ON LINE SUPPLEMENTARY MATERIAL

METHODS

This was a prospective longitudinal, multicentre observational study, performed from June 2011 to December 2013 at three Spanish university hospitals: Hospital Universitario Vall d'Hebron (Barcelona), Hospital Universitario Donostia (San Sebastián) and Hospital Universitario Virgen de la Arrixaca (Murcia).

Premature healthy infants born 30 weeks to 35 weeks+6 days gestation age (GA) and a control group of contemporaneous healthy term infants (≥37 weeks GA and birth weight ≥2.5 kg) were recruited at any time before 6 months postnatal age. GA in both infant groups was calculated from the date of maternal last menstrual period and confirmation by an ultrasound scan performed before 20 weeks of pregnancy. All infants were of singleton pregnancy.

Preterm babies were recruited from their hospitals of birth while the control group was recruited from a pool of healthy infants who attended a hospital clinic for cystic fibrosis screening (Barcelona) and also from primary care centres near the other two collaborating University hospitals (San Sebastián and Murcia).

None of the preterm infants required ventilatory support and had less than 24 hours supplemental oxygen following delivery. Infants with any of the following characteristics were excluded from the study: neonatal respiratory disease (respiratory distress syndrome, acute pneumonia, acute bronchiolitis), requiring invasive ventilation, or oxygen support for more than 24 hours during the neonatal period, having two or more episodes of bronchitis or a history of respiratory illness requiring hospitalization before the age of 6 months, frequent colds or upper respiratory illness making LF test results unsatisfactory, or with chronic diseases such as congenital heart diseases, congenital respiratory or gastrointestinal malformations, chromosomal abnormalities, chronic respiratory, renal, gastrointestinal and neurological diseases or primary or secondary immunodeficiencies.

Lung function data obtained from some of the term control infants had been included in a previous multicentre study to develop reference ranges for forced respiratory manoeuvres in infants.[17]

Local Research Ethics Committee approval was granted and written parental consent was obtained for all infants. The study was carried out in accordance to the international ethical recommendations for research and clinical studies in humans contained in the Declaration of Helsinki, standards of Good Clinical Practice, as well as the recommendations of the Spanish Agency for Medicines in the matter of clinical studies. The results obtained were stored electronically in accordance with current data protection laws.

Participating centres prospectively completed Case Record Forms (CRF) at recruitment and at each subsequent visit. A detailed perinatal history was compiled including: family history of atopic disorders and passive smoking (parents and siblings), data from pregnancy (gestational age, mother's age and ethnicity, number of fetuses), delivery data (mode of delivery, Apgar score, weight, length gender) and any peri – and postnatal pathology. The children respiratory symptoms or illnesses since birth and up to the age of 18 months were recorded, including the number of wheezing episodes.

Infant lung function tests.

All infants were tested at one of the three hospitals at around 6 [Test 1, (T1)] and 18 months [Test 2, (T2)] corrected age (Table 1). Lung function tests were done when infants were well or at least 3 weeks after any respiratory symptoms or infection. Data were recorded during quiet sleep, in supine position, and after administration of chloral hydrate orally (50-100 mg/kg; orally)[18,19]. Oxygen saturation and heart rate were monitored before sedation and continuously during testing. Weight and crown–heel length were measured using digital scales and a regularly calibrated stadiometer. weight and Length z-score were calculated by means of WHO reference values for child growth.[20]

Infant lung function was assessed using the Jaeger MasterScreen BabyBody System (v.4.65; Carefusion, Hoechberg, Germany). Measurements of tidal breathing, passive respiratory mechanics (total respiratory compliance, Crs, and total respiratory resistance, Rrs), tidal volume and raised volume forced expirations (tidal RTC and RVRTC, respectively) were undertaken according to ATS/ERS recommendations[21] and international guidelines.[22,23,24] The forced vital capacity (FVC), the forced expiratory volume at 0.5 seconds of FVC ($FEV_{0.5}$), the ratio $FEV_{0.5}$/FVC and the forced expired flows (FEF) at 50%, 75% and 25-75% of the FVC (FEF_{50}, FEF_{75}, FEF_{25-75}) were reported from the "best" out of three technically acceptable forced expiratory curve with the biggest sum of FVC and $FEV_{0.5}$.

Researchers from the three Spanish centres were trained by the infant lung function team at Respiratory, Critical Care & Anaesthesia section, UCL Great Ormond Street Institute of Child Health, London, United Kingdom. They developed identical study protocols for lung function testing, data analysis and quality control standards. The equipment software and the parameters measured were also standardized. AFH performed inter-laboratory visits for independent over-read of results to ensure quality control. [19,21]

Statistical analysis and sample size

Demographic and clinical data were collected prospectively on an electronic case report form (W3NEXUS, Fundació Institut Català de Farmacologia, Barcelona, Spain).

Lung function results were expressed as absolute values and converted to z-scores to adjust for gestation, age, length, weight, and sex, according to international reference equations.[17,25,26] Corrected age instead of chronological age was used for calculations of z-score in preterm infants.

Data were analysed by using SAS® 9.3 (SAS Institute Inc., Cary, NC, USA). Descriptive data are shown as mean (SD) for continuous variables and as n (%) for categorical ones. Comparisons between the preterm and the term infants were analysed using independent sample t-tests with 95% confidence intervals (CI) for continuous data and chi-square or Fisher exact tests where appropriate for categorical outcomes. A general lineal multivariable analysis was performed to adjust the comparisons according to maternal tobacco use during pregnancy and need for oxygen therapy the first 24 hours of life. Statistical significance was set at a *P* value of < 0.05.

It was estimated that 37 infants per group would provide 80% power to detect a difference of 0.67 z-scores (assuming SD 1 for both groups) between preterm and full-term infants for the lung function variables.

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Table S1: Proportion of valid tests at Test 1 ~6 months, and Test 2, ~18 months

	Test 1, ~6 months					Test 2, ~18 months				
			Valid tests					Valid tests		
	Test not done (a)	Non valid test (b)	Preterm (n=74)	Term (n=76)	Total (n=150)	Test not done (a)	Non valid test (b)	Preterm (n=57)	Term (n=61)	Total (n=118)
Tidal breathing, n (%)	9 (6%)	7 (4.7%)	65 (87.8%)	69 (90.8%)	134 (89.3%)	5 (4.2%)	4 (3.4%)	54 (94.7%)	55 (90.2%)	109 (92.2%)
Passive respiratory mechanics, n (%)	28 (18.7%)	57 (38%)	26 (35.1%)	39 (51.3%)	65 (43.3%)	13 (11%)	35 (29.7%)	36 (63.1%)	34 (55.7%)	70 (59.3%)
Tidal forced expiration, n (%)	6 (4%)	6 (4%)	70 (94.6%)	68 (89.5%)	138 (92.0%)	8 (6.8%)	0 (0%)	52 (91.2%)	58 (95.1%)	110 (93.2%)
Raised volume forced expiration, n (%)	34 (22.7%)	33 (22%)	43 (58.1%)	40 (52.6%)	83 (55.3%)	12 (10.2%)	10 (8.5%)	43 (75.4%)	53 (86.9%)	96 (81.4%)

a: infant did not sleep or awaked during the test, b: test did not accomplish quality control criteria

Table S2: Lung function tests at Test 1 ~6 months. Results reported as absolute values.

	Preterm	Term	*Difference (Preterm-term) (CI 95%)*	*P*
Tidal breathing tests (n)	68	72		
Respiratory rate, bpm, mean (SD)	33.26 (4.52)	32.89 (5.90)	0.37 (-1.39; 2.13)	0.677
Tidal volume, mL, mean (SD)	65.28 (9.84)	65.50 (10.84)	-0.22 (-3.69; 3.24)	0.900
Tidal volume/kg, (mL/kg, mean (SD)	9.04 (1.04)	8.78 (1.19)	0.26 (-0.11; 0.64)	0.171
Ti,, s, mean (SD)	0.74 (0.09)	0.76 (0.13)	-0.02 (-0.06; 0.02)	0.326
Te, s, mean (SD)	1.09 (0.18)	1.11 (0.26)	-0.02 (-0.04; 0.22)	0.629
tPTEF/tE, %, mean (SD)	25.49 (7.47)	27.24 (10.94)	-1.75 (-4.92; 1.41)	0.269
Passive respiratory mechanics (n)	26	40		
Crs, mL*kPa^{-1}, mean (SD)	87.49 (12.23)	97.83 (16.46)	-10.34 (-17.87; -2.81)	0.008
Rrs, kPa*L^{-1}*sec, mean (SD)	4.24 (1.35)	3.90 (1.44)	0.34 (-0.37; 1.05)	0.345
Tidal RTC (n)	70	68		
V'maxFRC, mL/s, mean (SD)	122.90 (59.71)	150.54 (67.95)	-27.64 (-49.16; -6.13)	0.012
Raised volume RTC (n)	43	40		
FVC, mL, mean (SD)	268.28 (48.41)	278.75 (46.46)	-10.46 (-31.22; 10.28)	0.318
$FEV_{0.5}$, mL, mean (SD)	211.77 (38.78)	223.87 (34.05)	-12.10 (-28.09; 3.89)	0.136
FEV0,5/FVC, mean (SD)	0.79 (0.08)	0.81 (0.07)	-0.01 (-0.05; 0.02)	0.393
FEF_{75}, mL/s, mean (SD)	224.50 (70.83)	245.28 (53.05)	-20.78 (-48.27; 6.71)	0.136
FEF_{25-75}, mL/s, mean (SD)	386.96 (102.40)	430.71 (92.68)	-43.75 (-86.52; -0.99)	0.045

SD: standard deviation; bpm: breaths/min**;** tPTEF/tE: time to peak tidal expiratory flow to expiratory time; ti: inspiratory time; te: expiratory time; V'maxFRC: maximal expiratory flow at forced residual capacity; Crs: compliance, Rrs: resistance, tidal RTC: tidal rapid thoracoabdominal compression; RVRTC; raised volume forced expiration; FVC: forced vital capacity; $FEV_{0.5}$: the forced expired volume at 0,5 seconds; FEF_{75}, $FEF_{25-75:}$ the forced expired flows at 50%, 75% and 25-75% of the FVC.

Table S3: Lung function tests at Test 2 ~18 months. Results reported as absolute values.

	Preterm	Term	*Difference (Preterm-term) (CI 95%)*	*P*
Tidal breathing tests (n)	54	55		
Respiratory rate, bpm, mean (SD)	26.69 (3.41)	24.99 (3.37)	1.70 (0.41; 2.99)	0.010
Tidal volume, mL, mean (SD)	108.95 (17.74)	119.02 (14.07)	-10.07 (-16.14; -4.00)	0.001
Tidal volume/kg, (mL/kg, mean (SD)	10.32 (1.36)	10.72 (1.46)	-0.39 (-0.93; 0.14)	0.147
Ti,, s, mean (SD)	0.90 (0.13)	0.96 (0.16)	-0.06 (-0.12; -0.01)	0.025
Te, s, mean (SD)	1.39 (0.21)	1.49 (0.22)	-0.10 (-0.19; -0.02)	0.016
tPTEF/tE, %, mean (SD)	24.11 (7.43)	25.54 (9.77)	-1.43 (-4.73; 1.87)	0.390
Passive respiratory mechanics (n)	36	34		
Crs, mL*kPa^{-1}, mean (SD)	164.96 (39.11)	166.46 (27.08)	-1.51 (-17.64; -14.62)	0.851
Rrs, kPa*L^{-1}*sec, mean (SD)	2.57 (0.58)	2.69 (0.55)	-0.12 (-0.39; 0.15)	0.369
Tidal RTC (n)	52	58		
V'maxFRC, mL/s, mean (SD)	252.37 (112.67)	245.10 (89.69)	7.26 (-31.57; 46.09)	0.708
Raised volume RTC (n)	43	53		
FVC, mL, mean (SD)	480.46 (79.78)	483.37 (85.59)	-2.91 (-36.75; 30.93)	0.864
$FEV_{0.5}$, mL, mean (SD)	354.67 (52.78)	354.19 (54.65)	0.48 (-21.46; 22.41)	0.966
FEV0,5/FVC, mean (SD)	0.75 (0.08)	0.74 (0.08)	0.01 (-0.03; 0.04)	0.818
FEF_{75}, mL/s, mean (SD)	365.82 (85.85)	364.08 (93.89)	1.74 (-35.10; 38.59)	0.926
FEF_{25-75}, mL/s, mean (SD)	612.90 (125.80)	604.92 (123.09)	7.98 (-42.68; 58.64)	0.755

SD: standard deviation; bpm: breaths/min; tPTEF/tE: time to peak tidal expiratory flow to expiratory time; ti: inspiratory time; te: expiratory time; V'maxFRC: maximal expiratory flow at forced residual capacity; Crs: compliance, Rrs: resistance, tidal RTC: tidal rapid thoracoabdominal compression; RVRTC; raised volume forced expiration; FVC: forced vital capacity; $FEV_{0.5}$: the forced expired volume at 0,5 seconds; FEF_{75}, $FEF_{25-75:}$ the forced expired flows at 50%, 75% and 25-75% of the FVC.

Table S4. Technically acceptable lung function results at Test 1, ~6 months and Test 2, ~18 months. Side by side results for easy comparison.

	Test 1, ~6 months						Test 1, ~18 months					
	Preterm n=74	**Term n=76**	***z-score Difference (Preterm-term) (CI 95%)***	**P**	***Adjusted z-score Difference (Preterm-term) (CI 95%)***	**P**	**Preterm n=57**	**Term n=61**	***z-score Difference (Preterm-term) (CI 95%)***	**P**	***Adjusted z-score Difference (Preterm-term) (CI 95%)***	**P**
Tidal breathing tests (n=)	68	72					54	55				
Respiratory rate, Z-score; mean (SD)	0.17 (0.86)	0.14 (1.26)	0.03 (-0.33; 0.39)	0.877	0.09 (-0.30; 0.48)	0.646	0.09 (0.83)	-0.26 (0.83)	0.35 (0.03; 0.66)	0.030	0.38 (0.05; 0.70)	0.025
Tidal volume, Z-score; mean (SD)	-0.47 (0.86)	-0.59 (1.18)	0.12 (-0.23; 0.47)	0.498	0.11 (-0.26; 0.47)	0.570	0.07 (1.02)	0.52 (0.86)	-0.46 (-0.81; -0.10)	0.013	-0.46 (-0.83; -0.09)	0.017
tPTEF/tE, Z-score; mean (SD)	-0.10 (0.86)	0.02 (1.11)	-0.12 (-0.45; 0.21)	0.480	-0.18 (-0.53; 0.17)	0.305	-0.29 (0.92)	-0.18 (1.14)	-0.11 (-0.50; 0.29)	0.591	-0.09 (-0.50; 0.32)	0.675
Passive respiratory mechanics (n=)	26	40					36	34				
Crs, Z-score; mean (SD)	-0.08 (0.34)	0.24 (0.51)	-0.32 (-0.53; -0.11)	0.003	-0.36 (-0.60; -0.12)	0.004	0.20 (0.43)	0.20 (0.28)	0.00 (-0.17; 0.18)	0.984	0.00 (-0.18; 0.19)	0.954
Rrs, Z-score; mean (SD)	0.14 (1.06)	-0.28 (1.27)	0.41 (-0.16; 0.99)	0.174	0.42 (-0.23; 1.07)	0.202	-0.97 (0.89)	-0.71 (0.85)	-0.27 (-0.68; 0.15)	0.205	-0.27 (-0.70; 0.16)	0.210
Tidal RTC (n=)	70	68					52	58				
V'maxFRC, Z-score; mean (SD)	-0.28 (0.92)	0.18 (0.90)	-0.47 (-0.77; -0.16)	0.003	-0.43 (-0.76; -0.11)	0.010	0.85 (1.24)	0.76 (0.98)	0.10 (-0.31; 0.52)	0.621	0.07 (-0.37; 0.50)	0.762
Raised volume RTC (n=)	43	40					43	53				
FVC, Z-score; mean (SD)	-0.49 (1.07)	-0.38 (0.96)	-0.12 (-0.56; -0.33)	0.603	-0.04 (-0.51; 0.43)	0.870	-0.27 (0.98)	-0.34 (1.00)	0.07 (-0.34; 0.47)	0.734	0.18 (-0.25; 0.60)	0.407
$FEV_{0.5}$, Z-score; mean (SD))	-0.59 (1.15)	-0.35 (0.92)	-0.24 (-0.70; 0.22)	0.303	-0.20 (-0.68; 0.28)	0.414	-0.09 (1.01)	-0.19 (1.09)	0.10 (-0.33; 0.53)	0.633	0.18 (-0.31; 0.53)	0.601
$FEV_{0.5}$/FVC, Z-score; mean (SD)	-0.07 (0.68)	0.07 (0.58)	-0.13 (-0.41; 0.14)	0.340	-0.16 (-0.44; 0.13)	0.278	0.11 (0.36)	0.11 (0.36)	0.00 (-0.15; 0.15)	0.983	-0.02 (-0.17; 0.13)	0.508
FEF_{75}, Z-score; mean (SD)	-0.36 (1.14)	-0.11 (0.79)	-0.25 (-0.68; 0.18)	0.249	-0.31 (-0.75; 0.14)	0.179	0.10 (0.93)	0.06 (1.04)	0.04 (-0.36; 0.45)	0.831	0.07 (-0.35; 0.48)	0.750
FEF_{25-75}, Z-score; mean (SD)	-0.57 (1.14)	-0.15 (0.95)	-0.42 (-0.88; 0.03)	0.069	-0.46 (-0.93; 0.02)	0.059	0.10 (0.97)	0.01 (0.98)	0.09 (-0.31; 0.49)	0.664	0.09 (-0.30; 0.49)	0.645

*Adjusted difference for smoking mother during pregnancy and the need for oxygen in the first 24 hours of life.
SD: standard deviation; tPTEF/tE: time to peak tidal expiratory flow to expiratory time; V'maxFRC: maximal expiratory flow at forced residual capacity; Crs: compliance, Rrs: resistance, tidal RTC: tidal rapid thoracoabdominal compression; RVRTC; raised volume forced expiration; FVC: forced vital capacity; $FEV_{0.5}$: the forced expired volume at 0,5 seconds; FEF_{75}, $FEF_{25-75:}$ the forced expired flows at 50%, 75% and 25-75% of the FVC.

Table S5: Catch-up of Lung function tests between Test 1, ~6 months and Test 2, ~18 months. Unadjusted data.

	Preterm ***z-score Difference (CI 95%)***	**P**	***Term*** *z-score Difference (CI 95%)*	**P**	***Preterm - term*** *z-score Difference (CI 95%)*	**P**
Tidal breathing tests (n)	68		72			
Respiratory rate [Z-score; mean (CI 95%)]	-0.12 (-0.39; 0.14)	0.363	-0.38 (-0.64; -0.12)	0.004	0.26 (-0.11; 0.63)	0.173
Tidal volume [Z-score; mean (CI 95%)]	0.56 (0.27-0.84)	< 0.001	1.06 (0.77; 1.34)	< 0.001	-0.50 (-0.90; -0.09)	0.016
tPTEF/tE [Z-score; mean (CI 95%)]	-0.16 (-0.46; 0.13)	0.277	-0.20 (-0.49; 0.08)	0.162	0.04 (-0.37; 0.46)	0.841
Passive respiratory mechanics (n)	26		40			
Crs [Z-score; mean (CI 95%)]	0.30 (0.13; 0.48)	0.001	-0.04 (-0.18; 0.11)	0.615	0.34 (0.11; 0.56)	0.004
Rrs [Z-score; mean (CI 95%)]	-1.14 (-1.66; -0.62)	< 0.001	-0.43 (-0.88; 0.03)	0.068	-0.72 (-1.41; -0.02)	0.042
Tidal RTC (n)	70		68			
V'maxFRC [Z-score; mean (CI 95%)]	1.04 (0.76; 1.33)	<0.001	0.58 (0.31; 0.86)	< 0.001	0.46 (0.07; 0.85)	0.022
Raised volume RTC (n)	43		40			
FVC [Z-score; mean (CI 95%)]	0.17 (-0.18; 0.52)	0.342	-0.00 (-0.36; 0.35)	0.981	0.17 (-0.33; 0.67)	0.489
$FEV_{0.5}$ [Z-score; mean (CI 95%)]	0.42 ((0.04-0.79)	0.030	0.20 (-0.17; 0.57)	0.292	0.22 (-0.31; 0.75)	0.411
$FEV_{0.5}$/FVC [Z-score; mean (CI 95%)]	0.17 (-0.02; 0.36)	0.073	0.09 (-0.09; 0.28)	0.322	0.07 (-0.19; 0.34)	0.577
FEF_{75} [Z-score; mean (CI 95%)]	0.39 (0.05; 0.72)	0.025	0.27 (-0.06; 0.60)	0.110	0.12 (-0.35; 0.59)	0.619
FEF_{25-75} [Z-score; mean (CI 95%)]	0.56 (0.22; 0.90)	0.001	0.24 (-0.09; 0.58)	0.153	0.32 (-0.16; 0.79)	0.190

SD: standard deviation; tPTEF/tE: time to peak tidal expiratory flow to expiratory time; V'maxFRC: maximal expiratory flow at forced residual capacity; Crs: compliance, Rrs: resistance, tidal RTC: tidal rapid thoracoabdominal compression; RVRTC; raised volume forced expiration; FVC: forced vital capacity; $FEV_{0.5}$: the forced expired volume at 0,5 seconds; FEF_{75}, FEF_{25-75}: the forced expired flows at 50%, 75% and 25-75% of the FVC.