T105403 Final Report

Actual *vs* original project plan

Thematics. Briefly, our research activity in the birth cohort layer was transformed into a bilateral collaborative follow-up research (Drakenstein birth cohort study, South Africa), which employed our infant lung function technique as a main methodological component immediately after its first feasibility testing in Szeged newborns. While we encountered serious limitations of infrastructural and logistic nature in building up longitudinal studies in neonates born at the Szeged University clinics, the South African cohort study provided us with an impressively organised research setting and a very competent use of our technique, in addition to the challenge of preclinical factors more accentuated in this low socio-economic environment. In turn, the Szeged group was able to concentrate more on the technical design and physiological exploration of measurements, and this resulted in a methodological development reaching far beyond the original plans. In particular, the intrabreath tracking mode of the forced oscillation technique (T-FOT), first introduced in our newborn infant studies, proved to be a cutting-edge technique in diagnosing preschooler asthma and adult COPD, and formed the basis of a Clinical Research Collaboration mentored by the European Respiratory Society (ERS). Enrichment in the FOT methodology has attracted partners abroad with new applications that were not included in the original project plan, such as lung mechanics monitoring via laryngeal airway masks employed in paediatric surgery (Perth, Australia), model measurements of infant upper airways on 3-D prints of reconstructed CT images (Amiens and Grenoble, France) and a follow-up study on 600 asthmatic pregnant women where measurements are made in the 2nd and 3rd trimester and postpartum (Newcastle, Australia).

Equipment. Initially, a methodological comparison involving a „standard" lung function method in parallel with our novel arrangement of the FOT was planned. Since the electrical impedance tomography (EIT) was no longer available commercially for infants, the multiple-breath washout technique (MBW) as an emerging method was selected. Unfortunately, the MBW has proven unsuitable in newborns eventually and is still a subject of growing concern in young (e.g. 6-wk-old) infants. Measurements made in the South African infants indicate that the lung clearence index estimated with the MBW technique does not correlate with the measures of mechanical inhomogeneity derived from the T-FOT data. Therefore, the MBW device will be utilised in future investigations on paediatric populations (e.g. in cystic fibrosis) and in methodological studies integrating the ultrasonic flowmeter into the FOT device. The EIT measurements, which were the original part of the physiological validation and interpretation of our technique will be implemented in the coming months in the framework of a collaborative study established in the meantime with Brisbane researchers.

Main results of the project

The major outcomes of the research are as follows.

<u>Advances in lung function methodology</u>. We developed a new measurement setup for lung function studies in infants during quiet sleep *(Hantos et al. Pediatr Pulmonol 2015)*. This device represents a significant advance to the few non-invasive techniques that are feasible in infants, as the measurements reveal the mechanical properties of the respiratory system during normal breathing, i.e. without interference with the spontaneous activity. In addition to the „classical" FOT approach that measures respiratory impedance (Zrs) over a range of frequencies to estimate respiratory resistance and elastance as average values for several breathing cycles, we developed the intrabreath tracking FOT (T-FOT), which focuses on the fast changes in Zrs as functions of respiratory flow and volume at a single oscillation frequency. Both the spectral and temporal modalities have been included in all

measurements in infants, demonstrating high feasibility in this age group *(Hantos et al. Pediatr Pulmonol 2015; Gray et al. Respirology 2015)*, as well as preschool-age children *(Czövek et al. Thorax 2016)*, COPD patients *(Lorx et al. Eur Respir J 2017)* and a healthy young adult cohort *(Radics et al., in preparation)*. The initial results with the T-FOT in particular have evoked both commercial interest and the need for continuing collection of normative data for this technique. The Clinical Research Collaboration Award No. 2013-02 (International Collaboration to Improve Respiratory Health in Children) from the ERS provided us with a formal framework for 3 years to organize multicentre studies on the intrabreath tracking technique in preschool-age children. The sites that installed our custom-made equipment for the collection of multicentre data (Szeged, Brisbane, Cape Town, Melbourne, Porto Alegre, Seattle and Indianapolis) have collected FOT measurements in more than 1,000 preschool-age (and 100 older) children, and some sites are also conducting special studies in children with asthma/wheeze (Brisbane, Melbourne) and cystic fibrosis (Porto Alegre and Indianapolis-Seattle). Because of these advances, the widening scope of these FOT studies and the fact that further centres (Grenoble, Cardiff) expressed their wish to join, another 3-years' framework support was obtained from the ERS. Overall, the success rate of the FOT measurements was 80% in children under 4 years of age and 92% in older children. On the basis of this development work coordinated by the Szeged group, the T-FOT technology and the collected normal values will be incorporated into a commercial device (*tremoFlo*®, Thorasys Inc, Montreal, Canada).

Longitudinal studies of lung function. (a) As indicated in the interim reports, we encountered logistic difficulties with the follow-up measurements in the Szeged newborns. The Drakenstein birth cohort study (Paarl, South Africa) launched in 2012 by the research group of the University of Cape Town introduced our forced oscillation technique (FOT), in addition to the MBW method, FeNO measurements and spontaneous breath analysis into its lung function methodology employed at the age of 6-10 weeks. Successful measurements with all of the methods were accomplished in 645 infants during natural sleep *(Gray et al. Thorax 2017)*; the follow-up studies using the same techniques at 1 and 2 years of age have been completed and the measurements in the children at the age of 3, 4 and 5 yrs in the sitting position are in progress (current numbers are 500, 420 and 51, respectively). (b) A short-term longitudinal study was conducted in a Szeged group of preterm neonates [32-36 wk gestational age (GA), n=45]. This study included, in addition to the first 3 days' measurements, follow-up sessions (2-3 days apart) during the hospitalization period and the infants were re-measured at 6 weeks of age. The preliminary results indicated significant day-to-day variations in lung function, slight changes in resistance and compliance, with no differences between groups GA<35 wk and GA≥35 wk at any time point; the analysis including intrabreath data still awaits completion. (c) Another South African study (Johannesburg) employing our FOT device investigates 400 1-yr-old infants with respiratory syncytial virus infection and 400 controls, and the 2-yr follow-up has just started; analysis of the FOT data are in progress.

Determinants of respiratory mechanics in infancy and early childhood. The preliminary study on healthy term newborn infants included measurements of spectral FOT in each of the first 3 days of life. The results on a subgroup of neonates born via vaginal delivery (n=38) have been published *(Hantos et al. Pediatr Pulmonol 2015)*. Resistance (R) and compliance (C) data from the other subgroup (Caesarean section, n=35) were not significantly different from that of the first group. Further main messages of this methodology paper were (a) the high success rate (~80%), (b) the physiologically novel and meaningful character of the Zrs data and (c) the between-day variability in R and C in the first days of life. This neonatal population has reached n=300 by now and re-analysis on this population (now including intrabreath data) are in progress.

Infants enrolled in a birth cohort study in South Africa had lung function measured at 6 to 10 weeks of age using our FOT setup *(Gray et al. Respirology 2015)*. Of the 219 infants tested with the spectral FOT, 198 (90%) infants had successful measurements. Gender was a determinant for R and C, while C was significantly affected by maternal smoking during pregnancy.

In the same South-African cohort, lung function was measured with our FOT method together with other techniques *(Gray et al. Thorax 2017)*. Acceptable and repeatable measurements were obtained in 293/333 (88%) infants for the forced oscillation technique. This study provides reference data for unsedated infant lung function in African infants and highlights the importance of developing reference equations that are population specific.

Revision of the definition of "healthy" for forced oscillation technique reference equations in 585 preschool-aged Caucasian children evoked considerable interest and the article *(Shackleton et al. Respirology 2017)* received an editorial. In this study, the impacts of postnatal factors are also addressed.

The intrabreath analysis of lung mechanics has demonstrated a striking advance to existing lung function tests. (a) The T-FOT was employed in healthy preschool-age children (n=75) and those with acute wheeze (n=31). The receiver operator characteristic (ROC) analysis revealed an outstanding performance of the resistance tidal change index (sensitivity and specificity of 92 and 89, respectively; ROC area of 0.95). The cut-off values obtained for airway obstruction were then successfully tested in children with recurrent wheeze (n=20) before and after administration of salbutamol *(Czövek et al. Thorax 2016)*. (b) These fascinating results have highlighted the need to establish normal values of intrabreath measures for the 3-6-yr age group; the manuscript on the study on 517 preschool-age children recruited from 3 ERS CRC project sites has been prepared for submission *(Shackleton et al. Am J Respir Crit Care Med)*. (c) Low lung function as characterised by the T-FOT measures at 6 weeks of age in 627 healthy South African infants predicted lower respiratory tract illness (LRTI) during the first year of life, with odds ratios from 2.5 to 5.3, depending on the number and severity of the forthcoming LRTI episodes. The predisposition to later LRTI was not signalled by conventional lung function measures including the classical FOT *(Czövek et al. Am J Resp Crit Care Med, under review)*.

Physiological studies. The mechanisms underlying the lung function in unsedated healthy term infants have been addressed in ~300 newborns in Szeged and via the analysis of the follow-up data from the Drakenstein study. In addition to the detection of intrapulmonary flow limitation often observed in the latter cohort, particular attention has been paid to the involvement of the upper airways (nasal passages and vocal chords). Other physiological techniques including breath sound recording and the simultaneous measurement of lung volume and regional ventilation via EIT are tested currently to further explore the determinants of dynamic respiratory mechanics manifested in the intrabreath tracking data.

Additional research involving the novel T-FOT approach

Studies outside the scope of this grant but employing the intrabreath FOT in adults have emerged, by using the analytical methods developed in the frame of the present grant support and setups appropriately scaled for the measurement setting. (1) In 55 patients with COPD and 20 age-matched controls, new FOT indices were determined to assess expiratory flow limitation and impaired lung homogeneity characterising mechanical abnormalities in COPD. Within-breath tracking has also proven an excellent approach to titrate positive pressure respiratory support in a subgroup of patients *(Lorx et al. Eur Respir J 2017)*. (2) In a West Australian birth cohort study launched 25 years ago, our FOT technique was included in the 23-yr follow up lung function measurements made before and after mannitol challenge *(White et al. Respirology 2016)*. Analysis of the FOT data obtained in 645 subjects is conducted by our group, and it is focused on the potential of our technique in the diagnosis and management support in asthma, which has an estimated prevalence of 10 % in this population *(Radics et al. Eur Respir J, in preparation)*.

Communication of results

Preliminary results of our research have been reported in 40 talks/posters at major international congresses (ERS, American Thoracic Society and the Thoracic Society of Australia and New Zealand),

several of them presented by the 3 PhD students (2 in Szeged and 1 in Brisbane, co-supervised by the PI of this grant) who joined our research group.

Eight journal articles indicating this grant support have been published (ΣIF=42.6). Because of the proliferation of the infant and preschooler studies using our technique the collected huge data sets still await for analysis; in particular, evaluation of the novel T-FOT data has to rely on the competence of the Szeged group. This projects a large number of forthcoming presentations and research papers.