06	0.263
CC16 (pg/mL) ¥	147	13.51 ± 21.85	153	22.06 ± 25.62	**0.003**
Aging biomarkers					
Telomere lenght (R/S ratio)	143	67.05±42.04	152	70.1±33.61	0.358
Mitochondrial DNA qPCR (12s/RNAsa P)	141	8.67±3.65	151	8.53±3.43	0.438

FIGURE LEGEND

Figure 1. Forest plot showing factors independently associated with FEV_1<LLN identified by the multivariate logistic regression in 299 individuals (152 FEV_1 > LLN and 147 FEV_1 < LLN). The C-index of the logistic regression was (0.722). The variables included in the model were the ones that minimized the Akaike information criterion (AIC), which indicates a better goodness-of-fit. OR are per increase of 1 SD in the values of the log-scaled biochemical and biomarkers measurements. For instance, the odds ratio for CC16 (0.59) indicates that for every 1 SD increase in CC16 levels, there is about a 41% decrease in the odds of having FEV_1< LLN. Abbreviations: BNDF=Brain-derived neurotrophic factor; CCL18=C-C Motif Chemokine Ligand 18; CCL19=C-C Motif Chemokine Ligand 19; CCL2=C-C Motif Chemokine Ligand 2; IL-8= Interleukine-8; sRAGE=Soluble Receptor for Advanced Glycation End Products; SP-D= Surfactant Protein D; vWFA-2= von Willebrand Factor 2; CC16=Club cell secretory protein.