# Non-contact Vital Signs Monitoring in Paediatric Anaesthesia – Current Challenges and Future Direction

Nicole Grech[1,*], Jean Calleja-Agius[2], Stephen Sciberras[1], Neil Micallef[3], Kenneth Camilleri[3], Owen Falzon[3]

ABSTRACT

Non-contact vital sign monitoring is an area of increasing interest in the clinical scenario since it offers advantages over traditional monitoring using leads and wires. These advantages include reduction in transmission of infection and more freedom of movement. Yet there is a paucity of studies available in the clinical setting particularly in paediatric anaesthesia. This scoping review aims to investigate why contactless monitoring, specifically with red-green-blue cameras, is not implemented in mainstream practise. The challenges, drawbacks and limitations of non-contact vital sign monitoring, will be outlined, together with future direction on how it can potentially be implemented in the setting of paediatric anaesthesia, and in the critical care scenario.

KEYWORDS

contactless; vital signs; paediatrics; anaesthesia; monitoring

AUTHOR AFFILIATIONS

[1] Department of Anaesthesia and Intensive Care Medicine, Mater Dei Hospital, Malta
[2] Department of Anatomy, Faculty of Medicine and Surgery, University of Malta
[3] Centre for Biomedical Cybernetics, Faculty of Engineering, University of Malta
* Corresponding author: Department of Anaesthesia and Intensive Care Medicine, Mater Dei Hospital, Malta; nicole.grech@gov.mt

Received: 21 January 2023
Accepted: 8 May 2023
Published online: 8 November 2023

Acta Medica (Hradec Králové) 2023; 66(2): 39–46
https://doi.org/10.14712/18059694.2023.14

## INTRODUCTION

Non-Contact Vital Signs Monitoring (NCVSM) is an area of science and healthcare that has garnered increasing interest in the past years due to its advantages both for patients and healthcare workers. Among the obvious advantages of monitoring vital signs without the use of leads and wires attached to patients is improved comfort and increased ability to mobilise (1). A more in-depth analysis highlights the potential for reduced transmission of multidrug resistant organisms (MDROs) through inadequately disinfected monitoring equipment; reduced issues of skin damage especially in vulnerable populations such as neonates, elderly frail people and burns patients; and reduced need for hospital staff to enter isolation rooms and waste precious personal protective equipment (PPE) just to replace a lead or sort out dislodged wires (2–6).

In children, especially those admitted to hospital, monitoring may prove difficult as paediatric patients may be stressed by the unfamiliar environment and the natural fear of upcoming procedures, or due to pain after such procedures. Children may not always cooperate with healthcare workers and the inability to keep monitoring leads and wires on for long enough to get a reliable reading of vital signs, is a common issue. Although inducing anaesthesia in a child who is not fully monitored is not desirable and increases risks of adverse events, it often ends up having to be done if the child is combative and will not stay still long enough for electrocardiography (ECG) leads and pulse oximeters to be appropriately positioned. Post-op, paediatric patients often wake up delirious and uncooperative. This may persist into the post-anaesthesia care unit (PACU), the intensive care unit (ICU), or the general wards. Once again, this renders paediatric patient care difficult and increases risks of deranged vital signs being detected at a much later stage (7–11).

The ability to monitor a child's vital signs in a non-contact manner therefore has obvious advantages in that it does not further distress the child, and may provide reliable readings for healthcare professionals in the case where the child is uncooperative and keeps removing monitoring equipment. In toddlers and infants, it may be possible that they even put leads in their mouth, posing a risk to themselves and also cause aberrant readings. Contactless monitoring also allows children to move more freely and be monitored while performing enjoyable tasks such as playing. This allows more accurate vital signs to be obtained since the sympathetic response to stress is reduced (12).

In the specific scenario of paediatric anaesthesia, NCVSM offers advantages even during maintenance of anaesthesia, apart from those already described relating to induction and emergence. Patient positioning in the operating theatre often dislodges leads and may cause monitoring equipment to become entangled, leading to potential damage to the internal structure of the wires. Once surgery has started and sterile surgical drapes are in place, access to monitoring equipment is very restricted. Attempts at manoeuvring and applying monitors under the drapes may lead to issues for the surgeon and displacement of surgical equipment, and is ultimately dangerous for the patient. Thus, any leads that get dislodged may lead to distorted readings of loss of monitoring capabilities. Use of diathermy often interferes with the quality of signals obtained, with ECG leads being particularly vulnerable to electrical interference (13). During long procedures, leads can put pressure on the fragile skin of small vulnerable children and in some interventions such as thoracic procedures they often need to be placed on the back, causing more pressure and potentially suboptimal monitoring (14, 15). Neurological complications such as acute brain injury, occuring for example during venoarterial extracorporeal membrane oxygenation (ECMO), may be detected better with non-invasive neuro-monitoring. Neuro-monitoring can be carried out via electroencephalography (EEG), somatosensory evoked potentials (SSEP) and near infrared spectroscopy (NIRS) (16). Cerebral desaturation measured using NIRS was associated with a poor short-term outcome in children undergoing ECMO (17). This is particularly important in an intensive care setting.

NCVSM can be carried out through vital sign extraction from red-green-blue (RGB) video recordings in real time (18). The beating heart produces rhythmically increased perfusion of the subcutaneous capillaries causing an increased pink tone in the skin, which is imperceptible to the naked eye. This occurs because the increased concentration oxygenated haemoglobin in these capillaries absorbs more of the blue spectrum of visible light and reflects red wavelengths. These changes can be extracted using multiple algorithms and they can be converted to a numerical value for heart rate (HR), and, subsequently, heart rate variability (HRV) over a period of time. This technique is termed reflectance photoplethysmography (rPPG) (19, 20). Commonly used algorithms include Eulerian video magnification (EVM), which essentially applies green filters to enhance the skin colour changes, and principal or independent component analysis (PCA and ICA respectively). The video is decomposed into different spatial frequency bands, where each spatial frequency band extracts the level of spatial detail required. All the bands are then filtered with the same time-domain filter to extract the motion detail required (e.g. heart rate perfusion), amplified and added to the original spatially filtered image frames, thus magnifying temporal changes in the video that may be imperceptible to the unaided eye (21–24). More recently, convolutional neural networks (CNNs) have gained huge popularity. These are very complex black box algorithms that can be trained to recognise a particular characteristic on a data set, often termed the "training" set, such as the skin colour changes in a set of data on healthy volunteers. Subsequently CNNs can be fed real world "test" data from which they should be able to detect the same changes that they learnt how to extract during training (25–27). The sensitivity and accuracy and reliability of these kind of systems can potentially be improved when used with machine learning in artificial intelligence (28).

Respiratory rate (RR) detection involves extraction of the rhythmic movements of the chest which translate to change in pixel intensity on RGB videos and images. To extract these signals, optical flow algorithms may be used as well as PCA or ICA with the focus being chest movements, and CNNs which can be trained to recognise the signal and are then applied to real world data (29–32).

One thing common to these algorithms is that a region of interest (ROI) must be selected, which is an area within the image, generally on the face of the patient, from which signals will be extracted. Other body parts may also be used such as hands and feet, but each poses its own challenges. In some algorithms, automatic recognition of the patients within the video frame and automatic selection of the best ROI for signal extraction is possible (33–35). While the technical details of data and image analysis are beyond the scope of this clinically oriented review, the limitations and how these affect clinical practise will be discussed.

In spite of the potential benefits to using NCVSM, this is not as yet routinely used in clinical practise. Experimental studies have been performed; however, there is a marked paucity of data on the use of these methods in terms of sensitivity and accuracy in the paediatric population, even more so in the perioperative period. The main aim of this review is to explore the limitations and challenges of NCVSM, in order to pave the way for increased uptake of this type of clinical monitoring in paediatrics.

## METHODOLOGY

This review involves an analysis of studies carried out since 2018 to date (May 2022) that involve monitoring of HR and RR by RGB cameras in real world hospital settings involving paediatric populations. The results obtained from these studies were analysed from a medical rather than a technical perspective, focusing on the limitations that may be contributing to the restricted use of these methods in everyday practise.

Boolean operators were used to search multiple search engines, namely Google Scholar, Medline, IEEE Explore, Cochrane Database, SCOPUS and CINAHL. In the case of Google Scholar, since over seventeen thousand results were obtained, the first hundred results were analysed. The search terms entered were the following: (contactless OR non-contact OR noncontact OR wireless OR RGB OR camera) AND (infant OR child OR baby OR paediatric OR neonate OR newborn) AND (heart OR heartbeat OR respiration OR breathing) NOT (wearable OR radar)

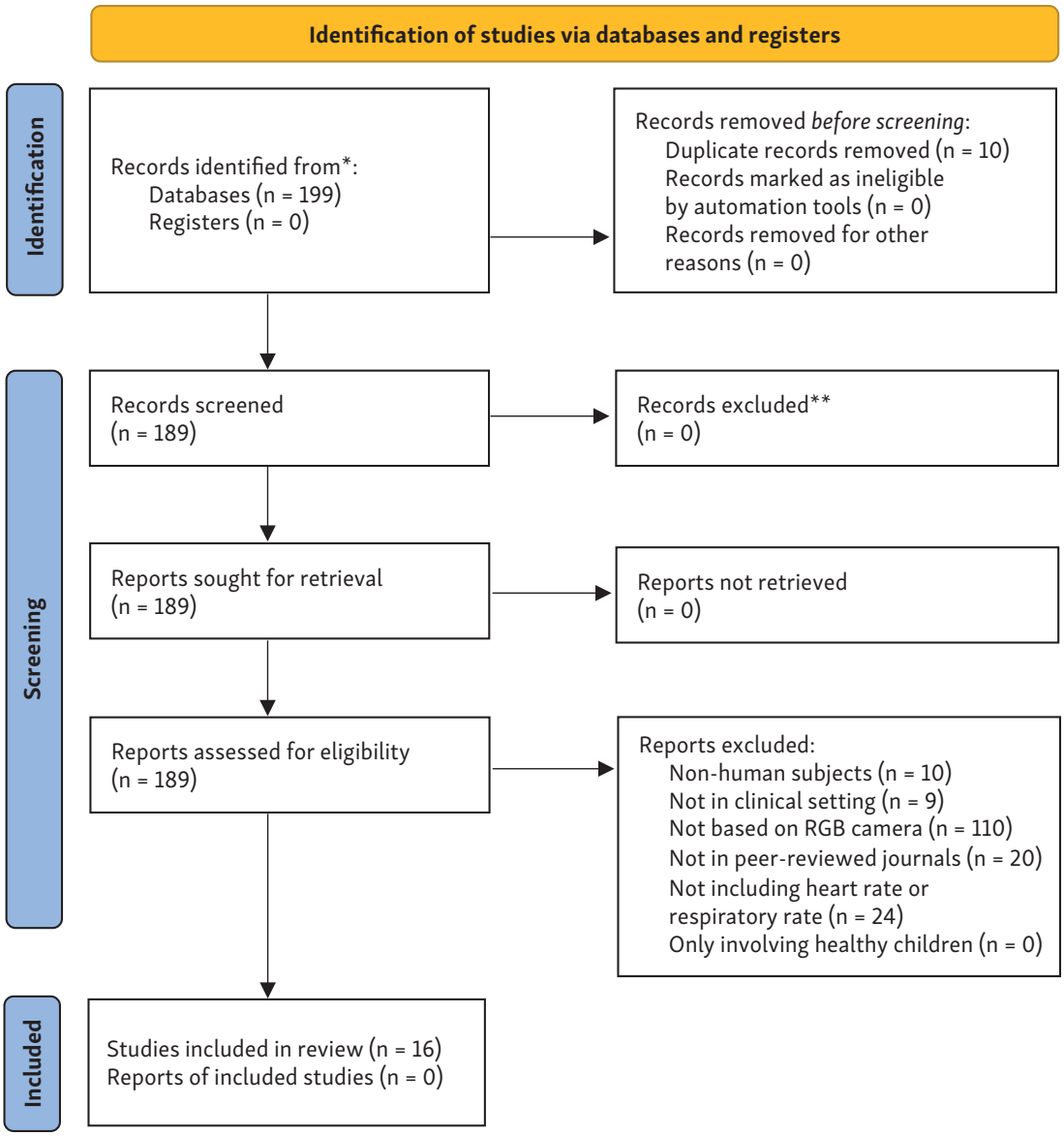


**Fig. 1** PRISMA flow diagram summarising search strategy and results (39).

Inclusion criteria were: studies published in peer-reviewed journals since 2018 and available in the English language; studies that include human subjects, performed on children less than eighteen years of age and carried out in clinical settings such as clinics or hospitals; inclusion of the use of RGB cameras and the extraction of HR or RR from RGB video data.

Exclusion criteria included studies published outside of peer review journals, including conference papers and symposia; studies published before 2018, not published in English and for which an English translation is not available; studies involving animals or simulators but no human subjects; involving only patients over eighteen years of age; performed outside of clinical settings; not involving RGB cameras or extraction of HR or RR data.

Studies involving RGB cameras were exclusively chosen for this review because these are the most widely available and are very cost effective. Some studies have in fact successfully extracted vital parameters using smartphone cameras, which in this day and age tend to be of very high quality and are overall very affordable (36, 37). Other types of cameras are used to extract vital signs from video data, such as depth cameras, structured light cameras and thermal cameras and although these have been successful in several trials, prohibitively high costs are a reason why they are not widely used. Therefore, these types of cameras were excluded from our review (38).

The PRISMA flow diagram as shown in Figure 1 summarises the search results obtained and the final full text papers included in this review.

The papers which have been included were analysed and the results obtained from the studies were categorised according to accuracy of vital signs obtained from video recordings and the various limitations posed by the clinical setting in which they were studied. This was done to attempt to understand what is holding back from the mainstream use of these NCVSM devices in clinical practice.

## RESULTS

Sixteen studies are included in this review. The included studies took place within hospitals, most commonly in neonatal intensive care units (NICUs) on children aged less than one year. Two of the included studies are reviews themselves and focus on NICUs, but do not include the most recent studies and are limited to NICU babies only (40, 41). Of the experimental studies, thirteen of them include a total of one hundred and fifty patients (42–55). This is a small sample size and the focus is mainly on just one clinical setting, albeit a complex one. No reports were identified in which children were studied specifically in the operating theatre setting or in the peri-operative period, and few studies included children who were older than the infant period, that is, above a year of age (56).

Table 1 summarises the studies included, the vital sign monitored, the population studied and its size, brief details of algorithms used and results obtained.

The first, and indeed, the most important factor to consider in any monitoring system is the accuracy of the system. In medicine, accurate readings of vital signs are essential in assessing and managing patients, even more so in anaesthesia when patients are unable to report symptoms (57). Studies that involve RGB cameras extract readings from videos taken in clinical scenarios and compare the vital signs readings extracted from these videos with those obtained by gold standard contact methods, most commonly the multiparameter monitors available in the hospital setting (55). A direct comparison between the results obtained by different research teams is difficult since some quote the root mean square error (RMSE), some the mean absolute error (MAE) for their dataset, and others quote other parameters such as the Pearson correlation coefficient (PCC). However, error values as low as an MAE of 1.8 beats per minute were reported for respiratory rate by Lucy et al. and MAE of 1.8 beats per minute was reported by Khanam et al. for their study involving heart rate extraction (55). Lucy et al. studied a group of five children known to have pneumonia and extracted their respiratory movements using colour component analysis in their video frames and then applied Fourier transformation to obtain respiratory rates (46). Khanam et al. used a deep neural network called YOLO 3 to automatically select the best ROI in their video frames obtained from seven NICU patients and to subsequently also extract colour changes corresponding to heart rates (55). These accuracy rates are excellent, since any deviation in heart rate or respiratory rate lower than two beats or breaths each minute is clinically not very significant. In paediatric surgery, small variations in parameters are usually not a cause for concern and do not prompt administration of drugs or any other changes (58). However, issues arise since this accuracy rate is not always maintained throughout long periods of time, with periods of time that involve changes in ambient illumination or movement of the patient affecting outcomes.

One major issue encountered in several studies that rely on RGB cameras to extract heart rates is concerning changes in ambient lighting (47, 49, 55). If low levels of light are available, not enough wavelengths may be reflected off the skin to obtain a significant result, and variations in ambient light during a video clip will cause worsening accuracy rates with values that begin to differ significantly from the gold standard monitoring (59).

During some surgeries, relative darkness is necessary, such as during laparoscopy when the laparoscopy monitor is the main source of light. Shadows cast by staff members moving around the patient also provide challenges and are noted to increase error values (42). One possible solution for this is to have a narrow beamlight source such as an LED or a bulb shining on the ROI chosen for the particular patient (49). The ROI selected in a theatre setting must allow for surgical drapes and equipment such as forced air blankets that often cover much of the body, and this in itself is another practical issue (45). However, uncovering a small part of the body far away from the operative site, such as the hands or feet in the case of abdominal surgery, or the forehead, will make this possible. Having a dedicated light source will add an extra piece of equipment to an often already cluttered anaesthetic space, and will also incur the extra cost associated with this particular equipment. However, overhead bulbs can be small and unobtrusive if placed strategically.

**Tab. 1** Summary of all studies included in this review with brief details of population studied, clinical setting and algorithms used with results obtained. Key to abbreviations: FFT – Fast Fourier Transform; PFF – Principal Flow Field; MSD – Micromotion and Stationary Detection; EEMD – Ensemble Empirical Mode Decomposition.

| Year | Authors | Population | Location | Algorithm Used | Parameter extracted | Error Value | Limitations |
|---|---|---|---|---|---|---|---|
| 2018 | Cobos Torres et al. | 9 preterm infants | NICU | FFT | HR and RR | PCC 0.94 for HR and 0.86 for RR | Movement, lighting |
| 2019 | Chaichulee et al. | 15 preterm infants | NICU | Deep learning framework | HR and RR | 98.8% accuracy results | Lighting |
| 2019 | Sun et al. | 5 preterm infants | NICU | Conventional optical flow and deep learning optical flow | RR | Cross-correlation coefficient of 0.70 for conventional optical flow and 0.74 for deep learning | Image resolution, background noise, lighting |
| 2019 | Gibson et al. | 10 premature infants | NICU | EVM and FFT | HR and RR | Mean difference of 4.5 bpm for HR and mean bias of 0.8 bpm for RR | Lighting, camera movement, |
| 2019 | Villarroel et al. | 30 premature infants | NICU | Convolutional neural network | HR and RR | MAE 2.3 bpm for HR and 3.5 bpm for RR | Background noise, motion |
| 2020 | Paul et al. | 19 neonates | NICU | Short time Fourier transform | HR | Segments of 3 bpm of difference obtained | Motion, light, ROI tracking |
| 2020 | Rossol et al. | 18 infants | NICU | EVM, PFF, MSD | RR | RMSE 6.36 bpm | Lighting and movement but MSD is robust to these |
| 2021 | Chen et al. | 9 neonates | NICU | EVM with majority voting | HR | MAE 3.39 bpm at rest and 4.34 bpm during movement | Unexpected head movement |
| 2021 | Khanam et al. | 7 neonates | NICU | CNN with automatic ROI selection and EEMD to reduce noise | HR and RR | MAE of 1.8 bpm for HR and 2.13 bpm for RR | Lighting, subject and camera movement |
| 2021 | Wieler et al. | 28 term neonates | Maternity hospital nursery | Publicly available software, FFT and manual ROI selection | HR | RMSE 20.4 bpm | Higher birth weight, movement |
| 2021 | Lorato et al. | 17 infants | Medium care unit | MATLAB software with labelling of motion in videos | RR | MAE 3.31 bpm on testing and 5.36 on validation dataset | Severe motion (although small motions compensated), clothing, suckling |
| 2021 | Lucy et al. | 5 children with pneumonia | Hospital | FFT and denoising algorithms | RR | MAE of 1.8 bpm | Motion, illumination changes |
| 2021 | Paul et al. | 1 baby | NICU | Feature maps to identify pulsatile signals, FFT | HR | Not mentioned | Shadows, reflective materials, movement |
| 2021 | Nagy et al. | 7 infants | NICU | CNN with detection of ROI and severe motion periods – 2 different algorithms | HR and RR | MAE 7.08 bpm and 6.19 bpm for HR when non-ideal conditions and ideal conditions were present respectively; MAE 5.08 bpm and 2.03 bpm for RR when non-ideal vs ideal conditions present respectively | Lighting, movement |

Patient movement is another major impediment mentioned in most studies. This is especially noticeable in studies involving the neonatal population since infants often exhibit uncoordinated and random movement. Movement may lead to the ROI selected moving out of the video frame as well as impeding the focus on a particular ROI if there is constant movement (41, 44, 46, 49–51, 54). In an operating theatre setting, intra-operatively, movement is often not an issue since patients are often paralysed, and almost all are heavily sedated enough that gross movement will not occur. This is, of course, completely different when the child is awake especially pre- and post-op, when distress may be significant and children often refuse to stay still for any appreciable length of time. This may be a valid reason

why these methods are still not in mainstream use and only advancements in the algorithms used for data analysis can overcome this issue. Many motion stabilisation algorithms are already being trialled and show promising results especiallu in terms of accuracy and reliability (53, 54).

Different skin tones reflect light wavelengths differently and may confound the interpretation of rPPG signals. Therefore, studies that include members of the population with different skin tones are important to develop robust algorithms following camera calibration on the skin (60). There is a paucity of data regarding the skin tones of children studied, and only one study identified in this review by Paul et al. specifically mentions the effect of skin tone on vital signs extraction (51). Including children with different skin tones should not be an issue especially in the operating theatre setting, since changes in light and patient movement can be practically abolished in anaesthesia, allowing investigation of skin tone as the sole confounding factor.

## DISCUSSION

This concise review aims to provide a brief overview of NCVSM, specifically heart rate and respiratory rate, by use of RGB cameras. RGB cameras are advantageous as they are inexpensive and widely available, with many studies successfully obtaining heart rate and respiratory rate measurements by using regular smartphone cameras. Other means of monitoring vital signs certainly exist, such as different types of radar, ultrasound Doppler and thermal imaging cameras, but these come with added costs and complexity and in some cases such as radar, potential health safety concerns about prolonged use abound, in view of possible increases in thermal energy transfer (61, 62).

Studies based on the paediatric population make up a significant proportion of non-contact vital sign studies carried out in clinical areas; however the studies are few and include small populations. Very few studies include patients with specific pathologies, and no study was identified that actually took place in the operating theatre or peri-operative environment, highlighting a need to explore this area further. Although these technologies show promise, there are still a number of limitations to tackle such as issues of patient or camera movement and changes in lighting. Many studies have in fact focused on excluding periods with a lot of noise signals such as during clinical intervention and accepting brackets of time in which the patient may not be monitored (43, 50). This, however, is often unacceptable in paediatric anaesthetic practise. A large lacuna exists regarding how sensitive, accurate and reliable these systems are or can be, especially in a paediatrics setting.

The theatre environment lends itself very well to such studies. The availability of camera-based monitoring would be of great advantage to vulnerable populations such as infants and patients with skin conditions or burns and so on, especially during prolonged procedures or surgeries where monitoring equipment such as ECG leads must be placed on the back, potentially leading to excessive pressure being applied to the patient leading to irritation and, in severe cases, ulceration (3). Patients undergoing surgery are also ideal candidates for data collection since they do not move, and illumination can be kept constant by dedicated light sources if the theatre lighting is inadequate (49).

One further point to consider which has not been deeply tackled in any of the studies identified as part of this review is the issue of privacy and data protection (63). RGB video data is easily identifiable and therefore data protection policies must be in place to ensure ethical handling of collected videos (64). The fact that a paediatric population is being studied and particularly at a time when the patient is most vulnerable in the peri-operative period makes such issues even more important to tackle and consent from the child's legal guardian or the children themselves if they are deemed to have capacity to consent is of utmost importance (65). This also makes sharing of data with other scientist teams more difficult and possibly ethically questionable and may affect the ability of different teams to build on each others' work and achieve better results.

## CONCLUSION

NCVSM is an exciting new area of medicine which offers unique advantages in clinical practise to both the patient and the attending clinicians. Although interest in the field is growing and progress is being made, there is still a long way to go in terms of obtaining adequate accuracy and sensitivity levels and overcoming practical obstacles to be able to incorporate NCVSM into everyday practise to give reliable and replicable results. The need for further studies within the hospital environment is highlighted in this review.

## FUNDING

The Malta Council for Science and Technology: MCST-CVP R&I-2018-004-V (2018)

## REFERENCES

1. Malasinghe LP, Ramzan N, Dahal, K. Remote patient monitoring: a comprehensive study. J Ambient Intell Humaniz Comput 2019; 10: 57–76.
2. WHO. Evidence of hand hygiene to reduce transmission and infections by multi-drug resistant organisms in health-care settings. http://whqlibdoc.who.int/publications, 2009.
3. Boric-Lubecke O, Lubecke VM, Droitcour AD, Park BK, Singh A. Doppler Radar Physiological Sensing. Wiley 2016.
4. Bella A, Latif R, Saddik A, Zahra Guerrouj F. Monitoring of Physiological Signs and Their Impact on The Covid-19 Pandemic: Review. E3S Web Conf 2021; 229: 01030.
5. Tsai C-Y, Chang N-C, Fang H-C, Chen Y-C, Lee S-S. A Novel Non-contact Self-Injection-Locked Radar for Vital Sign Sensing and Body Movement Monitoring in COVID-19 Isolation Ward. J Med Syst 2020; 44(10): 177.
6. Phua J, Weng L, Ling L, Egi M, Lim CM, Divatia JV, et al. Intensive care management of coronavirus disease 2019 (COVID-19): challenges and recommendations. Lancet Respir Med 2020; 8(5): 506–17.

7. Chorney JML, Kain ZN. Behavioral analysis of children's response to induction of anesthesia. Anesth Analg 2009; 109(5): 1434–40.
8. Fortier MA, Del Rosario AM, Martin SR, Kain ZN. Perioperative anxiety in children. Paediatr Anaesth 2010; 20(4): 318–22.
9. Watson AT, Visram A. Children's preoperative anxiety and postoperative behaviour. Paediatr Anaesth 2003; 13(3): 188–204.
10. Tan L, Frca BM, Meakin GH, Frca MD. Anaesthesia for the uncooperative child. Contin Educ Anaesth Crit Care Pain 2010; 10(2): 48–52.
11. Checketts MR, Alladi R, Ferguson K, Gemmell L, Handy JM, Klein AA, et al. Recommendations for standards of monitoring during anaesthesia and recovery 2015: Association of Anaesthetists of Great Britain and Ireland. Anaesthesia 2016; 71(1): 85–93.
12. Al-Khalidi FQ, Saatchi R, Burke D, Elphick H, Tan S. Respiration rate monitoring methods: A review. Pediatr Pulmonol 2011; 46(6): 523–9.
13. Mond HG, Haqqani HM. The Footprints of Electrocardiographic Interference: Fact or Artefact. Heart Lung Circ 2019; 28(10): 1472–83.
14. Takats Z, Strittmatter N, McKenzie JS. Ambient Mass Spectrometry in Cancer Research. Adv Cancer Res 2017; 134: 231–56.
15. Hewson DW, Hardman JG. Physical injuries during anaesthesia. BJA Educ 2018; 18(10): 310.
16. McDevitt WM, Farley M, Martin-Lamb D, Jones TJ, Morris KP, Seri S, Scholefield BR. Feasibility of non-invasive neuro-monitoring during extracorporeal membrane oxygenation in children. Perfusion 2023; 38(3): 547–56.
17. Vedrenne-Cloquet M, Lévy R, Chareyre J, Kossorotoff M, Oualha M, Renolleau S, Grimaud M. Association of Cerebral Oxymetry with Short-Term Outcome in Critically ill Children Undergoing Extracorporeal Membrane Oxygenation. Neurocrit Care 2021; 35(2): 409–17.
18. Allen, J. Photoplethysmography and its application in clinical physiological measurement. Physiol Meas 2007; 28(3): R1-39.
19. Přibil J, Přibilová A, Frollo I. Comparative Measurement of the PPG Signal on Different Human Body Positions by Sensors Working in Reflection and Transmission Modes. Eng Proc 2020; 2(1): 69.
20. Wadhwa N, Wu HY, Davis A, Rubinstein M, Shih E, Mysore GJ, et al. Eulerian video magnification and analysis. Commun ACM 2017; 60(1): 87–95.
21. Yang F, He S, Sadanand S, Yusuf A, Bolic M. Contactless Measurement of Vital Signs Using Thermal and RGB Cameras: A Study of COVID 19-Related Health Monitoring. Sensors (Basel) 2022; 22(2): 627.
22. Lauridsen H, Gonzales S, Hedwig D, Perrin KL, Williams CJA, Wrege PH, et al. Extracting physiological information in experimental biology via Eulerian video magnification. BMC Biol 2019; 17(1): 1–26.
23. A Step-by-Step Explanation of Principal Component Analysis (PCA). Built In [Internet]. Available from: https://builtin.com/data-science/step-step-explanation-principal-component-analysis
24. Independent Component Analysis (ICA) by Shawhin Talebi. Towards Data Science [Internet]. Available from: https://towardsdatascience.com/independent-component-analysis-ica-a3eba0ccec35
25. A Comprehensive Guide to Convolutional Neural Networks — the ELI5 way by Sumit Saha. Towards Data Science [Internet]. Available from: https://towardsdatascience.com/a-comprehensive-guide-to-convolutional-neural-networks-the-eli5-way-3bd2b1164a53
26. Albawi S, Mohammed TA, Al-Zawi S. Understanding of a convolutional neural network. Proc 2017 Int Conf Eng Technol ICET 2017. 2018 Mar 8.
27. How Do Convolutional Layers Work in Deep Learning Neural Networks? [Internet]. Available from: https://machinelearningmastery.com/convolutional-layers-for-deep-learning-neural-networks/
28. Currie G, Hawk KE, Rohren E, Vial A, Klein R. Machine Learning and Deep Learning in Medical Imaging: Intelligent Imaging. J Med Imaging Radiat Sci 2019; 50(4): 477–87.
29. Romano C, Schena E, Silvestri S, Massaroni C. Non-contact respiratory monitoring using an RGB camera for real-world applications. Sensors 2021; 21(15): 5126.
30. van Gastel M, Stuijk S, de Haan G. Robust respiration detection from remote photoplethysmography. Biomed Opt Express 2016; 7(12): 4941.
31. Valenzuela A, Sibuet N, Hornero G, Casas O. Non-contact video-based assessment of the respiratory function using a rgb-d camera. Sensors 2021; 21(16): 5605.
32. Turaga P, Chellappa R, Veeraraghavan A. Advances in Video-Based Human Activity Analysis: Challenges and Approaches. Adv Comput 2010; 80(C): 237–90.
33. Brüser C, Antink CH, Wartzek T, Walter M, Leonhardt S. Ambient and unobtrusive cardiorespiratory monitoring techniques. IEEE Rev Biomed Eng 2015; 8: 30–43.
34. Li P, Benezeth Y, Nakamura K, Gomez R, Yang F. Model-based Region of Interest Segmentation for Remote Photoplethysmography. Proceedings of the 14th International Joint Conference on Computer Vision, Imaging and Computer Graphics Theory and Applications (VISIGRAPP 2019); 2019: 383–8.
35. Kossack B, Wisotzky E, Hilsmann A, Eisert P. Automatic region-based heart rate measurement using remote photoplethysmography. Proc IEEE Int Conf Comput Vis 2021: 2755–9.
36. Coppetti T, Brauchlin A, Mu S, Attinger-toller A, Templin C, Scho F, et al. Accuracy of smartphone apps for heart rate measurement. Eur J Prev Cardiol 2017; 24(12): 1287–93.
37. Shao D, Liu C, Tsow F, Yang Y, Du Z, Iriya R, et al. Noncontact Monitoring of Blood Oxygen Saturation Using Camera and Dual-Wavelength Imaging System. IEEE Trans Biomed Eng 2016; 63(6): 1091–8.
38. Mcduff D. Camera Measurement of Physiological Vital Signs. 2018.
39. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. BMJ 2021; 372: n71
40. Harford M, Catherall J, Gerry S, Young JD, Watkinson P. Availability and performance of image-based, non-contact methods of monitoring heart rate, blood pressure, respiratory rate, and oxygen saturation: a systematic review. Physiol Meas 2019; 40(6): 06TR01.
41. Maurya L, Kaur P, Chawla D, Mahapatra P Non-contact breathing rate monitoring in newborns: A review. Comput Biol Med 2021; 132: 104321.
42. Cobos-Torres J-C, Abderrahim M, Martínez-Orgado J. Non-Contact, Simple Neonatal Monitoring by Photoplethysmography. Sensors 2018; 18(12): 4362.
43. Chaichulee S, Villarroel M, Arteta C, Mccormick K. Cardio-respiratory signal extraction from video camera data for continuous non-contact vital sign monitoring using deep learning. Physiol Meas 2019; 40(11): 115001.
44. Wieler ME, Murphy TG, Blecherman M, Mehta H, Bender GJ. Infant heart-rate measurement and oxygen desaturation detection with a digital video camera using imaging photoplethysmography. J Perinatol 2021; 41(7): 1725–31.
45. Lorato I, Stuijk S, Meftah M, Kommers D, Andriessen P, van Pul C, et al. Towards continuous camera-based respiration monitoring in infants. Sensors 2021; 21(7): 1–18.
46. Lucy FK, Suha KT, Dipty ST, Wadud SI, Kadir MA. Video based non-contact monitoring of respiratory rate and chest indrawing in children with pneumonia. Physiol Meas 2021; 42(10).
47. Nagy Á, Földesy P, Jánoki I, Terbe D, Siket M, Szabó M, et al. Continuous camera-based premature-infant monitoring algorithms for nicu. Appl Sci 2021; 11(16): 7215.
48. Sun Y, Wang W, Long X, Meftah M, Tan T, Shan C, et al. applied sciences Respiration Monitoring for Premature Neonates in NICU. Appl Sci 2019; 9(23): 5246.
49. Gibson K, Al-Naji, A, Fleet, J, Steen, M, Esterman, A, Chahl, J, Huynh, J, Morris, S. Non-contact heart and respiratory rate monitoring of preterm infants based on a computer vision system: a method comparison study. Pediatr Res 2019; 86: 738–41.
50. Villarroel M, Chaichulee S, Jorge J, Davis S, Green G, Arteta C, et al. Non-contact physiological monitoring of preterm infants in the Neonatal Intensive Care Unit. npj Digit Med 2019; 128: 1–18.
51. Paul M, Karthik S, Joseph J, Sivaprakasam M, Kumutha J, Leonhardt S, et al. Non-contact sensing of neonatal pulse rate using camera-based imaging: A clinical feasibility study. Physiol Meas 2020; 41(2): 024001.
52. Paul M, Behr SC, Weiss C, Heimann K, Orlikowsky T, Leonhardt S. Spatio-temporal and -spectral feature maps in photoplethysmography imaging and infrared thermograph. Biomed Eng Online 2021; 20(1): 1–54.
53. Rossol SL, Yang JK, Toney-Noland C, Bergin J, Basavaraju C, Kumar P, et al. Non-Contact Video-Based Neonatal Respiratory Monitoring. Children 2020; 7(10): 171.
54. Chen Q, Jiang X, Liu X, Lu C, Wang L, Chen W. Non-Contact Heart Rate Monitoring in Neonatal Intensive Care Unit using RGB Camera. In: Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBS. Institute of Electrical and Electronics Engineers Inc.; 2020: 5822–5.
55. Khanam FTZ, Perera AG, Al-Naji A, Gibson K, Chahl J. Non-contact automatic vital signs monitoring of infants in a neonatal intensive care unit based on neural networks. J Imaging 2021; 7(8): 122.
56. Infants (0–1 years). CDC [Internet]. Available from: https://www.cdc.gov/ncbddd/childdevelopment/positiveparenting/infants.html
57. Buhre W, Rossaint R. Perioperative management and monitoring in anaesthesia. Lancet 2003; 362(9398): 1839–46.
58. Eddahchouri Y, Peelen R V., Koeneman M, Touw HRW, van Goor H, Bredie SJH. Effect of continuous wireless vital sign monitoring on unplanned ICU admissions and rapid response team calls: a before-and-after study. Br J Anaesth 2022; 128(5): 857–63.
59. Lin YC, Lin YH. A study of color illumination effect on the SNR of

rPPG signals. Proc Annu Int Conf IEEE Eng Med Biol Soc EMBS 2017 Sep 13: 4301–4.
60. Nowara EM, Mcduff D, Veeraraghavan A. A Meta-Analysis of the Impact of Skin Type and Gender on Non-contact Photoplethysmography Measurements. Available from: http://data.un.org/
61. Hall T, Lie DYC, Nguyen TQ, Mayeda JC, Lie PE, Lopez J, et al. Non-contact sensor for long-term continuous vital signs monitoring: A review on intelligent phased-array doppler sensor design. Sensors (Basel) 2017; 17(1): 2632.
62. Barbosa Pereira C, Yu X, Czaplik M, Blazek V, Venema B, Leonhardt S. Estimation of breathing rate in thermal imaging videos: a pilot study on healthy human subjects. J Clin Monit Comput 2017; 31(6): 1241–54.
63. Choi P, Walker R. Remote Patient Management: Balancing Patient Privacy, Data Security, and Clinical Needs. Contrib Nephrol 2019; 197: 35–43.
64. Mondschein CF, Monda C. The EU's General Data Protection Regulation (GDPR) in a Research Context. Fundam Clin Data Sci 2018: 55–71. Available from: https://www.ncbi.nlm.nih.gov/books/NBK543521/
65. Lepola P, Needham A, Mendum J, Sallabank P, Neubauer D, De Wildt S. Informed consent for paediatric clinical trials in Europe. Arch Dis Child 2016; 101(11): 1017–25.
66. Gillick competence and Fraser guidelines. NSPCC Learning [Internet]. Available from: https://learning.nspcc.org.uk/child-protection-system/gillick-competence-fraser-guidelines

# Immunological Parameters in Patients Suffering from Atopic Dermatitis and Either Treated or Non-Treated with Dupilumab

Petra Boudková[1,*], Jarmila Čelakovská[2], Eva Čermáková[3], Ctirad Andrýs[1], Jan Krejsek[1]

ABSTRACT

Objective: The aim of the study is to analyze the absolute count of leukocytes, neutrophils, monocytes, eosinophils, T cells, natural killer cells, B cells and to evaluate the expression of functionally important CD23 and CD200 molecules on B cells in patients suffering from atopic dermatitis (AD), (with and without dupilumab therapy).

Materials and Methods: We examined 45 patients suffering from AD – 32 patients without dupilumab treatment (10 men, 22 women, average age 35.0 years), 13 patients with dupilumab treatment (7 men, 6 women, average age 43.4 years) and 30 healthy control (10 men, 20 women, average age 44.7 years). Immunophenotype was examined by flow cytometry (Navios Flow Cytometer – Beckman Coulter). The blood count was examined with a Sysmex XN 3000, Sysmex SP10, microscope DI60 for digital morphology evaluating cell division and microscope Olympus BX40. We compared the absolute count of leukocytes and their subsets, T cells (CD4, CD8), natural killers cells, absolute and relative count of B lymphocytes and expression of surface molecules CD23 and CD200 on B cells in AD patients and in control group. Non-parametric Kruskal-Wallis one-factor analysis of variance with post-hoc (follow-up multiple comparison) and Dunn's test with Bonferroni modification of significance level were used for statistical analysis.

Results: We confirmed the significantly higher number of neutrophils, monocytes and eosinophils and higher expression of CD23 and CD200 on B cells in peripheral blood of AD patients (either with or without dupilumab) therapy. We demonstrated the lower number of CD8+ T cells.

Conclusion: We demonstrated the difference in the count of white blood cells populations in patients suffering from AD compared with healthy control. There were a differences in the expression of immunoregulatory molecules CD23 and CD200 on B cells in AD patients (either with or without dupilumab therapy) in comparison to healthy controls.

KEYWORDS

atopic dermatitis; immunophenotyping; B cells; T cells; NK cells; CD23; CD200

AUTHOR AFFILIATIONS

[1] Department of Clinical Immunology and Allergology, Faculty Hospital and Faculty of Medicine, Charles University, Hradec Králové, Czech Republic

[2] Department of Dermatology and Venereology Faculty Hospital and Faculty of Medicine, Charles University, Hradec Králové, Czech Republic

[3] Department of Medical Biophysics, Faculty of Medicine, Charles University, Hradec Králové, Czech Republic

* Corresponding author: Department of Clinical Immunology and Allergology, Faculty Hospital and Faculty of Medicine, Charles University, Hradec Králové, 50005, Czech Republic; petra.boudkova@fnhk.cz

Received: 22 March 2023
Accepted: 9 June 2023
Published online: 8 November 2023

Acta Medica (Hradec Králové) 2023; 66(2): 47–54
https://doi.org/10.14712/18059694.2023.15

## INTRODUCTION

Atopic dermatitis (AD) is a common, chronic inflammatory skin disease characterized by immune abnormalities and a disturbed epidermal barrier, resulting in increased transepidermal water loss and increased penetration of allergens, irritants, and microbes. The key role of filaggrin (FLG), a protein present in the granular layer of the epidermis regulating the aggregation of keratin filaments, was evidenced in AD because some loss-of-function mutations in *FLG* gene resulting in FLG deficiency contribute to epidermal barrier dysfunction and is strongly associated with AD (1–4).

AD disease is characterized by a biphasic inflammation, evolving from an initial, acute phase dominated by Th2– and Th22 functionaly polarized T helper cells to a chronic phase characterized by the concomitant presence of various subsets of CD4+ T helper Th1, Th2 cells, and Th17 cells (1, 2). Excessive polarization of Th2 T cells leads to increased production of selected interleukins (IL) such as IL-4, IL-5 and IL-13. IL-4 has been shown to participate on the differentiation of naive CD4+ T cells into Th2 effector cells, while IL-13 plays an important role in goblet cell metaplasia, mucus hypersecretion, and smooth muscle contractility. Both cytokines also promote class switching to IgE and the chemotaxis of eosinophils (1, 2). Factors influencing the destruction of the epidermis, such as damage, infections (*Staphylococcus aures, Streptococcus spp.* and viral infections) or ongoing inflammation, stimulate keratinocytes to produce proinflammatory cytokines such as Thymic stromal lymphopoietin (TSLP), IL-25 and IL-33. They also contribute to the Th2-mediated immune response and activate innate lymphoid cells (subtype 2). TSLP, through its receptor, activates immature dendritic cells, enhances their maturation to the effective antigen-presenting cells (APCs) (2, 5–7). The Th2 cytokines interleukin 4 (IL-4) and IL-13 and the heterodimeric IL-4 receptor (IL-4R) complexes that they interact with, play a key role in the pathogenesis of allergic disorders. The multifaceted roles of IL-4 and Il-13 is an attractive target for treatment strategies. IL-4 is multifunctional cytokine, which promotes mature B cells activation and differentiation, proliferation and secretion of antibodies. IL-4 plays the role in prologing the survival of transitional B cells and promoting their maturation. There are multistep approaches to treat patients suffering from AD. The most effective therapy seems to be biological therapy. Dupilumab is a humane IgG4 monoclonal antibody that targets the IL-4 receptor alpha chain (IL-4Rα), common to both IL-4R receptors: type 1 (IL-4Rα/γc; IL-4 specific) and type 2 (IL-4Rα/IL-13Rα1; IL-4 and IL-13 specific) (5, 8–11).

Clinical studies with non specific and targeted therapeutics have helped to elucidate the contribution of various immune mechanisms to the disease phenotype. Besides skin lesions in AD patients, the blood components display specific inflammatory changes. The suppression of inflammatory changes is demonstrated in the case of dupilumab treatment, which inhibits the formation of key IL-4 and IL-13 cytokines. B cells proliferation and diferentiation is stimulated by IL-4, followed by terminal B cells differentiation to plasma cells. This cytokine increases expression of CD23 and supports isotype switching in antibodies (5, 6, 12–14).

The role of B cells in innate and adaptive immunity is rapidly evolving with acknowledgement of their complex multifactorial role in innate immunity through functions including antigen presentation, non-specific antibody secretion and cytokine secretion. A lot of new therapeutics for AD and other inflammatory diseases were derived from our understanding of T cells contribution to allergic inflammation. The function of B cells and their surface markers provided additional layers of complexity to understand of B cell function in normal and damaged skin. AD demonstrate high number of skin mature B cells (15). The other subtype of B cells, such as transitional B cells, represent a link between immature B cells in the bone marrow and mature B cells in peripheral blood. Transitional B cells represent one of the B cells in healthy subjects. Their count could be altered in patients with autoimmune immunopathological diseases such as multiple sclerosis, neuromyelitis optica spectrum disorders, systemic lupus erythematosus, rheumatoid arthritis and others. Transitional B cells can also produce homeostatic IL-10 and regulate proliferation of CD4+ T cells (6, 8). Activated Th2 cells produce IL-13. IL-13 could be also produced by basophils, natural killer and innate lymphoid cells (subtype 2). Activated eosinophils also produce IL-13 and during this process factors essential for polarization of eosinophils are induced and B cells are diferenciated for production of IgE (6, 8). IL-13 is involved to reshaping of B cells through the transitional B cells. However the function of transitional B cells remain largely unclear. This may partially be due to their low frequency in circulation (8, 13).

Activated B cells express CD23 surface molecule which is also expressed on monocytes and subsets of eosinophils. CD23 is low affinity immunoglobulin E, participating in the regulation of IgE synthesis and numerous pro-inflammatory activities. This molecule could trigger the release of proinflammatory cytokines, for example tumor necrosis factor alfa (TNF alfa), IL-1 beta, IL-6 (16–18). Transmembrane glycoprotein CD200 belong to the immunoglobulin superfamily. This molecule is expressed on lymphocytes (B-cells and T-cells) and endothelial cells. CD200 induces the downregulation of T-cells by interaction with its receptor CD200R. CD200 molecules demonstrated the inhibition of macrophage function, induction of regulatory T cells and suppression of the function of natural killer cells (19–22).

For these above mentioned facts that IL-4 and IL-13 are playing an important role in immunopathogenesis of AD and biological effect of these cytokines is blocked in patients treated by dupilumab, we focus on immunophenotype of blood cells as neutrophils, eosinophils, monocytes and lymphocytes. IL-4 supports switching B cells and subsequent output of antibodies and increased the expression of CD23 molecule. This cytokine play the role in formation of antibodies, the CD23 expression was followed in immunophenotyping analysis of peripheral blood of patients either treated or not with dupilumab. Assumed that the CD200 molecule could regulate myeloid cell activity and delivers an inhibitory signals for the macrophage lineage, this marker determination was also included in our immunophenotyping analysis (8, 14, 23, 24).

The aim of our study is to analyze the absolute count of leukocytes (neutrophils, monocytes, eosinophils), lymphocytes (T cells, B cells and NK cells) and relative count of transitional B cells and to evaluate the relation to the expression of CD23 and CD200 molecules on B cells in patients suffering from AD (with and without dupilumab therapy).

The evaluation of the expression of CD23 and CD200 surface molecules on B cells compared with absolute count of phagocytic cells could help us to asses the severity of AD and can reflect response to biological treatment with dupilumab.

## MATERIAL AND METHODS

### DERMATOLOGICAL EXAMINATION

Complete dermatological examination was performed in all patients included in the study. The diagnosis of atopic dermatitis was determined according to Hanifin-Rajka's diagnostic criteria. Severity of AD was scored in agreement with SCORAD (Scoring of Atopic Dermatitis), with assessment of topography (affected skin area), intensity criteria and subjective parameters and with the EASI system (Eczema Area and Severity Index). We also examined 30 healthy volunteers – blood donors (matched to age and sex).

The severity of atopic dermatitis was evaluated with SCORAD as a mild form to 25 points, as moderate over 25 to 50 points, as a severe form over 50 points. This examination was performed during one year every two month and the average SCORAD index was calculated.

The biological treatment (dupilumab) was indicated in patients with moderate or severe form of AD (SCORAD index = from 25 to 50 points and over). This is systemic treatment; the dose of dupilumab is 300 mg s.c. every two weeks. During the biological treatment these AD patients showed improvement of clinical signs to the mild form of AD (SCORAD index = to 25 points).

Inclusion criteria: 1) age 14 years and over 2) atopic dermatitis as defined by the criteria of Hanifin and Rajka. The severity of atopic dermatitis evaluated with SCORAD index and EASI score.

Exclusion criteria: pregnancy, breastfeeding, systemic therapy (cyclosporine A, systemic corticoids).

### EVALUATION OF THE IMMUNOLOGICAL PROFILE

The blood samples were collected from antecubical fossa vein into sample tubes pre-coated with EDTA – anticoagulant. The blood count was examined with a Sysmex XN 3000, Sysmex SP10, microscope DI60 for digital morphology evaluating cell division and microscope Olympus BX40.

Surface molecules expressed on immune cells were examined by flow cytometry using monoclonal antibodies labeled with fluorochromes purchased from Beckman Coulter. 5 µl of each fluorochrome-labelled monoclonal antibodies and 50 µl of peripheral blood was added to cytometric tube. Blood samples were incubated for 15 minutes with antibodies at room temperature in the dark. Then a lysis solution (OptiLyse C, Beckman Coulter) was added and samples were incubated for 10 minutes. The samples were measured with a Navios Flow Cytometer (Beckman Coulter). A minimum of 60,000 events (60,000 cells) were obtained for each stain and were supplied in list mode (LMD), which are necessarily for assessment. Multiple peripheral blood parameters were assessed as absolute and relative count.

The gating strategies for the different leukocytes and lymphocytes subsets assessed were as follows:

- leukocytes (CD45+), eosinophils (high SSC, CD49d+, CD15+), monocytes (CD45+, CD14+), neutrophils (CD15+, CD16+)
- lymphocytes (low SSC, CD45++), T cells (CD3+), helper T cells (CD3+, CD4+), cytotoxic T cells (CD3+, CD8+), natural killer (NK) cells (CD3–, CD56+ and/or CD16+), B cells (CD19+), transitional B cells (CD38+, CD24+, CD27–)
- B cells regulatory surface molecules CD23 and CD200

Monoclonal antibodies CD23 and CD200 were incorporated into immunophenotyping of B cells. We examined samples of peripheral blood in the period from October 2021 to February 2022 (out of pollen season).

This study was approved by Ethics commitee of Faculty Hospital Hradec Králové, Charles University, Czech Republic and it have been performed according to the Declaration of Helsinki. The informed consent has been obtained from all participants.

### STATISTICAL ANALYSIS

We compared the absolute count of leukocytes (neutrophils, monocytes, eosinophils) and lymphocytes (CD4+ T cells, CD8+ T cells, NK cells and B lymphocytes), relative count of transitional B lymphocytes and expression of CD23 and CD200 on B cells in patients suffering from AD (with or without dupilumab treatment) and in control group. For statistical analysis we used non-parametric Kruskal-Wallis one-factor analysis of variance with post-hoc (follow-up multiple comparison) and Dunn's test with Bonferroni modification of significance level. We used statistical software: NCSS 2021 Statistical Software (2021). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/ncss.

## RESULTS

### CHARACTERISTIC OF PATIENTS

During the period from October 2021 to February 2022 we examined 75 subjects: thirty-two patients suffering from AD without dupilumab treatment, thirteen patients with dupilumab treatment and thirty subjects as a heatlhy control. The characteristic of patients is recorded in Table 1.

The severity of atopic dermatitis was consistent in both group of AD patients before starting the dupilumab therapy. Patients on dupilumab therapy had suffered from a moderate and severe form of AD before starting the biological treatment. AD patients have been under dupilumab treatment at least 18 months; there was a significant improvement in the skin finding and we observe mild symptoms of AD in these patients (Table 1). The treatment

**Tab. 1** Characteristic of patients.

| | Number of patients | Age | SCORAD | | EASI | |
|---|---|---|---|---|---|---|
| AD patients with dupilumab treatment | **13** (7 males, 6 females) | **43.4** (38.6–48.3) | Before therapy with dupilumab | **36.1** (30.5–45.2) | Before therapy with dupilumab | **35.2** (30.1–44.2) |
| | | | after 1.5 years | **10.5** (7.1–18.2) | after 1.5 years | **10.1** (8.2–17.2) |
| AD patients without dupilumab treatment | **32** (10 men, 22 women) | **35.0** (27.2–48.7) | **33.2** (26.5–38.7) | | **32.1** (26.8–38.5) | |
| Control group | **30** (10 men, 20 women) | **44.7** (36.8–51.4) | 0 | | 0 | |

involves the use of moisturizers and application of dupilumab 300 mg s.c. every two weeks.

The changes in absolute counts of leukocytes (neutrophils, monocytes, eosinophils), lymphocytes (T cells, B cells and NK cells) and relative count of transitional B cells are shown in Table 2. In AD patients treated with dupilumab the absolute number of leukocytes was increased compared to control group. The absolute number of eosinophils, neutrophils and monocytes in both groups of AD patients was increased compared to healthy controls. Absolute counts of T cells, B cells and NK cells were not statistically significantly different in AD patients when compared to the control group. There was significantly decreased the absolute number of CD8+ T cells in patients with dupilumab treatment compared to control group.

The number of transitional B cells has not been changed for any analyzed group. However, the expression of CD23 and CD200 on B cells were increased. This change was apparent in patients with dupilumab treatment and in patients without dupilumab compared to controls. The expression of selected markers on B cells is shown in Table 3.

Absolute counts of leukocytes and expression of CD23 and CD200 markers are recorded in Figures 1–6.

## DISCUSSION

The Th2 pattern inflammatory pathway in AD atopic dermatitis is driven by activation of functionally polarised $CD4^+$ helper T cells and innate lymphoid type 2 cells (ILC2). The tissue infiltration is characterized by inflammatory cells such as eosinophils, mast cells, basophils, and production of proinflammatory cytokines, including IL-4, IL-5, and IL-13 (1, 2). The aim of our study was to evaluate the absolute number of leukocytes, T cells, B cells and NK cells in atopic dermatitis patients with and without

**Tab. 2:** Characterization of changes in absolute and relative counts of leukocytes (median values are recorded). Explanation: "DUP–" patients without dupilumab treatment, "DUP+" patients with dupilumab treatment, "KW test" results of Kruskal Wallis test, "MFI" mean fluorescence intensity. *p-value < 0.05* is considered as statistically significant.

| | DUP- | DUP+ | control | DUP+/DUP- | DUP-/control | DUP+/control |
|---|---|---|---|---|---|---|
| abs. count of leukocytes ($10^9$/l) | 6.64 | 7.30 | 5.640 | | | <0.050 |
| abs. count of neutrophils ($10^9$/l) | 4.20 | 4.94 | 3.200 | | <0.050 | <0.001 |
| abs. count of monocytes ($10^9$/l) | 0.50 | 0.55 | 0.390 | | <0.001 | <0.050 |
| abs. count of eosinophils ($10^9$/l) | 0.36 | 0.41 | 0.170 | | <0.050 | <0.050 |
| abs. count of T cells ($10^9$/l) | 1.31 | 1.41 | 1.210 | | | |
| abs. count of CD4+ T cells ($10^9$/l) | 1.10 | 1.32 | 0.075 | | | |
| abs. count of CD8+ T cells ($10^9$/l) | 0.48 | 0.36 | 0.590 | | | <0.050 |
| abs. count of B cells ($10^9$/l) | 0.18 | 0.21 | 0.200 | | | |
| rel. count of transitional B cell (%) | 1.00 | 1.00 | 0.800 | | | |
| abs. count of NK cells ($10^9$/l) | 0.18 | 0.20 | 0.175 | | | |

**Tab. 3:** Expression of markers CD23 and CD200 on B cells (median values are recorded). Explanation: "DUP-" patients without dupilumab treatment, "DUP+" patients with dupilumab treatment, "KW test" results of Kruskal Wallis test, "MFI" mean fluorescence intensity. *p-value < 0.05* is considered as statistically significant.

| | DUP- | DUP+ | control | KW test | DUP+/DUP- | DUP-/control | DUP+/control |
|---|---|---|---|---|---|---|---|
| expression CD23 B cells (MFI) | 10.50 | 9.54 | 6.46 | 0.0000 | | <0.001 | <0.001 |
| expression CD200 B cells (MFI) | 4.42 | 4.31 | 3.86 | 0.0202 | | <0.050 | <0.050 |

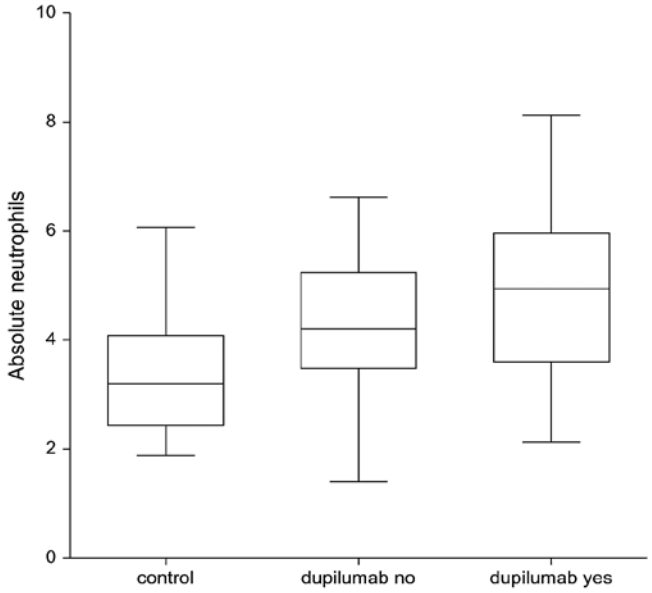


**Fig. 1** Absolute count of neutrophils ($10^9$/l).

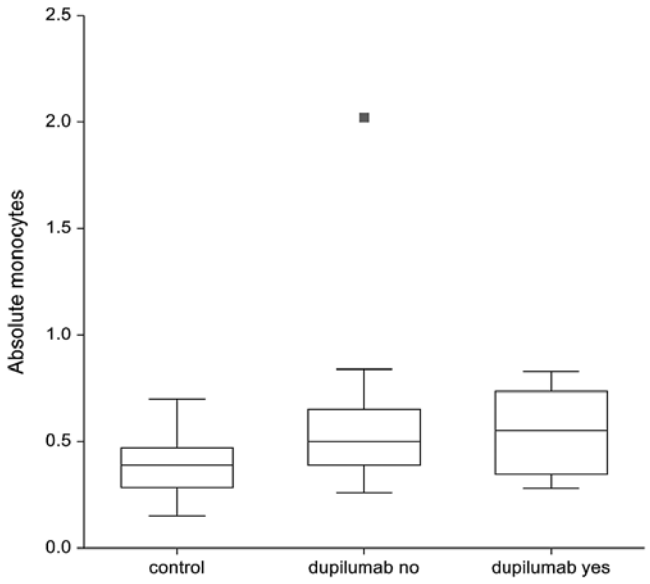


**Fig. 2** Absolute count of monocytes ($10^9$/l).

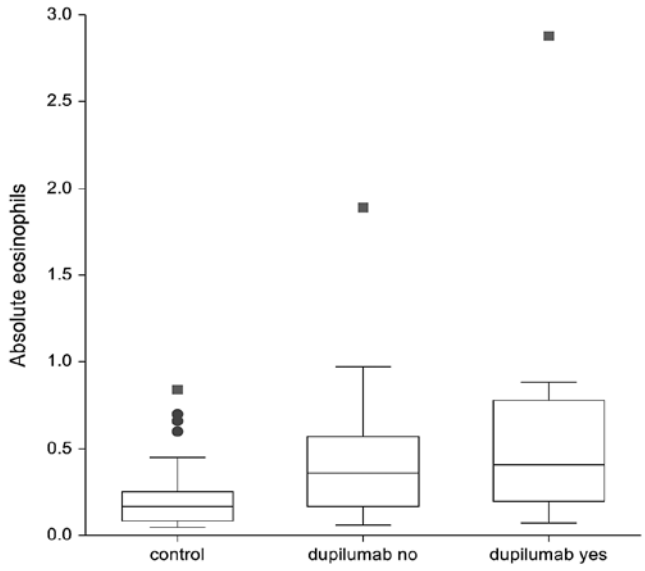


**Fig. 3** Absolute count of eosinophils ($10^9$/l).

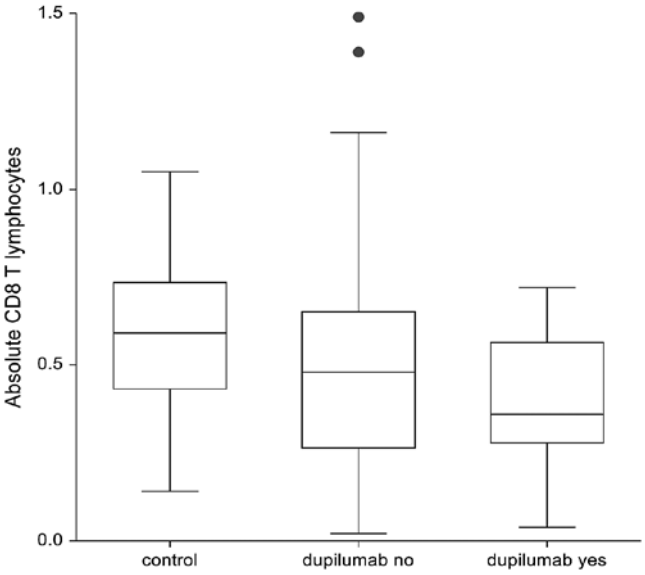


**Fig. 4** Absolute count of CD8 T lymphocytes ($10^9$/l).

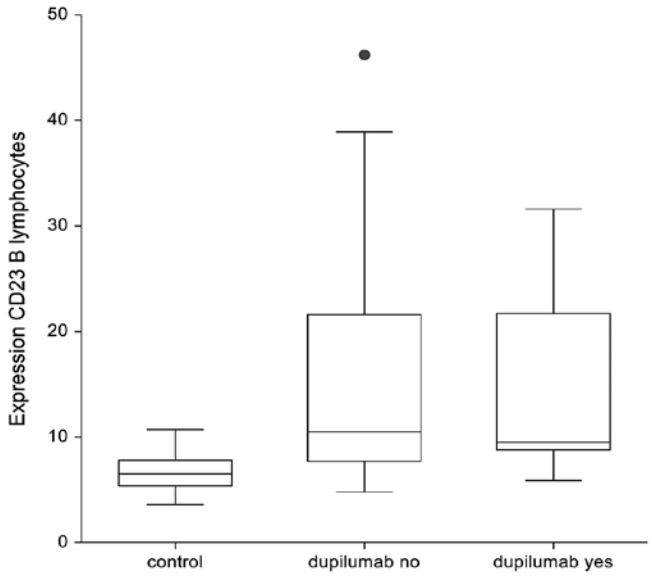


**Fig. 5** Expression of CD23 on B lymphocytes (MFI).

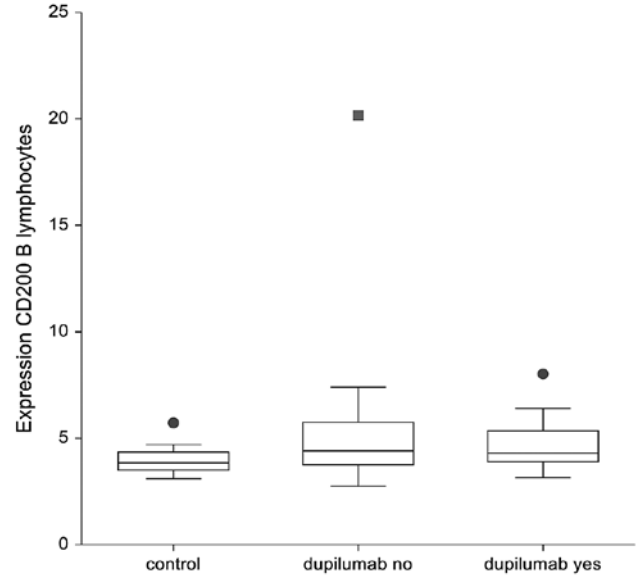


**Fig. 6** Expression of CD200 on B lymphocytes (MFI).

dupilumab treatment in comparison to healthy control. We focused on detailed immunophenotyping of B cells and we determined the expression of CD23 and CD200 regulatory molecules.

The study of Jiang (25) showed higher count of white blood cells, neutrophils and lymphocytes, which is in accord with results of our study. The inflammatory markers as a count of neutrophils, monocytes and eosinophils are increased in patients who were either treated with dupilumab or not. Eosinophilia has been shown to be present in majority of patients with AD and it correlated with the disease activity (25–28). Yamauchi et al. (29) demonstrated reduction of eosinophil number in peripheral blood in patient treated by dupilumab. In contrary, increased eosinophil counts have been reported in some dupilumab clinical trials. This increase generally occurred in the first few weeks and returned to baseline or is lower in the end of the treatment period (30). In our study, we recorded the higher absolute count of eosinophils in both group of patients (with or without dupilumab treatment).

The monocytes are a significant component of skin immunopathology such as atopic dermatitis or *psoriasis vulgaris*. These cells could invade the inflamed skin and differentiate there into macrophages. Macrophages can act as antigen-presenting cells in the skin lesion directly in patients with atopic dermatitis (31–33). IL-13 is a cytokine which is produced not only by stimulated Th2 lymhocytes, but also by CD8+ T cells, NK cells and keratinocytes. This cytokine acts on monocytes, but also stimulates the B cells proliferation (28, 33). Our study showed the difference in absolute count of CD8+ T cells in patients with dupilumab treatment. This result is in accord with results of Szymanski et al. (28), who claimed the IL-13 is also produced by CD8+ T cells. The absolute CD8+ T cells count in our study was reduced in patients with dupilumab treatment only. It could be the consequence of dupilumab treatment, because dupilumab blocks the subunit shared by receptors for IL-4/IL-13 (10, 28). Vestergaard et al. (31) showed the higher level of monocytes, which expressed CCR2 in patients with AD compared to healthy control. We analyzed monocytes without expression of CCR2 chemokine receptor, but the absolute number of monocytes was increased in both group of patients with AD compared with the control group. This result correlates with the study of Vestergaard et al. (23) and is reflecting ongoing inflammation. Apparently, in patients with dupilumab treatment the relief of clinical signs of disease activity is seen. However, the inflammatory response is still active as the monocytes and neutrophils counts are increased in patients with AD either treated or not treated with dupilumab (10, 26, 28, 32).

The higher count of leukocytes such as neutrophils, monocytes and eosinophils correlated positively with a diagnosis of AD. The result is similar to the study of Jiang et al. (25) who also found higher number of neutrophils and eosinophils in patients with AD. The neutrophils represent the most abundant population of circulating leukocytes in peripheral blood. These cells are indispensable for antimicrobial immunity. The activity of neutrophils is controlled by immune mechanism including chemotaxis of neutrophils to tissue-draining lymph nodes, resulting in antimicrobial immunity and inflammation. For this reason better understanding of the role of neutrophils in AD immunopathogenesis and impact of biological therapy of AD is warranted (34, 35).

There is the difference in the abundance of NK cells in the skin lesions compared with nonlesional skin in AD patients. NK cells are apparently more abundant in lesional skin (36). Mobus et al. (36) found, that the number of NK cells in skin lesions was upregulated after dupilumab treatment. We observed that the absolute count of NK cells in blood is not statistically significantly different in both examined patients groups compared to healthy control in our study. It could be caused by increased migration of NK cells from blood to skin lesions (28, 36–38).

The number of activity of B cells is also correlated with ongoing inflammation. Simon et al. (39) in their study claimed that the loss of B cells and their function as antigen presenting cells will ultimate to a lower T cell activation and consequently to decreased cytokines and mediators release. This could be the mechanism responsible for the clinical improvement in patients with AD. This statement is in accordance with effect of dupilumab treatment. The cytokine IL-4 is responsible for promoting Th2 cell functional polarisation and consequently the secondary production of IL-4 and IL-13, potent stimulators of IgE production by B cells (40, 41). No statistical difference in the absolute count of B cells in AD patients compared to healthy control was found in our study. However, there were the statistically significant differences in the expression of CD23 and CD200 molecules on B cells. It could be interpreted that B cells are activated and participate in the ongoing inflammation. The marker CD23 is expressed on B cells and IL-4 is required for its expression as found by Getahun et al. (42). In our study expression of CD23 on B cells was increased in both AD group compared to healthy control. It could be probably caused by increased level of IL-4, but this was not examinated by us (42).

Oligomerization of CD23 on the surface of B cells could enhance IgE binding through an avidity effect. The higher expression of CD23 on B cells could be caused by activation of B cells with effect of allergens and it leads to elevated IgE levels (43). This opinion of Engeroff et al. (43) could correlate with results of our study.

Furthermore, our study confirmed the higher expression of CD200 molecule on B cells in the patients with and without dupilumab treatment compared to the control group. It could be in accord with work of Mucha et al. (44) who evidenced that the genes *DOK2* and *CD200R1* contribute to AD risk (44). Also CD200 is expressed on the cell surface and this protein is considered as an immune checkpoint molecule. CD200 is present on the membrane of macrophages and other immune cells and this marker is responsible for the process leading to secretion of high level of IL-10. IL-10 is recognized as homeostatic cytokine preventing immune activation. Higher expression of CD23 and CD200 molecules as activation markers is correlating with increase of absolute count of neutrophils, monocytes and eosinophils adverts to ongoing phagocytosis presumably and general dysregulation of immune response (45, 46).

The laboratory results in both group of patients with AD (DUP+/DUP–) are almost comparable despite

of difference in SCORAD and EASI score. Whereas the SCORAD and EASI score include assessment of skin lesions, results of these scores are different in both group of patients with AD. In our study the immunophenotype was investigated from peripheral blood. There could be difference in immunological process in peripheral blood and skin lesions. Concurrently, the skin lesions were improved after dupilumab treatment, but inflammatory process is ongoing in peripheral blood. From this reason there could be unevenness between laboratory results and SCORAD and EASI score.

Patients who suffer from severe or persistent form of AD experience significant impairment in their quality of life which is also associated with substantial economic burden on society as a whole (7, 28). For that reason, the better understanding of immunopathological mechanisms in AD patients with or without dupilumab treatment related with B cells could be a hopeful step in improving the long term quality of their lives.

## CONCLUSION

The expression of CD23 and CD200 on B cells is elevated in both group of AD patients compared with controls but there was not the difference in absolute count of B cells. The absolute count of CD8+ T cell is lower in AD patients treated with dupilumab. Absolute number of leukocytes, neutrophils, monocytes and eosinophils are increased significantly in both groups of AD patients compared to healthy control. There is no statistically difference in the absolute count of NK cells and relative count of transitional B cells in blood in both groups of AD patients compared to controls. It is feasable that these cells (NK cells and trasitional B cells) could be localized in skin lesions in patients without dupilumab.

## ACKNOWLEDGEMENTS

This work was supported by the Cooperatio Program, research are IMMU.

## REFERENCES

1. Silverberg NB. Typical and atypical clinical appearance of atopic dermatitis. Clin Dermatol 2017; 35(4): 354–59.
2. Auriemma M, Giovina V, Paolo A, et al. Cytokines and T cells in atopic dermatitis. Eur Cytokine Netw 2013; 24(1): 37–44.
3. Paller A, Spergel J, Mina-Osorio P, et al. The atopic march and atopic multimorbidity: Many trajectories, many pathways. J Allergy Clin Immunol 2018; 143(1): 46–55.
4. Benedetto A, Kubo A, Beck LA. Skin barrier disruption: A requirement for allergen sensitization. J Invest Dermatol 2012; 132(3): 949–63.
5. Brauweiler AM, Goleva E, Leung DY. Th2 cytokines increase Staphylococcus aureus alpha toxin-induced keratinocyte death through the signal transducer and activator of transcription 6 (STAT6). J Invest Dermatol 2014; 134: 2114–21.
6. Brunner PM, Emma G, Donald YML. The immunology of atopic dermatitis and its reversibility with broad-spectrum and targeted therapies. J Allergy Clin Immunol 2017; 139(4): S65–S76.
7. Biedermann T, Skabytska Y, Kaesler S, et al. Regulation of T Cell Immunity in Atopic Dermatitis by Microbes: The Yin and Yang of Cutaneous Inflammation. Front Immunol 2015; 13(6): 353.
8. Zhou Y, Zhang Y, Han J, et al. Transitional B cells involved in autoimmunity and their impact on neuroimmunological diseases. J Transl Med 2020; 18(131): 1–12.
9. Fukuda K, Waka I, Tatsuma K, et al. Development of conjunctivitis with a conjunctival proliferative lesion in a patient treated with dupilumab for atopic dermatitis. Allergol Int 2019; 68(3): 383–4.
10. Nedoszytko B, Sokołowska-Wojdyło M, RuckemannDziurdzińska K, et al. Chemokines and cytokines network in the pathogenesis of the inflammatory skin diseases: atopic dermatitis, psoriasis and skin mastocytosis. Adv Dermatol Allergol 2014; 31: 84–91.
11. Brogger P, Lars B, Stine S, et al. Antagonism of the interleukin 4 receptor α promotes TH1-signalling among T cells from patients with atopic dermatitis after stimulation. Hum Immunol 2020; 91(1): 1–6.
12. Werfel T, Allam JP, Biedermann T, et al. Cellular and molecular immunologic mechanisms in patients with atopic dermatitis. J Allergy Clin Immunol 2016; 138: 336–49.
13. Kisich KO, Carspecken CW, Fieve S, et al. Defective killing of Staphylococcus aureus in atopic dermatitis is associated with reduced mobilization of human beta-defensin-3. J Allergy Clin Immunol 2008; 122: 62–8.
14. Mak T, Saunders M. Cytokines and cytokine receptors. Immune Response 2006; 2006(1): 463–516.
15. Frew JW, David G, Kristina N, et al. Beyond antibodies: B cells in Hidradenitis Suppurativa: Bystanders, contributors or therapeutic targets. Exp Dermatol 2019; 2020(29): 509–15.
16. Engeroff P, Vogel M. The role of CD23 in the regulation of allergic responses. Allergy 2021; 76(7): 1981–9.
17. Liu C, Richard K, Wiggins M, et al. CD23 can negatively regulate B-cell receptor signaling. Sci Rep 2016; 6(25629): 1–8.
18. Yao Y, Wang N, Chen CL, et al. CD23 expression on switched memory B cells bridges T-B cell interaction in allergic rhinitis. Allergy 2020; 75(10): 2599–612.
19. D'Arena G, De FV, Pietrantuono G, et al. CD200 and Chronic Lymphocytic Leukemia: Biological and Clinical Relevance. Front Oncol 2020; 26(10): 58442–7.
20. Gorczynski R, Khatri I, Lee L, et al. An interaction between CD200 and monoclonal antibody agonists to CD200R2 in development of dendritic cells that preferentially induce populations of CD4+CD25+ T regulatory cells. J Immunol 2008; 180: 5946–55.
21. Cherwinski HM, Murphy CA, Joyce BL, et al. The CD200 receptor is a novel and potent regulator of murine and human mast cell function. J Immunol 2005; 174: 1348–56.
22. Coles SJ, Wang ECY, Man S, et al. CD200 expression suppresses natural killer cell function and directly inhibits patient anti-tumor response in acute myeloid leukemia. Leukemia 2011; 25: 792–9.
23. Mannon P, Walter R. Interleukin 13 and its role in gut defence and inflammation. Gut 2012; 2012(61): 1765–73.
24. Barclay A, Wright G, Brooke G, et al. CD200 and membrane protein interactions in the control of myeloid cells. Trends Immunol 2002; 23(6), 285–90.
25. Jiang Y, Wencong MA. Assessment of Neutrophil-to-Lymphocyte Ratio and Platelet-to-Lymphocyte Ratio in Atopic Dermatitis Patients. Med Sci Monit 2017; 2017(23): 1340–6.
26. Brenninkmeijer EE, Schram ME, Leeflang MM, et al. Diagnostic criteria for atopic dermatitis: A systematic review. Br J Dermatol 2008; 158: 754–65.
27. Lee KY, Cho KJ, Kim YT, et al. Serum eosinophil-derived neurotoxin in childhood atopic dermatitis: A useful marker of disease activity? Ann Allergy Asthma Immunol 2009; 102: 532–4.
28. Szymanski L, Cioa A, Ciepielak M, et al. Cytokines and apoptosis in atopic dermatitis. Postepy Dermatol Allergol 2021; 38(1): 1–13.
29. Yamauchi T, Sasaki S, Lee ES, et al. Dupilumab treatment ameliorates clinical and hematological symptoms, including blood eosinophilia, in patients with atopic dermatitis. Int J Dermatol 2021; 60(2): 190–5.
30. Wenzel S, Castro M, Corren J, et al. Dupilumab efficacy and safety in adults with uncontrolled persistent asthma despite use of medium-to-high-dose inhaled corticosteroids plus a long-acting β2 agonist: a randomised double-blind placebo-controlled pivotal phase 2b dose-ranging trial. Lancet 2016; 388(10039): 31–44.
31. Vestergaard Ch, Helle J, BAUMGARTNER N, et al. Expression of CCR2 on monocytes nad macrophages in chronically inflamed skin in atopic dermatitis and psoriasis. Acta Derm Venereol 2004; 2004(84): 353–8.
32. Leung DY, Bieber T. Atopic Dermatitis. Lancet 2003; 361(9352): 151–60.
33. Katschke KJ Jr, Rottman JB, Ruth JH, Qin S, Wu L, LaRosa G, et al. Differential expression of chemokine receptors on peripheral blood, synovial fluid, and synovial tissue monocytes/macrophages in rheumatoid arthritis. Arthritis Rheum 2001; 44: 1022–32.

34. Ozcan A, Boyman O. Mechanisms regulating neutrophil responses in immunity, allergy and autoimmunity. Allergy 2022; 2022(77): 3567–83.
35. Amulic B, Cazalet Ch, Hayes G, et al. Mechanisms regulating neutrophil responses in immunity, allergy and autoimmunity. Annual review of Immunology 2012; 2012(30): 459–89.
36. Mobus L, Rodriguez E, Harder I, et al. Elevated NK-cell transcriptional signature and dysbalance of resting and activated NK cells in atopic dermatitis. Journal of Allergy and Clinical Immunology 2021; 147(5): 1959–65.
37. Shiraki Y, Ishibashi Y, Hiruma M, et al. Cytokine secretion profiles of human keratinocytes during Trichophyton tonsurans and Arthroderma benhamiae infections. J Med Microbiol 2006; 55: 1175–85.
38. Kabashima K, Weidinger S. NK cells as a possible new player in atopic dermatitis. The Journal of Allergy and Clinical Immunology 2020; 146(2): 276–7.
39. Simon D, Hosli S, Kostylina G, et AL. Anti-CD20 (rituximab) treatment improves atopic dermatitis. J Allergy Clin Immunol 2008; 121(1): 122–8.
40. Montes-torres A, Llamas-Velasco M, Perez-Plaza A, et al. Biological treatments in atopic dermatitis. J Clin Med 2015; 4(4): 593–613.
41. Gittler JK, Shemer A, Suarez-Farinas M, et al. Progressive activation of TH2/TH22 cytokines and selective epidermal proteins characterizes acute and chronic atopic dermatitis. J Allergy Clin Immunol 2012; 130: 1344–54.
42. Getahun A, Hjelm F, Heyman B. IgE enhances antibody and T cell responses in vivo via CD23 B cells. J Allergy Clin Immunol 2005; 175(3): 1473–82.
43. Engeroff P, Vogel M. The role of CD23 in the regulation of allergic responses. Allergy 2021; 76(7): 1981–9.
44. Mucha S, Baurecht H, Novak N, et al. Protein-coding variants contribute to the risk of atopic dermatitis and skin-specific gene expression. J Allergy Clin Immunol 2020; 145(4): 1208–18.
45. Baylet A, Laclaverie M, Marchand L, et al. Immunotherapies in cutaneous pathologies: an overview. Drug Discov Today 2021; 26(1): 248–55.
46. Xiong Z. CD200 checkpoint reversal: a novel approach to immunotherapy. Clin Cancer Res 2020; 26: 32–241.

# Evaluation of Diurnal Changes of Mental Fatigue Using a New Portable Device for Visual Cognitive Evoked Potentials

Huey Shin Chua[1], Katrin Ji-In Miller[1], Niha Akhtar Sayyeda[1], Marten Reda Youssef Ibrahim AttaAlla[1], Eithar Osama Eltayeb Babikir[1], Jan Kremláček[2], Miroslav Kuba[1,*]

ABSTRACT

In the age homogenous group of 13 healthy volunteers, we examined visual evoked potentials (VEP) visually evoked cognitive potentials (event-related potentials – ERP) and choice reaction time (CRT) five times during the day (from 10.00 a.m. up to midnight) to verify whether there are significant changes of the measured parameters of the cortical evoked potentials and CRT which might reflect the level of the mental fatigue. The electrophysiological testing was done with the use of a new portable VEP device named "VEPpeak" enabling to perform the examination outside standard labs in almost any conditions. It was found that the latency of ERP (P300 peak time) and CRT displayed significant prolongation toward midnight while VEP latency and all amplitudes did not change significantly. This pilot study supports our idea that the portable VEP device possibly might be used for the objective examination of mental fatigue that is needed in many situations. This should be confirmed in a larger study also including a comparison with non-electrophysiological fatigue testing.

KEYWORDS

visual evoked potentials (VEP); event related potentials (ERP); P300; mental fatigue; VEPpeak device

AUTHOR AFFILIATIONS

[1] Charles University, Faculty of Medicine in Hradec Králové, Dept. of Pathophysiology, Hradec Králové, Czech Republic
[2] Charles University – Faculty of Medicine in Hradec Králové, Dept. of Medical Biophysics, Hradec Králové, Czech Republic
* Dept. of Pathophysiology, Charles University – Faculty of Medicine in Hradec Králové, Šimkova 870, 500 03 Hradec Králové, Czech Republic; kuba@lfhk.cuni.cz

Received: 31 March 2023
Accepted: 17 August 2023
Published online: 8 November 2023

Acta Medica (Hradec Králové) 2023; 66(2): 55–60
https://doi.org/10.14712/18059694.2023.16

## INTRODUCTION

Fatigue of the central nervous system (CNS) is quite frequently tested as the exercise-induced central fatigue in various sport activities (1). Attempts for objective electrophysiological evaluation of mental fatigue are not so common (2) so far and the results are not too satisfactory (3–5). However, diurnal changes of event-related potentials (ERPs) were proved (e.g. 6) and confirmed the presumption that they might reflect the level of mental fatigue.

We came to the idea of evoked potentials application for fatigue testing when the development of the portable VEP device "VEPpeak" (7; https://www.veppeak.com/en/home-page/) was finished in our Electrophysiological Lab. This device allows visual evoked potential (VEP) and visual cognitive/event-related potential (ERP) examination outside the lab in almost any environment and it can be used also for simple self-monitoring. Thus, we decided to verify in this pilot study the possibility of this device to detect possible mental fatigue related to prolonged standard diurnal study activity in home conditions. The survey was performed by international students of our Faculty of Medicine who have been working for three years in the Electrophysiological lab in the form of students' research activity.

## MATERIAL AND METHODS

### SUBJECTS

Age homogenous group of 13 healthy volunteers, students of the Faculty of Medicine (6 men and 7 women – mean age of 23 years) served as experimental subjects after signing a written consent – agreed by the Ethical Committee of the University Hospital in Hradec Králové) that has approved the experimental person examinations accomplished in the study.

All procedures performed in our study were in accordance with the 2000 Helsinki Declaration and comparable ethical standards.

### CHARACTERISTICS OF THE PORTABLE DEVICE FOR VEP AND ERP EXAMINATION "VEPPEAK"

The prototype of the device constructed with the help of RCD Radiokomunikace Ltd. (Czech Republic) consists of a built-in LED visual stimulator, 4-channel low-noise EEG amplifiers, and a control unit. It includes a 3D accelerometer for the rejection of EEG epochs with head movement artifacts and a surrounding luminance detector for the possibility of adaptive regulation of visual stimuli luminance. Thus, the examination can be performed in various luminance environments. Digital inputs allow to detect the subject's reactions (via pressing a button) in cognitive evoked potential examination and triggering of external visual stimulators. All parts are built into a headset (see Fig. 1), which can be fixed on the head of the examined subject with an adjustable fastener band. The device with a total weight of 390 g is connected by a galvanically isolated USB interface with a control and evaluation unit, such as a laptop computer. Special software was prepared for visual stimuli generation, recording, and evaluation of VEPs and ERPs.

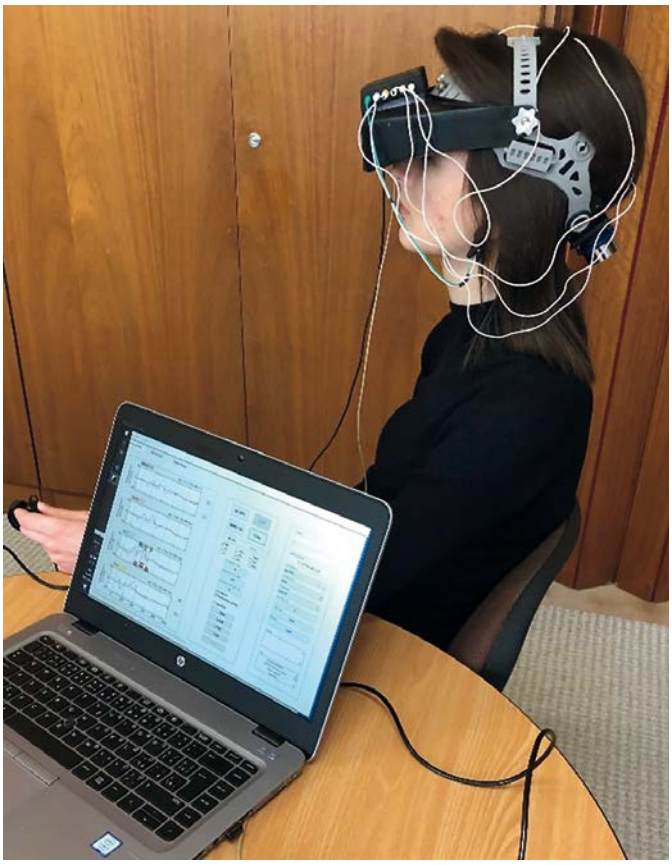

**Fig. 1** Experimental subject during the examination of VEP, ERP, and CRT.

Four unipolar channels were used for the recording of VEPs and ERPs. Two of them use dry electrodes incorporated in the fixing belt of the device. They are located in about Fp1 and Fp2 positions (non-hairy part of the head) and thus they do not require any special mounting they are ready immediately after fixing the device on the head. For the remaining two channels standard Ag/AgCl electrodes were used at locations Oz and Pz (relevant for the below specified visual and cognitive stimuli as it was learned based on the preliminary tests). EEG low-noise amplifiers (0.8–100 Hz) with attenuation of the high pass filter of 40 dB/decade, and that of the low pass filter of 60 dB/decade) and an integrated 16-bit A/D converter with a sampling frequency of 1 kHz provided a signal resolution of about 0.1 μV. The reference electrode and the electrode for noise suppression of the recorded signal (Czech Technical University in Prague – patent CZ 302454) were placed on the opposite sides of an earlobe clip. Signal smoothing with a Savitzky-Golay filter was applied (for more details about VEPpeak see Kuba et al. (7)).

### USED VISUAL STIMULI FOR VEP END ERP ACQUISITION

The built-in visual stimulator consists of a matrix of 32 color LEDs (diameter of 5 mm) placed in two horizontal rows (2 × 16) in the front part (peak) of the device at about

7.5 cm from the subject's eyes. Thus, the angular size of each LED is about 4°, and the total stimulus field subtends about 65° × 10°. It is possible to produce flashes, pattern reversals, the pattern on/off, apparent motion, color, or cognitive stimulations. Additional external stimulators can be eventually used for neuroophthalmological diagnostics where better parameters of visual stimulation are needed. On the basis of our previous testing of the available visual stimuli we selected the following two that provide enough robust reactions with low inter-individual variability, and they can be used with the built-in LED stimulator (more simple examination of VEPs and ERPs):

*Isoluminant (40 cd/m²) red/green LED stimuli* alternating in the full field with the frequency of 1 Hz. This kind of visual stimulation provided in the preliminary testing the largest VEPs with wide distribution over the head. Although this kind of VEPs does not belong to the standard set of VEPs used in neuro-ophthalmological diagnostics, because of its robustness and topography it seemed to be suitable for the detection of the fatiguability of CNS.

*Visual stimuli for cognitive potential (ERP – P300) examination* consisted of recognition of randomly presented isoluminant violet and green colors in the odd-ball paradigm (1:4 proportion of the rare/target and frequent/non-target stimuli) with the signaling of the target color by pressing a button, which also allowed the choice reaction time (CRT) measurement. Of the three opposite color pairs, red-green was chosen, but after adjusting for physiological color isoluminance, the resulting color was more violet than red.

We applied the cognitive task in the visual stimulation since ERP (P300) latency seems to better reflect changes in more complex (cognitive) information processing in CNS and it is reported that ERPs reflect better also CNS aging, psychic disorders, possibly also the level of fatigue (3, 8, 9).

## VEP, ERP AND CRT RECORDING

20 single VEPs (red/green alteration) and 15 ERPs were averaged for obtaining the average responses from all 4 recorded channels. Only the channel with the dominant response (largest interpeak amplitude) was evaluated. In the case of VEPs, it was almost exclusively the Oz lead which includes a reaction from the primary visual cortex, and in the case of cognitive potentials (ERPs), it was the reaction from Pz, although in a lot of subjects also prefrontal leads provided usable reactions with shorter latencies (see Fig. 2), which gives a good chance for ERP self-monitoring with built-in dry electrodes (when it is not necessary to make a montage of standard Ag-AgCl electrodes).

Electrophysiological examinations of the possible fatigue effect with the recording of the CRT started in all subjects at 10.00 a.m. and continued at 3.00 p.m., 8.00 p.m., 10.00 p.m., and 12.00 p.m. Subjects were prohibited from consuming caffeine, alcohol, and pharmacs potentially influencing their sleepiness, and from performing intense exercise one day before and during the day of the examination. During the whole time of the electrophysiological testing, subjects kept their standard diurnal weekend regime including predominantly learning activity (reading of textbooks). At each time all data recordings were repeated three times (altogether 15 VEPs, ERPs, and CRTs were evaluated in each subject) and averaged values of latencies and amplitudes of the dominant peaks (after the elimination of a few recordings containing artifacts) and average CRTs were used for statistical evaluation.

## STATISTICAL EVALUATION

All data were first tested for the normality of their distribution (Anderson-Darling test) which was not confirmed in the majority of cases. Thus besides the means and standard deviations also medians and percentiles are used in the descriptive statistics of P300 latency in Tab. 1.

Since the averaged values of the tested parameters from all particular times of the performed examinations did not differ significantly (graphs of the personal changes show that the trends of potential fatigue markers are interindividually different – see Fig. 3), time changes of relative individual values (related to their mean) were evaluated (for all parameters from all time intervals). The trends (slopes) were determined by linear regression and either the t-test or Wilcoxon test was performed in the group of trends, according to the data distribution. The alpha, significance level, was set at 5% for all comparisons.

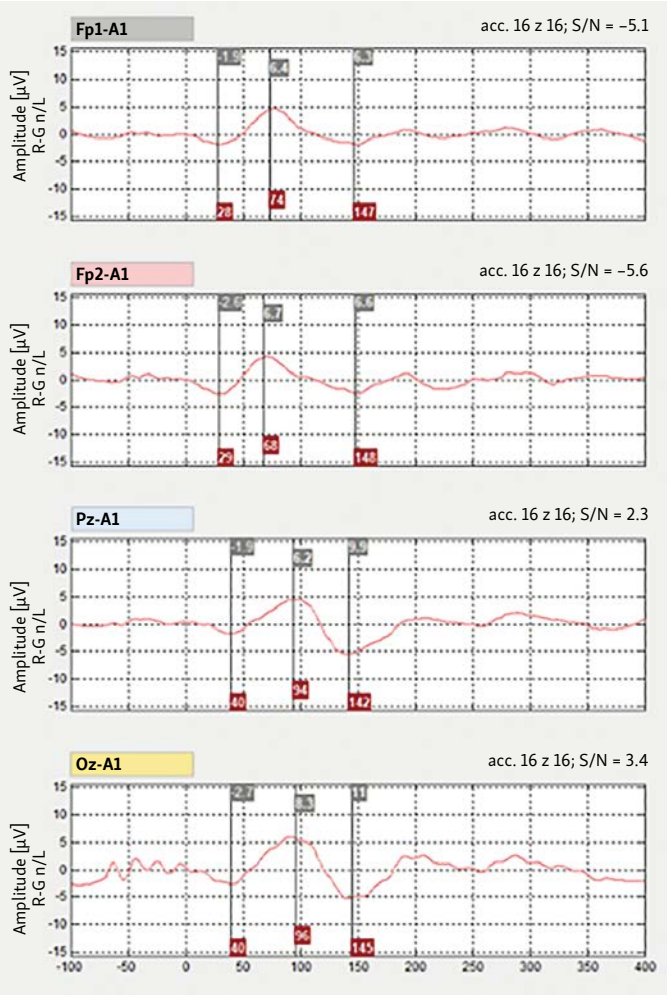


**Fig. 2** Example of an individual VEP with red-green stimulation.

**Tab. 1** P300 average latencies at all examination times [ms].

| Time | N | 25q | Median | 75q | Mean | SD | Min | Max |
|---|---|---|---|---|---|---|---|---|
| h10 | 13 | 339 | 358 | 374 | 359 | 27 | 322 | 414 |
| h15 | 13 | 343 | 368 | 394 | 372 | 48 | 301 | 493 |
| h20 | 13 | 345 | 355 | 366 | 358 | 22 | 322 | 403 |
| h22 | 13 | 330 | 343 | 370 | 349 | 21 | 320 | 387 |
| h24 | 13 | 344 | 355 | 376 | 361 | 29 | 313 | 431 |
| all | 65 | 339 | 355 | 374 | 360 | 31 | 301 | 493 |

## RESULTS

In the used VEPs with red-green stimulation, a positive peak with a latency of 105 ± 12 ms (Median 104; 25q 97; 75q 113) and amplitude of 6.2 ± 3.4 μV (Median 4.9; q25 4.0; q75 7.4) dominated in Oz lead (primary visual cortical area) in the majority of subjects (Fig. 2) but both parameters of this VEP did not display any significant changes during the whole-time span of examinations.

The dominant positive peak is present in the occipital-parietal cortical area but "usable" earlier reactions (signalizing "something happened") are detectable in this individual also in pre-frontal leads.

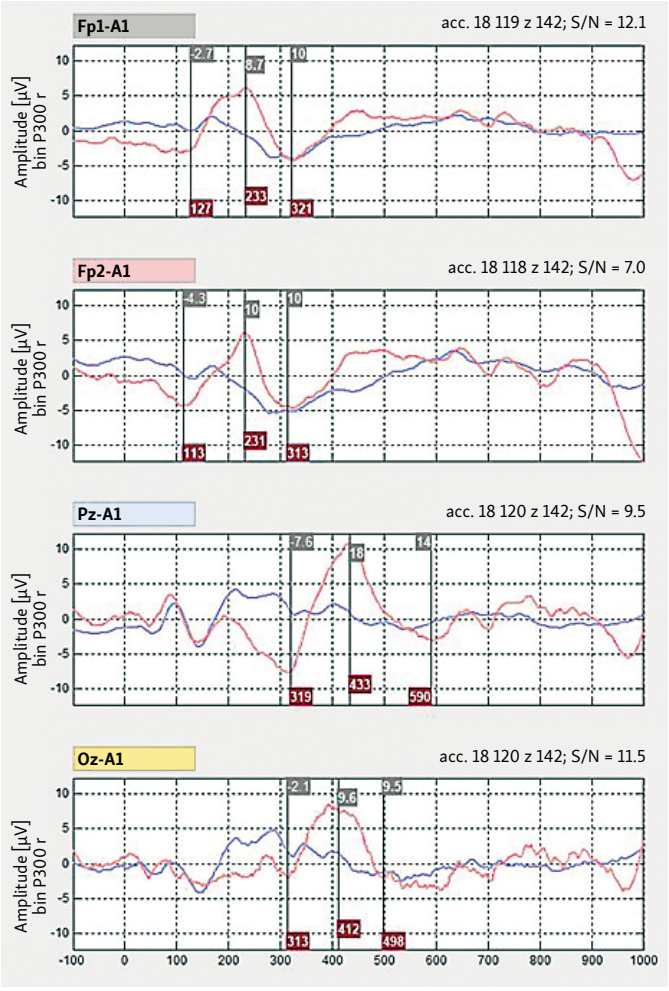


**Fig. 3** An example of individual ERP recordings from all four leads.

In all four channels, the number of accepted single trials (not including artifacts) and signal-to-noise ratio is indicated by the right upper corner.

ERP examination provided well recognizable P300 wave dominating in the Pz lead with an average latency of 360 ± 31 ms and the average amplitude of 13.7 ± 5.4 μV (see Tab. 1 and Fig. 3).

P300 peak latencies and inter-peak amplitudes are indicated in the responses to the target stimuli (in red). Blue traces represent responses to non-target stimuli, which are comparable only within 100–200 ms in the primary visual area (Pz). Dominant P300 in the Pz location has longer latency (over 400 ms) in this particular record due to fatigue. Earlier positive waves, also suitable for the detection of latency changes are present also in prefrontal leads.

The individual latencies displayed highly significant ($p = 0.006$) time-related changes (prolongation) signalizing a possible CNS fatigue development toward midnight. This is evident in Fig. 4 showing the individual time-dependent trends of the latencies. Eight subjects have latency prolongation but, in three cases, either no changes or even the opposite reaction – latency shortening in two subjects are recognizable, which might be interpreted as evidence of different (changed) diurnal regimes in these subjects – students of medicine.

Experimental subjects S1–S13. It is evident that the time dependence of the P300 latency (representing potentially the level of fatigue during the observed time) is not uniform but individually different. Subjects with latency prolongation (8) or shortening (2) are signed by symbols ↑ or ↓, individuals with inconsistent latency changes are signed as ↑↓ (3).

Although there was also a predominant trend of amplitude decrease with increasing fatigue toward midnight in most subjects, this effect was not significant. Probably due to the higher intraindividual variability of amplitudes compared to latencies (see the Discussion).

The CRT values were on average 329 ± 48 ms, which means CRT was shorter than P300 latencies (see the Discussion for explanation) but with higher variability, mainly for some (probably not fully cooperating) subjects. The average CRT variation coefficient (from all examination times) was 29% compared to 9% for P300 latency and the highest was at 3:00 p.m. (both for CRT and P300 latency). Despite it, on average CRT also exhibited significant ($p = 0.007$) prolongation which can be related to fatigue. However, there was no significant correlation between the P300 latencies and CRT.

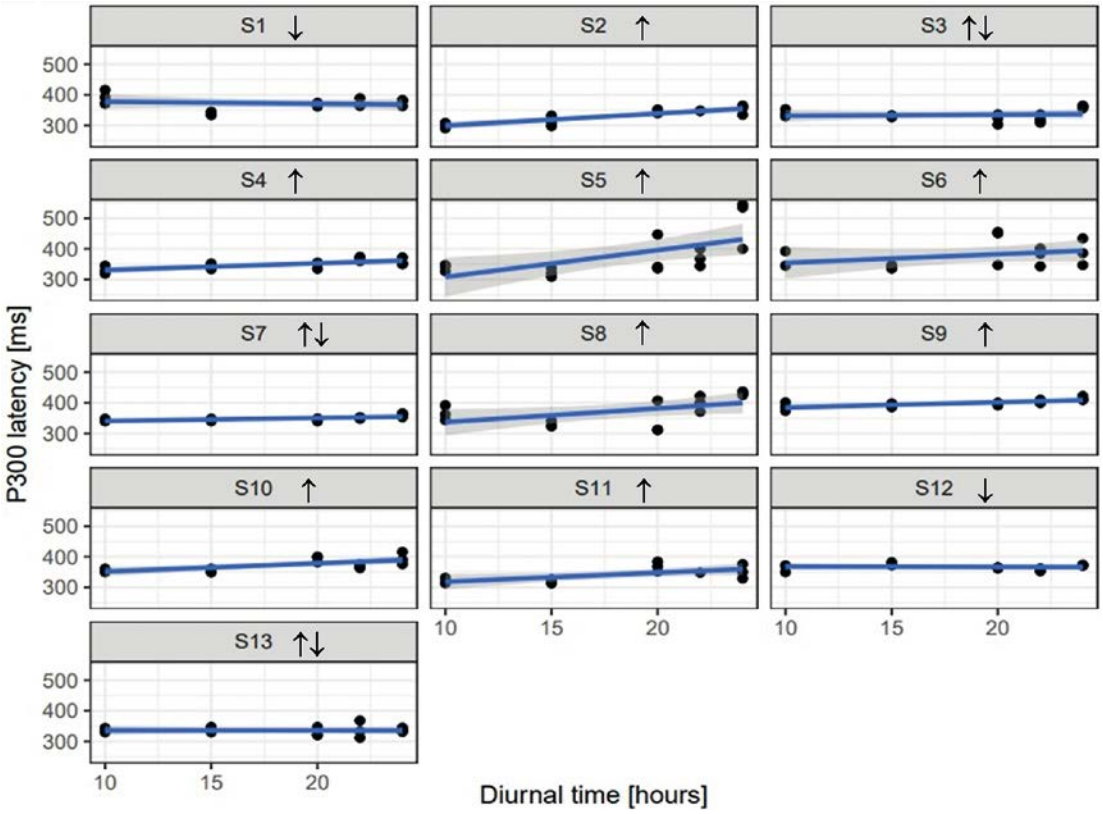


**Fig. 4** Individual changes of P300 latency in 13 subjects between 10.00 a.m. and 24.00 p.m.

## DISCUSSION

We believe that the demonstrated prolongation of P300 latencies and CRT during the day by midnight can be considered a sensitive tool for the evaluation of a potential increase in CNS fatigue as it was already reported (6, 10, 11).

It was mentioned in the Results that there was no significant correlation between the found prolongation of P300 latencies and prolonged CRT. Although it might be expected that both these parameters will react similarly during the development of CNS fatigue, their independence is a common observation (12) since the P300 represents a much more complex reaction (corresponding to activation of the larger part of the brain), compared to the CRT that on the other hand also includes the executive (motoric) reaction. It is probably also the reason, why surprisingly CRT is typically shorter than the P300 peak latency in most subjects. This is more consistent with parallel rather than serial models of information processing. Simultaneous measurement of the P300 latency and CRT may probably differentiate whether a delay is related to neuronal networks concerned with stimulus evaluation or to those concerned with response execution (13).

Possible considering whether CRT (that is simpler for an examination compared to electrophysiological testing) cannot be preferable for fatigue detection in comparison with ERP (P300) latency, should take into account that CRT is more inter- and intraindividually variable (more dependent on good cooperation and attention) and thus it is not so objective parameter as P300 latency. Moreover, as above explained, CRT does not represent so complex CNS activity as it is considered for the P300 wave, so it need not be so sensitive to CNS fatigue but could be influenced by some pathology of the motor pathway (13). On the other hand, if an efficient brain network is associated with reduced CRT variability (14), this parameter should also be considered as a potential marker of fatigue, as well as some other reported changes in ERPs (e.g. 15).

The fact that amplitudes of VEPs and ERPs were not significantly changed due to fatigue (despite some trend to their reduction) is not surprising, since in all evoked potentials, amplitudes have larger inter- and also intra-individual variability compared to latencies (9) depending on a lot of personal characteristics and fluctuation of attention paid to stimulation. Thus, diagnostic applications of cortical potentials amplitude changes are rather limited.

It is important to mark that our examination performed with the use of the mobile device VEPpeak seems to be well applicable in almost any conditions, comfortable for subjects, and fully comparable with a standard way of VEP, ERP, and CRT examination in electrophysiological labs. When the examination can be done almost anywhere, it significantly helps with its practical use for the tested purpose. It could be very useful in many human activities to check/monitor the level of CNS fatigue to prevent failures of personnel or to signalize critical levels of brain functions. Since the device can be used for self-examination (without any assistance), mainly when only the built-in recording electrodes fixed to the forehead provide sufficient information, it gives a chance for a fully automated evaluation with software providing signalization of critical limits of the tested parameters.

We found interindividual differences in the circadian trends of P300 latency (eight subjects lengthened them,

two shortened them, and three subjects had inconsistent changes) and as it is partially recognizable from Fig. 4, the subjects with prolonged latencies display also larger intraindividual variability. Our subjective evaluation is that it fits well with the recognized "morning-type, evening-type, and intermediate-type" individuals in the population according to their mental performance and sleepiness at different times (6). If it would be confirmed in the planned larger study, it could represent an objective confirmation of this subjective characteristic of people.

We are aware of the limited validity of the results of this pilot study which must be enlarged not only by a higher number of experimental subjects but also by repeated examinations over several days in combination with some standard subjective questionnaires and psychophysical tests for individual detection and quantification of fatigue. For more reliable testing of the electrophysiological approach to quantitative fatigue detection, it would also be useful to use only experienced experimental subjects.

## CONCLUSION

This pilot study supports our idea that the new portable device VEPpeak (enabling examination of visual evoked potentials (VEP) and event-related cortical potentials (ERP) outside electrophysiological labs) can be used for the evaluation of CNS fatigue in almost any environment. Prolongation of ERP latencies, tested this way, seems to be a simple and sensitive tool for its objective detection.

## ACKNOWLEDGEMENTS

The authors are grateful for the financial support from the Ministry of Health of the Czech Republic (Grant AZV No. NV18-08-00314) and by the project of Charles University Cooperatio – NEUR.

## REFERENCES

1. Tornero-Aguilera JF, Jimenez-Morcillo J, Rubio-Zarapuz A, Clemente-Suárez VJ. Central and Peripheral Fatigue in Physical Exercise Explained: A Narrative Review. Int J Environ Res Public Health 2022; 19: 3909.
2. Lamti HA, Khelifa MMB, Hugel V. Mental fatigue level detection based on event related and visual evoked potentials features fusion in virtual indoor environment. Cogn Neurodyn 2019; 13: 271–85.
3. Polich J, Herbst KL. P300 as a clinical assay: rationale, evaluation, and findings. Int. J. Psychophysiol 2000; 38: 3–19.
4. Lee MH, Williamson J, LeeYE, Lee SW. Mental fatigue in central-field and peripheral-field steady-state visually evoked potential and its effects on event-related potential responses. Neuroreport 2018; 29: 1301–8.
5. Sabeti M, Boostani R, Rastgar K. How mental fatigue affects the neural sources of P300 component? J Integrat Neurosci 2018; 17: 93–111.
6. Huang H, Katsuura T, Shimomura Y, Iwanaga K. Diurnal Changes of ERP Response to Sound Stimuli of Varying Frequency in Morning-type and Evening-type Subjects. J Physiol Anthropol 2006; 25: 49–54.
7. Kuba M, Kremláček J, Vít F et al. VEP examination with new portable device. Doc Ophthalmol 2023; 146: 79–91.
8. Pavarini SCI, Brigola AG, Luchesi BM et al. On the use of the P300 as a tool for cognitive processing assessment in healthy aging – a review. Dement Neuropsychol 2018; 12: 1–11.
9. Kuba M, Kremlacek J, Langrova J, Kubova Z, Szanyi J, Vit F. Aging effect in pattern, motion and cognitive visual evoked potentials. Vision Res 2012; 62: 9–16.
10. Kaseda Y, Jiang C, Kurokawa K, Mimori Y, Nakamura S. Objective evaluation of fatigue by event-related potentials. J Neurol Sci 1998; 158: 96–100.
11. Higuchi S, Liu Y, Yuasa T, Maeda A, Motohashi Y. Diurnal variation in the P300 component of human cognitive event-related potential. Chronobiology Internat 2000; 17: 669–78.
12. Duncan-Johnson CC, Donchin E. The Relation of P300 Latency to Reaction Time as a Function of Expectancy. Progress in Brain Res 1980; 54: 717–22.
13. Kraiuhin C, Yiannikis C, Coyle S et al. The relationship between reaction time and latency of the P300 event-related potential in normal subjects and Alzheimer's disease. Clin Exp Neurol 1989; 26: 81–8.
14. Machida K, Murias M, Johnson KA. Electrophysiological Correlates of Response Time Variability During a Sustained Attention Task. Front Hum Neurosci – Sec. Brain Health and Clin Neurosci 2019; 13: 363.
15. Zhou Y, Wang W, Yan L, Yang B. Research on the Relationship between Fatigue and P300 Potential in Multi-Stage RSVP Small Target Detection. Proceedings of the 10th International Conference on Computing and Pattern Recognition 2021: 92–98.

# Associations of Serum Total Homocysteine Levels with Various Demographic, Clinical and Genetic Characteristics in Healthy Greek Adults

Elias E. Mazokopakis[1,2,*], Maria G. Papadomanolaki[3], John A. Papadakis[4]

ABSTRACT

Aim: The aim of this study was to investigate the association of serum total Hcy (tHcy) levels with various demographic, clinical and genetic characteristics in healthy Greek adults.

Methods: Anthropometric characteristics (height, weight), systolic and diastolic blood pressure, complete blood count and biochemical assessments, were recorded and measured among 383 Greek adults (199 men). Serum folate, Cobalamin (Cbl) and tHcy levels were determined using immunoassays methods. The MTHFR C677T and A1298C gene polymorphisms were genotyped using polymerase chain reaction and reverse hybridization.

Results: MTHFR C677T gene polymorphism, serum folate and Cbl levels were correlated with serum tHcy levels independently. The individuals with 677TT genotype had significantly higher serum tHcy levels than individuals with 677 CC or CT genotypes. Regarding the MTHFR C677T gene polymorphism, the existence of the T allele was associated with statistically significantly lower serum folate and higher serum tHcy levels than C allele. Regarding the MTHFR A1298C gene polymorphism, the existence of the C allele was associated with statistically significant lower serum tHcy levels than A allele. Furthermore, there was no significant correlation between the serum tHcy levels and demographic (except age) or clinical characteristics (sex, BMI, smoking status, SBP, DBP, HGB, HCT, TC, TG, HDL-C, LDL-C, TC/HDL-C).

Conclusions: Serum tHcy levels are influenced by the existence of MTHFR C677T gene polymorphism (mainly 677TT genotype), serum folate and Cbl levels. Individuals with hyperhomocysteinemia should be further investigated for the existence of MTHFR C677T gene polymorphism, with the aim to determine the suitable treatment.

KEYWORDS

cobalamin; folate; homocysteine; MTHFR C677T; MTHFR A1298C

AUTHOR AFFILIATIONS

[1] Department of Internal Medicine, Naval Hospital of Crete, Chania, Greece
[2] Private Medical Office of Internal Medicine, Chania, Greece
[3] School of Production Engineering and Management, Technical University of Crete, Chania, Greece
[4] Department of Internal Medicine, University Hospital of Heraklion, Heraklion, Greece
* Correspondence address: Elias E. Mazokopakis, M.D., Ph.D., 36 K. Mitsotaki street, Chania 73 132, Crete, Greece; emazokopakis@yahoo.gr

Received: 27 October 2022
Accepted: 21 August 2023
Published online: 8 November 2023

Acta Medica (Hradec Králové) 2023; 66(2): 61–67
https://doi.org/10.14712/18059694.2023.17

## INTRODUCTION

Homocysteine (Hcy) is a sulfur-containing non-essential amino acid produced as an intermediary of methionine metabolism. It is either metabolized to cysteine by the transsulfuration pathway with *cystathionine β-synthase* (CBS) as the main enzyme with the contribution of vitamin B6, or converted back to methionine by the remethylation pathway with the action of the enzymes methionine synthase (MS) and $N_5$,$N_{10}$-methylene tetrahydrofolate reductase (MTHFR) and the contribution of $N_5$-methyltetrahydrofolate (5-MTHF) and cobalamin (Cbl, vitamin B12) (1–6) (Fig. 1). The function of the MTHFR enzyme is of great importance for the regulation of available 5-MTHF which is the predominant and biologically active form of circulating folate (1, 7). Genetic defects in the enzymes or dietary deficiency of B-vitamin cofactors and their substrates could be responsible for elevations in the serum total Hcy (tHcy) levels (3-6). Between the two well-known polymorphisms of the MTHFR gene, the C677T (substitution of C to T at the residue 677) and the A1298C (transversion of A to C at nucleotide position 1298), which affect the MTHFR enzymatic activity, only the first appears to be associated with increased serum tHcy levels (5). The difference in the functional properties of these variants is due to their respective positions in the protein, such as the C677T being located in the catalytic domain whereas the A1298C is located in the regulatory domain. Individuals with 677TT or 677CT genotype have, respectively, approximately 30% and 65% the MTHFR enzyme activity of those with the 677CC genotype (5).

It is known that a prolonged exposure to elevated serum tHcy levels, referred to as hyperhomocysteinemia (HHcy) (typically defined as serum tHcy levels ≥15 μmol/L)(4), is associated with a wide range of health problems and conditions including cardiovascular disease, deep vein thrombosis or pulmonary embolism, neurocognitive disorders, pregnancy complications (preeclampsia, placental abruption, pregnancy loss), birth defects, osteoporotic fractures, etc. (4–6, 8–11). The aim of this study was to investigate the associations of serum tHcy levels with various demographic, genetic and clinical characteristics in healthy Greek adults.

## SUBJECTS AND METHODS

Our study population included 383 healthy Greek individuals, residents of Chania, Crete, who had visited the Outpatient Clinic of Internal Medicine of the Naval Hospital of Crete or a private medical office of Internal Medicine between January 2016 and December 2018 in the framework of their periodic medical examination (military personnel) or check-up (non-military personnel). The subjects met the following five criteria: (1) age ≥18 years old; (2) normal renal and thyroid function; (3) absence of a

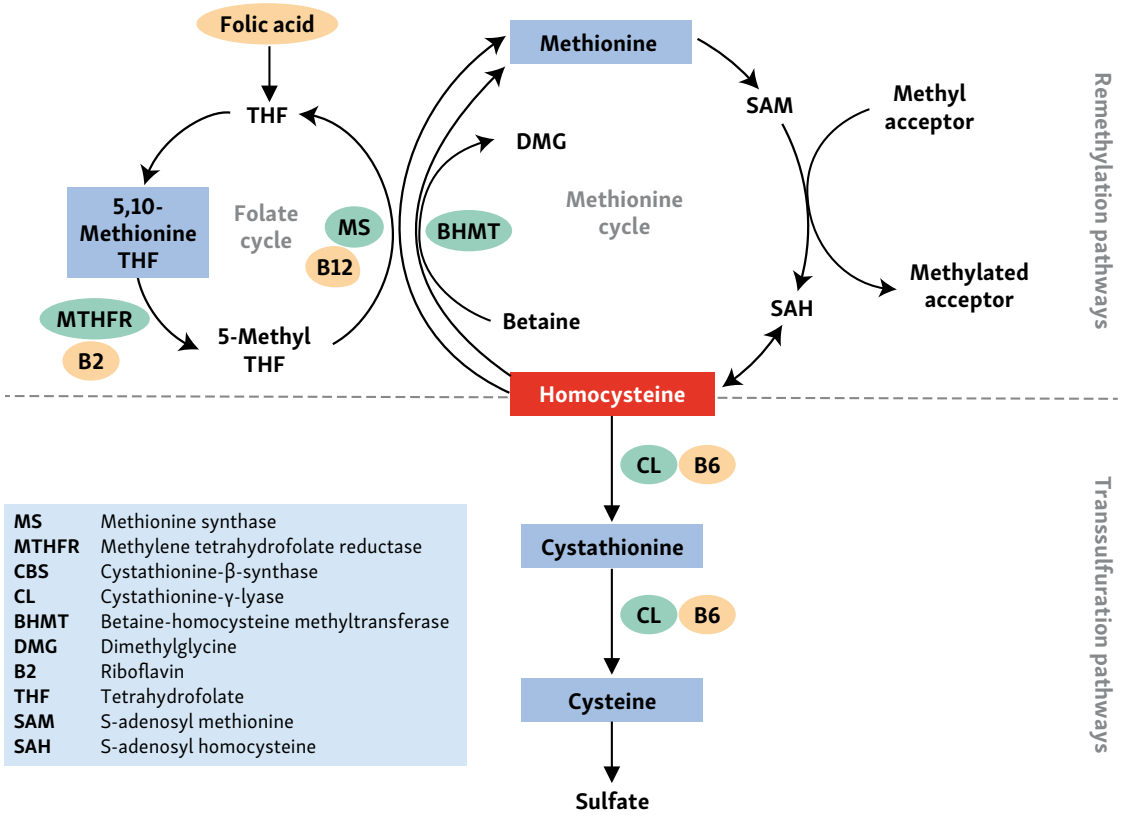


**Fig. 1** Pathways of homocysteine (Hcy) metabolism (2). Hcy is metabolized by one of two divergent pathways: transsulfuration; and remethylation. The transsulfuration of Hcy to cysteine is catalysed by cystathionine-b-synthase (CBS), a process that requires pyridoxal phosphate (vitamin B6) as a cofactor. Remethylation of Hcy produces methionine. This reaction is catalysed either by methionine synthase or by betaine-homocysteine methyltranferase. Vitamin B12 (Cbl) is the precursor of methylcobalamin, which is the cofactor for methionine synthase.

known gastrointestinal disorder (pernicious anemia, gastrectomy/bariatric surgery, inflammatory bowel disease, gastritis, autoimmune metaplastic atrophic gastritis, malabsorption syndrome, Helicobacter pylori infection), diabetes mellitus, coronary heart disease, stroke, malignancy or alcoholism; (4) no consumption of vitamin supplements or drugs affecting folate or Cbl metabolism (i.e. methotrexate, sulphasalazine, antiepileptic drugs, statins, metformin, proton-pump inhibitors) during the last semester, and regarding females (5) no pregnancy or lactation. The subjects were interviewed using a structured form, which included sociodemographic data, lifestyle and dietary habits, such as medical history. Anthropometric parameters of all subjects consisting of height, body weight (BW) and body mass index (BMI), as well as arterial systolic and diastolic blood pressure (SBP, DBP), had been measured and recorded. Records were made of sitting SBP and DBP (two measurements averaged) with a mercury-free sphygmomanometer (*A&D* Medical, model UM-102) by the auscultatory method, standing body height (measured without shoes to the nearest 0.5 cm) with a rigid height meter and BW (without shoes and tunic) with a calibrated balance scale (Fazzini, model S7350HR). BMI was calculated as the BW (kg) divided by the height (m) squared (kg/m$^2$) (12). Blood samples were collected after overnight fasting. A complete blood count and various biochemical parameters including renal and liver function tests, serum glucose, total cholesterol (T-C), high density lipoprotein-cholesterol (HDL-C), triglycerides (TG), folate, Cbl and tHcy (the sum of free and protein-bound homocysteine, homocysteine and homocysteine-cysteine mixed disulfide) were determined by the following standard laboratory procedures. Low density lipoprotein-cholesterol (LDL-C) and atherogenic index (AI; TC/HDL-C ratio) were calculated using the Friedewald (13) and Lauer (14) equations, respectively. Serum folate and Cbl levels were measured with a chemiluminescent microparticle immunoassay method (CMIA) (Architect i1000 System®, Abbott Laboratories, USA). Serum tHcy levels was measured with a fluorescence polarization immunoassay (FPIA) using the commercial kit in the AXSYM® System (Abbott Laboratories, USA). Individuals with serum tHcy levels ≥15 μmol/L were considered to have HHcy.

Total genomic DNA was extracted using standard phenol-chloroform procedures. Screening of the MTHFR C677T and A1298C gene polymorphisms was performed by polymerase chain reaction (PCR) and reverse hybridization (CVD StripAssay® Testing Strip, Vienna Lab, CE IVD kit). Subjects with one copy of the mutant allele on the MTHFR gene were called "heterozygous", but subjects with two copies of the same mutant allele were called "homozygous". Thus, our subjects for C677T and/or A1298C polymorphisms in the MTHFR gene were divided to three genotypes (normal or wild-type (677CC; 1298AA), heterozygous (677CT; 1298AC) and homozygous (677TT; 1298CC).

## STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS software package (version 20, Inc., USA). Data are presented as number (%) for categorical variables and mean ± standard deviation (SD) for continuous variables. Categorical data were analyzed with Fisher's exact test. Continuous variables were compared using Student's t-test for normally distributed variables. One-way analysis of variance (ANOVA) with Bonferroni post hoc test for the significant differences was performed to examine the influence of serum folate levels, serum Cbl levels and the MTHFR C677T and A1298C genotypes on the serum tHcy levels. Correlation between serum Hcy levels and demographic or clinical characteristics was assessed by Pearson's correlation (r). A multivariate linear logistic regression model was developed in order to evaluate the independent effect of the above parameters on serum tHcy levels. All tests were two-tailed and p-values <0.05 were considered to be significant.

**Tab. 1** Demographic, clinical and genetic characteristics of the study population by sex. Values are expressed as mean ± SD and/or No (%).

| Characteristic | Men (n = 199) | Women (n = 184) | p |
|---|---|---|---|
| Age, years | 41.9 ± 14.8 | 43.1 ± 14.6 | 0.448 |
| *Smoking status* | | | |
| Yes | 47 (23.7%) | 42 (22.8%) | 0.904 |
| *Anthropometric values* | | | |
| BW (kg) | 85.0 ± 12.9 | 72.4 ± 12.6 | <0.001 |
| Height (cm) | 177.0 ± 6.0 | 168.0 ± 6.0 | <0.001 |
| BMI (kg/m$^2$) | 27.3 ± 3.9 | 25.5 ± 4.1 | <0.001 |
| *Blood pressure* | | | |
| SBP (mmHg) | 121.1 ± 7.1 | 120.7 ± 7.5 | 0.634 |
| DBP (mmHg) | 69.7 ± 10.3 | 70.0 ± 8.5 | 0.819 |
| *Fasting plasma values* | | | |
| HCT | 43.9 ± 2.8 | 40.6 ± 3.4 | <0.001 |
| HGB | 14.7 ± 1.1 | 13.4 ± 1.3 | <0.001 |
| TC (mg/dl) | 195.5 ± 39.3 | 193.9 ± 34.5 | 0.683 |
| TG (mg/dl) | 124.7 ± 75.2 | 102.8 ± 47.0 | 0.001 |
| HDL-C (mg/dl) | 48.4 ± 11.9 | 55.0 ± 15.3 | <0.001 |
| LDL-C (mg/dl) | 122.2 ± 33.9 | 118.1 ± 30.9 | 0.215 |
| AI | 4.2 ± 1.3 | 3.8 ± 1.2 | <0.001 |
| Folate (ng/mL) | 3.15 ± 1.1 | 3.21 ± 1.1 | 0.560 |
| Cbl (pg/mL) | 275.4 ± 102.8 | 292.0 ± 114.3 | 0.133 |
| tHcy (μmol/L) | 15.07 ± 9.1 | 13.6 ± 6.9 | 0.087 |
| *MTHFR C667T genotypes* | | | |
| Normal (CC) | 41 (20.6%) | 32 (17.4%) | 0.645 |
| Heterozygous (CT) | 105 (52.8%) | 97 (52.7%) | |
| Homozygous (TT) | 53 (26.6%) | 55 (29.9%) | |
| *MTHFR A1298C genotypes* | | | |
| Normal (AA) | 138 (69.3%) | 125 (67.9%) | 0.936 |
| Heterozygous (AC) | 53 (26.6%) | 52 (28.3%) | |
| Homozygous (CC) | 8 (4.0%) | 7 (3.8%) | |

BW: body weight; BMI: body mass index; HCT: hematocrit; HGB: hemoglobin; TC: total cholesterol; TG: triglycerides; HDL-C: high density lipoprotein-cholesterol; LDL-C: low density lipoprotein-cholesterol; AI: atherogenic index (TC/HDL-C); Cbl: cobalamin; tHcy: total homocysteine; SBP: systolic blood pressure; DBP: diastolic blood pressure; MTHFR: $N_5$,$N_{10}$-methylene tetrahydrofolate reductase

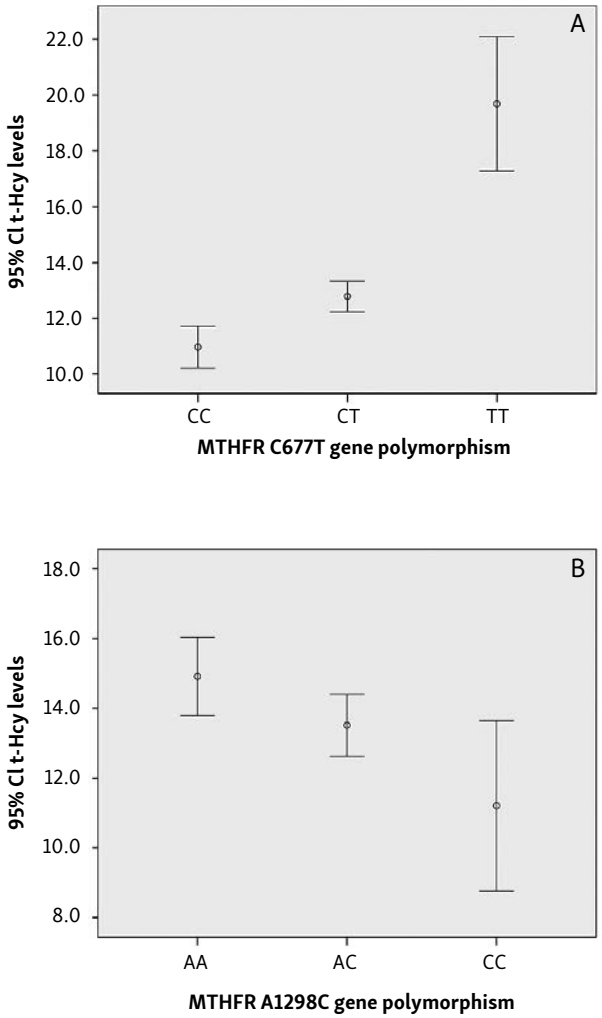


**Fig. 2** Serum tHcy levels according to MTHFR C677T (A) and A1298C (B) gene polymorphisms.

## RESULTS

The demographic, clinical and genetic characteristics of 383 individuals (199 men, 52%) with mean age ± SD: 37.3 ± 8.2 years who participated in our study, divided in 2 groups by sex, are presented in table 1. The serum tHcy levels according to MTHFR C677T and A1298C gene polymorphisms are showed in Fig. 2. The overall C and T allele frequency for the MTHFR C677T gene polymorphism was 45.4% and 54.6%, respectively, and the overall A and C allele frequency for the MTHFR A1298C gene polymorphism was 82.3% and 17.6%, respectively.

Serum tHcy levels had a statistically significant reverse correlation with serum folate and serum Cbl levels, as well as a positive correlation with age ($p < 0.001$, $p < 0.001$ and $p = 0.039$, respectively) (Table 2). Additionally, both MTHFR C677T and A1298C gene polymorphisms had a statistically significant impact on serum tHcy levels. Multivariate linear regression analysis (Table 3) revealed that among them only MTHFR C677T gene polymorphism, serum folate and serum Cbl levels, and age were correlated with tHcy levels independently. As showed in table 4, Bonferroni post hoc test revealed that serum tHcy levels were statistically significantly higher among the subjects with the MTHFR 677TT genotype than the subjects with MTHFR 677CC or 677CT genotypes. Additionally, serum tHcy levels

**Tab. 2** Correlation between the serum tHcy levels and other demographic and clinical characteristics.

| | $r_s$ | p |
|---|---|---|
| Age | 0.105 | 0.039 |
| Sex | 0.088 | 0.087 |
| Smoking status | 0.010 | 0.852 |
| SBP | -0.007 | 0.899 |
| DBP | 0.008 | 0.883 |
| BMI | 0.057 | 0.263 |
| Folate levels | -0.249 | <0.001 |
| Cbl levels | -0.198 | <0.001 |
| TC | 0.043 | 0.399 |
| TG | 0.005 | 0.923 |
| HDL-C | -0.035 | 0.497 |
| LDL-C | 0.069 | 0.178 |
| AI | 0.059 | 0.251 |
| Ht | 0.056 | 0.277 |
| Hb | 0.074 | 0.151 |
| MTHFR C677T gene polymorphisms | 0.388 | <0.001 |
| MTHFR A1298C gene polymorphisms | -0.109 | 0.039 |

$r_s$: Spearman's correlation; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; other abbreviations: see table 1

**Tab. 3** Multivariate linear regression analysis.

| Model | | Coefficients[a] | | | t | Sig. |
|---|---|---|---|---|---|---|
| | | Unstandardized | | Standardized | | |
| | | B | Std. Error | Beta | | |
| 1 | (Constant) | 12.156 | 2.300 | | 5.285 | <0.001 |
| | MTHFR C677T gene polymorphisms | 2.314 | 0.297 | 0.389 | 7.784 | <0.001 |
| | Folate | -1.381 | 0.333 | -0.189 | -4.142 | <0.001 |
| | Cbl | -0.013 | 0.003 | -0.173 | -3.809 | <0.001 |
| | Age | 0.052 | 0.025 | 0.094 | 2.072 | 0.039 |
| | MTHFR A1298C gene polymorphisms | 0.421 | 0.363 | 0.058 | 1.160 | 0.247 |

a. Dependent Variable: t-Hcy. For abbreviations: see table 1.

**Tab. 4** Serum tHcy levels distributed by MTHFR genotypes and by serum tHcy levels groups ≥15 or <15). Values are expressed as mean ± SD or No (%).

| MTHFR C677T genotypes | tHcy | tHcy | | p-value |
|---|---|---|---|---|
| | | <15 (n = 258) | ≥15 (n = 125) | |
| CC (n = 73) | 10.96 ± 3.21a | 67 (26.0%) | 6 (4.8%) | <0.001* |
| CT (n = 202) | 12.79 ± 3.91b | 152 (58.9%) | 50 (40.0%) | |
| TT (n = 108) | 19.68 ± 12.59c | 39 (15.1%) | 69 (55.2%) | |
| *Bonferroni post hoc test* | | | | |
| CC vs TT | p < 0.001 | | | |
| CC vs CT | p < 0.001 | | | |
| CT vs TT | p < 0.001 | | | |
| **MTHFR A1298C genotypes** | **tHcy** | **tHcy** | | **p-value** |
| | | <15 (n = 258) | ≥15 (n = 125) | |
| AA (n = 263) | 14.92 ± 9.25 | 177 (68.6%) | 86 (68.8%) | 0.074* |
| AC (n = 105) | 13.5 ± 4.6 | 67 (26.0%) | 38 (30.4%) | |
| CC (n = 15) | 11.2 ± 4.4 | 14 (5.4%) | 1 (0.8%) | |

One-way analysis of variance (ANOVA) with Bonferroni post hoc test test; P < 0.001 by column (a *vs* b *vs* c). * Fisher exact test. For abbreviations: see table 1.

were statistically higher among the subjects with MTHFR 677CT genotype than those with 677CC genotype (Table 4). Regarding the MTHFR C677T gene polymorphism, the existence of the T allele was associated with statistically significant lower serum folate and higher serum tHcy levels than C allele.

Interestingly, even though MTHFR A1298C gene polymorphism had no significant impact on serum tHcy levels (p = 0.09), only one subject out of 15 (6.7%) with MTHFR 1298 CC genotype had serum tHcy levels ≥15 μmol/L. Furthermore, regarding the MTHFR A1298C gene polymorphism, the existence of the C allele was associated with statistically significant lower serum tHcy levels than A allele (p = 0.02).

As showed in table 2 there was no significant correlation between the serum tHcy levels and other demographic or clinical characteristics (sex, BMI, smoking status, SBP, DBP, HGB, HCT, TC, TG, HDL-C, LDL-C, AI).

## DISCUSSION

In the present study of 383 Greek healthy adults, only the MTHFR C677T polymorphism, serum folate and serum Cbl levels were significantly associated independently with serum tHcy levels. Among these three independent factors, MTHFR C677T polymorphism had the greatest influence on those. To our knowledge, this is the first study which was conducted to investigate independent factors affecting the serum tHcy levels in healthy Greek adults.

In our study, the individuals with 677TT genotype had significantly higher serum tHcy levels than individuals with the 677CC or 677CT genotypes, as other studies have also revealed (15–17). This finding could be attributed to thermolability induced in the enzyme MTHFR which results in its lower activity (approximately 70% for 677TT genotype) and therefore inability to efficiently convert 5,10-methylene-THF to 5-MTHF, a conversion necessary for the remethylation of Hcy to methionine (4–6, 16). The significant contribution of the T allele on elevated serum tHcy levels and the statistically significant lower frequency of MTHFR 677CC genotype among individuals with serum tHcy levels ≥15 μmol/L than individuals with serum tHcy levels <15 μmol/L can justify the further investigation of HHcy individuals for a possible existence of MTHFR C677T polymorphism. The significant negative correlation between the serum levels of tHcy and folate or Cbl in our study population was expected considering the participation of folate and Cbl as cofactors in Hcy metabolism (Fig. 1), and justifies on the one hand why two-thirds of cases of elevated serum tHcy levels are due to low serum folate and/or Cbl levels, and on the other hand the administration of folate and Cbl supplements among patients with elevated serum tHcy levels (18). The weak contribution of serum Cbl levels on serum tHcy levels is probably due to the fact that in our study population the majority of individuals (51.7%) had serum Cbl levels between 200 and 300 pg/mL (indicative levels of possible Cbl deficiency) and only 17% had serum Cbl levels <200 pg/mL (indicative levels of Cbl deficiency) (2).

The results of our research did not reveal a significant influence of MTHFR A1298C gene polymorphism on serum tHcy levels. This finding could be explained by the fact that this mutation, despite affecting MTHFR activity, does not result in the synthesis of a thermolabile protein (5, 19, 20). Thus, the investigation of MTHFR A1298C gene polymorphism among individuals with elevated serum tHcy levels is rather unnecessary.

Clinically, the *MTHFR C677T* genotyping mainly among the individuals with HHcy is not only important, it is critical for determining the appropriate folate supplement (folic acid or folinic acid or 5-MTHF) in order to successfully decrease or normalize the elevated serum tHcy concentrations without increasing the levels of unmetabolized folic

acid (UMFA) in the peripheral circulation (18, 21, 22). It is recommended that patients with MTHFR 677TT genotype who have HHcy be treated with 5-MTHF (400-800 μcg daily) and not with folic or folinic acid (22). Moreover, it is well-known that high levels of UMFA may promote the growth of existing cancers and have been associated with decreased natural killer cell activity and increased cancer risk (23–25). Except the monitoring of serum folate levels, the monitoring of serum Cbl levels among HHcy individuals is also necessary considering that low Cbl levels increase the serum tHcy levels because of the impaired activity of Cbl-dependent enzyme MS which regenerates methionine from Hcy (3). Also, a Cbl deficiency can lead to a specific reaction, called methylfolate trap. This trap results from the fact that 5-MTHF can neither be metabolized via the MS pathway, nor reconverted to its precursor 5,10-methylene-THF (Fig. 1), which leads to the 5-MTHF becoming metabolically trapped and unable to be employed anymore (26).

In contrast to other studies (4, 16, 27–33), the serum tHcy levels in our population were not associated with sex, BMI, BP, smoking status, and lipids. A possible explanation for these controversial results may be the differences in studies populations, sample size and the concomitant drugs or diseases of participants. The significant effect of age on serum tHcy concentration in our population is in accordance with other studies (32, 33).

The present study has some limitations. First of all, the sample size was relatively small. Second, other genetic or environmental (e.g. vitamin B6 deficiency) factors involving in the development of HHcy (6) were not evaluated.

## CONCLUSIONS

Serum tHcy levels are influenced by the existence of MTHFR C677T gene polymorphism (mainly 677TT genotype), serum folate and serum Cbl levels. We suggest that the individuals with HHcy should be further investigated for the existence of MTHFR C677T gene polymorphism, with aim to determine the suitable treatment.

## ACKNOWLEDGMENTS

The authors thank all the subjects who participated in this study. They also express their gratitude to the director and laboratory staff of the Department of Biopathology and Microbiology of the Naval Hospital of Crete, the Blood Donation Laboratory of the General Hospital of Chania "St. George", the private Clinical Laboratory "BIOEREYNA DIAGNOSTIC LABORATORIES", and the private Molecular Genetics Laboratory "ELEFTHO" in Chania, Crete, Greece.

## DISCLOSURE STATEMENT

This study was conducted independently; no company or institution supported it financially.The authors declare that they have no conflicts of interest.

## REFERENCES

1. Blom HJ, Smulders Y. Overview of homocysteine and folate metabolism. With special references to cardiovascular disease and neural tube defects. J Inherit Metab Dis 2011; 34: 75–81.
2. Mazokopakis EE, Starakis IK. Recommendations for diagnosis and management of metformin-induced vitamin B12 (Cbl) deficiency. Diabetes Res Clin Pract 2012; 97: 359–67.
3. Hiraoka M, Kagawa Y. Genetic polymorphisms and folate status. Congenit Anom (Kyoto) 2017; 57: 142–9.
4. Tinelli C, Di Pino A, Ficulle E, Marcelli S, Feligioni M. Hyperhomocysteinemia as a risk factor and potential nutraceutical target for certain pathologies. Front Nutr 2019; 6: 49.
5. Brustolin S, Giugliani R, Félix TM. Genetics of homocysteine metabolism and associated disorders. Braz J Med Biol Res 2010; 43: 1–7.
6. Lopes CI. Hyperhomocysteinemia: How does it affect the development of cardiovascular disease?. Int Arch Cardiovasc Dis 2018; 2: 008.
7. Pietrzik K, Bailey L, Shane B. Folic acid and L-5-methyltetrahydrofolate: comparison of clinical pharmacokinetics and pharmacodynamics. Clin Pharmacokinet 2010; 49: 535–48.
8. Ganguly P, Alam SF. Role of homocysteine in the development of cardiovascular disease. Nutr J 2015; 14: 6.
9. Moll S, Varga EA. Homocysteine and MTHFR mutations. Circulation 2015; 132: e6–9.
10. Moretti R, Caruso P. The controversial role of homocysteine in neurology: from labs to clinical practice. Int J Mol Sci 2019; 20: E231.
11. Mazokopakis EE, Papadomanolaki MG. Methylene tetrahydrofolate reductase (MTHFR) gene polymorphisms among Greek women with medical history of recurrent pregnancy loss. Arch Gynecol Obstet 2020; 302: 1555–6.
12. WHO (World Health Organization), Obesity: preventing and managing the global epidemic. WHO/NUT/NCD/98.1. Geneva, Switzerland: World Health Organization, 1998.
13. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972; 18: 499–502.
14. Lauer RM, Lee J, Clarke WR. Factors affecting the relationship between childhood and adult cholesterol levels: the Muscatine Study. Pediatrics 1988; 82: 309–18.
15. Colson NJ, Naug HL, Nikbakht E, Zhang P, McCormack J. The impact of MTHFR 677 C/T genotypes on folate status markers: a meta-analysis of folic acid intervention studies. Eur J Nutr 2017; 56: 247–60.
16. Ni J, Zhang L, Zhou T, et al. Association between the MTHFR C677T polymorphism, blood folate and vitamin B12 deficiency, and elevated serum total homocysteine in healthy individuals in Yunnan Province, China. J Chin Med Assoc 2017; 80: 147–53.
17. Waskiewicz A, Piotrowski W, Broda G, Sobczyk-Kopciol A, Ploski R. Impact of MTHFR C677T gene polymorphism and vitamins intake on homocysteine concentration in the Polish adult population. Kardiol Pol 2011; 69: 1259–64.
18. Kang SS, Rosenson RS. Analytic approaches for the treatment of hyperhomocysteinemia and its impact on vascular disease. Cardiovasc Drugs Ther 2018; 32: 233–40.
19. Li WX, Dai SX, Zheng JJ, Liu JQ, Huang JF. Homocysteine metabolism gene polymorphisms (MTHFR C677T, MTHFR A1298C, MTR A2756G and MTRR A66G) jointly elevate the risk of folate deficiency. Nutrients 2015; 7: 6670–87.
20. Weisberg IS, Jacques PF, Selhub J, et al. The 1298A–>C polymorphism in methylenetetrahydrofolatereductase (MTHFR): in vitro expression and association with homocysteine. Atherosclerosis 2001; 156: 409–15.
21. Scaglione F, Panzavolta G. Folate, folic acid and 5-methyltetrahydrofolate are not the same thing. Xenobiotica 2014; 44: 480–8.
22. Servy E, Menezo Y. The Methylene Tetrahydrofolate Reductase (MTHFR) isoform challenge. High doses of folic acid are not a suitable option compared to 5 Methyltetrahydrofolate treatment. Clin Obstet Gynecol Reprod Med 2017; 3: 1–5.
23. Sawaengsri H, Wang J, Reginaldo C, et al. High folic acid intake reduces natural killer cell cytotoxicity in aged mice. J Nutr Biochem 2016; 30: 102–7.
24. Kim YI. Folic acid supplementation and cancer risk: point. Cancer Epidemiol Biomarkers Prev 2008; 17: 2220–5.
25. Ledowsky C (2015). Folic Acid vs 5-MTHF in treating MTHFR deficiency. MTHFR Support Australia. Available on: https://mthfrsupport.com.au/2015/05/folic-acid-vs-5-mthf-treating-mthfr-deficiency/.
26. Smulders YM, Smith DE, Kok RM, et al. Cellular folate vitamer distribution during and after correction of vitamin B12 deficiency: a case for the methylfolate trap. Br J Haematol 2006; 132: 623–9.

27. Nygård O, Refsum H, Ueland PM, Vollset SE. Major lifestyle determinants of plasma total homocysteine distribution: the Hordaland Homocysteine Study. Am J Clin Nutr 1998; 67: 263–70.
28. Al-Bayyari N, Hamadneh J, Hailat R, Hamadneh S. Total homocysteine is positively correlated with body mass index, waist-to-hip ratio, and fat mass among overweight reproductive women: A cross-sectional study. Nutr Res 2017; 48: 9–15.
29. Daly C, Fitzgerald AP, O'Callaghan P, Collins P, Cooney MT, Graham IM; COMAC Group. Homocysteine increases the risk associated with hyperlipidaemia. Eur J Cardiovasc Prev Rehabil 2009; 16: 150–5.
30. Momin M, Jia J, Fan F, Li J, Dou J, Chen D, Huo Y, Zhang Y. Relationship between plasma homocysteine level and lipid profiles in a community-based Chinese population. Lipids Health Dis 2017; 16: 54.
31. Wu H, Wang B, Ban Q, et al. Association of total homocysteine with blood pressure in a general population of Chinese adults: a cross-sectional study in Jiangsu province, China. BMJ Open 2018; 8: e021103.
32. Xu R, Huang F, Wang Y, Liu Q, Lv Y, Zhang Q. Gender- and age-related differences in homocysteine concentration: a cross-sectional study of the general population of China. Sci Rep 2020; 10: 17401.
33. Han L, Liu Y, Wang C, et al. Determinants of hyperhomocysteinemia in healthy and hypertensive subjects: A population-based study and systematic review. Clin Nutr 2017; 36: 1215–30.

# COVID-19 Can Unveil Brugada: A Rare Case

Savalan Babapoor[1,*], Zachary Port[2], Philip C. Wiener[3],
Roozbeh Tarighati Rasekhi[4], Ali Babapour Farrokhran[5], Sumeet K. Mainigi[2]

ABSTRACT
Brugada syndrome (BRS) is a channelopathy with three characteristic electrocardiogram patterns and an increased risk of sudden cardiac death (SCD), in the absence of gross structural heart disease. Fever is shown to precipitate ventricular arrhythmias in patients with BRS. Here, we report a rare case of Brugada pattern in a patient with Coronavirus Disease 2019 (COVID-19) without fever. A baseline ECG should be considered for patients with COVID-19, even in the absence of fever. COVID-19 by itself may be a factor that can induce Brugada pattern ECGs.

KEYWORDS
Brugada Syndrome; Corona Virus Disease-19; ST-Elevation myocardial injury

AUTHOR AFFILIATIONS
[1] Leon H. Charney Division of Cardiology, Cardiac Electrophysiology, NYU Langone Health, New York University School of Medicine, NY, USA
[2] Department of Medicine, Division of Cardiology, Einstein Medical Center, Philadelphia, PA, USA
[3] Department of Internal Medicine, Division of Cardiology, Washington University in St. Louis, St. Louis, MO, USA
[4] Department of Neurology, SUNY Upstate, Syracuse, NY, 13210, USA
[5] Urmia University of Medical Sciences, Urmia, Iran
* Correspondending author: Leon H. Charney Division of Cardiology, Cardiac Electrophysiology, NYU Langone Health, New York University School of Medicine, NY, USA; Savalan.BabapoorFarrokhran@NYULANGONE.org

Received: 12 May 2023
Accepted: 22 May 2023
Published online: 8 November 2023

Acta Medica (Hradec Králové) 2023; 66(2): 68–71
https://doi.org/10.14712/18059694.2023.18

## INTRODUCTION

First described in 1992, Brugada syndrome (BRS) is an inherited arrhythmia disorder which leads to an increased risk of sudden cardiac death in a structurally normal heart (1).

Nearly half of Brugada cases are caused by loss of function mutations in the genes encoding the cardiac sodium channel, SCN5A and SCN10A (2). Patients with BRS are often otherwise healthy and unaware of their genetic predisposition. As a result, the diagnosis of BRS is often made after a syncopal episode or an aborted sudden cardiac arrest (3). In the case where a patient is found to have the characteristic electrocardiographic changes without any prior history of sudden cardiac death, ventricular arrhythmias, or syncopal episodes, the patient is diagnosed with the "Brugada pattern" rather than the syndrome phenotype.

A febrile state, electrolyte abnormalities such as hyper/hypokalemia, substance use such as alcohol or cocaine, and sodium channel blocking medications are some of the provocative triggers that may unmask the ECG manifestation and induce an arrhythmia in patients with BRS (4). Arrhythmias and conduction system abnormalities are one of the major complications of COVID-19 (5). Here, we report a rare case of type 2 Brugada pattern in a patient with Coronavirus Disease 2019 (COVID-19) without fever.

## CASE REPORT

A male patient in his 40s with a history significant for diabetes and hypertension, presented to the emergency department (ED) reporting a one-week history of fever, chills, and headache. In the ED, his nasal swab sample tested positive for COVID-19 by reverse transcriptase polymerase chain reaction (RT-PCR). He was discharged home due to stable vital signs and clinical condition with no new medications.

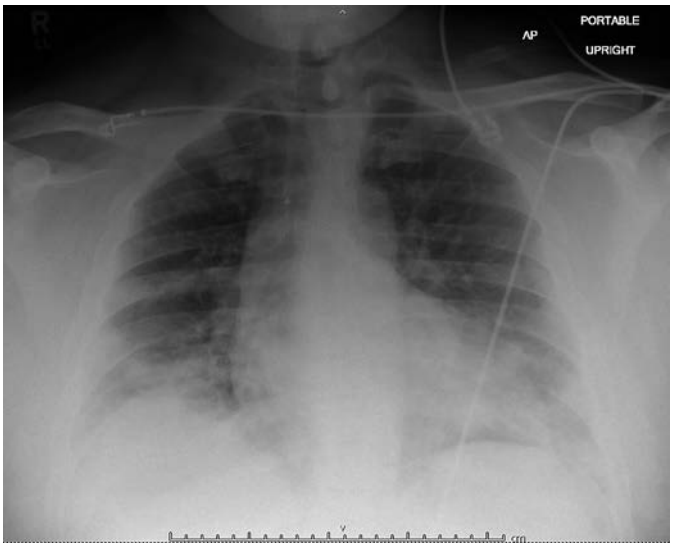


**Fig. 2** Chest X-Ray

Two days following discharge, he reported dyspnea on exertion, shortness of breath, with worsening headache and myalgias and underwent re-evaluation in the ED. On presentation the patient was afebrile (36.9 °C), had a pulse of 84 bpm, respiratory rate of 19, and an oxygen saturation of 92% on room air. He was placed on 2 liters of supplemental oxygen and his oxygen saturation improved to 98%. In discussion with the patient, he denied recent or remote syncope or a family history for sudden cardiac death (SCD).

An electrocardiogram (ECG) was obtained that showed ST elevation in lead V1 and V2 (Figure 1). There were no prior ECGs for comparison. Chest x-ray demonstrated diffuse interstitial and patchy ground-glass opacities throughout both lungs consistent with COVID pneumonia (Figure 2). He had a normal echocardiogram with normal ejection fraction and no significant structural or valvular abnormalities. There was no pericardial effusion.

The patient re-tested for COVID-19 with nasal swab RT-PCR at this second evaluation and remained positive. Computed tomography (CT) scan of the chest with and

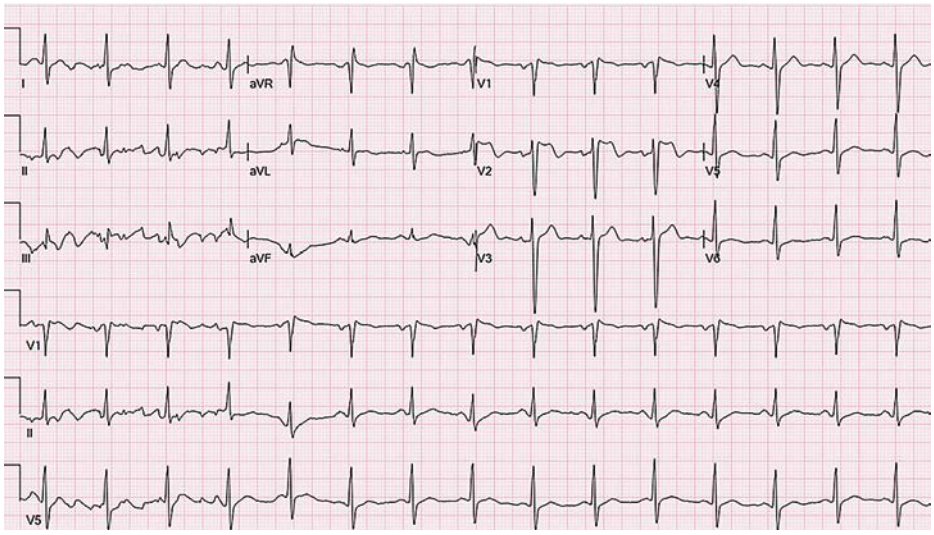


**Fig. 1** ECG: Presentation electrocardiogram (ECG) for our patient demonstrating sinus rhythm with incomplete right bundle branch block pattern and ST elevation in precordial leads > 2 mm with "saddle back" conformation indicative of the type 2 Brugada pattern.

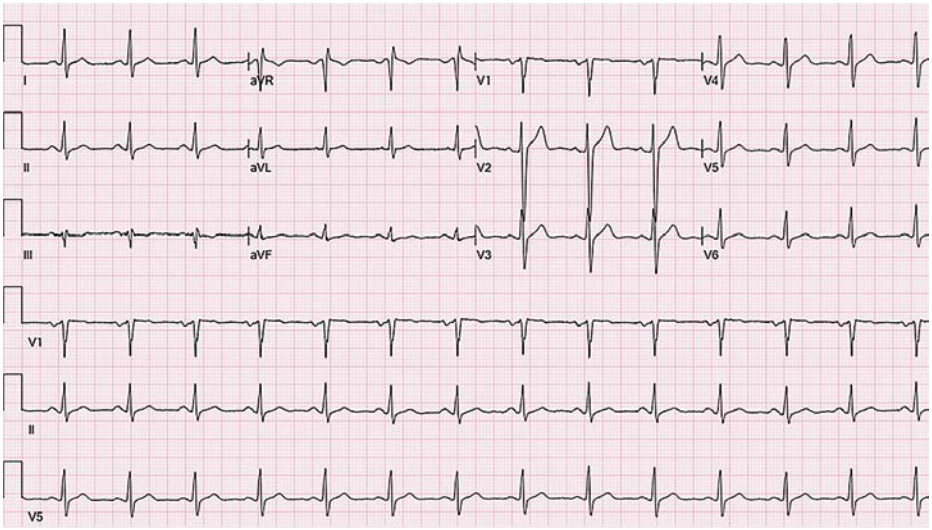


**Fig. 3** ECG Prior to discharge: Electrocardiogram prior to discharge demonstrating sinus rhythm with resolution of precordial ST elevation and Brugada pattern.

without contrast showed no evidence of pulmonary emboli and confirmed multi-lobar pneumonia. His admission laboratory tests were within normal limits except for an elevated creatinine of 1.22 mg/dl, ferritin of 1305 ng/ml (normal range of 22–275 ng/ml) and lactate dehydrogenase of 470 IU/L (normal range of 125-220 IU/L).

Based on an initial concern for an ST elevation myocardial infarction (STEMI), he received aspirin 325mg, sublingual nitroglycerine, and one dose of dexamethasone. CT coronary angiogram showed clear coronaries. Troponin I (high sensitivity troponin) levels were trended three times with all 3 resulting <0.03 ng/mL. The patient was treated with steroids and remdesivir. On day 2 of admission, the ST elevation resolved and returned to normal baseline (Figure 3). His renal function also returned to normal with a creatinine of 0.86 mg/dl. The patient remained afebrile on admission, throughout hospitalization, and during each ECG evaluation. No arrhythmia was noted on telemetry monitoring during his hospital stay.

The patient's home medications were unchanged prior to or during admission. These medications included glimepiride, irbesartan, dexamethasone, and atorvastatin.

On post discharge follow up appointments, patient had ECG pattern similar to the ECG prior to discharge with no evidence of Brugada pattern

## DISCUSSION

BRS is a channelopathy with three characteristic electrocardiogram patterns and an increased risk of SCD, in the absence of gross structural heart disease (6). Type 2 pattern is characterized by ST-segment elevation of ≥ 2 mm with a saddleback morphology in ≥ 1 right precordial leads (Figure 1). Fever is shown to precipitate ventricular arrhythmias in patients with BRS. One study showed that more than half of their participating patients experienced syncope or cardiac arrest in the setting of a fever (7). It is known that COVID-19 results in multiple cardiac rhythm abnormalities and conduction system disorders via multiple mechanisms including direct damage to the myocytes and conduction system via inflammation and altered electrolyte channel function.

Fever is the most common clinical presentation in patients testing positive for COVID-19. However, in our case the patient was afebrile when the ECGs were obtained making fever induced Brugada pattern less likely. In addition to that he was not taking any antipyretics that would mask fever. Medications, another possible etiology for unmasking Brugada, did not appear to be a culprit as the home medications for our patient remained unchanged prior to and during admission. Dexamethasone and remdesivir have not been reported to provoke Brugada. The potential mechanism for why COVID caused Brguada pattern in this case can be explained by myocardial inflammation, interstitial edema leading to electrophysiological and structural remodeling, altered intercellular coupling, and action potential abnormalities.

Although type 2 Brugada pattern may be seen spontaneously and the ECG phenotype can be variable from day to day, in this case, ECG pattern disappearance with symptom resolution and absence of Brugada pattern on follow up ECGs post hospital discharge favors COVID-19 to be a potential trigger for revealing Brugada pattern.

All in all, based on the history and above findings, the likelihood of this patient having inherited arrhythmia is not excluded and it is likely that COVID-19 unmasked the Brugada pattern regardless of having inherited arrhythmia.

## CONCLUSION

Multiple cases of different electrophysiological complications of COVID-19 have been reported and the number is still growing (5, 8). Brugada pattern and ventricular arrhythmias more commonly occur in patients with fever. In this report we describe a patient with Brugada pattern ECG in the setting of COVID-19 infection without fever. A baseline ECG should be considered for patients with

COVID-19, even in the absence of fever. As demonstrated by this case, Brugada pattern ECG can be seen in COVID-19 patients even in the absence of fever. COVID-19 can be considered as a provocative trigger for Brugada pattern ECG. Cardiac monitoring may be particularly important in patients with suspected or history of BRS.

## LIMITATIONS

The patient did not undergo cardiac MRI to better assess cardiac structure and rule out microstructural abnormalities. No drug provocation test with sodium channel blockers, and genetic testing were done.

## DECLARATIONS

*Funding*
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

*Competing interests*
The authors declare no competing interests.

*Ethics approval*
Research conducted according to ethical guidelines.

## REFERENCES

1. Brugada P, Brugada J. Right bundle branch block, persistent ST segment elevation and sudden cardiac death: a distinct clinical and electrocardiographic syndrome. A multicenter report. J Am Coll Cardiol 1992; 20(6): 1391–6.
2. Hu D, Barajas-Martínez H, Pfeiffer R, et al. Mutations in SCN10A Responsible for a Large Fraction of Brugada Syndrome Cases. J Am Coll Cardiol 2014; 64(1): 66.
3. Priori SG, Napolitano C, Gasparini M, et al. Natural history of Brugada syndrome: insights for risk stratification and management. Circulation 2002; 105(11): 1342–7.
4. Antzelevitch C, Brugada P, Borggrefe M, et al. Brugada Syndrome: Report of the second consensus conference. Circulation 2005; 111(5): 659–70.
5. Babapoor-Farrokhran S, Rasekhi RT, Gill D, Babapoor S, Amanullah A. Arrhythmia in COVID-19. SN Compr Clin Med 2020; 2(9): 1430–5.
6. Sieira J, Brugada P. The definition of the Brugada syndrome. Eur Heart J 2017; 38(40): 3029–34.
7. Amin AS, Meregalli PG, Bardai A, Wilde AAM, Tan HL. Fever increases the risk for cardiac arrest in the Brugada syndrome. Ann Intern Med 2008; 149(3): 216–8.
8. Babapoor-Farrokhran S, Port Z, Wiener PC, Amanullah A, Mainigi SK. Polymorphic Ventricular Tachycardia with a Normal QTc Interval in a Patient with COVID-19 and Fever: Case Report. SN Compr Clin Med 2020; 2(11): 2387–90.

# Sodium-Hyaluronate Cystic-like Lesion in the Anterior Chamber Following Cataract Surgery: A Case Report

Christina Mitsi[1,*], Minas Bakirtzis[1], Eirini-Kanella Panagiotopoulou[1], Georgios Labiris[1]

ABSTRACT
This is a case report describing the uncommon finding of a cystic-like lesion and its management in the anterior chamber of a male patient after cataract surgery.

KEYWORDS
anterior chamber cyst; free-floating cystic lesion; cataract surgery

AUTHOR AFFILIATIONS
[1] Department of Ophthalmology, University Hospital of Alexandroupolis, 68100 Dragana, Alexandroupolis, Greece
* Corresponding author: Department of Ophthalmology, University Hospital of Alexandroupolis, 68100 Dragana, Alexandroupolis, Greece; e-mail: mitsi.christ@gmail.com

Received: 20 June 2023
Accepted: 12 September 2023
Published online: 8 November 2023

Acta Medica (Hradec Králové) 2023; 66(2): 72–74
https://doi.org/10.14712/18059694.2023.19

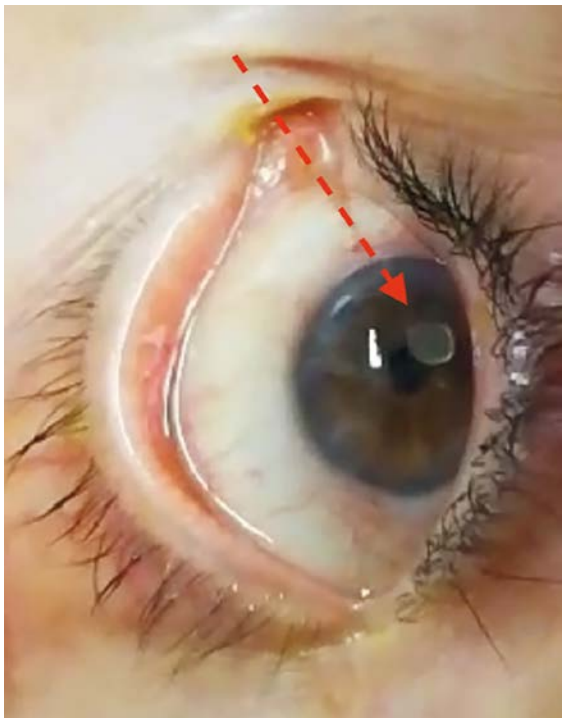

**Fig. 1** Grade 1 conjunctival redness and cystic lesion.

## INTRODUCTION

There is limited literature regarding free-floating anterior chamber cysts and cystic-like lesions with most of them being pigmented iris cysts (1–6). In this case report we describe a sodium hyaluronate free-floating cyst-like lesion in the anterior chamber following uncomplicated phacoemulsification.

## CASE REPORT

An 83-year-old man underwent uncomplicated phacoemulsification for stage 2 LOCS-III cataract, and received a foldable monofocal intraocular lens (IOL) in his left eye. Following surgery, he was prescribed fixed combination of tobramycin & dexamethasone (FCTD) q.i.d. and 0.1% sodium hyaluronate q.i.d. For non-medical reasons, the patient missed his first postoperative follow-up examination (1 week postop) and scheduled for the 2nd postoperative visit at 4 weeks. However, 20 days postoperatively, he urgently visited our outpatient service due to transient blurring of vision, and ocular redness. He reported that blurred vision was aggravated during certain activities, such as leaning down or lying in bed. On the other hand, he reported no visual disturbances when standing.

Uncorrected Distance Visual Acuity (UDVA) was logMAR 0.1 and intraocular pressure was 18 mmHg. Slit-lamp biomicroscopy revealed a round, clear, non-pigmented, free-floating cystic-like structure in the anterior chamber. With the patient standing, the cystic structure was almost hidden at the top (12th hour) of the anterior chamber; on the other hand, during certain head postures, it occasionally obstructed the visual axis. No inflammatory cells could be detected in the anterior chamber. Despite that fact, grade 1 conjunctival redness could be detected. Anterior segment optical coherence tomography (AS-OCT) visualized the free-floating structure; however, it presented as a uniform, solid structure and not a cyst (Figures 1, 2).

Immediate surgical extraction was decided, and the cystic-like lesion was successfully removed. When extracted from the anterior chamber it was dissolved in a clear gelatinous substance (Figures 3, 4). Histopathological examination returned no organic cells, but sodium hyaluronate. Three months following extraction surgery, UDVA remains at logMAR 0.1 with symptom-free quality of vision.

## DISCUSSION

A variety of cystic-like lesions have been reported in the anterior chamber. Differential diagnosis includes an iris cyst, a non-keratinized squamous epithelium cyst and cysticercosis. In our case, lack of the pigmentation of the inner wall (1) made the diagnosis of an iris cyst less probable (6), as well as its appearance on OCT imaging, presenting as an equally hyperreflective lesion, internally and externally, with typical OCT findings of hyperreflective walls and hypo-reflective internal structure being the most common (7–8). On the other hand, cysticercosis cysts show characteristic contracting and expanding movements with the presence of a live scolex as a dense white spot (9). Histopathological examination confirmed sodium-hyaluronate, which, to our knowledge, is the first incident to be reported and should be taken into consideration as a rare complication in an otherwise uncomplicated phacoemulsification.

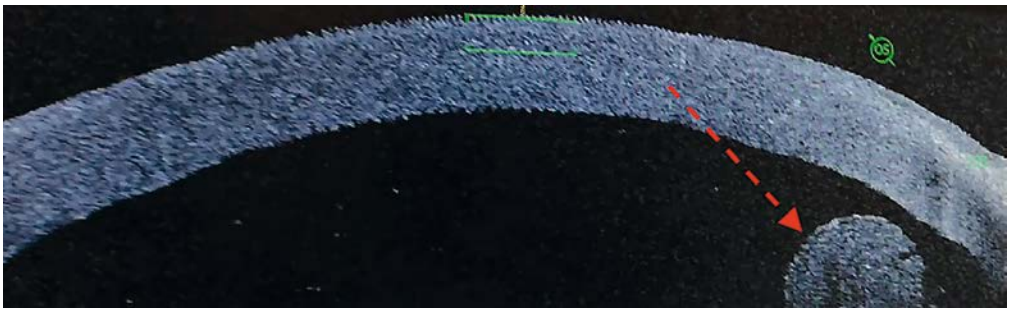

**Fig. 2** AS-OCT appearance of the lesion.

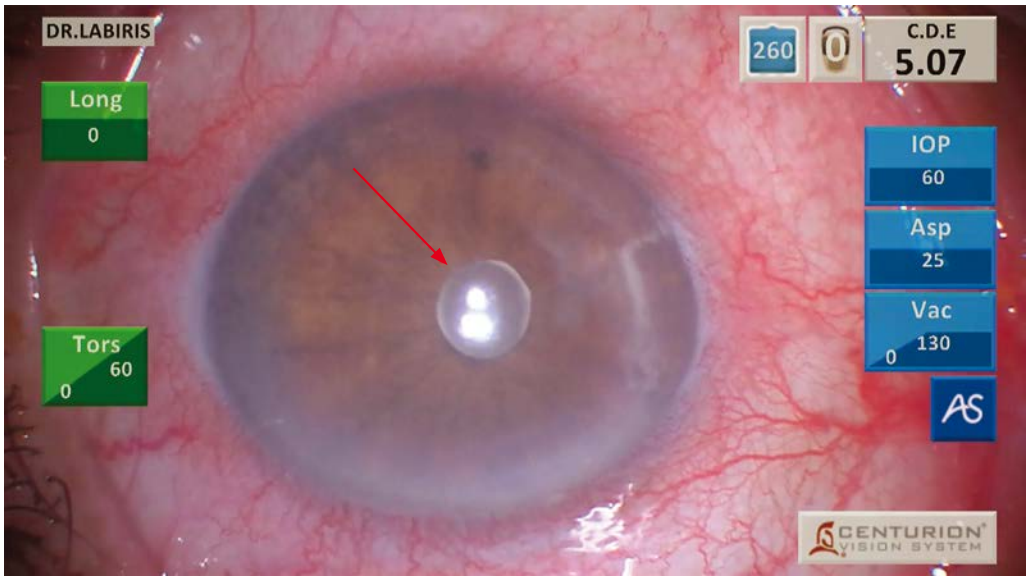


Fig. 3 Photograph of the lesion on surgery day.

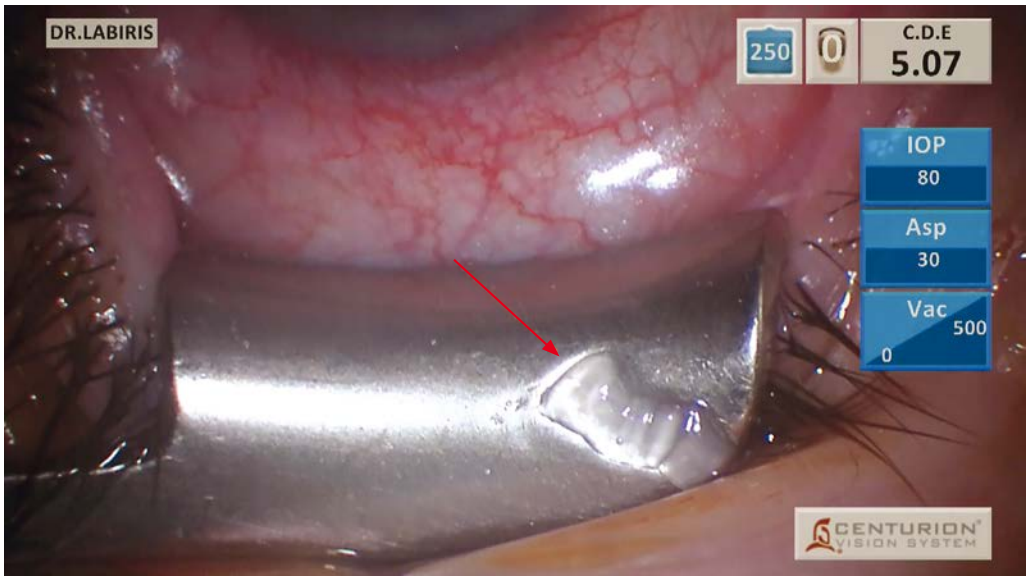


Fig. 4 Cystic lesion dissolved in a clear gelatinous substance.

## CONCLUSIONS

Such lesions have not been reported in the literature. The appearance of cysts after cataract surgery is relatively rare and usually consist of epithelial cells. In our case, the prognosis was good and the patient made a full recovery.

## FINANCIAL DISCLOSURE

No financial support was received for this case report. None of the authors has any proprietary interests or conflicts of interest related to this submission. It is not simultaneously being considered for publication at any other journal.

## REFERENCES

1. Singh G, Narendran K, Saravanan VR, Narendran V. Pigmented free-floating iris cysts. Br J Ophthalmol 2007; 91(8): 1037.
2. Paysse EA, Coats DK. Free-floating cyst in the anterior chamber. Arch Ophthalmol 1998; 116: 1256–7.
3. Shields JA, Shields CL, DePotter P, Wagner RS, Caputo AR. Free-floating cyst in the anterior chamber of the eye. J Pediatr Ophthalmol Strabismus 1996; 33: 330–1.
4. Lu CW, Liu XF, Qu LM. Pigmented Free-Floating Iris Cyst in the Anterior Chamber. Ophthalmology 2022; 129(10): 1151.
5. Arora M, Satpute K, Verma S, Singh A. Free-floating iris cyst in the anterior chamber BMJ Case Reports CP 2022; 15: e253527.
6. Malta JB, Banitt M, Soong HK. Free floating cyst in anterior chamber after cataract surgery. Br J Ophthalmol 2008; 92(7): 911.
7. Köse HC, Gündüz K, Hoşal MB. Iris Cysts: Clinical Features, Imaging Findings, and Treatment Results. Turk J Ophthalmol 2020; 50(1): 31–6.
8. Georgalas I, Petrou P, Papaconstantinou D, Brouzas D, Koutsandrea C, Kanakis M. Iris cysts: A comprehensive review on diagnosis and treatment. Surv Ophthalmol 2018; 63(3): 347–64.
9. Takkar B, Mehdi MU, Ahmed NR, Chandra P, Vanathi M. Anterior segment optical coherence tomography of live ocular cysticercosis. Clin Experiment Ophthalmol 2014; 42: 896–8.

# Abstracts from 20th International Medical Doctoral Conference (2023) – New Horizons for PhD Students in Medical Research

Hradec Králové, 29 November – 1 December 2023



Acta Medica (Hradec Králové) 2023; 66(2): 75–99
https://doi.org/10.14712/18059694.2023.20

## ARE RESTORATIONS MANUFACTURED BY CAD/CAM READY TO FIXATION WITHOUT ADJUSTMENTS?

R. Mounajjed[#,1,2], T. Taylor[3], O. Hamadah[4], I. Voborná[2], M. Al-Akkad[#,2]

[1] DCM Clinic, Hradec Králové, Czech Republic
[2] Institute of Dentistry and Oral Sciences, Faculty of Medicine and Dentistry, Palacký University Olomouc, Olomouc, Czech Republic
[3] Department of Reconstructive Sciences, UConn School of Dental Medicine, Farmington, Connecticut, United States of America
[4] Department of Oral Medicine, Faculty of Dental Medicine, Damascus University, Damascus, Syria
[#] Contributed equally.

**Introduction (aim of the study):** The master cast is the gold standard for the control and eventual adjustment of restorations produced by conventional procedures. Some digital workflow bypasses the master cast and relies completely on the precision of the CAD/CAM restoration.

The study aims to examine the reproducibility of the margins of CAD/CAM restorations generated from a single digital scan. Also, to check the readiness of these restorations for delivery directly after fabrication without adjustment on a master cast and thereby eliminate the need for the master cast.

**Methods:** A total of 18 metal substructures made from cobalt chrome alloy were fabricated utilizing a single STL file. The circumference was divided into eight zones. The vertical marginal discrepancy (VMD) was measured at each zone of each metal substructure, with optical microscopy at ×200 magnification.

**Results:** Measurements of vertical marginal discrepancy were in a range of (−94: 300) with a mean of 62 ± 60 µm. A one-way ANOVA test revealed that the mean VMD is significantly different among the 18 substructures ($F_{17, 1,134} = 63.948$, $p < 0.001$).

**Discussion:** Although all the received substructures were fabricated from the same scan file, they were not identical and varied widely, and they were going outside the acceptable range in some zones.

**Conclusions:** Within the limitations of this study, the marginal fit can be improved by extraoral adjustments on the master cast. Thus, skipping the master cast deprives the dentist of delivering a restoration of higher quality.

## SOLUTION TO PROTECT ESOPHAGEAL NITI STENT FROM THE CORROSIVE ENVIRONMENT OF THE HUMAN BODY AND NEW APPROACHES IN THEIR TESTING

D. Alferi[1], J. Hanuš[1], J. Fojt[2]

[1] Charles University, Fakulty of Medicine in Hradec Králové, Czech Republic
[2] UCT Praha, Czech Republic

**Introduction:** Due to its properties such as mechanical properties, biocompatibility or corrosion resistance, NiTi alloy is used in a wide range of medical fields from orthodontic wires to spinal clips and stents. A typical use of NiTi stents is to mitigate the impact of esophageal cancer, where the stent causes patency of the esophagus. Corrosion resistance is crucial to ensuring the safety and efficacy of the stent. The key to obtaining relevant information on corrosion resistance is to establish an electrolyte that better reflects the actual environment of esophagus. There is currently no standard that defines the environment for measuring the corrosion resistance of esophageal stents.

**Methods:**

*1. Comparison of different environment.* NiTi esophageal stents from 4 anonymized manufacturers (A-D) using 3 types of wires from different suppliers (1-3) were used for the measurements. The samples were exposed in an environment SGF and PBS. Cyclic polarization according to ASTM F2129-19a standard.

*2. Influence of input material and production processes on corrosion resistance.* The 6-week immersion of stents in SGF was performed. With a subsequent assessment of the surface condition by X-Ray photoelectron spectroscopy (XPS) and scanning electron microscopy (SEM). The amount of ions released from the stents was monitored by atomic absorption spectroscopy (AAS).

**Results:**

Ad 1

- Breakdown potentials ($E_b$) decrease in SGF and at the same time to an increase in the corrosion current density ($j_{corr}$).
- The integrity of the stents was compromised in the SGF.

Ad 2

- Corrosion initiation occurs on the free surface of the stents and not in the wire crossing
- There was a decrease in nickel concentration from the surface of the stents.
- Stents with a higher concentration of nickel on the surface before exposure release more nickel ions.

**Discussion:** Based on the comparison of cyclic polarizations, the environment determined by the above-mentioned standard can give distorted results about the corrosion resistance of stents in the esophagus (higher Eb values and lower jcorr values in PBS).

The 6-week exposure of the stents shows that the production process significantly affects the corrosion resistance. An inappropriate combination of input material and production process, a significant reduction in corrosion resistance can occur.

**Conclusion:** From current research, a corrosion testing environment has been established that provides relevant information on corrosion resistance.

From the 6-week immersion, it was found that a poorly chosen combination of wire and production process can rapidly deteriorate corrosion resistance, but also that there is a production process in which the quality of the input material has a negligible effect on the final corrosion resistance of the stent.

## THE RELATIONSHIP BETWEEN NUTRITIONAL STATUS AND THE OCCURRENCE OF FRAILTY AND QUALITY OF LIFE IN PATIENTS WITH CHRONIC KIDNEY DISEASE UNDERGOING HEMODIALYSIS – A PILOT STUDY

M. Andreew[1,2]

[1] Doctoral School, Faculty of Health Sciences in Katowice, 12 Medyków Street, 40-752 Katowice, Medical University of Silesia in Katowice, Poland

[2] Scientific Study Group at the Department of Internal Nursing, Department of Internal Medicine, Faculty of Health Sciences in Katowice, Ziołowa 45/47 Street, 40-635 Katowice, Medical University of Silesia in Katowice, Poland

**Introduction:** Chronic Kidney Disease (CKD) is often in Poland disease syndrome classified as a chronic non-communicable disease. Hemodialysis is one of the methods of renal replacement therapy. People with CKD often have abnormal body weight, protein-calorie malnutrition, muscular sarcopenia, and frailty syndrome. The specificity of hemodialysis treatment requires regular visits to the dialysis center, which worsens the quality of life of patients. The main purpose of the study was to define the relationship between nutritional status and the risk of frailty syndrome and quality of life of patients with CKD undergoing hemodialysis (HD) treatment.

**Methods:** The study was conducted at a dialysis station at a hospital in Katowice with the participation of chronic hemodialysis patients and consisted of a total of 34 patients aged 30 to 90. Men constituted the majority (N = 22) in comparison with women (N = 12). Body Mass Index (BMI), muscle strength, arm circumference, lean body mass after dialysis, adipose tissue mass after dialysis, % of adipose tissue after dialysis and phase angle after dialysis, were measured. Standardized questionnaires KDQOL 1.3, Tilburg, were used. An additional research tool was a self-constructed questionnaire containing questions on age, education, place of residence, vascular access.

**Results:** The study involved 34 patients aged 30 to 90 (M = 63.06; SD = 14.70), treated with HD. As strength increases muscle quality of the examined persons, the quality of life index in the physical area increased moderately functioning and symptom and problem list ($p < 0.05$). Positive associations of moderate strength occurred also between lean body mass after dialysis and physical functioning, sf 12 mental composite, symptom and problem list, cognitive function, energy/fatigue and role physical ($p < 0.05$).

**Discussion:** Other authors also cite the relationship between muscle strength and the quality of life and patient satisfaction (Hoshino, 2021). Malnutrition, in turn, is perceived as the most significant determinant of the quality of life domains of the KDQOL-SF questionnaire (Visiedo et al., 2022). Saitoh et al. (2020) suggested that a lower phase angle value was associated with a higher risk of protein-calorie malnutrition and frailty in HD patients.

**Conclusions:** The strongest relationship between the nutritional status assessment parameters used in the study and the quality of life domains was obtained between: muscle strength and the quality of the quality of life index in the physical area and functioning and symptom and problem list, between lean body mass after dialysis and physical functioning, sf 12 mental composite, symptom and problem list, cognitive function, energy/fatigue and role physical.

## ASPIRIN THERAPY INCREASES LIKELIHOOD OF OPERABILITY AND REDUCES METASTATIC RISK IN PANCREATIC ADENOCARCINOMA

A. M. Banni[1], S. John[1], I. Sirák[1], M. Vošmik[1]

[1] Department of Oncology and Radiotherapy; Charles University, Faculty of Medicine in Hradec Králové, Czech Republic

**Introduction:** Blood platelets (PLT) are known to support tumorigenesis, angiogenesis and metastasis. Acetylsalicylic acid (ASA) is a known PLT inhibitor and has been shown to reduce cancer incidence, metastatic rates and improve

survival in some cancer types. This study aims to assess the effect of ASA on the clinical outcome in pancreatic ductal adenocarcinoma (PDAC) and determine whether use of ASA may offer survival benefits or therapeutic advantages.

**Methods and patients:** Retrospective analysis of data collected from a cohort of 182 patients with PDAC in a 6-year period was performed. The effects of ASA use for two or more years on operability, TNM stage, overall survival, disease-free survival and time to progression was assessed. Furthermore, the effect of ASA on metastasis in subgroups of patients was evaluated.

**Results:** According to our data, in the group of patients without ASA therapy (non-ASA), 75% presented with inoperable tumours and 57% had metastasis upon diagnosis. We observed a significant difference in the ASA group, where 46% patients presented with inoperable tumours and 21% had metastases upon diagnosis. Among the patients with tumours localized in the head of the pancreas, 49% in comparison to 21% had metastases when diagnosed, in non-ASA and ASA group respectively. Similarly, in the subgroup of patients with tumours localized outside the head of the pancreas, 74% in comparison to 33% of patients presented with metastasis at diagnosis in non-ASA and ASA group, respectively. These findings were shown to be statistically significant using logistic regression and age was found to have no correlation with operability or risk of metastasis. We observed no significant difference in either T or N stage between both groups, as well as no significant effect on over-all survival, disease free survival or time to progression using log-rank test.

**Discussion:** In this study, we demonstrate that ASA use is associated with a higher probability of operability. The reason for this finding could be reduction of metastatic spread through the hematogenous route rather than the lymphatic route for tumours involving the head of the pancreas, or outside of the head (body, tail and uncinate process). Although ASA treatment did not influence survival endpoints, it may offer clinical and therapeutic advantages and allow a higher probability for presenting in curable or treatable stages of PDAC. Limitation of this study, however, is its single-centred character and relatively small sample size.

**Conclusion:** Our retrospective analysis shows that patients treated by ASA for two or more years were nearly twice more likely to present in operable stages upon diagnosis of PDAC and over two times less likely to present with metastasis.

## HULL'S MAGIC BOX: ARGININE METHYLATION INHIBITORS AS A POTENTIAL NOVEL THERAPY FOR GBM

A. Barry[1], S. Samuel[1], I. Hosni[1], A. Moursi[1,2], C. Sennett[1], S. Deepak[1,2], S. Achawal[2], C. Rajaraman[2], L. Feugere[1], I. Scott[3], A. Iles[1], V. Green[1], L. Stead[4], M. Wade[1], J. Greenman[1], P. Beltran-Alvarez[1]

[1] Diabetes and Metabolism Theme, Biomedical Institute for Multimorbidities, Centre for Biomedicine, Hull-York Medical School, UK
[2] Hull University Teaching Hospitals NHS Trust, UK
[3] Neuroscience Laboratories, The Walton Centre NHS Foundation Trust, Liverpool, UK
[4] University of Leeds, UK

**Introduction:** GBM tumours are diffuse, necrotic and the most common and aggressive form of glioma in the central nervous system (Louis et al. 2021). Patients undergo the Stupp regimen of surgical resection, ionizing radiation and chemotherapy using temozolomide (TMZ) (Stupp et al. 2009). This has remained unchanged for 15 years and median life expectancy for these patients is around 14 months from diagnosis (Zhu et al. 2017), highlighting the urgent requirement for novel therapeutics. Research into the effect of arginine methylation in GBM has provided new therapeutic targets (Samuel et al. 2021) and protein arginine methyltransferase (PRMT) inhibitors, such as GSK3368715 have recently entered clinical trials for a range of other cancers (Fedoriw et al. 2019) and could be promising new therapies for GBM.

**Methods:** GBM biopsies from Hull Royal Infirmary, or healthy mouse brain tissues, were maintained in a novel fluidics system, pioneered in Hull, for 8-days and perfused with GSK3368715-treated media, at 3 µl/min, mimicking the *in vivo* environment and synergising with personalised patient care and precision medicine.

**Results and Discussion:** GSK3368715 causes apoptosis of GBM tissue *ex vivo* but not of healthy tissue. Immunohistochemistry using apoptotic inhibitor cleaved-PARP indicated a 2.17 ± 1.1-fold increase in apoptosis in GSK3368715-treated GBM samples, showing that PRMT inhibition causes cell death in GBM *ex vivo*.

RNA-sequencing determined thousands of differentially expressed genes in GBM tissues resulting from GSK3368715 treatment, showing highly significant GO-term-enrichment in ribosome and translation pathways, suggesting decreased protein synthesis capacity after GSK3368715 treatment. My data also indicated a reduction in variation of differentially expressed genes upon PRMT inhibition, from more aggressive to less aggressive phenotypes in principal component analysis. Additionally, several hundreds of genes were found to be undergoing alternative splicing (AS), compatible with a mechanism where changes in the arginine methylation pattern of spliceosome member fused in sarcoma (FUS), upon GSK3368715 treatment, contribute to AS. GO-term-enrichment of these AS events was found in DNA damage and cell death, giving credence to my results which highlight GSK3368715 as a cause of apoptosis in GBM maintained *ex vivo*.

**Conclusion:** My results highlight an exciting new potential therapeutic target for GBM, *via* induction of alternative splicing pathways, through arginine methylation inhibition, which may underly the initiation of apoptotic pathways and tumour cell death of a disease with clear clinical needs.

## CHANGES IN MUCIN SECRETION IN THE RATS' STOMACH WITH TYPE 2 DIABETES AND UNDER CORRECTION

I. Chebernina[1], T. Kerimov[1], K. Vakhnovska[1]
Supervisor: S Smirnov[1]

[1] State Establishment "Lugansk State Medical University", Rivne, Ukraine

**Introduction:** Type 2 diabetes mellitus (T2DM) is one of the most common diseases in the world. The disease affects all organ systems and, in particular, gastrointestinal. There are some studies, that display such changes in morphology and the functional activity of the gastrointestinal tract. For instance, atrophy of the gastric mucosa, antibodies to parietal cells, a decrease in tone and motility, abnormalities in the vessels of the villi, and microbiome misbalance. The influence of T2DM on the gastrointestinal tract is generally studied, although, the changes in the functional activity of stomach granulocytes are insufficiently studied. Therefore, the aim of the study is to observe the changes in the mucin secretion of the gastric mucosa of rats against the background of the course of diabetes mellitus 2 and its correction by the combination of metformin with propionic acid.

**Methods:** T2DM was simulated by streptozocin injections and food challenge in 24 white laboratory rats, which were divided into 4 groups: the 1st group – T2DM without treatment, the 2nd group – T2DM+metformin, the 3rd group – T2DM + propionate, and the 4th group – T2DM and metformin + propionate. The group received drugs for 14 days. For histological examination, the stomach was taken for histochemical examination. Statistical data processing was done using the StatPlus program ver. 7.3.0. The difference between groups was considered significant at $p \leq 0.05$.

**Results:** The gastric glands of rats from the control group had the localization of mucin both on the surface and in the cells. There are mucopolysaccharides in all parts of the fundal glands. The 1st and the 2nd groups had similar changes, which included the reduction of mucin in the cells by 2.6 times compared to the control, and mucopolysaccharides were not found in the deep parts of the glands. In the 3rd group against the background of treatment by propionate are increase in mucin synthesis by 25% ($p \leq 0.05$) was observed in comparison with the indicators of the 1st, 2nd, and 4th groups. However, the amount of mucus-producing cells also increased in the 4th group that used the combination of metformin and propionic acid. It is believed that a sharp decrease in the amount of mucin occurs as a result of inhibition of the activity of synthetic processes and, in particular, glycoproteins, against the background of type 2 diabetes.

**Conclusions:** A significant decrease in mucin production in the base of the gastric glands of rats by 2.6 times was established, which is associated with a decrease in the number of cells producing mucopolysaccharides. Metformin did not affect the increase in the production of mucin in the stomach in patients with type 2 diabetes, but the use of propionate both alone and in combination with metformin increased the production of mucopolysaccharides.

## THE EFFECT OF THE COMBINATION OF TEMOZOLOMIDE AND FLUBENDAZOLE ON GLIOBLASTOMA CELLS

K. Dvořáková[1], B. Vítovcová[1], V. Skarková[1]
Supervisor: E. Rudolf[1]

[1] Department of Medical Biology and Genetics, Faculty of Medicine in Hradec Králové, Charles University, Czech Republic

**Introduction:** Glioblastoma multiforme (GBM) is the most common and malignant primary brain tumor. Infiltrative growth, many different genetic and epigenetic variants as well as low plasma concentration of the alkylating drug temozolomide (TMZ) in the tumor and quickly developing chemoresistance are the reasons of therapy failure.

One of the strategies of increasing the treatment effectiveness is to combine drugs with different mechanisms of action. Flubendazole (FLU), the member of benzimidazoles group, exhibits anticancer effect based on its ability to interact with cell microtubules. Cell microtubules play important role in proliferation and invasion of cancer cells. Higher expression of βIII-tubulin found in higher grade gliomas indicates possible treatment target.

The aim of this study was to investigate the effect of the combination of TMZ and FLU on the viability and survival of stabilized GBM cell lines (A172, T98G and U118MG) and to verify this effect in an *in vivo* model.

**Methods:** Cell viability and proliferation were evaluated biochemically (WST-1 assay) and cytometrically (phase contrast microscopy). Drug synergism was determined using CompuSyn software. Changes in microtubule morphology were examined by fluorescence microscopy. Quantification of TMZ, FLU and their metabolites in cells was carried out using LC/MS analysis, while expression of selected cell cycle markers was determined by RT-PCR and Western blot.

**Results:** The combination of TMZ and FLU decreased cell viability and proliferation in exposed cell lines more effectively than with individual drug. Furthermore, changes in cell morphology and microtubule structure were noted as well as differently expressed cell cycle markers, such as cdc2 or cyclin B. Use of this combination also increased the amount of TMZ and FLU accumulated in tested GBM cell lines as well as in the tumor and brain of *in vivo* model organism.

**Discussion:** In our study, we combined the commonly used chemotherapeutic drug TMZ and a candidate anticancer drug FLU, which fits in a currently used concept aiming the improvement of the treatment efficiency in various types of cancers. Our obtained data clearly demonstrate improved efficiency of our drug combination in *in vitro* and *in vivo* GBM models. Since the use of tested drug combination also resulted in an increase of drug accumulation in GBM cells, this strategy should be the subject of further investigation.

**Conclusions:** Our results demonstrate that FLU improves TMZ antiproliferative effect in all tested GBM cell lines. Moreover, this drug combination caused significant changes in cell morphology, microtubule cytoskeleton and drug accumulation in cells. Obtained results indicate

possible strategic advantage of combination therapy in GBM and should be further investigated.

**Acknowledgement:** This study was supported by Ministry of Health, Czech Republic, project No. NU20-03 -00360.

## CEREBRAL PALSY RISK FACTORS, COMORBID CONDITIONS AND TYPES IN GEORGIAN INFANTS

T. Giuashvili[1,2], N. Tatishvili[1,2], S. Tatishvili[1,3], A. Bedoshvili[2,3]

[1] David Tvildiani Medical University, Tbilisi, Georgia
[2] M. Iashvili Children's Central Hospital, Tbilisi, Georgia
[3] Neurodevelopmental Centre, Tbilisi, Georgia

**Introduction:** Cerebral palsy is the most common motor disability among children and has many comorbidities. Diagnosing cerebral palsy is not easy in some cases. According to available literature data and guidelines, cerebral palsy is diagnosed before 6 months of age in only 25% of cases. Furthermore, looking for new methods to improve the early diagnosis of the disease is ongoing. Early diagnosis leads to early intervention, thus provides a better outcome. The study aims to develop appropriate diagnostic tools and effective measures for improving the early diagnosis of children under 9 months of age with cerebral palsy risk factors in the Georgian population for better outcomes.

**Methods:** Prospective/Longitudinal Cohort Study – At least 80 infants at high risk of cerebral palsy (extremely premature, extremely low birth weight, HIE, other neurological risk factors) are being screened and followed for 24 months. The infants at high risk of CP are assessed with Prechtl's general movements, Hammersmith Infant Neurological Evaluation, MRI. Additionally, with BSID III-IV or ASQ and GMFCS.

**Results:** The research is ongoing, at this stage 114 children have been evaluated, and have 12-month data in 53.5%. The most common risk factor for the development of CP in our research group is birth asphyxia – 43, prematurity – 48, infections – 23, seizures – 19, NICU administration – 79.

According to HINE assessment, study group was divided into non-walkers 23.8% (<40 score) and walkers 49.5% (40–62 score). Percentage distribution according GMFCS levels: I – 32.8%, II – 22.9%, III – 11.5%, IV – 26.2%, V – 6.6%. According to motor limitation, uni-, 3 limb-spasticity, and bi-spasticity are the most common 29.5%, 26.2%, and 22.9% important portion has mixed form and hypotonicity – 13.2% and 6.6%. Vision and cognitive dysfunction are predominant among comorbidities, also the detectable part has epilepsy and hearing problems.

**Discussion:** Modern scientific literature shows us very disturbing statistics and the difference between developed and developing countries in relation to the early diagnosis of the disease. The average age of diagnosis of cerebral palsy is about 18 months in high-income countries. The diagnosis of cerebral palsy is further delayed in low- and middle-income countries, for several reasons that include cultural, historical, geographic, and economic challenges. The mean age of diagnosis of cerebral palsy in low and middle-income countries varies from 2.5 years to 6.5 years and most children are described as GMFCS levels III to V.

**Conclusions:** Among the 12-month-old patients included in the Georgian population study, the proportion of the ambulant type of cerebral palsy is high, which indicates that early diagnosis and appropriate intervention significantly improve the clinical outcome and lessen the burden of CP in patients and their families.

## GENE EXPRESSION PROFILING OF PRIMARY LOW- AND HIGH-GRADE BLADDER CANCER IN RELATION TO CIRCULATING TUMOR CELL DISSEMINATION

A. Gregušová[1], J. Šonský[1], L. Molnárová[2], K. Kološtová[2], V. Bobek[2], P. Klézl[1], R. Grill[1]

[1] Department of Urology, FNKV and 3. LF UK, Prague, Czech Republic
[2] Laboratory of personalized medicine, Department of Oncology, FNKV and 3. LF UK, Czech Republic

**Introduction:** Evaluation of the presence of circulating tumor cells (CTCs) in the peripheral blood (PB) of non muscle-invasive bladder cancer patients and analysis of gene expression of both primary tumor (PT) cells and CTCs isolated from peripheral blood (PB) could help define the aggressiveness of tumours and could be prognostic marker and marker assessing the efficacy of therapy (e.g. BCG vaccine). The aim of this study is to test the presence of CTCs in the blood of patients prior to transurethral resection (TUR) of primo-resected disease and to evaluate the expression of selected genes in the resected PT and correlating CTCs by quantitative PCR (qPCR).

**Methods:** Patients with primo-finding of BC were included in the study (n = 12, mean age = 72.5). Before TUR, blood sample (2x 8mL, EDTA) was collected. During TUR, a small sample of pathological tissue is collected for gene expression analysis (GEA). Blood samples are examined for the presence of CTC using a cell-size-base separation method (MetaCell®). Subsequently, enriched CTC are stained with vital fluorescent dyes for cytomorphological analysis, including assessment of CTC metabolism and their quantity. RNA isolated from the PT and CTC is subsequently transcribed into cDNA. The expression of 16 selected genes related to BC pathophysiology was measured in the tested cDNA samples. Taqman® chemistry and Taqman® gene probes were used for qPCR for the following genes: ACTB, CD274, CK20, GLUT1, TWIST, ZEB1, TRPM4, EGFR, HER2, SOX2, POUSF1, NANOG, ALDH1, AURKA, CD24, CD44).

**Results:** A total number of 12 PT samples and 30 CTC samples are currently being evaluated . There was no difference in numbers of CTCs in compared groups (LG vs. HG). GEA data were compared for both LG and HG groups and genes with elevated expression were identified for HG/LG.

In PT samples, there is increased expression of EGFR, TRPM4 and GLUT1 in LG group, and increased expression of HER2, AURKA, NANOG and CD24 in HG group. In CTCs

samples there is increased expression of EGFR, TRPM4 and GLUT1in LG and increased expression of POUSF1, NANOG, TWIST and CD274 (PD-L1) in HG group.

HG groups displayed significantly increased expression of stemness-associated genes.

**Discussion:** The data indicate that there is a significant portion of stem cells present in HG patient blood and primary tumor, which might cause the aggressive behavior of tumor cells. CTCs enriched from LG patients are more differentiated as seen by the gene expression profile.

**Conclusion:** There is a difference in gene expression in LG and HG group. HG group has significantly increased expression of stemness-associated genes. Changes in the expression profile of CTCs over time could mirror the effect of administered therapy (BCG-vaccine) in HG patients.

## PHARMACOKINETICS OF INTRAMUSCULARLY ADMINISTERED THERMORESPONSIVE POLYMERS

O. Groborz,[1,2] K. Kolouchová, J. Pankrác, P. Keša, J. Kadlec, T. Krunclová, A. Pierzynová, J. Šrámek, M. Hovořáková, L. Dalecká, Z. Pavlíková, P. Matouš, P. Páral, L. Loukotová, P. Švec, H. Beneš, L. Štěpánek, D. Dunlop, C. V. Melo, L. Šefc, T. Slanina, S. van Vlierberghe, R. Hoogenboom, M. Hrubý, J. Beneš

[1] Ústav biofyziky a informatiky 1. LF UK, Czech Republic
[2] Ústav organické chemie a biochemie, AV ČR, Czech Republic

**Introduction:** Aqueous solutions of some polymers exhibit a lower critical solution temperature (LCST); that is, they form phase-separated aggregates when heated above a threshold temperature. Such polymers found many promising medical applications, including in situ thermogelling with controlled drug release, polymer-supported radiotherapy (brachytherapy), immunotherapy, and wound dressing, among others. Yet, despite the extensive research on medicinal applications of thermoresponsive polymers, their biodistribution and fate after administration remained unknown. Thus, herein, we studied the pharmacokinetics of four different thermoresponsive polyacrylamides after intramuscular administration in mice.

**Method:** We synthesized 4 different thermoresponsive polymers, each with two different molar masses, and labelled them with a covalently bound fluorescent dye. Next, we administered solutions of these polymers into hind legs of BALB/c mice and observed their dissolution over time with *in vivo* fluorescence imaging. Finally, we evaluated the dissolution kinetics of these polymers.

**Results and discussion:** In vivo, these thermoresponsive polymers formed depots that subsequently dissolved with a two-phase kinetics (depot maturation, and slow redissolution) with half-lives 2 weeks to 5 months, as depot vitrification prolonged their half-lives. Additionally, the decrease of $T_{CP}$ of a polymer solution increased the density of the intramuscular depot. Moreover, we detected secondary polymer depots in the kidneys and liver; these secondary depots also followed two-phase kinetics (depot maturation and slow dissolution), with half-lives 8 to 38 days (kidneys) and 15 to 22 days (liver).

**Conclusions:** In our study, we elucidated the fate or thermoresponsive polymers in body and our head-to-tail study compared the dissolution kinetics of 8 different polymers. Overall, these findings may be used to tailor the properties of thermoresponsive polymers to meet the demands of their medicinal applications. Our methods for assessing polymers distribution and dissolution may become a benchmark for future studies of polymer biodistribution.

Further reading at https://doi.org/10.1002/adhm.202201344.

## SPECTROSCOPY OF BLOOD PLASMA AS A TOOL FOR EARLY DIAGNOSTICS OF HEPATOCELLULAR CARCINOMA

P. Hříbek[1,2], O. Vrtělka[3], L. Habartová[3], T. Kupsa[2], V. Setnička[3], P. Urbánek[1]

[1] Military University Hospital Prague, Department of Medicine 1st Faculty of Medicine Charles University and Military University Hospital Prague, U Vojenské nemocnice 1200, 169 02 Praha, Czech Republic
[2] Department of Medicine, University of Defense, Faculty of Military Health Sciences in Hradec Králové, Třebešská 1575, 500 01 Hradec Králové, Czech Republic
[3] Department of Analytical Chemistry, University of Chemistry and Technology Prague, Technická 5, 166 28 Praha, Czech Republic

**Introduction (Aim of the Study):** Hepatocellular carcinoma (HCC) is the most common primary liver malignancy. The main risk factor for its development is liver cirrhosis of different etiologies or chronic HBV infection even in pre-cirrhotic stages. None of the biomarkers studied so far in the HCC area has yielded higher sensitivity than the liver ultrasonography examination in the early stage diagnosis (approx. 60%). There is an urgent clinical need for establishing a laboratory marker for HCC that meets the high sensitivity and specificity requirements for the screening and early diagnosis of at-risk patients. As various pathological processes, including carcinogenesis, may cause changes in both the concentration and the structure and spatial arrangement of body biomolecules, the spectroscopic analysis of blood-based derivatives appears to be an appropriate tool for early detection. In our research, the focus is on the identification of novel biomarkers in blood plasma that would exhibit sufficient sensitivity and specificity to detect early and potentially curable HCC stages, and that would be potentially useful for routine screening of this disease in well-defined at-risk groups. We hypothesized that spectroscopic methods can reach sensitivity of 65%, and specificity of 75%, and thus will be more efficient than liver ultrasound.

**Methods:** We included 60 subjects in the study (20 with liver cirrhosis, 20 with proven HCC, and 20 healthy volunteers). The blood plasma of the subjects was examined by Raman optic activity, Raman spectroscopy, infra-red spectroscopy, and electronic circular dichroism. To reduce the high computational time of classification while using whole spectra, mean-centered data from individual methods were subjected to a dimensionality reduction

method, namely principal component analysis (PCA). The classification was performed using partial least square discriminant analysis. Then we made models for sample discrimination.

**Results:** The combined model involving all spectroscopic methods reached for discrimination samples between HCC patients and cirrhotics with a sensitivity of 85%, and specificity of 95% (AUC-ROC = 0.958).

**Conclusion:** Based on our data the combination of advanced spectroscopic analysis of blood plasma could be a promising tool in HCC diagnosis.

## IN VITRO STUDIES USING COLLAGEN MEMBRANES: A SYSTEMATIC REVIEW

A. Imani[1], L. Panahipour[2], R. Gruber[3]

[1] PhD Student of Oral Biology, School of Dentistry, Medical University of Vienna, Austria
[2] Department of Oral Biology, School of Dentistry, Medical University of Vienna, Austria
[3] Department of Oral Biology, School of Dentistry, Medical University of Vienna, Austria

**Introduction:** Collagen membranes are widely used in regenerative dentistry to stabilize defect areas and to shield them from the soft tissue. Apart from serving as a barrier membrane, collagen membranes become a temporary scaffold for immigrating local cells. It is thus reasonable to assume that the immigrating cells respond to the collagen membranes.

**Materials:** This clinical situation is simulated under laboratory conditions aiming to understand the direction the cells response takes once they get into contact with the collagen membranes. Our aim was therefore to summarize current knowledge based on laboratory-based in vitro research using cell culture models.

**Results:** Based on the search terms "collagen membranes" and "in vitro" we could identify 170 abstracts, however, only 42 abstracts were related to "dentistry". Based on our systematic search, we identified collagen membranes tested were typically of xenogenic origin and either remained native or were cross-linked. We identified various cell types to respond to the collagen membranes including oral fibroblasts, osteogenic cells, epithelial cells and cells of the haematopoietic lineage such as macrophages and osteoclasts.

**Discussion & conclusions:** The in vitro cell response was related to viability, proliferation, osteogenic differentiation, osteoclastogenesis, and the adsorption of growth factors including TGF-β1 and BMP-2, as well as of antimicrobial compounds. Overall, this systematic review identifies a spectrum of in vitro bioassays helping to better understand the biological properties of collagen membranes.

## MIRACULOUS FAT – HOW LIPOTRANSFER CAN ALTER THE PERIPROSTHETIC TISSUE. IN VIVO ANIMAL MODEL

A. Jaźwiec[1], E. Timek[2], B. Drozdzowska[1]

[1] Medical University of Silesia in Katowice, Department of Patomorphology in Zabrze, Poland
[2] Advanced Plastic Surgery, Grand Rapids, Michigan, USA

**Introduction:** Since the first reported successful fat transfer in 1893 by Dr Neuman, we have learned much more about the properties of this fascinating tissue and its ability to remodel and rejuvenate the environment through its surprising density of the viable stromal cells. Nowadays, after mastering fat grafting techniques and gaining the wide knowledge about its histological and physiological properties lipotransfer is known for a wide range of possible applications both in aesthetic and reconstructive surgery. The aim of this study was to investigate the histological effect of lipotransfer on the tissue surrounding the implant.

**Methods:** 20 male Sprague-Dowley rats underwent 3 separate surgical procedures: 2 mini-tissue expanders implantation, periprosthetic fat grafting and saline injections serving as control, and harvesting of the implant with capsule and surrounding tissue (3 and 12 weeks after the primary procedure). Subsequently, the tissues were stained and analyzed histologically. The data obtained was statistically analyzed (Stata v.15.1).

**Results:** Statistically higher inflammation score ($P < 0.001$) with edema, granulation tissue and mixed inflammatory cells was noted among study group. Fat grafting was associated with a significant reduction in capsule density ($P = 0.001$). Moreover, fat-grafted samples had a fiber orientation score 67-fold lower than the controls ($P < 0.001$). Lipotransfer led to 28.7% reduction in dense collagen ($P = 0.001$). Control group samples displayed dense collagen bands firmly adjacent to the implant, rather than disorganized collagen bands infiltrating into fat in study group. Synovial metaplasia occurred in 25% of periprosthetic tissue, but no significant difference was found between the groups.

**Discussion:** Our histological analysis supports the hypothesis that collagen beads are the preferred scaffold for the conversion of adipose stem cells into fat, because the collagen fibers in graft are entangled in a disordered manner with reduced alignment of the collagen fibers.

The observations from our experiment have direct clinical implications. The arrangement of collagen fibers is believed to be a key feature of capsular contracture. Disruption of collagen fibers is a crucial intervention to reduce the severity of capsular contracture. As demonstrated in our study, early fat grafting can prevent formation of a dense and organized capsule.

**Conclusions:** The successful clinical lipotransfer application in prosthetic surgery might be supported by data confirming that enhancement of peri-prosthetic tissue with fat grafting decreases collagen content, density, and fiber alignment of implant capsules.

## REMOTE HEART FAILURE SYMPTOM ASSESSMENT AFTER MYOCARDIAL INFARCTION IDENTIFIES PATIENTS AT RISK OF DEATH

D. Jenča[1,2], P. Wohlfahrt[3,4], V. Melenovský[1], J. Stehlík[5], J. Mrázková[6], M. Šramko[1,4], M. Kotrč,[1] M. Želízko[1], V. Adámková[3], J. Piťha[1], J. Kautzner[1,7]

[1] Department of Cardiology, Institute for Clinical and Experimental Medicine (IKEM), Prague, Czech Republic
[2] Third Faculty of Medicine, Charles University, Prague, Czech Republic
[3] Department of Preventive Cardiology, Institute for Clinical and Experimental Medicine (IKEM), Prague, Czech Republic
[4] First Faculty of Medicine, Charles University, Prague
[5] University of Utah School of Medicine, Salt Lake City, UT, USA
[6] Experimental Medicine Centre, Institute for Clinical and Experimental Medicine (IKEM), Prague, Czech Republic
[7] Medical and Dentistry School, Palacký University, Olomouc, Czech Republic

**Introduction:** Despite improvements in the treatment of myocardial infarction (MI), heart failure (HF) remains the most common complication associated with increased mortality. Evaluation of HF symptoms early after hospital discharge is not routinely performed, and validated tools do not exist.

Whether evaluation of HF symptoms after MI hospital discharge can improve stratification of patient mortality risk is unknown. The present study aimed to evaluate the predictive value of the 23-item Kansas City Cardiomyopathy Questionnaire (KCCQ) and its components for all-cause mortality in patients after acute coronary syndrome (ACS).

**Methods:** Our study examined 1829 consecutive patients hospitalized for ACS at a large tertiary cardiac centre between June 2017 and September 2022. A total of 1,174 patients (aged 65 ± 12 years, aged 65 ± 12 years) completed KCCQ one month after hospital discharge. Cox regression was used to test the association of four categories of the KCCQ overall summary score (<25, 25–49, 50–74, ≥75) and the 23 individual items with all-cause mortality.

**Results:** KCCQ score <25 and 25-49 were independently associated with increased mortality risk compared to the reference group KCCQ ≥75. We developed a novel HF score utilizing three items of KCCQ (and their respective HR), that were independently associated with mortality risk: walking impairment, leg swelling, and change in symptoms over the last 2 weeks. Compared with reference group (HF score 3), patients with HF score 4–5, 6–7, 8–10 had significantly higher mortality risk.

**Discussion:** We examined the prognostic significance of patient-reported outcomes (as measured by KCCQ) one month after hospital discharge in large, prospective cohort of MI patients. To the best of our knowledge, this is the first study that evaluates the impact of KCCQ on long-term prognosis of consecutive patients with MI across the full range of left ventricular ejection fraction.

In the present study, KCCQ scores < 50 were present in approximately 13% of patients; with 68% of them were in Killip class I during hospital stay. Thus, large proportion of these patients developed HF symptoms after hospitalization, or Killip class is insensitive to HF signs. Remote assessment of patients' health status may decrease the burden on both patients and medical staff. It may identify high-risk patients who require closer follow-up and targeted therapy.

**Conclusion:** Evaluation of HF symptoms by KCCQ in post-MI patients after hospital discharge can identify a group at increased mortality risk. The novel HF score, which evaluates three symptom domains, is a simple tool that can further improve risk stratification.

## GLYCOPROFILING OF PROTEINS APPLICABLE AS BIOMARKERS OF PROSTATE CANCER

M. Jirásko[1,2], R. Kučera[1,2], T. Bertók[3], J. Tkáč[3]

[1] Department of Pharmacology and Toxicology, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic;
[2] Department of Immunochemistry Diagnostics, University Hospital and Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic
[3] Glycanostics, Ltd., Kudlakova 7, Bratislava, Slovak Republic

**Introduction:** Yearly incidence rate of prostate cancer (PCa) is over milion new cases worldwide with mortality rate over 300 000. In the Czech Republic in 2015 the incidence rate was 70 new cases per 100 000 people and the mortality over 12 per 100 000. Although the number of diagnosed cases is increasing, the mortality rate remains stable. This is mostly due to highly sensitive diagnostic methods and care provided to patients suffering from PCa. Prostate-specific antigen (PSA) and Zn-alpha-2-glycoprotein (ZA2G) are proteins whose glycosylated forms change significantly with the development and progression of PCa and such forms could be used to further increase the specificity of the diagnostic algorithm. The aim of our study was to conduct the glycoprofiling of those two proteins to prove their applicability as PCa biomarkers.

**Methods:** Changes in glycan composition were detected using lectins. Specific glycoprofiling was performed using magnetic particle (MP) modified with horseradish peroxidase and antibodies that selectively enriched fPSA and ZA2G in human serum samples. Subsequently, the proteins attached to the MP-antibodies were incubated on lectin-modified ELISA plates.

**Results:** Two clinical validation studies were performed, one for fPSA glycoprofiling and the other for ZA2G glycoprofiling. The results were presented in the form of a reciever operating characteristic curve. Glycoprofiling of fPSA showed better specificity and sensitivity (AUC = 0.84, n = 501) over fPSA alone (AUC = 0.76) in PCa diagnostics as well as in accompanying diagnostics, namely to differentiate between PCa patients undergoing treatment and those who do not. The analysis also revealed in a subset of serum samples (n = 215) that glycoprofiling of fPSA surpassed the prostate health index test (AUC = 0.69). Furthermore, out of 392 negative biopsies considered to be avoidable, 70-73% could have been prevented with the use of fPSA glycoprofiling compared with 52–53% in common fPSA test. No significant advantages were found in ZA2G glycoprofiling over commonly used diagnostic methods.

**Discussion:** PSA-based tests are quantitative methods commonly used for PCa screening and accompanying diagnostics. The false negative/positive rates of these test are high, therefore its use in screening and diagnostics is limited. In our study, we confirmed that two glycoforms of fPSA recognised by specific lectins are cancer-specific and thus useful in distinguishing malignant from benign cases and can outperform other tests applied in PCa screening and diagnostics.

**Conclusions:** While ZA2G glycoprofiling showed little promise as a potential biomarker of PCa, fPSA glycoprofiling has significant clinical potential as an early diagnosis biomarker of PCa as well as in accompanying diagnostics.

## OUTCOME OF CONTINUOUS POSITIVE AIRWAY PRESSURE ADHERENCE BASED ON NASAL ENDOSCOPY AND MEASUREMENT OF NASAL PATENCY

Z. Knížek[1,2], M. Kotulek[1], P. Brothánková[1,3], E. Pecháčková[3], J. Vodička[1,3]

[1] Department of Otorhinolaryngology and Head and Neck Surgery, Hospitals of Pardubice region – Hospital in Pardubice, Czech Republic
[2] Faculty of Medicine in Hradec Králové, Charles University, Hradec Králové, Czech Republic
[3] Faculty of Health Studies, University of Pardubice, Pardubice, Czech Republic

**Introduction:** The gold standard for treating obstructive sleep apnea in adults is continuous positive airway pressure (CPAP). However, it can be difficult to convince patients to adhere to therapy. The aim of this study is to assess the relationship between nasal endoscopy findings and nose patency measured by flow measurement and CPAP adherence.

**Methods:** A cohort of 450 consecutive patients suspected to have OSA were prospectively enrolled. For further analyses, 47 subjects with OSA being treated with CPAP treatment, were selected (13 females and 34 males, average age 65.3, BMI 34.1, apnoe-hypopnoe index AHI 51.0). Patients were divided into groups: two groups based on rhinoendoscopic findings (patient with and without endoscopically significant nasal obstruction) and two groups based on measurement of flow in nasal cavity (patients with more or less than 4.57 V of flow measured by flowmeter). The influence of nasal endoscopy and flow measurement on CPAP adherence and CPAP-related nasal complaints questionairre was explored.

**Results:** We found a statistical independence between CPAP compliance and rhinoendoscopic findings ($p = 0.498$) and nasal patency measured by flow measurement ($p = 0.754$). We found a statistical independence between CPAP-related nasal complaints and rhinoendoscopic findings ($p = 0.588$) and nasal patency measured by flow measurement ($p = 0.657$).

**Discussion:** Nasal patency improvement can be useful for reducing the level of PAP when treating OSA, resulting in better adherence to CPAP therapy. However, the positive effect of surgery on CPAP tolerance was not confirmed in every study. Therefore, it is very important to carefully select patients for possible nasal intervention before inciating CPAP. Our results were in agreement with conclusions of authors (Brimioulle 2022), but in conflict with results published by several other authors (Inoueet 2019, Park 2017, Sugiuraet 2007).

**Conclusions:** In our studied sample, sleep monitoring data, endoscopic findings and nose patency measured by flow measurement are not predictors for CPAP non-adherence in the first year of the CPAP treatment. Our data show that although an endoscopic finding in the nasal cavity could indicate a more severe obstruction, compliance to CPAP therapy is not reduced in these patients. Neither is compliance to CPAP therapy reduced with the decrease in nasal flow, according to our observation.

## PREDICTION OF TREATMENT RESPONSE USING PET/MRI EXAMINATION PERFORMED DURING TREATMENT IN PATIENTS WITH LOCALLY ADVANCED CERVICAL CANCER TREATED WITH CONCURRENT CHEMORADIOTHERAPY

P. Kovářová[1], R. Vojtíšek[1], J. Baxa[2]

[1] Department of Oncology and Radiotherapy, Charles University in Prague, Faculty of Medicine and University Hospital in Pilsen, Czech Republic
[2] Department of Imaging Methods, Charles University in Prague, Faculty of Medicine and University Hospital in Pilsen, Czech Republic

**Introduction:** The standard treatment approach for patients with locally advanced cervical cancer is radical concurrent chemoradiotherapy, consisting of external beam radiotherapy and concurrent chemotherapy, followed by MRI-guided brachytherapy. PET/MRI (positron emission tomography/magnetic resonance imaging) examination is a hybrid imaging method that combines metabolic imaging with the advantages of magnetic resonance imaging, providing morphological, functional and metabolic parameters in a single examination.

**Aim of the study:** We aimed to find morphological, functional and metabolic characteristics of the tumour to predict failure to achieve a complete metabolic remission by PET/MRI examination performed during treatment in patients with locally advanced cervical cancer treated with concurrent chemoradiotherapy.

**Methods:** We evaluated 66 patients treated between August 2015 and November 2019 who underwent PET/MRI examination as part of staging (pre-PET/MRI), then at the end of external chemoradiotherapy (mid-PET/MRI) and 3 months after completion of the whole treatment (post-PET/MRI). We divided the whole group of patients into a group of responders (patients who achieved a complete metabolic remission) and a group of non-responders (patients who did not achieve a complete metabolic remission) according to RECIST (Response Evaluation Criteria in Solid Tumors) and PERCIST (PET Response Criteria in

Solid Tumors) criteria. We compared these two groups based on parameters obtained from pre-PET/MRI and mid-PET/MRI. The evaluated parameters were: pre-SUV (standard uptake value) max, pre-SUVmean, pre-MTV (metabolic tumour volume), mid-SUVmax, mid-SUV mean, mid-MTV.

**Results:** A statistically significant difference in the evaluated parameters between responders and non-responders was found for the parameter mid-MTV ($p = 0.006$).

**Discussion:** The number of recurrences after concurrent chemoradiotherapy for locally advanced cervical cancer remains high. Therefore, it is important to identify patients who are unlikely to achieve a complete remission. These patients could be treated more intensively based on selected predictive factors identified prior to treatment. Although posttreatment imaging parameters have been proven to predict survival, they do not allow early therapeutic adjustments. It is advisable to obtain information on whether persistence or early recurrence of the disease can be expected with high probability in the period just before the decision moment on possible therapeutic intensification, i.e. optimally before the end of the whole treatment.

**Conclusion:** Using a PET/MRI examination performed during treatment, we were able to identify a metabolic parameter (mid-MTV) suitable for predicting failure to achieve a complete metabolic remission.

## VALIDATION OF THE CZECH VERSION OF THE VOICE HANDICAP INDEX

J. Krtičková[1], J. G. Švec[2], J. Haviger[3], K. V. Phadke[2], J. Držata[4], L. Školoudík[4], M. Homoláč[4], A. Švejdová[4], J. Mejzlík[4], L. Hodačová[5], V. Chrobok[4]

[1] Department of Otorhinolaryngology and Head and Neck Surgery, University Hospital Hradec Králové, Hradec Králové, Czech Republic; Faculty of Medicine in Hradec Králové, Charles University, Hradec Králové, Czech Republic
[2] Voice Research Laboratory, Department of Experimental Physics, Faculty of Science, Palacky University, Olomouc, Czech Republic
[3] Department of Informatics and Quantitative Methods, Faculty of Informatics and Management, University of Hradec Králové, Hradec Králové, Czech Republic
[4] Department of Otorhinolaryngology and Head and Neck Surgery, University Hospital Hradec Králové, Hradec Králové, Czech Republic; Faculty of Medicine in Hradec Králové, Charles University, Hradec Králové, Czech Republic
[5] Department of Preventive Medicine, Charles University, Faculty of Medicine in Hradec Králové, Hradec Králové, Czech Republic

**Introduction:** The present study aims to evaluate the reliability and construct validity of the Czech version of the Voice Handicap Index and determine the cut-off value to distinguish dysphonic from nondysphonic patients.

**Methods:** Prospective study, parallel group design. The study included 51 healthy subjects in the control group, and 100 adult patients with dysphonia (out of these 25 were used for test-retest analysis, 45 to determine the responsiveness to change. All 151 individuals completed the VHI-CZ and were examined with the videostroboscopy. The internal consistency, the test-retest reliability, and the construct validity were analyzed and the normative cut-off value was determined.

**Results:** The internal consistency of the VHI-CZ was excellent (Cronbach $\alpha = 0.984$), test-retest reliability was excellent (ICC = 0.95, $P < 0.001$). The correlation between the self-assessed severity of the voice disorder and the VHI-CZ score was strong (Spearman's $\rho = 0.877$, $P < 0.001$). The VHI scores differences between dysphonic and nondysphonic were statistically significant (Mann-Whitney U test, $P < 0.001$). The differences among the three etiological subgroups (neurogenic, functional, and structural) were statistically significant (Kruskal-Wallis test, $P < 0.001$). The differences in the VHI-CZ total scores between pretreatment and posttreatment were statistically significant (Wilcoxon test, $P < 0.001$). The cut-off score of 13 points was found, by the analysis of the Receiver Operating Characteristic.

**Discussion:** According to the European Laryngological Society, comprehensive assessment of voice disorders should involve auditory-perceptual evaluation, videostroboscopy, acoustics, aerodynamics, and self-assessment of the patient.

**Conclusions:** The Czech VHI is a useful and valid monitoring tool for clinicians.

## VALIDATION OF FRANCISELLA TULARENSIS PEPTIDES AS IMMUNOGENIC T-CELL EPITOPES

P. Lásková[1], S. Porkertová[1], O. Ballek[2], I. Pávková[1], M. Link[1], J. Stulík[1]

[1] Department of Molecular Pathology and Biology, Faculty of Military Health Sciences, University of Defence, Hradec Králové, Czech Republic
[2] Laboratory of Immunobiology, Institute of Molecular Genetics of the Czech Academy of Sciences, Vídeňská 1083, 142 20 Prague 4, Czech Republic

**Abstract:** *F. tularensis* is a gram-negative, facultative intracellular and highly virulent bacterium. In the mouse model of infection, the survival of sublethal *F. tularensis* LVS infection is dependent on the correct functioning and cooperation of innate and adaptive immune system, and the immunity mediated by T-cells is critically required for long-term survival and clearance of *Francisella*. However, the particular T-cell antigens/epitopes are not well documented yet and we established experiments for identification of *Francisella* CD4+ T-cell targets.

This study follows up immunopeptidomics experiments which identified bacterial peptides presented on MHC-II molecules after *in vitro* infection of murine BMDCs by *Francisella* LVS (accompanying abstract "MHC-II peptidome of *F. tularensis* LVS infected dendritic cells"). The design and first results of experiments verifying if these peptides also operate as CD4+ T-cell epitopes and enhance the immune response in mice are presented.

Cell suspensions or isolated CD4+ T-cells were obtained from spleens and lymph nodes of mice immunized with sublethal dose of *F. tularensis* LVS. In initial screening

experiments, these cells were stimulated with synthetic *Francisella* peptides arranged into three peptides pools (PP). By IFN-γ Elispot, the following order of reactivity, PP-3 >> PP-2 > PP-1 was found. The PP-3 was consistently reactive in most replicates and stimulated most IFN-γ producing T-cells, whereas the reactivity of peptide pools 2 and 3 was either not so consistent across replicates or the production of IFN-γ was lower. This hierarchy of reactivity was observed at 3, 4 and 5 weeks post infection. Production of selected cytokines and cell proliferation after peptides restimulation was also assessed by FACS analysis. The results were however inconclusive due to very low frequency of responding cells and interference from nonspecific spontaneous cell stimulation. The screening of immune responses following stimulation with individual peptides was therefore based on IFN-γ Elispot. The results of early experiments pointed to 5-7 reactive *Francisella* peptides as potential CD4+ T-cell targets. This results could bring more knowledge and possibilities into immune monitoring of infection and identification of possible immune protective antigens.

**Funding:** Specific Research grant of Ministry of Education, Youth and Sports of Czech Republic (*SV/FVZ202202*); Ministry of Defence of the Czech Republic – DRO of the University of Defence, Faculty of Military Health Sciences Hradec Králové, Czech Republic – Medical issues of WMD II, (DZRO-FVZ22-ZHN II).

## PLASMA VITAMIN D LEVEL IN GERM CELL TUMOUR PATIENTS IS ASSOCIATED WITH SURVIVAL OUTCOME AND DISEASE CHARACTERISTICS

P. Lesko[1], B. Vlkova[4], K. Kalavska[3], V. DE Angelis[1], V. Novotna[2], J. Obertova[1], Z. Orszaghova[1], P. Palacka[1], K. Rejlekova[1], Z. Sycova-Mila[1], B. Kollarik[1], R. Aziri[1], D. Pindak[1], J. Mardiak[1], M. Chovanec[1], P. Celec[4], M. Mego[1,3]

[1] 2nd Department of Oncology, Faculty of Medicine, Comenius University and National Cancer Institute, Bratislava, Slovakia
[2] 1st Department of Oncology, FMCU and St.Elisabeth Cancer Institute, Bratislava, Slovakia
[3] Translation Research Unit, Comenius University, National Cancer Institute, Bratislava Slovakia
[4] Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia

**Background:** Testicular cancer is the most common malignancy among young men. Vitamin D has various effect in cancer pathogenesis and plays role in metastatic cascade. The aim of this study is to assess Vitamin D and its association with clinico-pathological finding along with prognostic impact of vitamin D among certain populations in our studied group.

**Methods:** This study included 120 newly diagnosed and/or relapsed germ cell tumor patients treated in April 2013 to July 2020 for whom plasma was available in the biobank. Blood samples were drawn at the time before 1st chemotherapy cycle as well as before 2ndcycle. Study also included 21 age-matched healthy donors. Plasma vitamin D level was measure by ELISA and correlated with disease characteristics and patient's outcome. For survival analysis it was dichotomized to "low" and "high" based on median value.

**Results:** Mean ± standard error of mean [SEM] plasma level of vitamin D in GCTs patients was 15.87 ± 0.68. Vitamin D level was not associated with disease characteristics except of brain metastases, where patients with brain metastases had significantly lower D vitamin level compare to patients without brain metastases (10.9 ± 3.05 vs 15.99 ± 0.7, p = 0.034). Lower vitamin D level was also detected among GCT patients with unfavorable response compared to favorable responses (mean + SEM, 11.26 ± 1.84 vs 16.47 ± 0.74, p = 0.016) and in patients, that experienced disease recurrence. Patients with "low" vitamin D level had inferior progression free survival but not overall survival compared to patients with "high" vitamin D level (HR = 3.02, 95% CI (1.36–6.71), p = 0.014 for PFS and HR = 2.06, 95% CI (0.84–5.06), p = 0.135 for OS, respectively).

**Discussion:** Mean vitamin D level among all studied patients was observed in range of hypovitaminosis, thus, most of patients were characterized by vitamin D insufficiency. The observation of prognostic impact of vitamin D is consistent with studies evaluating prognostic impact of vitamin D and PFS in different types of cancer, however, prognostic significance in overall survival was not statistically significant. The reason could be change of vitamin D level over the time of treatment period.

**Conclusion:** Our study for the first time revealed prognostic value of pretreatment vitamin D level in GCTs patients. Moreover, low plasma vitamin D level was associated with unfavorable response to therapy and disease recurrence, however, it remains to elucidated whether it mirror biology of disease or if its supplementation could affect patient outcome.

## TRANSCRIPTION-FACTOR AP-1 IN COLORECTAL CANCER

Ella Leugner[1], Nikolai Schleußner[1,2], Thomas Schmidt[3], Uraz Yasar[1], Elena Dahm[1], Justus Gauckel[1], Natalia Giese[1], Martin Schneider[4]

[1] Heidelberg University
[2] DKFZ
[3] Cologne University
[4] Gießen University

**Introduction:** Colorectal cancer still is a burden for humanity as it is one of the most common cancerous diseases globally, with the second highest mortality rate across all genders. Although great progress was achieved over the past decades in treating and screening for colorectal cancer, the metastasized stage has a 5-year survival rate of only 14% in Germany. These numbers indicate the importance of further research regarding the mechanisms that lead to metastasizing, to eventually identify new therapeutical targets for advanced stages.

For tumour cells to colonize a new organ, a sequence of processes must occur that causes the tumour cells to first lose their cell-cell contacts and adhesion to the

extracellular matrix. In the next step, invasion into blood or lymphatic vessels must take place. For this the cells must be able to survive in the bloodstream. Thus, cells with previously epithelial differentiation take on a more mesenchymal phenotype. This describes the so-called epithelial to mesenchymal transition (EMT), which is an important step in the process of developing metastasis.

Different signalling pathways and transcription factors induce these changes in tumour cells, of which some are already well known, whereas others need further investigation. Among these transcription factors presumably involved in the processes of tumorigenesis and metastasis is the activator protein-1 (AP-1).

AP-1 is a multigenic transcription factor complex, containing several protein families. The most prominent being the Jun, Fos, MAF and BATF family. AP-1 members are basic leucin zippers, forming homo- or heterodimers. The protooncogenic potential of AP-1 has already been shown for several tumour entities. Some of the AP-1 factors also seem to be relevant for the development of colorectal carcinoma. CJUN for example has been shown to have a growth-promoting function in colorectal cancer, but its role in progression of colorectal cancer is not understood yet. This study's aim is to investigate the very complex but seemingly relevant transcription factor family AP-1 and its role in the metastasizing process of colorectal cancer.

**Methods:** To start with, it was crucial to show the expression profile of several AP-1 members in colorectal cancer. For this purpose, investigations on protein level using immunohistochemical staining and western blotting were conducted. A total of 11 matched primary tumour and liver metastasis tissue samples were used for immunohistochemical staining. The lysates of nine colorectal cancer cell lines, as well as lysates of patient derived organoids were used for western blot analysis. For biological characterization a stable CRISPR/Cas 9 knock out system was established and a stable knock out cell line created for each member of the Jun and Fos family. Different assays were performed to investigate the proliferative and migrative properties of colorectal carcinoma cells after knockout. Cell viability after treatment with an AP-1 inhibitor was also tested, as well as a global knockout of AP-1 using the dominant negative AFOS. To put the *in vitro* data into clinical context, the experimental part was followed up by a retrospective correlation between AP-1 expression profile in colorectal carcinoma and clinical outcome.

**Results:** As data analysis is not completed yet, only preliminary results can be presented. The expression profile of AP-1 families across nine different colorectal cancer cell lines, including two metastasis cell lines, shows an overall high expression of the Jun family, whereas expression of the Fos family members differs more. The expression of the BATF family is very low throughout all cell lines. Preliminary data of immunohistochemical stainings show the upregulation of certain AP-1 members in primary tumour and liver metastasis compared to healthy mucosa. Expression is particularly high in the more invasive properties of tumour and metastasis. The knockout of single AP-1 members led to a decrease in cell viability and the ability to form colonies. Migratory properties seem to also be affected by the knockout of single AP-1 members. The global knock out of AP-1 showed a significant decrease in cell viability and cloning ability. The pharmaceutical inhibition of AP-1 in a 2D-culture did only show a tendency to reduce cell viability. The correlation of AP-1 expression and clinical outcome is still pending but will be presented at the conference.

**Discussion:** The expression data generated on protein level in primary tumour coincide with findings in older publications. This study now gives further insight into the expressional patterns of the Jun and Fos family members in liver metastasis compared to the matched primary tumour. The in vitro data also supports the hypothesis regarding the involvement of AP-1 in the epithelial to mesenchymal transition.

To be able to make a more differentiated statement on AP-1's role in tumour progression double knock outs should be performed in future studies, as the function of single AP-1 members differs, depending on their dimerization partner.

As this study only generated *in vitro* data, it can show tendencies and give direction for further investigation. The correlation of clinical outcome of colorectal cancer patients and the expression profile on cDNA level could give deeper insight into the clinical relevance of AP-1. To round of this study *in vivo* experiments would be necessary. A mouse model using a highly invasive murine colorectal cancer cell line to induce tumour growth and metastasizing is planned for future projects.

**Conclusion:** AP-1's expression profile in primary tumour and liver metastasis indicates the importance of this transcription factor complex in the progression of colorectal cancer. In *vitro data* confirms AP-1's protooncogenic properties and suggests an involvement in the process of metastasizing. Pharmaceutical inhibition of AP-1 did not show significant effects, further investigation using 3D culture models might be insightful. AP-1 seems to have the potential to transpire as a future therapeutical target for colorectal cancer, but further investigations are necessary.

## COMPUTER ANALYSIS OF GAIT DISORDERS

D. Matyáš [1], O. Vyšata[2]

[1] Faculty of Medicine in Hradec Králové, Charles University, Czech Republic
[2] Department of Neurology, University Hospital Hradec Králové, Czech Republic

**Introduction:** Gait disorders are common manifestations of many neurological diseases. Diagnosis, determination of its gravity, and the monitoring of its progression or regression in time are burdened with interpersonal differences. Our aim is to use objective data provided by commercially available accelerometer for effective diagnosis and treatment. To do this, it is necessary to identify key features that could be used for reliable classification.

**Methods:** The Random Forest method with 100 trees was used for analysis. The dataset consisted of 605 samples with 15 different features. Samples were taken from

50 healthy controls and 100 patients with neurological gait disorders.

**Results:** Certain features, such as RMS ML Accel, RMS AP Accel, RMS Pitch Velocity, and RMS Roll Velocity, were identified as significantly important for classification.

**Discussion:** While some features were identified as key, future research might explore additional potential features and their combinations for even more accurate classification.

**Conclusions:** The analysis confirmed the significance of certain kinematic features in distinguishing healthy subjects from patients with gait disorders.

## SEQUENATION OF DNA METHYLATION OF HMGCR GENE COULD BE PREDICTOR OF MORBIDITY AND MORTALITY IN PATIENTS ON HEMODIALYSIS

M. Mokrejšová[1,2], S. Dusilová Sulková[1,2], J. Kalous[3], M. Vágnerová[2]

[1] Charles University, Faculty of Medicine in Hradec Králové, Czech Republic
[2] University Hospital Hradec Králové, Czech Republic
[3] Institute of Animal Physiology and Genetics, Academy of Science of the Czech Republic

**Introduction:** Chronic kidney disease [CKD] is a one of the fastest growing health problem. Patients with CKD exhibit a pronounced risk for cardiovascular mortality; the most vulnerable group enrolls diabetic patients on hemodialysis.

Although a risk of cardiovascular events in general population has significantly decreased after launching of cholesterol lowering medication – inhibitors of 3-hydroxymethyl-3-glutaryl Coenzyme A reductase [HMGCR], unfortunately in patients undergoing dialysis no meaningful benefit of this therapy was demonstrated.

The incidence of complications in CKD patients correlates with changed epigenetic mechanisms, the most studied epigenetic phenomenon is DNA methylation. The principle of DNA methylation is modification of DNA structure through binding of methyl group at the cytosine. This makes change in chromatin conformation: methylated parts are more compact and no readily accessible to the transcriptional. On the contrary unmethylated genes are easily transcribed, e.g. unmethylated HMGCR gene is more expressed and cholesterol synthesis is enhanced.

The aim of this pilot study was to elucidate the methylation status of HMGCR gene and evaluate its impact on cardiovascular morbidity and mortality in patients receiving hemodialysis.

**Methods:** We analyzed the plasma of 12 dialyzed patients suffering from type 2 diabetes. DNA was isolated from samples before and after dialysis, then bisulfite conversion was carried out to distinguish methylated and unmethylated form of DNA. The primers of HMGCR genes promotors were designed, consequently methylation specific PCR and sequential analysis of PCR products were performed.

**Results:** Although the methylation rate of HMGCR gene dropped in part during the hemodialysis session, no statistical significance was confirmed.

Hypomethylated promotor of HMGCR gene was found in 6 patients, who either underwent amputation of lower extremity due to arterial ischemia or has deceased due to cardiovascular event during 6 month of follow-up period.

**Discussion:** These results claim for the importance of early recognition of dialyzed patients in high cardiovascular risk, since hypomethylation of HMGCR gene has consequence in higher rate of cardiovascular morbidity and mortality. The impact of dyslipidemia in hemodialysis patients has not been adequately elucidated yet and underestimation of HMGCR activity has been presumable and imminent.

The limitation of our pilot study is a low number of participants, nevertheless the ongoing investigations are performed to expand the range.

**Conclusions:** This pilot study suggests that both DNA demethylation and consecutive increased expression of HMGCR gene are predictors of high risk of cardiovascular morbidity and mortality in hemodialyzed diabetic patients.

**Funding:** Supported by the grant GAUK 249423.

## ASSESSMENT OF ALLERGIC REACTION AND SPECIFIC IMMUNOGLOBULIN E LEVEL IN NASAL SECRETION OF LOCAL ALLERGIC RHINITIS PATIENTS

M. M. Mortada[1], M. Kurowski[1]

[1] Department of Immunology and Allergy, Central Teaching Hospital of the Medical University of Lodz, Poland

**Introduction:** Local allergic rhinitis (LAR) is characterized by similar symptoms as allergic rhinitis (AR): nasal itching, sneezing, rhinorrhea, and nasal congestion. However, specific Immunoglobulin E (sIgE) sensitization cannot be ascertained neither through skin prick test nor through serum sIgE assessment. Unfortunately, the delayed LAR diagnosis leads to prolonged suffering of the patients. Our study aims to assess the allergic inflammation processes and IgE-mediated reaction that may occur only at the level of the local nasal mucosa.

**Methods:** Suspected LAR subjects (n = 23) are included in the study. Subjects are assessed through rhinoscopy for nasal symptoms. TNSS/VAS questionnaires were completed during the period of increased exposure to mites allergens (Fall/Winter) and before/after nasal provocation test (NPT) with HDM allergens (Dermatophagoides pteronyssinus; *D. p* and Dermatophagoides farinae; *D. f*) and then analyzed. Nasal secretions were collected from the subjects via the nasal lavage method and sIgE levels to D. *p* and *D. f* were measured during the exposure period (n = 12) and before and after NPT (n = 15).

**Results**: TNSS/VAS scores were higher in subjects during the exposure period when compared to the same subjects during the off-season. For patients who underwent NPT, TNSS/VAS scores were higher after provocation in

subjects with positive NPT, lower after provocation in subjects with negative NPT, and the same or slightly higher after provocation in subjects with doubtful NPT results. 3 sIgE measurements were excluded due to technical errors. During the exposure period, 80% of the subjects showed higher levels of sIgE for *D. p* when compared to *D. f* and the remaining 20% showed the same concentration. The level of sIgE for *D. p* was higher after NPT in 8 subjects (57.14%), the same in 2 subjects (21.42%), and lower in 2 subjects (21.42%). On the contrary, levels of sIgE for *D. f* showed less variation with 7 subjects (57.3%) having the same level, 2 subjects (15.38%) with a lower concentration, and 3 subjects (23.07%) with increased levels after NPT.

**Discussion:** NPT is the gold standard in LAR diagnosis; however, results may be inconclusive or inconsistent, and combining the clinical assessment and TNSS/VAS may provide more specificity in the evaluation of LAR subjects. More information can be acquired through sIgE assessment in nasal secretions as we saw in our results with the increase noted in the level of sIgE for *D. p*.

**Conclusion:** Albeit our study showed promised results, increasing the studied population and assessing sIgE and additional inflammatory mediators levels in nasal secretions acquired through other methods (e.g. Nasosorption) and in serum would possibly provide a more conclusive and consistent assessment of LAR subjects.

## SYNDECAN-1 LEVELS IN ORGAN DONORS AND KIDNEY TRANSPLANT RECIPIENTS

P. Navrátil[1,2], S. Sahi[3], P. Hrubá[4], A. Tichá[5], K. Timková[6], O. Viklický[6], V. Černý[2,7–10], D. Astapenko[2,9,10]

[1] Department of Urology, University Hospital Hradec Králové, Czechia
[2] Charles University, Faculty of Medicine in Hradec Králové, Czechia
[3] Department of Medicine, Division of Nephrology and Hypertension, Mayo Clinic, Rochester, Minnesota, USA
[4] Transplant Laboratory, Institute for Clinical and Experimental Medicine, Prague, Czechia
[5] Department of Clinical Biochemistry and Diagnostics, University Hospital Hradec Králové, Czechia
[6] Department of Nephrology, Institute for Clinical and Experimental Medicine, Prague, Czechia
[7] Department of Anesthesia, Pain Management and Perioperative Medicine, Dalhousie University, Halifax, Nova Scotia, Canada
[8] Department of Anaesthesiology, Perioperative Medicine and Intensive Care, Bory Hospital Bratislava, Slovakia
[9] Department of Anaesthesiology and Intensive Care, University Hospital Hradec Králové, Czechia
[10] Faculty of health sciences, Technical University in Liberec, Czechia

**Introduction:** Kidney transplantation remains the optimal treatment for end-stage renal disease, offering improved quality of life and increased survival rates compared to long-term dialysis. However, despite advances in surgical techniques, immunosuppression regimens, and post-operative care, there are still significant challenges in predicting the long-term outcomes of transplantation. Among the many factors that influence graft survival, the quality of the donated organ plays a fundamental role. Syndecan-1 is found in the endothelial glycocalyx and shed in a higher rate into the blood during systemic pathological conditions. In this study, we aimed to investigate the potential role of syndecan-1 as a biomarker of kidney donor organ quality. We hypothesized that elevated syndecan-1 levels in donors correlate with short-term outcome in kidney recipients. To test this hypothesis, we analyzed a cohort of kidney transplant donors and recipients and evaluated the relationship between syndecan-1 levels in the donor organ and various clinical and laboratory parameters in recipients, including graft function.

**Methods:** In this single-center, retrospective, observational cohort study we investigated 80 donors' serum syndecan-1 levels and correlated them with donor characteristics and short-term outcome in corresponding 104 kidney recipients.

**Results:** Higher syndecan-1 levels correlated with last creatinine before organ harvest and were marginally higher in donors with acute kidney injury. However, syndecan-1 levels were not associated with short-term outcome in kidney recipients (delayed graft function and renal function at 3 months post kidney transplantation).

**Discussion:** Numerous pathophysiological phenomena are evidently connected to structural and functional aberrations of the endothelial glycocalyx. The most substantial proof for this connection is found in the context of organ damage following ischaemia, sepsis and inflammation, diabetic vascular disease, atherosclerosis, and kidney diseases. Our understanding of the role of syndecan-1 in kidney transplantation is just beginning to be elucidated. There is an apparent need for more robust, prospective studies that investigate the relationship between donor syndecan-1 levels and post-transplant kidney function at different times. Gaining a better understanding of this correlation could open the door for improvements in donor organ selection, pre-transplant risk assessment, and post-transplant patient management, potentially leading to better patient outcomes.

**Conclusion:** Our findings suggest that syndecan-1 could serve as a novel indicator of graft quality, ultimately contributing to a more personalized and effective approach in kidney transplantation. However, this finding needs validation in larger cohorts.

**Acknowledgments:** This work was supported by MH CZ – DRO (UHHK, 00179906), the Charles University COOPERATIO Program and Ministry of Health of the Czech Republic grant NU21-06-00021.

## DIAGNOSTIC ACCURACY OF CAROTID PLAQUE INSTABILITY BY NONINVASIVE IMAGING: A SYSTEMATIC REVIEW AND META-ANALYSIS

D. Pakizer[1], J. Kozel[1], P. Taffé[2], J. Elmers[3]; J. Feber[1], P. Michel[4], D. Školoudík[1], G. Sirimarco[4]

[1] Center for Health Research, Faculty of Medicine, University of Ostrava, Czech Republic
[2] Center for Primary Care and Public Health, Division of Biostatistics, University of Lausanne, Switzerland

[3] Medical Library, Lausanne University Hospital and University of Lausanne, Switzerland
[4] Stroke Center, Service of Neurology, Department of Clinical Neurosciences, Lausanne University Hospital, Switzerland

**Introduction:** There is increasing evidence that plaque instability in the extracranial carotid artery may lead to an increased stroke risk independently of the degree of stenosis. We aimed to determine diagnostic accuracy of vulnerable and stable plaque using noninvasive imaging modalities when compared to histology in patients with symptomatic and asymptomatic carotid atherosclerosis.

**Methods:** Medline Ovid, Embase, Cochrane Library, and Web of Science were searched for diagnostic accuracy of noninvasive imaging modalities (CT, MRI, US) in the detection of 1) vulnerable/stable plaque, and 2) vulnerable/stable plaque characteristics, compared to histology. The quality of included studies was assessed by QUADAS-2 and univariate and bivariate random-effect meta-analyses were performed.

**Results:** We included 36 vulnerable and 5 stable plaque studies in the meta-analysis, and out of 211 plaque characteristics from remaining studies, we classified 169 as vulnerable and 42 as stable characteristics (28 CT, 120 MRI, 104 US characteristics). We found that MRI had high accuracy (90% [95% CI: 82–95%]) in the detection of vulnerable plaque, similar to CT (86% [95% CI: 76–92%]; $p > 0.05$), whereas US showed less accuracy (80% [95% CI: 75–84%]; $p = 0.013$). CT showed a high diagnostic accuracy to visualize characteristics of vulnerable or stable plaques (89% and 90%) similar to MRI (86% and 89%; $p > 0.05$); however, US had lower accuracy (77%, $p < 0.001$ and 82%, $p > 0.05$).

**Discussion:** High accurate CT and MRI may be used as the first-choice modality to select high-risk patients for clinical trials aiming to reduce the rate of stroke recurrences and the burden of new ischemic lesions on follow-up MRI. All characteristics of a vulnerable plaque were detected by MRI with high accuracy, suitable for assessing the effect of specific treatments targeting the vulnerable plaque characteristics, such as lipid-lowering and anti-inflammatory strategies.

**Conclusions:** CT and MRI have a similar, high performance to detect vulnerable carotid plaques, whereas US showed significantly less diagnostic accuracy.

**Registration:** PROSPERO ID CRD42022329690

## ROTATIONAL THROMBOELASTOMETRY AND PSORIASIS

M. J. Péč[1], J. Jurica[1], J. Benko[1], M. Péčová[2], J. Žolková [2], T. Bolek[1], K. Vorčáková[3], T. Hurtová[3], I. Škorňová[2], J. Sokol[2], J. Staško[2], P. Galajda[1], M. Samoš[1], M. Mokáň[1]

[1] Department of Internal Medicine I, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Slovakia
[2] Department of Haematology and Transfusiology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Slovakia
[3] Department of Dermatovenerology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Slovakia

**Background:** It has been repeatedly described that patients with severe psoriasis are at higher risk of thrombotic complications. Rotational thromboelastometry (ROTEM) is a viscoelastic hemostatic test that enables sophisticated evaluation of hemostasis in whole blood sample. The aim of this study was to evaluate changes in hemostasis in psoriatic patients using ROTEM.

**Methods:** We performed a pilot, observational, prospective study. Totally 60 patients with severe psoriasis (PASI > 15) were enrolled in this study. The control group consisted of 11 healthy blood donors. Blood samples were tested with ROTEM using INTEM, EXTEM and FIBTEM reagents.

**Results:** We detected significant changes in INTEM clot forming time, INTEM maximum clot firmness, EXTEM clotting time, FIBTEM clotting time and FIBTEM maximum clot firmness compared to healthy controls.

**Conclusion:** We observed abnormal changes in hemostasis using rotational thromboelastometry in patients with severe psoriasis.

**Keywords:** severe psoriasis; rotational thromboelastometry; ROTEM

## APPLIANCE OF CONTINUOUS GLUCOSE MONITORING SYSTEM AS AN EDUCATIONAL TOOL IN PATIENTS WITH TYPE 2 DIABETES IN THE EARLY STAGE OF DISEASE

M. Płonka-Stępień[1]

[1] Jagiellonian University Medical College, Świętej Anny 12, 31-008, Kraków Cracow, Poland

**Introduction (aim of the study):** Type 2 diabetes mellitus is an economical, medical and social problem. Patients with type 2 diabetes live shorter compared to peers without diabetes, mainly because of cardiovascular diseases. The basis of the treatment of these illness is multifactorial therapy focused on maintaining healthy levels of blood glucose and prevention of cardiovascular complications. Continuous glucose monitoring (CGM) is a small device, which measures glucose level in tissue fluid every few minutes and allows to observe glucose trends on the graph. One of the most popular systems is Freestyle Libre, it is based on scanning. A sensor is placed on the posterolateral part of the arm and then a mobile phone or reader with the NFC function is brought close to the sensor to obtain a reading. Compared to people using glucometers, patients using CGM systems, including Freestyle Libre, achieve better diabetes control, have fewer hypoglycaemic episodes, and their quality of life improves. The hypothesis that the temporary, educational use of the CGM system may have clinical significance in patients in the early stage of type 2 diabetes requires verification. The aim of the study is to evaluate the effectiveness of an educational intervention involving the periodic usage of the Freestyle Libre 2 CGM system in patients in the early stage of type 2 diabetes.

**Methods:** Both qualitative and quantitative methods are used. In-Depth Interview is conducted among men to

access the impact of the sensor on motivation to long-term lifestyle change. The quantitative methods belong laboratory blood tests, anthropometric measurements, and psychological assessment. Psychological tools such as QoL-Q Diabetes, Diabetes Distress Scale (DDS), Athenian Scale of Insomnia are used.

**Results:** The study is still in progress. In-Depth Interviews are analysed. Freestyle Libre is perceived as small and useful tool. A few patients consider using continuous glucose monitoring system on their own temporarily, without refund. Patients report a desire to change lifestyle, compare food products and introduce physical activity.

**Discussion:** Recent years have provided convincing evidence that CGMS use results in clinical benefits, namely improvement in glycemic control and quality of life, in both T1DM and T2DM treated intensively with insulin. Sparse data exists concerning patients with T2DM on less intensive therapeutic models, such as oral medications.

**Conclusions:** The results of the study may influence the guidelines and clinical practice in diabetes by providing the evidence that short-term use of CGM soon after T2DM diagnosis improves long term glycemic control.

## INVESTIGATING HOST-BACTERIA INTERACTIONS IN AGING SKIN USING LONG-READ SEQUENCING

N. Rocha[1], M. Rudden[1], H. Wilkinson[1], M. Hardman[1]

[1] Centre for Biomedicine, Hull York Medical School, Hull, United Kingdom

Our skin serves as a habitat for millions of microorganisms that play vital roles in both health maintenance and disease. This bacterial community can be influenced by diverse factors, including antibiotics, diet, exercise, and age. The latter is known to have a significant impact on microbiota composition, yet the intricate cause-effect relationship remains poorly understood. Our current study aims to unravel the link between human skin ageing and skin microbes. To achieve this, we recruited healthy volunteers from two age groups: young individuals aged 18 to 30 and older adults aged 60 and above. For our investigation, we collected swabs from the forehead, arm, and foot aiming to isolate bacteria and perform metagenomic profiling via long-read Nanopore sequencing. Concurrently, we accessed a range of age-associated biophysical parameters at each body site using the multi-probe DermaLab Combo device. Our analysis revealed *Staphylococcus* species as the prominent constituents of the foot microbiome in both age groups. Interestingly, *Corynebacterium* exhibited a threefold increase in abundance on the aged forehead skin. Moreover, older individuals displayed a lower abundance of *Cutibacterium* and *Malassesia*, in line with prior findings. The forearm site exhibited highly diverse bacterial profiles, where the prevalence of *Staphylococcus* was reduced in the older cohort alongside a higher prevalence of *Acinetobacter* and *Moraxella*. While alpha and beta diversity, as well as biophysical parameters, did not show statistically significant differences among the groups, a strong negative correlation was notably observed between thickness and collagen intensity in the forehead and arm. Further analysis of the species and strain level is still required to solidify our conclusions. Ongoing studies are currently exploring functional differences in bacterial isolates from old versus young individuals through the analysis of biofilm formation, UV resistance, and influence on host/bacteria. This project is significantly advancing our understanding of the aged skin microbiome. Our findings will provide a foundation for future perspectives and applications, from managing strategies to address skin age to understanding the potential contribution of bacteria to impaired wound healing and skin disorders in the elderly.

## TOWARDS NEW BIOMARKERS OF PSORIATIC ARTHRITIS – THE ROLE OF MICROTRAUMA, ALARMINS AND LIPID MEDIATORS IN DEVELOPMENT AND RESOLUTION OF INFLAMMATION

A. Sherri[1], J. Makowska[1], A. Lewandowska-Polak[1]

[1] Department of Rheumatology, Medical University of Lodz, Poland

**Introduction:** Psoriatic arthritis (PsA) is the most prevalent chronic systemic inflammatory arthritis. Because of significant heterogeneity and lack of autoantibodies that can help pose the diagnosis, it remains a clinical challenge for practitioners. Recent studies suggest that that balance between biomarkers of microtrauma at entheseal sites leads to inflammation, which then spreads to secondarily involved structures. Among those mediators are Lipid mediators, which have both pro and anti-inflammatory properties like Leukotrienes, lipoxins, and Alarmins. The general goal of the study has been divided into several specific objectives with 3 research models. The aim of this presented study is to compare the influence of lipoxin A4 on the synthesis of pro-inflammatory cytokines by PBMCs from patients with PsA and healthy controls.

**Methods:** The peripheral blood will be collected from patients with psoriatic arthritis (n = 12) and healthy controls (n = 12). PBMC isolated from peripheral blood will be further stimulated with LPS and the modulatory effect of analogue of lipoxin A4 on generation of cytokines will be assessed. The level of cytokines in PBMC culture supernatants will be quantified with Cytometric Bead Array kit. The samples will be measured on BD LSR Fortessa flow cytometer and the results were analyzed with BD FCAP Array software.

**Results:** A set of preliminary experiments funded by the Medical University of Lodz was performed in our group. The basal and LPS-induced pro-inflammatory cytokine production by PBMCs was lower in PsA patients as compared to healthy subjects. Strikingly, co-stimulation with LXA4 resulted in a significant increase in pro-inflammatory cytokine secretion in PsA, in contrary to the reduction observed in healthy subjects.

**Discussion:** In one study lipoxin A4 was postulated to be important factor in the resolution of tendon inflammation in patients experiencing pain before and after surgical subacromial decompression treatment of supraspinatus

tendon. However, it is important to stress that role of lipoxins has not been studied up till now in pathogenesis of psoriatic arthritis. The following results would possibly help in establishing the role of lipoxin A4 in resolution of inflammation in Psoriatic arthritis.

**Conclusion:** Psoriatic arthritis is one of the most common inflammatory arthritis, leading to deterioration in the quality of life and disability. Understanding the process which initiates inflammation in PsA will potentially enable rapid therapeutic intervention and contribute to the prognosis improvement.

## LOW FIBRINOGEN LEVELS IN THE TREATMENT OF ISCHAEMIC STROKE BY INTRAVENOUS THROMBOLYSIS: A POSSIBLE PREDICTOR OF HAEMORRHAGIC INTRACRANIAL COMPLICATIONS?

L. Šimůnek[1], V. Kunešová[2], R. Mikulík[2,3], R. Jura[4], D. Václavík[5], P. Geier[6], M. Šrámek[7], J. Fiksa[8], R. Herzig[1]

[1] Department of Neurology, Comprehensive Stroke Center, Charles University, Faculty of Medicine and University Hospital in Hradec Králové, Hradec Králové, Czech Republic
[2] Cerebrovascular Research Program, International Clinical Research Centre, St. Anne's University Hospital, Brno, Czech Republic
[3] Department of Neurology, T. Baťa Regional Hospital, Zlín, Czech Republic
[4] Departmentof Neurology, Masaryk University, Faculty of Medicine and University Hospital, Brno, Czech Republic
[5] Department of Neurology, Faculty of Medicine, University of Ostrava and University Hospital, Ostrava, Czech Republic
[6] Department of Neurology, Faculty of Health Studies, Pardubice University and Pardubice Hospital, Pardubice, Czech Republic
[7] Department of Neurology, Central Military Hospital in Prague, Prague, Czech Republic
[8] Department of Neurology, Charles University, 1st Faculty of Medicine and General University Hospital, Prague, Czech Republic

**Introduction:** Intravenous thrombolysis (IVT) is the recommended recanalization therapy for ischaemic stroke. One complication of IVT is intracranial haemorrhage. Its incidence as a consequence of IVT is 5–12% in stroke centres in the Czech Republic.

The aim of this study is to evaluate whether a decrease in fibrinogen levels below the reference range of 1.8 g/l is associated with a higher likelihood of intracranial haemorrhage in patients with ischaemic stroke treated with IVT.

**Methods:** This multicentre retrospective study was conducted in 7 centres of the National Stroke Research Network STROCZECH. The study population consists of 280 patients enrolled in the observational group (divided into subgroups according to the type of haemorrhagic complication after IVT) and the control group (without haemorrhagic complication) according to the model 1:1 principle (same NIHSS, age, sex). Parameters monitored include personal and pharmacological history, laboratory and neurological parameters and results of selected imaging examinations. For statistical analysis, a non-parametric one-way ANOVA test with the Friedman and Dunns tests and a non-paired non-parametric Mann-Whitney test were selected.

**Results:** In all treatment groups (all observational subgroups as well as the control group), a decrease in fibrinogen levels was noted after IVT administration, with varying degrees of signification. A statistically significant difference ($p = 0.0394$) was found at 6 h after IVT administration in patients with the most clinically serious form of bleeding (parenchymal haematoma type 2), fibrinogen value 1.84 g/l vs 2.53 g/l.

An interesting finding, where signification was not reflected, concerns the baseline fibrinogen (i.e. before IVT administration), which is higher in all subgroups of patients with haemorrhagic complications compared to the control group.

**Discussion:** Current guidelines for the intravenous thrombolytic therapy include the examination of coagulation parameters, including fibrinogen levels, before IVT, 6 hours and 24 hours after IVT, but there is no official recommendation on when to substitute fibrinogen, and whether to do so at all. Substitution is available in hospitals (Haemocomplettan i.v.). Fibrinogen substitution may be a way to prevent the development of bleeding complications during IVT treatment.

**Conclusions:** Patients with the clinically most severe form of haemorrhagic complication (parenchymal haematoma type 2) had significantly lower fibrinogen levels than those in the control group 6 hours after IVT administration.

The study also leads us to a new hypothesis: a haemorrhagic complication of IVT could be associated with a relative decrease in fibrinogen levels from baseline, despite the fact that the values are still within the physiological range.

## IN VITRO ACTIVITY OF NEW SMALL-MOLECULE PERK INHIBITOR IN PARKINSON'S DISEASE CELLULAR MODEL

N. Siwecka[1], Z. Granek[1], K. Saramowicz[1], G. Galita[1], W. Rozpędek-Kamińska[1], D. Pytel[2], J. A. Diehl[3], I. Majsterek[1]

[1] Department of Clinical Chemistry and Biochemistry, Medical University of Lodz, Poland
[2] Department of Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston, SC, USA
[3] Department of Biochemistry and Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, OH, USA

**Introduction:** Abnormal aggregation of α-synuclein is the major molecular event underlying Parkinson's disease (PD) pathogenesis. Accumulated α-synuclein induces Endoplasmic Reticulum (ER) stress and activates the Unfolded Protein Response signaling pathway, that ultimately leads to PERK-dependent apoptosis and neurodegeneration. Therefore, the aim of the present study was to determine an in vitro effect of new small-molecule PERK inhibitor LDN-27339 (LDN) in cellular models of PD.

**Methods:** The study was carried out using SH-SY5Y and DI TNC1 cell lines with dopaminergic and astrocytic phenotype, respectively. The cytotoxic effect of LDN was evaluated by the colorimetric XTT assay. Cells were treated with LDN in wide concentration range (0.75 μM – 0.5 mM) or with vehicle (0.01% DMSO) and incubated for 16, 24 and 48 h. Untreated cells constituted a positive control, and cells treated with 99.9% DMSO served as a negative control. After incubation, the XTT/PMS mixture was added to each well and the absorbance was measured after 3 h. The inhibitory activity of the tested compound was evaluated by Western blotting. Cells were pretreated with LDN at the concentration range of 3–50 μM for 1h and then exposed to an ER stress inducer, thapsigargin (Th), at 500 nM for 2h. Untreated cells constituted a negative control, and cells treated with Th only served as a positive control. The detection of immune complexes was performed by means of chemiluminescence.

**Results:** LDN significantly inhibited PERK-dependent phosphorylation of eIF2α in SH-SY5Y cells at 25 μM and in DI TNC1 cells at 50 μM. The inhibitor had negligible cytotoxic effect at any concentration and incubation time in both tested cell lines.

**Discussion:** The findings indicate high activity of LDN against PERK in both SH-SY5Y and DI TNC1 cell lines, and the inhibitory effect was stronger in dopaminergic cells. At the same time, the compound did not significantly impair cell viability at the effective concentrations.

**Conclusions:** The inhibitor may rescue neural and glial cells from ER stress-mediated apoptosis in the course of PD, in which the PERK-dependent pathway is overactivated. Further studies are needed to assess the full potential of LDN and its possible application in novel therapeutic strategies against PD. This work was supported by the Medical University of Lodz, Poland (grant no. 503/1-156-07 /503-11-001) and by the National Science Centre, Poland (grant no. 2021/43/O/NZ5/02068).

## BINOCULAR VIDEO HEAD IMPULSE TEST: NORMATIVE DATA STUDY

M. Střiteská[1,2], M. Chovanec[2], T. Steinmetzer[3], V. Chrobok[1], O. Profant[2,4], E. Schneider[3], J. Kremláček[5], M. Vališ[6]

[1] Department of Otorhinolaryngology and Head and Neck Surgery, University Hospital Hradec Králové, Charles University, Faculty of Medicine in Hradec Králové, Hradec Králové, Czechia
[2] Department of Otorhinolaryngology, Third Faculty of Medicine, Charles University and University Hospital Kralovske Vinohrady, Prague, Czechia
[3] Institute of Medical Technology, Brandenburg University of Technology Cottbus-Senftenberg, Cottbus, Germany
[4] Department of Auditory Neuroscience, Institute of Experimental Medicine (ASCR), Prague, Czechia
[5] Department of Medical Biophysics, Faculty of Medicine in Hradec Králové, Charles University, Hradec Králové, Czechia
[6] Department of Neurology, University Hospital Hradec Králové, Charles University, Faculty of Medicine in Hradec Králové, Prague, Czechia

Published as original research article in Front Neurol 2023; 14: 1153102.

**Introduction:** The video head impulse test (vHIT) evaluates the vestibulo-ocular reflex (VOR). It's usually recorded from only one eye. Newer vHIT devices allow a binocular quantification of the VOR.

**Purpose (Aim):** To investigate the advantages of simultaneously recorded binocular vHIT (bvHIT) to detect the differences between the VOR gains of the adducting and the abducting eye, to define the most precise VOR measure, and to assess gaze dys/conjugacy. We aimed to establish normative values for bvHIT adducting/abducting eye VOR gains and to introduce the VOR dysconjugacy ratio (vorDR) between adducting and abducting eyes for bvHIT.

**Methods:** We enrolled 44 healthy adult participants in a cross-sectional, prospective study using a repeated-measures design to assess test–retest reliability. A binocular EyeSeeCam Sci 2 device was used to simultaneously record bvHIT from both eyes during impulsive head stimulation in the horizontal plane.

**Results:** Pooled bvHIT retest gains of the adducting eye significantly exceeded those of the abducting eye (mean (SD): 1.08 (SD = 0.06), 0.95 (SD = 0.06), respectively). Both adduction and abduction gains showed similar variability, suggesting comparable precision and therefore equal suitability for VOR asymmetry assessment. The pooled vorDR here introduced to bvHIT was 1.13 (SD = 0.05). The test–retest repeatability coefficient was 0.06.

**Conclusion:** Our study provides normative values reflecting the conjugacy of eye movement responses to horizontal bvHIT in healthy participants. The results were similar to a previous study using the gold-standard scleral search coil, which also reported greater VOR gains in the adducting than in the abducting eye. In analogy to the analysis of saccade conjugacy, we propose the use of a novel bvHIT dysconjugacy ratio to assess dys/conjugacy of VOR-induced eye movements. In addition, to accurately assess VOR asymmetry, and to avoid directional gain preponderance between adduction and abduction VOR-induced eye movements leading to monocular vHIT bias, we recommend using a binocular ductional VOR asymmetry index that compares the VOR gains of only the abduction or only the adduction movements of both eyes.

## DIAGNOSTIC FEATURES OF POLYNEUROPATHY IN PATIENT WITH 2 TYPE DIABETES MEIIITUS

D. Sushetska[1]

[1] Kharkiv National Medical University, Ukraine

**Introduction:** Polyneuropathy is the most common and prognostically unfavorable complication of diabetes mellitus (DM). Distal symmetrical polyneuropathy (DSPN) is the most common type of peripheral neuropathy in patients with type 2 diabetes. Electroneurophysiological and clinical manifestations DSPN precede the manifestations of the main disease and appear 2–8 years before the appearance of the first clinical symptoms.

Most polyneuropathies are of metabolic origin, including DSPN, which is accompanied by an axonal type

of nerve damage. However, patients with DM also have a demyelinating type of nerve damage, which makes it necessary to differentiate DSPN from chronic inflammatory demyelinating polyneuropathy (CIDP).

**Methods:** We examined 32 patients with type 2 diabetes and a concomitant diagnosis of polyneuropathy of the lower extremities of varying degrees of severity. All patients underwent a neurological examination, evaluation of NISS-LL, TSS scales and stimulation ENMG examination of the nerves of the upper and lower extremities.

**Results:** All patients were divided into three groups according to ENMG data. The first group included patients with a predominantly demyelinating form of nerve damage – 16 patients. The second group with predominant axonal degeneration – 12 patients. The third group includes 4 patients with a mixed type (axonal-demyelinating). The sums of points in patients according to diagnostic scales (NISS-LL, TSS) depending on the stage of polyneuropathy increased with increasing severity of DPNP. During the analysis of disease histories, it was found that the experience of diabetes among patients of the 1st group is 1–5 years, they have a controlled blood glucose level and regularly do sports. In the II group, the majority are patients with diabetes for more than 5 years, whose blood glucose level is insufficiently controlled, episodes of hypo- or hyperglycemia are noted. Patients of this group are also characterized by moderate or low physical activity. In the III observation group, most patients have been sick for more than 10–13 years, they have an elevated blood glucose level, with frequent hypo- or hyperglycemic states, excess body weight and very low physical activity.

**Conclusion:** Based on the results of our study, we can conclude that the development of severe axonal-demyelinating polyneuropathy is directly correlated with diabetes experience and blood glucose control. Adequate physical activity of patients also plays an important role. At the same time, identification of the morphological type of polyneuropathy is important for prescribing specific therapy.

## SEEING BEYOND THE SURFACE: ADVANCED ENDOSCOPY IN LARYNGEAL MALIGNANCY CLASSIFICATION

A. Švejdová[1,2], M. Homoláč[1,2], J. Šatanková[1,2], J. Krtičková[1,2], M. Černý[1,2], L. Zeinerová[1], V. Chrobok[1,2]

[1] Department of Otorhinolaryngology and Head and Neck Surgery, University Hospital Hradec Králové, Czech Republic
[2] Department of Otorhinolaryngology and Head and Neck Surgery, Charles University, Faculty of Medicine in Hradec Králové, Czech Republic

**Introduction:** Enhanced contact endoscopy (ECE) – combination of contact endoscopy and NBI (Narrow band imaging) or IMAGE1S, is a non-invasive optical technique used for assessment of superficial vascular changes of mucosal lesions in high magnification. The aim of our study was to evaluate the diagnostic value of ECE in an intraoperative settlement.

**Methods:** Patients with laryngeal lesions underwent microlaryngoscopy with a structured assessment of the lesion using white light, NBI and ECE. Lesions were classified according to the European Laryngological Society Classification that divides the vascular pattern changes into longitudinal (unsuspicious) and perpendicular (suspicious). Evaluation was correlated with histopathology.

**Results:** 60 patients with 76 lesions were enrolled. Sensitivity, specificity, and accuracy for NBI assessment reached 71.4%, 100% and 78.6%, resp., Kappa index of 0.556. Sensitivity, specificity, and accuracy for ECE 86.4%, 89.5% and 87.3%, resp., Kappa index of 0.716. Additional 12% (9/76) of the lesions could be assessed with ECE compared to NBI.

**Conclusions:** Our data support the assumption that ECE is a useful tool for pre-histological examination of mucosal lesions, however it cannot fully replace biopsy sampling. ECE shows higher accuracy in detecting malignant lesions compared to NBI and can be especially helpful in the assessment of vocal fold leukoplakia.

**Keywords:** enhanced contact endoscopy; narrow band imaging; squamous cell carcinoma; laryngeal lesion; leukoplakia

**Funding:** The study was supported by the Charles University, project GA UK No. 506122.

## INCREASED CARBOHYDRATE INTAKE DOES NOT WORSEN DIABETES COMPENSATION IN OLDER TYPE 2 DIABETICS

M. Tichá[1], O. Sobotka[1], P. Skořepa[1], L. Sobotka[1]

[1] 3rd Department of Medicine, Faculty of Medicine in Hradec Králové, Charles University, Hradec Králové, Czech Republic

**Introduction:** Especially in seniors the carbohydrate (CHO) intake is usually reduced due to high prevalence of type 2 diabetes (T2DM). However, low CHO intake can lead the lack of building blocks for regeneration and other anabolic processes that are accompanied by insulin resistance. The aim of insulin resistance can be inhibition of glucose oxidation and redistribution of glucose for growing ans regenerating tissues. The aim of our study was to assess the effect of increased CHO intake on blood levels of glucose, insulin and C-peptide in older patients with and without type 2 diabetes.

**Methods:** Twenty-eight patients were enrolled to prospective study (20 T2DM patients, and 8 non-diabetic). They received standard hospital diet during the first week of study. Throughout the second week they received 150 g of maltodextrin (Fantomalt Nutricia) divided to 3 daily doses immediately after the normal meal. Blood glucose level was monitored continuously by a subcutaneous sensor Medtronic. Mean glycaemia, time in range (TIR) and calculated HgA1 were evaluated. Additionally, plasmatic levels of C-peptide, insulin were measured on the first, seventh and fourteenth day in 7 subjects.

**Results:** Increased CHO intake led to transient increase of blood glucose levels in T2DM patients during the day

1–3. After 3 days the postprandial peak hyperglycaemias were blunted. CHO did not influence the percentual range of high glucose level and decreased a risk of hypoglycaemia. The change of plasmatic levels of C-peptide and insulin was minimal after addition CHO to diet.

**Discussion:** We found that increased CHO intake gradually decreased insulin resistance. This supports the idea that insulin resistance cause by preferred non-oxidative glucose metabolism can be improved by increased CHO intake. Therefore, CHO intake should not be decreased in patients who require nutritional support due to muscle loss and sarcopenia.

**Conclusion:** Liberal intake of CHO in diet can be recommended in malnourished patients with type 2 diabetes.

## CLINICAL PATTERNS OF PSYCHICAL DISORDERS CREATED IN THE COURSE OF A FULL-SCALE INVASION OF UKRAINE IN MILITARY PERSONNEL

K. O. Tsebriuk[1]

[1] Kharkiv National Medical University

**Relevance:** In recent times due to the situation in Ukraine related to the defense of its territorial integrity, starting with the situation in eastern Ukraine in 2014 with the operation of the Joint Forces (JF), in which tens of thousands of people are involved. Fighting in the country is characterized by a high level of stress. More than 85 thousand servicemen have been in the active combat zones of the country. It is important to determine what influence on the psyche has their military experience.

Objective: to study the peculiarities of the clinical picture of mental disorders acquired during the period of environmental protection.

**Materials and methods:** Taking into account the rules of bioethics and deontology and in the presence of informed consent for participation in the study, 40 patients were examined in 2023 on the basis of the Military Medical Clinical Center of the Northern Region of the Ministry of Defense of Ukraine. The age of all male patients ranged from 18 to 53 years. The following psychodiagnostic techniques were used: Mississippi scale of posttraumatic stress disorder, CES-D scale and traumatic events assessment scale.

**Results:** according to the results of psychodiagnostic study of Mississippi scale of post-traumatic stress disorder 30% of respondents (12 people) had a good level of adaptation with scores 35–96, and 70% (28 people) had an adaptation disorder with scores 100 and more when taking this test. According to the results of psychodiagnostic survey on CES – D scale 20% (8 people) of respondents had low level of fighting (10–13 points), 16% (7 people) average level (14–17 points), 28% (11 people) elevated level (18–21 points), 28% (11 people) with high level (22–25 points) and 8% (3 people) with very high level (26–28 points). Based on the results of the traumatic event impact assessment scale, 17% of respondents (7 people) had avoidance symptoms, 33% (13 people) had obsessive compulsive symptoms, and 50% (20 people) had agitation symptoms.

**Conclusion:** The combat experience of the majority of patients in the active combat zone ranged from medium to high. Only 8% (3 people) had a very high level of combat experience and 28% (11 people) had a high level of combat experience. The respondents' length of stay in the active combat zone ranged from 60 to 340 days. 17% of the respondents (7 people) had avoidance symptoms, 33% (13 people) had obsessive compulsive symptoms, and 50% (20 people) had arousal symptoms. Therefore, further in this direction the targeting of psychotherapeutic and psychoeducational programs in this category of patients will be considered.

## LONG-STAY ICU PREDICTORS AFTER BENTALL PROCEDURE: RETROSPECTIVE STUDY

V. Tymoshenko[1,2], O. Zelenchuk[1,2], S. Maruniak[1,2,3], B. Todurov[1,2]

[1] Heart Institute Ministry of Health of Ukraine, Kyiv, Ukraine
[2] Shupyk National Healthcare University of Ukraine, Kyiv, Ukraine
[3] Paracelsus Medical University, Nuremberg, Germany

**Aim:** To analyze the frequency of early postoperative complications and predictors of long-stay patients in the intensive care unit (ICU) after the Bentall procedure.

**Materials and methods:** This retrospective study is based on the obtained medical records of adult patients (aged 18–75), who underwent Bentall procedure for an ascending aortic aneurysm between 2012 and 2021. Depending on the length of ICU stay, all patients were divided into two groups: length of ICU stay up to 3 days (group 1) and length of ICU stay longer than 3 days (group 2). Univariate and multivariate analysis (logistic regression) was used to determine prognostic risk factors.

**Result:** Patients of the second group were characterized by reliably older age ($p = 0.005$), more frequent presence of arterial hypertension ($p = 0.044$) among concomitant diseases ($p = 0.044$) and significantly lower baseline glomerular filtration rate (GFR) ($p = 0.045$). In case of the second group patients in comparison with the first one, cell saver was used 3.6 times ($p = 0.0005$) more often and almost 6 times ($p = 0.0037$) more often, as well as the need for rethoracotomy due to bleeding, 4.3 times ($p = 0.0002$) more often acute renal failure renal failure and 3.3 times ($p = 0.0004$) more often acute respiratory failure. Multivariate logistic analysis revealed two independent predictors of long-stay ICU: duration of mechanical ventilation (OR 1.204 (CI 1.053–1.377), $p = 0.007$) and development of acute renal failure renal failure (OR 4.069 (CI 1.040–15.923), $p = 0.044$).

**Discussion:** Aortic reconstruction can pose a significant risk of postoperative complications and, accordingly, a long-stay ICU. Among postoperative complications after surgery for AAA, the prevalence of arrhythmias was 33.7%, acute renal failure – 14.6%, bleeding/tamponade – 14.6%, while hospital mortality was recorded at 15.6% [3]. In our opinion, such a high mortality rate, in contrast to our work, is due to the high frequency of emergency operations (43%) and the presence of concomitant

cardiac surgery. At the same time, the prevalence of acute renal failure and arrhythmias in the postoperative period was relatively similar to our study – 52.4% and 18.4%, respectively.

**Conclusions:** All patients must be treated in the same way to avoid per- and postoperative complications, but patients with risk factors for long-stay ICU should be under special attention to avoid additional complications and, subsequently, to reduce length of ICU stay.

**Keywords:** Bentall's procedure, length of ICU stay, ascending aortic aneurysm, risk factors.

## EIF4F CONTROLS AMPK ACTIVITY IN MALIGNANT MELANOMA

N. Vadovičová[1], B. Valčíková[1], K. Kozdonová[1], D. Potěšil[2], Z. Zdráhal[2], S. Uldrijan[1,3]

[1] Department of Biology, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[2] Central European Institute of Technology, Masaryk University, Brno, Czech Republic
[3] International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic

**Introduction:** Malignant melanoma is an aggressive form of cancer with poor prognosis, often caused by mutations in the MAPK ERK signaling cascade. Clinically relevant small-molecule drugs targeting BRAF and MEK kinases can prolong survival, but resistance rapidly emerges. Recently, the eukaryotic translation initiation complex (eIF4F) has been reported as the nexus of resistance to BRAF/MEK inhibitors. Furthermore, simultaneous inhibition of BRAF and eIF4F synergized in killing cancer cells. Therefore, we aimed to precisely characterize the crosstalk between the ERK and eIF4F pathways in melanoma.

**Methods:** We used MS-based proteomic approach and small-molecule MEK/eIF4F inhibitors to identify common targets of the ERK and eIF4F pathways in NRAS- and $BRAF^{V600E}$-mutant melanoma cells. Then we validated the targets using RNA interference and western blotting.

**Results:** The proteomic analysis revealed a significant overlap of ERK and eIF4F targets in both melanoma subtypes. Interestingly, apart from the cell cycle and DNA repair regulators, we found regulators of the primary cellular energy sensor, AMP-dependent protein kinase (AMPK).

MO25, part of an AMPK-activating complex (LKB1-STRAD-MO25), and PP2Aα, an AMPK-inhibiting phosphatase, were found to be downregulated. Upon eIF4F inhibition, we observed ERK pathway activation and surprisingly, AMPK activation despite the downregulation of LKB1, the canonical activator of AMPK. This was also confirmed in LKB1-deficient $BRAF^{V600E}$-mutant melanoma cells. These results suggest the existence of a novel, LKB1-independent mechanism of AMPK activation in melanoma cells.

Furthermore, PP2Aα downregulation seems to play an essential role, as RNAi-mediated knockdown of PP2Aα and a small-molecule PP2A inhibitor both potently promoted AMPK activity.

**Conclusion**: Our findings reveal new insights into the molecular mechanisms of melanoma resistance and indicate the cooperation of the ERK and eIF4F pathways in controlling essential cellular processes. eIF4F inhibition promoted non-canonical, LKB1-independent AMPK activation with simultaneous ERK activation. We describe a novel mechanism of AMPK activity control in $BRAF^{V600E}$-mutant melanoma cells.

## LACTATE: THE ENEMY OF THE NEURONS IN THE PRESENCE OF GLUTAMATE TOXICITY – A STUDY IN CORTICAL PRIMARY CELLS

A. Vaglio-Garro[1], A. Weidinger[1], A. V. Kozlov[1]

[1] LBG Ludwig Boltzmann Institute for Traumatology. The Research Center in Cooperation with AUVA, Vienna, Austria

**Introduction:** Limited substrate availability because of the blood brain barrier has made the brain develop specific molecular mechanisms to survive, using lactate synthesized by astrocytes, as an energy source in neurons. In primary cortical cells, lactate showed a beneficial and deleterious effect against glutamate toxicity depending on the conditions.

**Methods:** To understand if lactate was used as a preference energy source to rescue neurons after inducing glutamate shock, we treated primary cortical cells with oxamate (inhibitor of lactate dehydrogenase (LDH)), 50 mM glutamate, and lactate and/or glucose for 24 hours. LDH activity was detected in single neurons linked or not linked to glia cells by a redox reaction. The total cell number was counted in three random fields. Nuclei were stained with Dapi and the viable neurons were detected by MAP2.

**Results:** We showed that neurons linked to glial cells presented a higher LDH activity compared to not-linked neurons, supporting the hypothesis that lactate shuttle exists and interacts between astrocytes and neurons. Next, we examined the effect of glucose, lactate, and oxamate on neuron viability. In the presence of glucose, inhibition of LDH by oxamate decreased viability of neurons. However, addition of oxamate to cells incubated in media containing lactate elevated survival of neurons.

**Discussion and preliminary conclusions:** Considering mechanisms related to the tricarboxylic acid cycle, we assume that these effects of lactate are the result of switching between pyruvate and glutamate consumption in mitochondria. For example, the addition of oxamate in media with lactate decreases the formation of pyruvate and as a compensation, increases consumption of glutamate, reducing glutamate toxicity. In the brain, when neurons do not have access to glucose, lactate is transported to neurons from astrocytes. However, this energy source is deleterious in the presence of toxic glutamate concentrations because it facilitates uptake of pyruvate by mitochondria.

## PLATELET FUNCTION TESTING – A SINGLE CENTRE EXPERIENCE

P. Vigláš[1,2], F. Cihlář[1], J. Raupach[2], P. Bradáčová[3], A. Hejčl[4], D. Černík[5], L. Lejnarová[1]

[1] Radiologická klinika Fakulty zdravotnických studií UJEP a Krajské zdravotní a.s. – Masarykovy nemocnice v Ústí nad Labem, o.z., Česká republika
[2] Radiologická klinika LF UK a FN, Hradec Králové, Česká republika
[3] Oddělení klinické hematologie, Krajská zdravotní a.s. – Masarykova nemocnice v Ústí nad Labem, o.z., Česká republika
[4] Neurochirurgická klinika Fakulty zdravotnických studií UJEP a Krajské zdravotní a.s. – Masarykovy nemocnice v Ústí nad Labem, o.z., Česká republika
[5] Neurologické oddělení, Krajská zdravotní a.s. – Masarykova nemocnice v Ústí nad Labem, o.z., Česká republika

**Introduction:** Aim of the study is to evaluate the laboratory effectiveness of the most used antiplatelet drugs in our centre (clopidogrel, ticagrelor, aspirin) and to assess the effectiveness of the changed treatment in patients in whom we found in vitro resistance, or high on-treatment platelet reactivity (HTPR) to clopidogrel.

**Methods:** In our set of patients, the effectiveness of antiplatelet drugs was tested using the light transmission aggregometry (LTA) method from 16. 9. 2020 to 31. 12. 2022. The effectiveness of treatment in patients switched to an alternative medicine was monitored and the success of the change in therapy was evaluated.

**Results:** In the monitored period, a total of 529 blood samples were tested for clopidogrel (n = 216), ticagrelor (n = 59) and aspirin (n = 254). The efficacy of clopidogrel treatment was 45.4%. On the other hand, the efficacy of ticagrelor was 93,2%, statistically significantly higher ($p < 0.000$). In case of conversion from clopidogrel to ticagrelor, treatment was successful in 49 of 54 patients (90.7%).

**Discussion:** To effectively prevent thromboembolic events during endovascular procedures, it is necessary to correctly identify patients in whom ineffective antiplatelet treatment could cause thrombosis, occlusion of an implanted stent or an ischemic stroke. Currently, there is no standardization among neurointerventional centers in testing the effectiveness of antiplatelet treatment, nor antiplatelet therapy guidelines. Identifying the incidence of HTPR in our population is the first step on the way to standardization.

**Conclusion:** For the antiplatelet drugs tested, statistically significant differences in the effectiveness were demonstrated. In the case of clopidogrel, ticagrelor is a suitable alternative, significantly reducing incidence of HTPR. The number of patients with HTPR undergoing stent/flow diverter implantation can be significantly reduced by including platelet function testing in the algorithm of endovascular procedures.

## CIRCADIAN LOCOMOTOR DYSRHYTHMIA AND THERAPEUTIC POTENTIAL OF METHYLPHENIDATE IN A RAT MODEL OF SPORADIC ALZHEIMER'S DISEASE

D. Virag[1], J. Homolak[1], A. B. Perhoč[1], A. Knezović[1], J. Osmanović Barilar[1], M. Šalković-Petrišić[1]

[1] Laboratory for Molecular Neuropharmacology, Department of Pharmacology, University of Zagreb School of Medicine, Croatia

**Introduction:** Early diagnosis of sporadic Alzheimer's disease (sAD) presents a difficult challenge, with only recently emerging disease-modifying therapy. Epidemiological and animal studies have highlighted circadian dysrhythmia and an Attention Deficit Hyperactivity Disorder-like phenotype as risk factors and plausible etiopathogenetic factors, scarcely investigated in AD patients and inadequately modelled in animal AD models. We aimed to investigate these parameters in a rat model of AD induced by intracerebroventricular streptozotocin (STZ) and how they are affected by oral methylphenidate (MPH).

**Methods:** Three-month old male Wistar rats (n = 40) were injected intracerebroventricularly with citrate buffer (control/CTR) or STZ (3 mg/kg) split in two doses 48 hours apart after which MPH therapy was initiated daily in a dual-bottle dosage regimen (4 and 10 mg/kg) for 6 weeks to half of CTR and STZ groups. Cognitive ability and locomotor activity were assessed by Novel Object Recognition (NOR) and Open Field (OF) at 2, 4, and 6 weeks after STZ. Baseline and 6-week continuous measurements of cognition, locomotion and impulsivity were done by custom home cage apparatus in an operant conditioning task (VlaDiSlav test), and the Multicage InfraRed Open Source Locomotor Activity eValuator (MIROSLAV).

**Results:** At 2 weeks, STZ demonstrated worse performance than CTR (NOR) but this deficit was ameliorated by MPH. No change in NOR were observed at 4 and 6 weeks between the groups. Learning curves in continuous testing using VlaDiSlav show a severely diminished learning rate in STZ group, however, STZ+MPH learning rate was similar to CTR values. STZ+MPH group also showed reduced impulsivity compared to all other groups. OF thigmotaxis was slightly increased in CTR relative to other groups, with no differences in total distance traversed. Preliminary data (MIROSLAV) demonstrated reduced nocturnal and increased diurnal activity in the STZ group.

**Discussion:** VlaDiSlav continuous measurement gives insight into the learning process suggesting that STZ induced a severe learning deficit that might be normalized by MPH treatment. These novel findings also highlight the benefits of supplementing conventional behavioural testing with continuously operating, automatised, programmable home cage-based devices. Preliminary MIROSLAV data indicate the presence of a circadian dysrhythmia with similar parameters to those observed in AD patients, unlike those observed in other AD animal models.

**Conclusion:** Novel non-invasive methodology points to preliminary robust beneficial effects of MPH on cognitive performance in the STZ model of sAD,but further

analysis is necessary to better describe the complex STZ phenotype and the effects of MPH.

**Acknowledgement:** Supported by Croatian Science Foundation (HRZZ-DOK-2021-02-6419) and Medice.

## DISTANCE FROM MAIN ARTERIES IS ASSOCIATED WITH MICROSTRUCTURAL AND FUNCTIONAL BRAIN TISSUE CHARACTERISTICS

V. Weiss[1,2], V. Kokošová[1,3], Z. Valenta[4], I. Doležalová[1], M. Baláž[1], S. Mangia[5], S. Michaeli[5], L. Vojtíšek[6], R. Herzig[2,7], P. Filip[5,8]

[1] First Department of Neurology, Faculty of Medicine, Masaryk University and University Hospital of St. Anne, Brno, Czech Republic
[2] Department of Neurology, Charles University Faculty of Medicine, Hradec Králové, Czech Republic
[3] Department of Neurology, Faculty of Medicine, Masaryk University and University Hospital Brno, Brno, Czech Republic
[4] Department of Statistical Modelling, Institute of Computer Science of the Czech Academy of Sciences, Prague, Czech Republic
[5] Center for Magnetic Resonance Research (CMRR), University of Minnesota, Minneapolis, MN, USA
[6] Central European Institute of Technology (CEITEC) Masaryk University, Neuroscience Centre, Brno, Czech Republic
[7] Department of Neurology, Comprehensive Stroke Center, University Hospital Hradec Králové, Czech Republic
[8] Department of Neurology, Charles University, First Faculty of Medicine and General University Hospital, Prague, Czech Republic

**Introduction:** Given the substantial dependence of neurons on continuous supply of energy, the distribution of major cerebral arteries opens a question whether the distance from the main supply arteries constitutes a modulating factor for the microstructural and functional properties of brain tissue.

**Methodology:** To tackle this question, multimodal MRI acquisitions of 102 healthy volunteers over the full adult age span were utilised to quantify microstructural (cellularity, myelin density, iron concentration) and functional (connectivity and neural activity) tissue characteristics in various brain regions and ascertain their dependence on the distance from the closest major artery.

**Results:** Our result point to higher iron concentration and higher cellularity in areas more distant to main arterial trunks. In the cortex, these findings were combined with lower intracellular volume fraction and higher myelin density, suggesting relatively higher abundance of smaller cells and/or myelinated small axons farther away from main arteries. In the white matter, bigger distance from main arteries was associated with relatively higher intracellular volume fraction and increased myelin density. Furthermore, age emerged as a substantial factor influencing this pattern.

**Conclusion:** With higher age, cortical areas more distant from arteries show lower intracellular volume fraction, pointing to their potentially higher vulnerability. All in all, this pilot study provides a novel insight on brain regionalisation based on artery distance, which merits further investigation to validate its biological underpinnings.

## THE EFFECTS OF PRENATAL PRAVASTATIN TREATMENT IN THE RABBIT FETAL GROWTH RESTRICTION MODEL

K. Zapletalová[1,2], I. Valenzuela[1], M. Greyling[1], Y. Regin[1], C. Frigolett[3], L. Krofta[2], J. Deprest[1,4], J. van der Merwe[1,4]

[1] Department of Development and Regeneration, Cluster Woman and Child, Group Biomedical Sciences, KU Leuven, Leuven, Belgium
[2] Institute for the Care of Mother and Child, Third Faculty of Medicine, Charles University, Prague, Czech Republic
[3] Department of Public Health and Primary Care, Leuven Statistics Research Centre, KU Leuven, Leuven, Belgium
[4] Department of Obstetrics and Gynecology, Division Woman and Child, University Hospitals Leuven, Leuven, Belgium

**Introduction:** Fetal growth restriction (FGR) remains one of the main contributors to perinatal mortality and morbidity. Although there is some clinical evidence supporting the benefit of early postnatal strategies, no prenatal strategies have accomplished positive clinical results. Research on murine FGR models suggests the potential benefits of prenatal pravastatin. Given that the rabbit hemodichorial placenta more closely resembles the human condition, we investigated the effects of prenatal maternal pravastatin administration in the rabbit FGR model.

**Methods:** On gestational age 25 (term 31 d) pregnant dams (n = 14) underwent partial uteroplacental vessel ligation (UPVL) in one uterine horn to induce FGR, leaving the other horn as control. Dams were randomized to either receive pravastatin 5 mg/kg/d dissolved in their drinking water or normal drinking water until delivery. On GA 30d rabbits were delivered and grouped into four categories: controls without pravastatin (C/NoPrav) (n = 33), FGR without pravastatin (FGR/NoPrav) (n = 36), FGR with pravastatin (FGR/Prav) (n = 30), and controls with pravastatin (C/Prav) (n = 32). Newborn rabbits underwent pulmonary function assessment using the FlexiVent system, neurobehavioral assessment, and were harvested for alveolar morphometry or neuropathology. Placentas were analyzed for histological and RNA expression.

**Results:** Birthweight was lower in the FGR groups (FGR/Prav, FGR/NoPrav), but there was no difference between FGR/Prav and C/NoPrav. No differences were observed in placental zone proportions; however, eNOS in FGR/Prav placentas and VEGFR-2 in FGR/Prav and C/Prav were upregulated. There were no differences in pulmonary function assessment and alveolar morphometry. FGR/Prav kittens had increased neurosensory scores on postnatal day 1, but there were no differences in neuromotor tests, neuron density, apoptosis, and astrogliosis.

**Discussion:** Hydrophilic pravastatin primarily affects maternal and placental tissue. Thus, improvement of fetal or neonatal outcomes would likely depend on enhanced placental function. However, in our study, this was not the case, as our primary outcome, birthweight,

was not improved. Additionally, no differences were observed in pulmonary function, alveolar morphometry, or brain neuron density. On the other hand, pravastatin was associated with a small but significant increase in neurosensory scores. Although this study was not powered to detect small effects on secondary outcomes, these findings warrant further investigation.

**Conclusions:** In the rabbit FGR model, pravastatin upregulated expression of VEGFR-2 and eNOS in FGR placentas and was associated with higher neurosensory scores, without a measurable effect on birthweight, pulmonary function and morphology, or neuron density.

## LYMPHOCYTE SUBPOPULATION CHANGES IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA

K. Žibřidová[1,3], O. Souček[2], L. Kujovská Krčmová[4], K. Jankovičová[2], M. Gančarčíková[4], M. A. Pejková[1,3], J. Drugda[1,3], D. Nováková[1,3], M. Košťál[1,3]

[1] 4th Department of Internal Medicine – Hematology, University Hospital Hradec Králové, Sokolská 581, 500 05 Hradec Králové, Czech Republic
[2] Department of Clinical Immunology and Allergology, University Hospital Hradec Králové, Sokolská 581, 500 05 Hradec Králové, Czech Republic
[3] Charles University, Faculty of Medicine in Hradec Králové, Šimkova 870, 500 03 Hradec Králové, Czech Republic
[4] Department of Clinical Biochemistry and Diagnostics, University Hospital Hradec Králové, Sokolská 581, 500 05 Hradec Králové, Czech Republic

**Background:** Immune thrombocytopenia (ITP) is an autoimmune bleeding disorder caused by increased platelet destruction and altered production. Although some patients achieve long-term remission after 1st line therapy, almost 80% ITP patients require further treatment. Current guidelines consist of monotherapy with different drugs, with no tool to predict individual response.

**Aim:** In our study, we evaluated laboratory parameters in patients with ITP with the aim of identifying a prognostic marker for a poor response leading to chronicity and/or refractoriness to treatment.

**Methodology:** To our project, we collected data from 35 patients with primary ITP (newly diagnosed or relapsed disease). We assessed blood count, antiplatelet autoantibodies, and T lymphocyte subsets in peripheral blood at 0, 3, 6 and 12 months during follow-up. Using multicolour flow cytometry, we determined relative and absolute numbers of lymphocytes, T cells, and their subpopulations: CD4+ helper T cells (including IFN-γ+/CD4+ T cells, IL-4+/CD4+ T cells, IFN-γ+/IL-4+/CD4+ T cells and IL-17A+/CD4+ T cells), CD8+ cytotoxic T cells (including IFN-γ+/CD8+ T cells, IL-4+/CD8+ T cells and IFN-γ+/IL-4+/CD8+ T cells) and ratio of CD4 + to CD8+ T cells.

**Results:** Our data show that the value of antiplatelet autoantibodies, the percentage of cytotoxic T lymphocytes, and the immunoregulatory index (IRI, CD4+/CD8+ T lymphocyte ratio) differ significantly by treatment response. Responders have a higher IRI ($P = 0.04$), higher antiplatelet autoantibodies ($P = 0.01$) and lower CD8+ T cells relative count ($P = 0.2$) before treatment.

**Discussion:** The results suggest that antiplatelet autoantibodies and subsets of T lymphocytes in peripheral blood (especially CD8+ CD3+ lymphocyte relative count of CD8+ CD3+ lymphocytes and ratio CD4+ / CD8+) could be used as a prognostic tool for a worse clinical outcome in patients with ITP.

**Conclusion:** These biomarkers could be utilised for stratification and eventually selection of treatment preferring combination therapy in patients with unfavourable immunological setting.