RESULTS OF THE RESEARCH PROJECT, FULFILLMENT OF OBJECTIVES

According to the research plan, the research activity focused on the characterization of the effects of cardiovascular support on respiratory mechanics, lung ventilation and oxygenation. This general goal was addressed in the research protocols through a better understanding of the roles of the respiratory and cardiovascular systems in lung functional changes in animal models and in human investigations. Since the cardiovascular support is a key therapy of patients with compromised haemodynamics and the lungs are among the most affected organs, our research addressed an area with ample research and clinical importance. Our results contributed to better understand the consequences of cardiovascular support by elaborating the **following main findings**:

i) compensating blood volume loss with crystalloids in 1:1 ratio restores systemic haemodynamics and is favourable over a conventional ratio of 1:3 (I/1);
ii) dopamine ameliorates both cholinergic and histaminergic bronchoconstriction (I/2);
iii) red blood cells contribute significantly to the overall lung tissue viscoelasticity, which is of major importance in fluid therapies affecting the blood haematocrit (I/3);
iv) levosimendan exerts a K_{ATP}-channel-mediated potential to prevent bronchoconstriction and prohibits adverse changes both in the small bronchi and the pulmonary parenchyma (II/1);
v) acute normovolemic hemodilution leads to detrimental cardiorespiratory changes in piglets, gradual developments of bronchoconstriction, lung tissue extravasation and stiffening (II/2);
vi) positive inotropes exhibit great differences in the pulmonary effects: adrenaline, dopamine and levosimendan exhibit bronchodilatory action, while dobutamine and milrinone are not (II/3);
vii) volumetric capnography is important monitoring modality to detect ventilation/perfusion mismatch subsequent to various interventions including fluid resuscitation (III/1);
viii) the chest wall contribution should be considered when lung mechanics is assessed in intact chest, which has importance in fluid resuscitation affecting tissue mechanics (III/2).

In parallel with the experimental work, the development concerning the **methodological advancement** of the forced oscillatory setup and the software design to evaluate the expiratory capnogram traces were also performed successful in this project, with major achievement of:

i) a semi-automatic evaluation of the capnogram shape factors and dead space parameters;
ii) hardware and software developments of the forced oscillatory system to base its platform on an up-to-date information technology. This assured the continuous technical support to cope with the rapidly changing computer hardware and software environment;
iii) mechanical properties of the airways and the respiratory tissues can be reliably assessed using a supraglottic airway device to secure the airways (III/3);
iv) ventilator sharing is feasible as a lifesaving modality in emergency situations, such as encountered during the COVID-19 pandemic (IV/6).

According to the approved research plan, research conducted in **clinical environment** has led to the following main findings:

i. near-infrared spectroscopy has particular monitoring value in patients with diabetes, which has particular importance during cardiovascular support in this frequent metabolic disease (IV/3);
ii. dopamine has a potential benefit on the gas exchange abnormalities after weaning cardiac surgery patients from cardiopulmonary bypass (IV/7).

PUBLICATION OF THE SCIENTIFIC RESULTS

The success of the research work is well reflected in the 18 full-length peer-reviewed papers in English with direct acknowledged support of the current grant published in the most prestigious

international journals of the area with a total impact factor of 60.714. Furthermore, with the support of the current grant, scientific papers and popular science articles were also published in Hungarian, as well as more than 21 international congress abstracts in English.

MODIFICATIONS IN THE ORIGINAL RESEARCH PLAN AND THEIR RATIONALE

The research even opened areas for further investigations originally not included in the project. Accordingly, the actual research activity overfulfilled the commitments outlined in the approved research plan. While these activities were not planned in the original proposal, they provided highly relevant data in the main topic of the project. Our research achievements were also completed in the following complimentary results:

i. measurement of ventilation dead spaces requires mainstream capnography, while sidestream capnography provides adequate information on ventilation-perfusion mismatch (I/4.a);
ii. capnographic parameters from early expiration are linked to the pulmonary elastance, whereas the effect of airway patency on the third phase dominates over the lung tissue stiffness (I/4.b);
iii. diabetes is related to a reduced airway and lung tissue viscoelasticity, resulting in alveolar collapsibility that can be compensated by increasing PEEP (IV/4);
iv. hypoxic pulmonary vasoconstriction counterbalances diabetes-induced intrinsic mechanical abnormalities, maintaining intrapulmonary shunt and lung oxygenation ability (IV/5);
v. obesity causes similar detrimental changes in respiratory mechanics and alveolar heterogeneity to diabetes, and these alterations also compromised gas exchange (IV/5).

Due to the difficulties in performing experimental research during the COVID-19 pandemic, an additional year was allowed to complete the research plan. This complementary year allowed us to extend our investigations towards closely related topics with a primary focus on the pulmonary effects of metabolic disorders, such as obesity and diabetes mellitus (IV/3, IV/4). Furthermore, the worldwide challenge posed by COVID-19 pandemic stimulated the initiation of a research work assessing the theoretical possibility and practical implementation of shared ventilation (IV/6).

ROLE OF RESEARCH PARTICIPANTS

The tasks outlined in the research plan were fully completed by the participants. Barna Babik contributed to the organizing the data collection in the clinical studies by recruiting patients, assessing clinical symptoms, performing data collection and manuscript drafting. Gergely Fodor, Ádám Balogh and Álmos Schranc contributed to the data collection both in the experimental studies and in clinical environment, in the planning of the animal models, and in the data analyses and statistical evaluation. Ferenc Rárosi and Katalin Virág development of novel data processing algorithms with particular interest in the analyses of the expiratory capnogram signals and statistical evaluations. József Tolnai had a key role in the data collection and analyses, with particular focus on the development of custom-made software for the detailed examination of the dynamics of the expired CO_2 traces. The financial resources for personnel costs were used as planned. The exception was the slight rearrangement of the financial support of non-research staff to colleagues contributed to the research by preparing animals, designing measuring equipments, and providing administrative background. This was approved by the financial authorities.

PARTCIPATING RESEARCH INSTITUTIONS

In agreement with the approved work plan, the experimental and clinical protocols were conducted in collaboration with several local and international research institutes. With the support of previous and the current OTKA grants, the supervisor set up an independent research laboratory at

the Institute of Medical Physics and Medical Informatics of the University of Szeged, which provided the conditions for performing experiments on small laboratory animals. Animal experiments with special needs were performed by involving national and international collaborator (Walid Habre) at the University of Geneva, Faculty of Medicine, Unit for Anaesthesiological Investigations (Switzerland) under the personal direction of the supervisor. Measurements performed in a clinical environment were performed at the Department of Anaesthesiology and Intensive Therapy (Barna Babik).

DETAILED SCIENTIFIC ACTIVITIES PERFORMED IN THE RESEARCH PROJECT

I. Research performed in the 1st year of the project

I/1. Blood replacement with crystalloid: effects volume ratio on lung function changes

We provided evidence in this study for the optimal fluid replacement ratio when considering crystalloids for fluid replacement following blood loss. A ratio of 1:1 was more appropriate than the historical approach (1:3) since it is able to meet the needs for maintaining homeostasis while avoiding the adverse effects related to fluid extravasation. We suggest that considering crystalloid fluid replacement in a ratio of 1:1 to compensate continuous occult bleeding maintains appropriate organ perfusion without the adverse consequences of fluid extravasation. These results were presented at the Euroanaesthesia Congress in 2018 and the manuscript has been published in *BMC Anesthesiol. 19: 21, 2019 (IF: 1.695*).

I/2. Characterization of the bronchial effects of dopamine

We revealed the ability of dopamine to ameliorate both cholinergic and histaminergic bronchoconstriction (Fig. 1). The concomitant presence of the already established beneficial cardiovascular effects of dopamine and its dose- and stimulus-independent bronchodilator activity may have importance when the circulatory and respiratory systems are both impaired, such as in patients with heart failure associated with bronchial hyperreactivity or in those with an elevated bronchial tone subsequent to impaired circulatory function. The results were presented at the European Respiratory Society congress in 2016, and the manuscript summarizing these results was published in *Respir Physiol Neurobiol 259:156-161, 219 (IF: 1.591).*

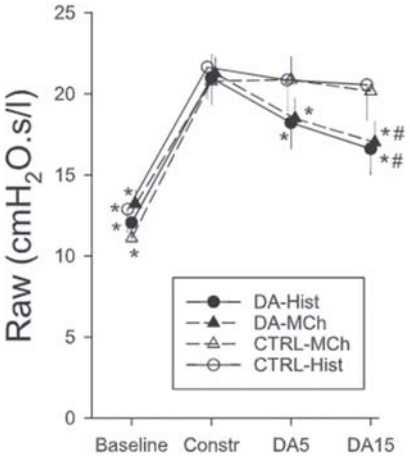

Figure 1. Effects of dopamine (5 and 15 μg/kg/min) on the histamine- or methacholine -induced airway resistance

I/3. Effects of blood rheology on the lung mechanics

Additional insight into the participation of the pulmonary vascular capillary network in the elastic and dissipative properties of the lung tissue was revealed in this study performed in isolated perfused rat lungs. Decreasing haematocrit in the pulmonary circulation below physiological level diminished the viscous and elastic parameters of the pulmonary tissues, therefore demonstrating

that the energy dissipation and storage displayed by the red blood cells are significant contributing factors in the total lung viscoelasticity (Fig. 2). The paper including these findings was presented at the 2016 American Thoracic Society congress in San Francisco and published in *J Appl Physiol. 121:261-7, 2016 (IF: 3.351).*

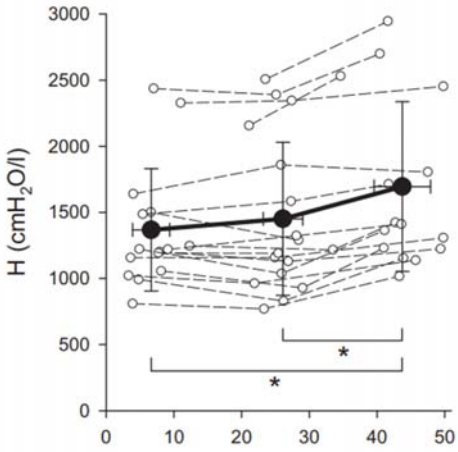

Figure 2. Lung elasticity during lung perfusions with low, intermediate, or normal levels of blood hematocrit.

I/4. Application of translational research to clinics

I/4.a. Mainstream vs. sidestream capnography

Mainstream and sidestream time and volumetric capnography were performed to compare several factors in anaesthetized, mechanically ventilated patients undergoing elective heart surgery. Our results revealed that sidestream capnography provides adequate quantitative bedside information about uneven alveolar emptying and ventilation–perfusion mismatch, but mainstream capnography is required for a reliable measurement of volumetric parameters. The findings were presented at the European Respiratory Society congress in 2016, and the paper summarizing these results was published in *Br J Anaesth. 117: 109-17, 2016 (IF: 6.238).*

I/4.b. Assessing the correspondence with capnographic and mechanical indices

We, also set out to characterize how the mechanical properties of the airways and lung tissues modify the indices obtained from the different phases of the time and volumetric capnograms and how the lung mechanical changes are reflected in the altered capnographic parameters after a cardiopulmonary bypass. Results obtained in a large cohort of cardiac surgery patients revealed that the capnographic parameters from the early phase of expiration are linked to the pulmonary elastic recoil, whereas the effect of airway patency on phase 3 dominates over the lung tissue stiffness. Our results were published in *Anesth Analg. 122: 1412-20, 2016 (IF: 4.014).*

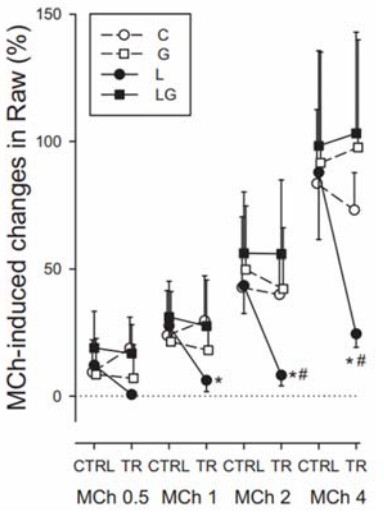

Figure 3. Changes in airway resistance (Raw) to methacholine in groups control (C), glibenclamide (G) Levosimendan (L) and combined (LG) at control (CTRL) and treatment (TR) stages.

II. Research performed in the 2nd year of the project

II/1. Levosimendan prevents against bronchoconstriction

The strong ability of levosimendan was demonstrated in this study to protect against methacholine-induced changes in respiratory system mechanics. Levosimendan prevents central airway constriction (Fig. 3), prohibits the enhancement of ventilation heterogeneities, and inhibits elevations in respiratory tissue damping and stiffness. This effect was caused by opening K_{ATP} channels. This multimodal cardiorespiratory benefit of levosimendan has a potential of shortening the time of invasive ventilation following its application. The results were presented in 2016 at the European Respiratory Society (ERS) congress, and published in *Am J Physiol Lung Cell Mol Physiol 313: L950-L956, 2017(IF: 4.092).*

II/2. Acute normovolemic haemodilution in a paediatric and adult experimental models

The results of this experimental study demonstrated prompt effects of acute normovolemic haemodilution on the cardiorespiratory function with the severity of adverse symptoms depending greatly on the age of the subject. The age-dependent cardiorespiratory responses to acute normovolemic haemodilution suggest a better tolerability of this intervention in young individuals; however, a clinical study is needed to clarify the applicability of these findings to humans. These results are published in *Anesth Analg 126: 995-1003, 2018 (IF: 3.489).*

II/3. Reversal of bronchoconstriction by inotropes

In this study, adrenaline, dopamine, and levosimendan exert a bronchodilatory effect in the presence of cholinergic elevation of the airway smooth muscle tone via different pathways (Fig. 4). Conversely, dobutamine and milrinone had no bronchodilatory properties. The multifactorial effect of these commonly used inotropic agents may have relevance in patients with concomitant impairments in the circulatory and respiratory systems, for which cardiovascular support and improved respiratory function have mutual importance. These results were presented in 2017 at the European Respiratory Society congress, and the paper summarizing these data is published in *Anesth Analg 129: 745-752, 2019 (IF: 3.463).*

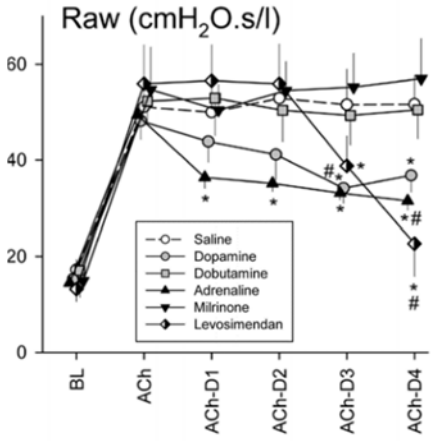

Figure 4. Changes in airway resistance. Ach: acetylcholine alone; ACh+D1–D4: increasing doses of saline or inotropic agents during ACh infusions.

III. Research performed in the 3rd year of the project

III/1. Ventilation/perfusion mismatch in injured lungs

This experimental investigation highlighted the limitations of time capnography as an on-line noninvasive bedside monitor to detect factors leading to the ventilation/perfusion mismatch that develops in lung injury. Conversely, volumetric capnography revealed an excellent ability to detect uneven ventilation/perfusion caused by both increased dead space and intrapulmonary shunt. We concluded the necessity to promote volumetric capnography as a routine monitoring modality in clinical practice both in anaesthesia and intensive care settings. The paper summarizing these results is accepted for publication in *Front Physiol 12;9:1805., 2018 (IF: 3.201).*

III/2. Lung and chest wall contribution to respiratory mechanics in small laboratory animals

This study was motivated by the fact that changes in lung mechanics following various interventions including fluid resuscitation are frequently inferred from intact-chest measures of total respiratory system mechanics, without consideration of the chest wall contribution. Thus, we compared these contributions in intact-chest mice, rats and rabbits. The contribution from the chest wall should be considered when intact chest measurements are used to estimate lung parenchymal mechanics in small laboratory models, particularly at elevated lung volumes. Part is these data were presented at the 2018 European Respiratory Society Congress, and the paper summarizing these results has been published in *J Appl Physiol 127: 198-204, 2019 (IF: 3.044).*

III/3. Forced oscillatory assessment of respiratory mechanics across a laryngeal mask airway

This feasibility study was based on the recognition that laryngeal mask airway is increasingly used to secure the airways in subjects under sedation or general anaesthesia, but the forced oscillation technique as the method of choice for assessment of respiratory tissue mechanics has not been applied in this setting. We proved that the mechanical properties of the airways and the respiratory tissues can be reliably assessed wia this airway device. However, the involvement of a longer

laryngo-tracheo-bronchial pathway and upper airway shunting should also be considered. These data were presented at the 2018 European Respiratory Society congress, and the results were published in *Physiol Meas 40:065001, 2019 (IF: 2.309)*.

IV. Research performed in the 4th and 5th (complimentary) year of the project

IV/1. Assessment of shape factors and dead space indices by volumetric capnography in rats

In this experimental protocol, the value of the sidestream capnography was characterized in a rat model that is commonly used to study various respiratory diseases and resuscitation strategies. Our results demonstrated that adaptation of sidestream volumetric capnography to rats has the potential to assess capnogram shape and dead space parameters. Thus, it has the value to study lung diseases affecting ventilation-perfusion mismatch in small rodents, if the biasing effect of Fowler dead space and gas sampling are considered. These results have been presented at the European Respiratory Society congress in 2018, and the manuscript summarizing the results is under preparation.

IV/2. The value of 3D printing in adapting a human capnography device for small-animals

In another experiment, a new 3D-printed adapter was designed to have transparent sampling window for the spectrophotometric measurement of the expired CO_2 concentration with an instrumental deadspace appropriate for rodent ventilation. The validation findings demonstrate that the 3D-printed airway adapter yields in close to the physiological assessment of capnographic parameters. This work was presented at the American Thoracic Society congress and the development of the device is currently undergoing.

IV/3. Central venous and cerebral tissue oxygen saturations in patients with diabetes

The aim of the cardiovascular support is to increase cardiac output and thereby to improve tissue perfusion and oxygenation. Therefore, a further important aspect of the cardiovascular support is related to the characterization of the differences between central venous and cerebral tissue oxygen saturation in healthy patients and in patients with abnormal vasculature. Our results indicate the wide gap between central and regional oxygen saturation indices and their uncoupled relationship in patients with diabetes (Fig 5). Thus, the monitoring value of near-infrared spectroscopy in diabetes is advocated, which has particular importance during cardiovascular support. This paper has been accepted for publication in *Sci Rep 24;9: 19740, 2019. (IF: 3.998)*.

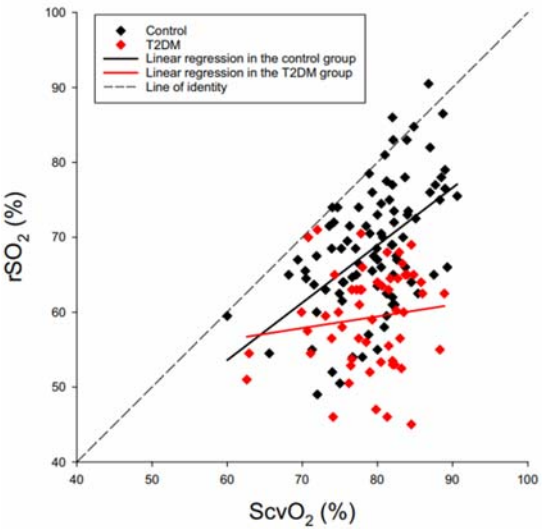

Figure 5. Correlation between cerebral oxygen saturation (rSO_2) and central venous oxygen saturation ($ScvO_2$). Regression in **diabetes** (p=0.34, r=0.13) and in **control** group (p<0.0001, r=0.52).

IV/4. Effects of diabetes on the respiratory function

In this protocol, animal models of type 1 and type 2 diabetes showed evidence of detrimental changes in airway and tissue compartments. The decline in airway function was reflected in elevated airway resistance and abnormal adaptation of the airways to exogenous constrictor stimuli. Lung tissue remodelling was manifested in compromised viscoelastic tissue mechanics. These findings may contribute to an improved understanding of the pulmonary consequences of diabetes and hold promise for the advancement of therapeutic interventions. The results have been presented

at the annual congress of the European Respiratory Society and the paper summarizing these results is published in *Respir Res 9; 21:82, 2020 (IF: 5.631)*.

IV/5. Obesity and diabetes: respiratory mechanical and gas exchange defects

We demonstrated in this clinical research that diabetes affected central and peripheral airway function and the dissipative and elastic properties of the respiratory tissues, leading to ventilation heterogeneities and enhanced lung collapsibility (Fig. 6). These mechanical abnormalities were counterbalanced by the increased contractile response of the pulmonary vasculature to hypoxic stimuli, which was able to maintain the normal intrapulmonary shunt fraction and lung oxygenation. Obesity, otherwise, deteriorated the global respiratory mechanics in a similar manner, and the external trigger worsened gas exchange. These pathophysiological changes highlight the importance of lung protective ventilation not only in obesity but also in patients with diabetes. The paper summarizing these findings is published in *Am J Physiol Lung Cell Mol Physiol 320(3): L368-L376, 2020 (IF: 5.464)*.

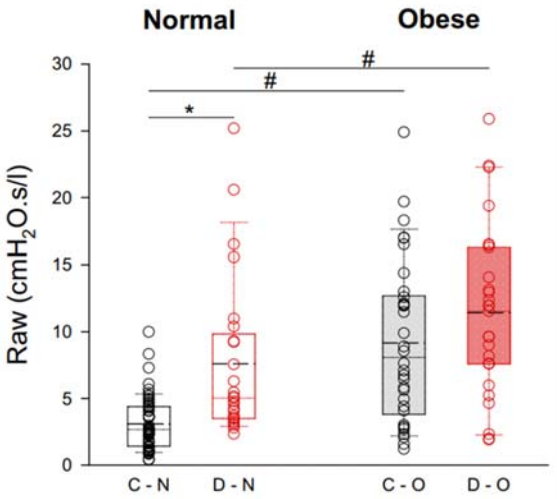

Figure 6. Airway resistance obtained in patients without (C) and with (D) type 2 diabetes mellitus associated with normal body shape (N) or obesity (O).

IV/6. Ventilator sharing in the COVID-19 era

This research was stimulated by the recent situation in the COVID-19 pandemic, that has led to acute shortage of ventilators. Two model lungs were ventilated with one ventilator to i) test the adequacy of individual tidal volumes via capnography, ii) assess cross-breathing between lungs, and iii) offer a simulation-based algorithm for ensuring equal tidal volumes. The findings demonstrate that capnography serves as simple method for controlling the adequacy of split ventilation and thus, the feasibility of ventilator sharing in emergency situations. These results were reported in *Respir Physiol Neurobiol 285:103611, 2021. (IF: 1.931)*

IV/7. Clinical research: pulmonary effects of dopamine

The data collection in the clinical environment to clarify the effects of dopamine on the lung mechanics was initiated in the first year of the project and was ongoing during the entire period. The results demonstrated the ability of dopamine to alleviate compromised airway function and ventilation heterogeneities that were triggered by cardiopulmonary bypass without an evidence of any disadvantage on the gas exchange abnormalities (Fig 7). Therefore, this inotrope can be safely recommended in the post-bypass period to improve cardiac function and to mitigate the compromised lung function, without the risk for disadvantageous consequences on ventilation/perfusion matching. The paper summarizing these results is accepted for publication in the *J Cardiothorac Vasc Anesth. (in press, IF: 2.628)*.

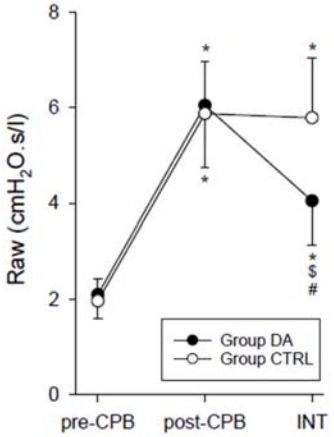

Figure 7. Airway resistance in patients with (DA) or without (CTRL) dopamine. CPB: cardiopulmonary bypass, INT: intervention.