

Inflammatory imbalance in tracheal aspirate of very preterm newborns is associated with airway obstruction and lung function deficiencies at school-age: a cohort study

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Online supplement

Methods

Tracheal aspirates were analysed, as previously described [S1], for CC16 (using the Human Club cell Protein ELISA kit from Biovendor, Modrice, Czech Republic), proinflammatory and modulatory cytokines (IL-1 β , IL-6, IL-10, monocyte chemoattractant protein (MCP)-1 and TNF- α) and vascular endothelial growth factor (VEGF) using a Bio-Plex Human Cytokine assay (Bio-Rad Laboratories, Hercules, CA), the protease MMP-9 (using Milliplex Human MMP panel, Merck Millipore, Darmstadt, Germany), and total protein (using Bradford Protein assay, Bio-Rad Laboratories).

Lung function: predicted values at 12 years of age

Predicted individual normal values and limits of normal for spirometry, static lung volumes and diffusing capacity were calculated from age, height and sex according to the Global Lung Initiative, GLI [S2-S4]. We report these results as percent of predicted and z-score (median and interquartile range) as well as proportion of children outside of normal limits. For each test, GLI defines the individual lower limit of normal (LLN) as -1.645 z-scores, and the upper limit of normal (ULN) as +1.645 z-scores. For impulse oscillometry, airway resistance by body plethysmography, and multiple breath washout, normal values according to GLI are not available. For these tests, we chose to define limits of normal as the 5th and 95th centiles of 56 children born at term and examined by us [S5], also at 12 years of age (median 12.0, range 11.7 - 12.7): Impulse oscillometry; R₅: ULN=0.71 kPa*s/L, R₂₀: ULN=0.53 kPa*s/L, R₅-R₂₀: ULN=0.22 kPa*s/L, X₅: LLN= -0.21 kPa*s/L, F_{res}: ULN=23.52 1/s, AX: ULN=1.70 kPa/L. Body plethysmography; R_{in}: ULN=0.65 kPa*s/L, R_{ex}: ULN=0.87 kPa*s/L and R_{tot}: ULN=0.65 kPa*s/L. Multiple breath washout: LCI_{2.5}: ULN=7.44, LCI_{5.0}: ULN=5.38, S_{cond}: ULN=0.046 and S_{acin}: ULN=0.11.

References

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Supplementary tables

Table S1. Lung function results at the age of 12 in all 41 children who performed lung function tests and in the 20 children from whom tracheal aspirate was collected at birth compared to the 21 children from whom no tracheal aspirate was collected.

	Lung function results in all children	Lung function results in children with tracheal aspirate	Lung function in children without tracheal aspirate	P-value
Spirometry	(n=41)	(n=20)	(n=21)	
FEV1, L	2.19 (2.06 to 2.45)	2.19 (2.06-2.56)	2.19 (1.95 to 2.43)	0.63
FEV1, %pred	89 (83-98)	92 (85-98)	89 (80 to 98)	0.53
FEV1, z-score	-0.94 (-1.48 to -0.18)	-0.67 (-1.28- -0.20)	-0.97 (-1.68 to -0.17)	0.53
FEV1 < LLN, n	9 (22%)	4 (20%)	5 (24%)	1.0
FVC, L	2.71 (2.47 to 3.06)	2.71 (2.50-3.08)	2.71 (2.40 to 2.92)	0.85
FVC, z-score	-0.38 (-1.05 to 0.23)	-0.18 (-0.91-0.21)	-0.43 (-1.12 to 0.26)	0.55
FVC < LLN, n	3 (7%)	2 (10%)	1 (5%)	0.61
FEV1/FVC	0.83 (0.78 to 0.86)	0.84 (0.78-0.85)	0.83 (0.76 to 0.87)	0.77
FEV1/FVC, z-score	-0.85 (-1.62 to -0.14)	-0.84 (-1.18- -0.11)	-0.94 (-1.69 to -0.14)	0.58
FEV1/FVC < LLN, n	9 (22%)	3 (15%)	6 (29%)	0.45
FEF ₂₅₋₇₅ , L/s	2.13 (1.74 to 2.73)	2.28 (1.86-2.73)	1.92 (1.67 to 2.77)	0.47
FEF ₂₅₋₇₅ , z-score	-1.46 (-2.00 to -0.41)	-1.34 (-1.68- -0.48)	-1.68 (-2.28 to -0.41)	0.36
FEF ₂₅₋₇₅ < LLN, n	15 (38%)	5 (25%)	10 (50%)	0.10
VC, L	2.74 (2.52 to 3.09)	2.77 (2.54-3.14)	2.74 (2.43 to 2.93)	0.70
VC, z-score	-0.59 (-1.35 to -0.19)	-0.60 (-1.13- -0.19)	-0.50 (-1.44 to -0.21)	0.68
VC < LLN, n	3 (7%)	1 (5%)	2 (10%)	1.0
Impulse oscillometry	(n=41)	(n=20)	(n=21)	
R _s , kPa*s/L	0.52 (0.44 to 0.61)	0.48 (0.44-0.60)	0.55 (0.47 to 0.63)	0.26
R _s >95th perc, n	5 (12%)	2 (10%)	3 (14%)	1.0
R ₂₀ , kPa*s/L	0.39 (0.32 to 0.44)	0.37 (0.32-0.43)	0.40 (0.32 to .46)	0.38
R ₂₀ >95th perc, n	5 (12%)	1 (5%)	4 (19%)	0.34
R _s -R ₂₀ , kPa*s/L	0.15 (0.08 to 0.18)	0.14 (0.07-0.20)	0.16 (0.09 to 0.18)	0.65
R _s -R ₂₀ >95th perc, n	7 (17%)	3 (15%)	4 (19%)	1.0
X _s , kPa*s/L	-0.18 (-0.20 - -0.14)	-0.16 (-0.20- -0.14)	-0.19 (-0.22 to -0.15)	0.30
X _s , <5th perc, n	9 (22%)	3 (15%)	6 (29%)	0.45
F _{res} , Hz	17.9 (15.3-20.2)	17.0 (15.2-20.2)	18.3 (15.4 to 20.3)	0.60

F _{res} >95th perc, n	3 (7%)	2 (10%)	1 (5%)	0.61
AX, kPa/L	1.13 (0.57-1.40)	0.88 (0.55-1.53)	1.15 (0.78 to 1.40)	0.61
AX >95th perc, n	6 (15%)	4 (20%)	2 (10%)	0.41
Body plethysmography	(n=39)	(n=18)	(n=21)	
R _{insp} , kPa*s/L	0.47 (0.39-0.58)	0.43 (0.34-0.55)	0.53 (0.43 to 0.59)	0.06
R _{insp} >95th perc, n	5 (13%)	2 (11%)	3 (14%)	1.0
R _{exp} , kPa*s/L	0.72 (0.60-0.83)	0.69 (0.58-0.86)	0.74 (0.63 to 0.82)	0.75
R _{exp} >95th perc, n	6 (15%)	4 (22%)	2 (10%)	0.39
R _{tot} , kPa*s/L	0.57 (0.46-0.65)	0.49 (0.45-0.64)	0.61 (0.51 to 0.66)	0.19
R _{tot} >95th perc, n	11 (28%)	4 (22%)	7 (33%)	0.44
	(n=39)	(n=17)	(n=21)	
TLC, L	3.65 (3.22-3.93)	3.70 (3.21-4.06)	3.51 (3.21 to 3.89)	0.40
TLC, z-score	-0.35 (-0.78-0.19)	-0.09 (-0.70-0.36)	-0.38 (-0.80 to -0.20)	0.19
TLC < LLN, n	0 (0%)	0 (0%)	0 (0%)	n.a.
RV, L	0.88 (0.71-1.02)	0.97 (0.71-1.29)	0.83 (0.66 to 0.97)	0.13
RV, z-score	0.09 (-0.47-0.44)	0.22 (-0.33-0.80)	-0.001 (-0.62 to 0.22)	0.11
RV > ULN, n	1 (3%)	1 (6%)	0 (0%)	0.45
RV/TLC	0.25 (0.20-0.29)	0.26 (0.22-0.32)	0.24 (0.19 to 0.27)	0.23
RV/TLC, z-score	0.37 (-0.24-0.83)	0.47 (-0.14-1.11)	0.27 (-0.36 to 0.65)	0.23
RV/TLC > ULN, n	1 (3%)	1 (6%)	0 (0%)	0.45
Diffusion capacity	(n=39)	(n=18)	n=21	
DL _{CO} , mmol/(min*kPa)	6.0 (5.6-6.8)	6.1 (5.6-7.0)	5.8 (5.6 to 6.7)	0.43
DL _{CO} , z-score	-0.13 (0.78-0.31)	0.12 (-0.50-0.35)	-0.29 (-0.85 to 0.33)	0.27
DL _{CO} , < LLN, n	1 (3%)	0 (0%)	1 (5%)	1.0
Alveolar volume, L	3.5 (3.2-3.9)	3.6 (3.2-4.1)	3.5 (3.1 to 3.8)	0.48
Alveolar volume, z-score	-0.04 (-0.59-0.48)	0.16 (-0.33-0.70)	-0.15 (-0.65 to 0.26)	0.22
Alveolar volume, < LLN, n	0 (0%)	0 (0%)	0 (0%)	n.a.
K _{CO} , mmol/(min*kPa*L)	1.7 (1.6-1.8)	1.8 (1.6-1.8)	1.7 (1.6 to 1.8)	0.81
K _{CO} , z-score	-0.15 (-0.66-0.25)	-0.07 (-0.57-0.24)	-0.15 (-0.66 to 0.29)	0.81
K _{CO} < LLN, n	1 (3%)	0 (0%)	1 (5%)	1.0
Multiple breath washout	(n=39)	(n=19)	n=20	
LCl _{2.5}	7.04 (6.58-7.35)	7.05 (6.61-7.62)	6.96 (6.51 to 7.24)	0.15
LCl _{2.5} >95 th perc, n	6 (15%)	6 (32%)	0 (0%)	0.008
LCl _{5.0}	5.07 (4.70-5.29)	5.16 (4.72-5.43)	4.99 (4.69 to 5.26)	0.22
LCl _{5.0} >95 th perc, n	8 (21%)	6 (32%)	2 (10%)	0.13
S _{cond} x VT	0.038 (0.027-0.046)	0.038 (0.032-0.053)	0.037 (0.026-0.045)	0.59
S _{cond} x VT >95 th perc, n	9 (23%)	5 (26%)	4 (20%)	0.72
S _{acin} x VT	0.052 (0.035-0.071)	0.063 (0.035-0.088)	0.049 (0.034-0.069)	0.35
S _{acin} x VT >95 th perc, n	2 (5%)	1 (5%)	1 (5%)	1.0

Lung function results are presented as median (interquartile range) or as proportions, n (%). The actual number (n) is given for the respective test, except for FEF₂₅₋₇₅ where data from one child is missing.

Z-score and %pred (=percent of predicted) are calculated according to the Global Lung Initiative (GLI); perc=percentile; FEV₁=forced expiratory volume in 1 sec; FVC=forced vital capacity; FEF₂₅₋₇₅= mean forced expiratory flow between 25% and 75% of FVC; R₅, R₂₀= respiratory resistance at 5 and 20 Hz, respectively; R₅-R₂₀= frequency dependence of resistance; X₅= respiratory reactance at 5 Hz; F_{res}=resonant frequency; AX= area under the reactance curve between 5 Hz and F_{res}; R_{insp}= inspiratory flow resistance of the airways; R_{exp}= expiratory flow resistance of the airways; R_{tot}= total flow resistance of the airways; LCl_{5.0} and LCl_{2.5}= lung clearance index, defined as the number of lung volume turnovers required to reach 5% and 2.5% of initial end-tidal N₂-concentration; S_{cond} x VT= an index of ventilation inhomogeneity in conductive airways; S_{acin} x VT= an index of ventilation inhomogeneity in intra-acinar airways; LLN and ULN= lower and upper limit of normal according to GLI. P-value=difference between children who had tracheal aspirate collected at birth compared to those who had not tracheal aspirate collected at birth, tested using Mann-Whitney U-test or Fisher's exact test when appropriate.

Table S2. Correlations of additional lung function results at 12 years of age in relation to CC16, IL-1 β , and TNF- α (and ratios) in tracheal aspirate at birth.

	CC16 in tracheal aspirate (n=20)	IL-1 β in tracheal aspirate (n=18)	TNF α in tracheal aspirate (n=18)	IL-1 β /CC16 ratio in tracheal aspirate (n=18)	TNF- α /CC16 ratio in tracheal aspirate (n=18)
Spirometry					
FVC, L	r=-0.37, p=0.11	r=-0.25, p=0.31	r=-0.53, p=0.023	r=-0.044, p=0.86	r=-0.084, p=0.74
FVC, z-score	r=-0.11, p=0.64	r=0.19, p=0.43	r=-0.13, p=0.62	r=-0.35, p=0.15	r=-0.13, p=0.60
VC, L	r=-0.17, p=0.47	r=-0.34, p=0.28	r=-0.42, p=0.08	r=-0.29, p=0.25	r=-0.15, p=0.55
VC, z-score	r=0.10, p=0.68	r=0.22, p=0.39	r=0.10, p=0.69	r=0.15, p=0.55	r=0.13, p=0.62
Body plethysmography					
TLC, L	r=-0.49, p=0.85	r=-0.30, p=0.25	r=-0.23, p=0.40	r=-0.24, p=0.36	r=0.015, p=0.96
TLC, z-score	r=0.28, p=0.28	r=0.11, p=0.69	r=0.42, p=0.11	r=0.047, p=0.86	r=0.30, p=0.26
RV, L	r=0.01, p=0.98	r=-0.21, p=0.45	r=0.15, p=0.59	r=-0.060, p=0.82	r=0.26, p=0.33
RV, z-score	r=0.03, p=0.91	r=-0.01, p=0.97	r=0.34, p=0.19	r=0.16, p=0.56	r=0.43, p=0.099
RV/TLC	r=-0.002, p=0.99	r=-0.15, p=0.59	r=0.22, p=0.41	r=0.029, p=0.91	r=0.30, p=0.25
RV/TLC, z-score	r=-0.002, p=0.99	r=-0.16, p=0.56	r=0.24, p=0.38	r=0.029, p=0.91	r=0.30, p=0.25

The table shows lung function variables where less than 10% of the children had results outside the normal range. Lung function results are presented as median (interquartile range). The actual number of children who completed the procedure (n) is given for the respective test. Correlations were calculated by Spearman's rho, and P-values <0.05 were considered significant and are marked as bold. FVC=forced vital capacity; VC= (slow) vital capacity; TLC= total lung capacity; RV= residual volume; DL_{CO}=diffusion capacity of the lung for carbon monoxide; K_{CO}= diffusion coefficient for carbon monoxide; z-scores are according to the Global Lung Initiative (GLI).