Праці НТШ Медичні науки
2022, Том 66, № 1
ISSN 2708-8634 (print) www.mspsss.org.ua

Proc Shevchenko Sci Soc Med Sci
2022, Vol. 66, 1
www.mspsss.org.ua ISSN 2708-8642 (online)

Дослідження фізіологічних та біохімічних параметрів для виявлення простих, надійних та інформативних біомаркерів для клінічних досліджень у пацієнтів з ПТСР: обґрунтування, елементи дизайну та контекст війни в Україні

Вікторія Сергієнко[1], Клаус Хольцман[2], Сергій Голота[3,4], Зеновій Деркач[5], Армен Нерсесян[2], Станіслав Мельник[6], Олександра Черниш[7], Остап Яцкевич[8], Мірослав Мішік[2], Володимир Бубало[9], Ольга Стрільбицька[10], Богдана Вацеба[11], Олег Лущак[10], Зіґфрід Кнасмюллєр[2], Андрій Черкас[12†]

[1]*Кафедра ендокринології, Львівський національний медичний університет імені Данила Галицького, м. Львів, Україна*
[2]*Центр дослідження раку, Медичний університет Відня, м. Відень, Австрія*
[3]*Кафедра фармакологічної, органічної та біоорганічної хімії, Львівський національний медичний університет імені Данила Галицького, м. Львів, Україна*
[4]*Кафедра органічної хімії та фармації, Волинський національний університет імені Лесі Українки, м. Луцьк, Україна*
[5]*Військово-медичний клінічний центр західного регіону, м. Львів, Україна*
[6]*Медичний факультет №1, Львівський національний медичний університет імені Данила Галицького, м. Львів, Україна*
[7]*Університет Монпельє, м. Монпельє, Франція*
[8]*Кафедра внутрішньої медицини №1, Львівський національний медичний університет імені Данила Галицького, м. Львів, Україна*
[9]*Науковий центр превентивної токсикології, харчової та хімічної безпеки імені академіка Л.І. Медведя МОЗ України, м. Київ, Україна*
[10]*Прикарпатський національний університет імені Василя Стефаника, м. Івано-Франківськ, Україна*
[11]*Івано-Франківський національний медичний університет, м. Івано-Франківськ, Україна*
[12]*Колишня адреса: кафедра внутрішньої медицини №1, Львівський національний медичний університет імені Данила Галицького, м. Львів, Україна*

†Теперішня адреса: Відділ ранніх проектів цукровий діабет 1-го типу, терапевтична галузь "Діабет та серцево-судинна медицина", Санофі-Авентіс-Дойчланд, м.Франкфурт-на-Майні, Німеччина

OPEN ACCESS

DOI: 10.25040/ntsh2022.02.14

Адреса для листування: Кафедра ендокринології, Львівський Національний Медичний Університет імені Данила Галицького, вул. Острозького, 1, Львів, Україна, 79010

Твіттер: @VictoriyaSerh
Е-пошта: serhiyenkov@gmail.com

Надійшла до редакції: 15.10.2022
Прийнята до друку: 14.12.2022
Опублікована: 30.12.2022

ORCID IDs
Вікторія Сергієнко: https://orcid.org/0000-0002-6414-0956
Клаус Хольцман: https://orcid.org/0000-0003-4077-3377
Армен Нерсесян: https://orcid.org/0000-0001-5571-7904
Сергій Голота: https://orcid.org/0000-0002-9892-437X
Зеновій Деркач: https://orcid.org/0000-0001-6195-8456
Станіслав Мельник: https://orcid.org/0000-0002-6156-5000
Олександра Черниш: https://orcid.org/0000-0003-0737-274X
Остап Яцкевич: https://orcid.org/0000-0001-5146-2553
Мірослав Мішік: https://orcid.org/0000-0002-8182-3695
Володимир Бубало: https://orcid.org/0000-0003-0230-9354
Ольга Стрільбицька: https://orcid.org/0000-0003-3277-2294
Богдана Вацеба: https://orcid.org/0000-0002-5794-8754
Олег Лущак: https://orcid.org/0000-0002-4627-1987
Зіґфрід Кнасмюллєр: https://orcid.org/0000-0002-1638-4438
Андрій Черкас: https://orcid.org/0000-0002-6652-6983

Конфлікт інтересів: автори заявляють про відсутність конфлікту інтересів.

Особистий внесок авторів:
Створення концепції: Вікторія Сергієнко, Клаус Хольцман, Сергій Голота, Олег Лущак, Зіґфрід Кнасмюллєр, Андрій Черкас;
Результати дослідження: Зеновій Деркач, Остап Яцкевич, Станіслав Мельник;
Написання: Вікторія Сергієнко, Станіслав Мельник, Андрій Черкас;
Редагування та затвердження остаточного варіанту: усі автори.

Дозвіл комісії з питань біоетики: Комісія з біоетики Львівського національного медичного університету імені Данила Галицького, протокол № 9 від 22.11.2021 р.

Фінансування: Двосторонній Австро-Український проект No. UA 08/2020 Австрійського федерального міністерства освіти, науки та досліджень "Дослідження фізіологічних та біохімічних параметрів, які пов'язані зі здоров'ям, для встановлення простих, надійних та відповідних біомаркерів, які можна використовувати для терапевтичних втручань серед пацієнтів з ПТСР "

Вступ. Зростання поширеності посттравматичного стресового розладу (ПТСР) через війну в Україні набуває все більшого значення, оскільки цей розлад є серйозним чинником ризику зловживання алкоголем/наркотиками, депресії, самогубства та функціональних порушень роботи внутрішніх органів, зокрема серцево-судинної, ендокринної, імунної систем. Також ПТСР може призводити до соціальної дизадаптації в сім'ї та на роботі. Таким чином ПТСР дуже важливою соціальною проблемою в Україні, спричиняє значні економічні збитки, оскільки більшість пацієнтів з ПТСР є молодими та працездатними.

Метою цієї статті є опис дослідження, його організації, методології та майбутніх перспектив для проекту "Дослідження фізіологічних та біохімічних параметрів, які пов'язані зі здоров'ям, для встановлення простих, надійних та відповідних біомаркерів, які можна використовувати для лікування пацієнтів з ПТСР".

Методи. Дослідження часових та частотних параметрів ВСР проводилося за допомогою короткотривалих записів ЕКГ (5 хвилин у положенні лежачи та 6 хвилин у положенні стоячи), для запису використовувався цифровий електрокардіограф "КАРДІОЛАБ" (Харків, Україна) із відповідним програмним забезпеченням.

Результати. Характер змін ВСР вказує на пригнічення активності парасимпатичної нервової системи, низьку загальну спектральну потужність та переважання симпатичного контролю в автономній нервовій системі у пацієнтів з ПТСР.

Висновки. Наразі немає ефективного лікування ПТСР, а ефективність найкращих клінічних методів не перевищує 50%. Реалізація клінічного дослідження, описаного у цій статті, допоможе з'ясувати механізми, що лежать в основі системних наслідків ПТСР, допоможе підібрати методи лікування ПТСР, ефективність яких дозволить з'ясувати наступні клінічні дослідження.

Ключові слова: посттравматичний стресовий розлад (ПТСР), Україна, війна, варіабельність серцевого ритму (ВСР), імунна система, ендокринна система, автономна нервова система, клінічне дослідження.

An exploratory study of physiological and biochemical parameters to identify simple, robust and relevant biomarkers for therapeutic interventions for PTSD: study rationale, key elements of design and a context of war in Ukraine

Viktoria Serhiyenko[1], Klaus Holzmann[2], Serhii Holota[3,4], Zenovii Derkach[5], Armen Nersesyan[2], Stanislav Melnyk[6], Oleksandra Chernysh[7], Ostap Yatskevych[8], Miroslav Mišík[2], Volodymyr Bubalo[9], Olha Strilbytska[10], Bohdana Vatseba[11], Oleh Lushchak[10], Siegfried Knasmüller[2], and Andriy Cherkas[12†]

[1] *Department of Endocrinology, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine*
[2] *Center for Cancer Research, Medical University of Vienna, Vienna, Austria*
[3] *Department of Pharmaceutical, Organic and Bioorganic Chemistry, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine*
[4] *Department of Organic Chemistry and Pharmacy, Lesya Ukrainka Volyn National University, Lutsk, Ukraine*
[5] *Military Medical Clinical Center of the Western region, Lviv, Ukraine*
[6] *Faculty of Medicine #1, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine*
[7] *University of Montpellier, Montpellier, France*
[8] *Department of Internal Medicine #1, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine*
[9] *L.I. Medved's Research Center of Preventive Toxicology, Food and Chemical Safety, MHU, Kyiv, Ukraine*
[10] *Vasyl Stefanyk Precarpathian National University, Ivano-Frankivsk, Ukraine*
[11] *Ivano-Frankivsk National Medical University, Ivano-Frankivsk, Ukraine*
[12] *Former Department of Internal Medicine #1, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine*

†Present address: Team Early Projects Type 1 Diabetes, Therapeutic Area Diabetes and Cardiovascular Medicine, Research & Development, Sanofi -Aventis Deutschland GmbH., Industriepark Höchst-H831, Frankfurt am Main, Germany

OPEN ACCESS

DOI: 10.25040/ntsh2022.02.14

For correspondence: Department of Endocrinology, Danylo Halytsky Lviv National Medical University, str. Ostroz'koho 1, Lviv, Ukraine, 79010

Twitter: @VictoriyaSerh
E-mail: serhiyenkov@gmail.com

Received: 15 Oct, 2022
Accepted: 14 Dec, 2022
Published: 30 Dec, 2022

ORCID IDs
Viktoria Serhiyenko: https://orcid.org/0000-0002-6414-0956
Klaus Holzmann: https://orcid.org/0000-0003-4077-3377
Serhii Holota: https://orcid.org/0000-0002-9892-437X
Zenovii Derkach: https://orcid.org/0000-0001-6195-8456
Armen Nersesyan: https://orcid.org/0000-0001-5571-7904
Stanislav Melnyk: https://orcid.org/0000-0002-6156-5000
Oleksandra Chernysh: https://orcid.org/0000-0003-0737-274X
Ostap Yatskevych: https://orcid.org/0000-0001-5146-2553
Miroslav Mišík: https://orcid.org/0000-0002-8182-3695
Volodymyr Bubalo: https://orcid.org/0000-0003-0230-9354
Olha Strilbytska: https://orcid.org/0000-0003-3277-2294
Bohdana Vatseba: https://orcid.org/0000-0002-5794-8754
Oleh Lushchak: https://orcid.org/0000-0002-4627-1987
Siegfried Knasmüller: https://orcid.org/0000-0002-1638-4438
Andriy Cherkas: https://orcid.org/0000-0002-6652-6983

Disclosures: The authors declared no conflict of interest.

Author Contributions:
Conceptualization: Viktoria Serhiyenko, Klaus Holzmann, Serhii Holota, Oleh Lushchak, Siegfried Knasmüller, Andriy Cherkas;
Results of study: Zenovii Derkach, Ostap Yatskevych, Stanislav Melnyk;
Writing: Viktoria Serhiyenko, Stanislav Melnyk, Andriy Cherkas;
Review, editing and approval: All authors.

Ethical approval: The bioethics committee of Danylo Halytsky Lviv National Medical University, protocol № 9 of 22.11.2021.

Funding: Bilateral Austrian-Ukrainian project No. UA 08/2020 of the Austrian Federal Ministry of Education, Science and Research „Exploratory study of health related physiological and biochemical parameters to identify simple, robust and relevant biomarkers for therapeutic interventions for PTSD patients".

Introduction. The incidence of post-traumatic stress disorder (PTSD) is increasing due to war in Ukraine is of growing importance as this disorder is a serious risk factor for alcohol/drug abuse, depression, suicide and functional dysregulation of internal organs, in particular cardiovascular, endocrine, immune systems. PTSD can also lead to social inadaptation in family and at work. This makes PTSD a very important social problem in Ukraine that causes significant economic damage, since most of the PTSD patients are young and in working age.

Objectives. The purpose of this paper is to describe the rationale, key elements of design, methodology and future prospects of the ongoing project "Exploratory study of health related physiological and biochemical parameters to identify simple, robust and relevant biomarkers for therapeutic interventions for PTSD patients".

Methods. Short time ECG records (5 minutes in supine position and 6 minutes in orthostatic test) recorded by "CARDIOLAB" (Kharkiv, Ukraine) digital ECG device with software to analyze HRV was used to calculate time-domain and frequency-domain parameters of HRV.

Results. The pattern of HRV changes indicates suppression of parasympathetic activity, low overall spectral power and dominance of sympathetic branch of autonomic nervous system in participants with PTSD.

Conclusions. PTSD has no effective treatment so far and the best clinical practices rarely reach efficacy of 50%. The realization of the exploratory clinical study described in this paper will provide insights in the mechanisms underlying systemic consequences of PTSD and will build the evidence enabling clinical trial(s) for studying treatment intervention for patients with PTSD.

Keywords: Post-traumatic stress disorder (PTSD), Ukraine, war, heart rate variability (HRV), immune system, endocrine system, autonomic nervous system, clinical study.

Introduction

Since the 24th of February 2022 the outbreak of the full-scale invasion phase of the war of Russia against Ukraine caused severe level of distress in the general population. The largest war in Europe since the World War II is associated with the use of modern heavily destructive weaponry, is fully covered in conventional and the social media. Exposure to a high degree of violence, continuous threat of terrorist rocket/drone attacks even in remote locations with regard of active battlefield, destruction of energy generation and supply infrastructure reinforced by unprecedented level of disinformation and propaganda from the Russian side creates the perfect background for development of mental disorders referred to as posttraumatic stress disorder (PTSD) at the level of whole country. This takes place in already compromised population due to more latent war in the East of Ukraine which started in 2014, occupation of some areas of Donetsk and Luhansk regions and occupation with annexation of Crimean Peninsula by Russia, COVID-19 pandemic and related economic crisis. The acute phase of the war causes severe suffering and distress, at the same time it presents itself an exceptional opportunity to observe and scientifically assess the changes taking place as the part of natural experiment.

Severe distress due to ongoing Russian military aggression in the East of Ukraine started in 2014. Millions of citizens were exposed to various traumatic factors. These include being hit by war either directly (military personnel, civil populations in cities and villages that were or remain a battlefield, volunteers, medical personnel, journalist), or indirectly (e.g. forced resettling to unaffected areas very often with families, death or injuries of family member(s), relatives and/or friends, loss of properties, jobs, source of income, limited access to basic goods and supplies, lack of electricity and heating due to damage to infrastructure and other factors). According to current estimates about 5 to 10% of military personnel have subthreshold or probable PTSD in time of peace[1] and 20-30% of soldiers participating in the war suffer from PTSD that makes up hundreds of thousands affected subjects among military staff alone, while among affected civilians PTSD may be as high as 32%[2]. This may increase the number of patients with PTSD to the numbers of tens of millions in Ukraine. PTSD affects also Russian soldiers and officers, as well as, although in lesser degree, general population.

At the global scale PTSD is an emerging challenge as the COVID-19 pandemic caused a significant distress in large population groups, primarily affected patients themselves and healthcare workers on a frontline (medical doctors, nurses, paramedics etc.) as well as in members of other professions such as su-

permarket cashiers, bus and taxi drivers, police officers and many others[3]. There is also increasing evidence that extended lockdowns often accompanied with stress related to loss of income (economic crisis) and more recently acceleration of inflation may contribute to long term deteriorations of mental health in vulnerable populations including young individuals, women, etc. [4]. These factors make PTSD a global challenge.

Although PTSD has a high prevalence in Ukraine, the awareness of this serious disorder remains rather limited and up-to-date diagnostic and treatment options are often not available. The issue of massive PTSD rise is of growing importance as this disorder is a serious risk factor for alcohol/drug abuse[5], depression, suicide and functional dysregulation of internal organs, in particular cardiovascular [6,7], endocrine [8], immune[9,10] systems. PTSD can also lead to social inadaptation in family and at work [11]. This makes PTSD a very important social problem in Ukraine that influences its defense capacity and causing significant economic damage, since most of the PTSD patients are young and in working age.

Scientific rationale

Despite availability of multiple pharmacological and non-pharmacological methods that have been used to treat patients with PTSD there is no effective treatment that could be widely used so far. Therefore, there exist a huge unmet medical need [12]. This is, to large extend, due to very limited knowledge on systemic effects of PTSD that include persistent activation of sympathetic nervous system (psycho-emotional stress) that leads to deteriorations in immune system, increases release of stress hormones (catecholamines, corticosteroids) and causes changes in metabolism clearly demonstrated in a detailed metabolomic study in US military veterans with PTSD [13]. Very close and highly regulated interactions between nervous, immune system and metabolism [14] are linking stress to inflammation and to the metabolic syndrome, cancer [15] and other conditions [16]. It is well established that low grade inflammation is associated with metabolic deteriorations. This effect includes dysregulation of neuro-immuno-endocrinological mechanisms of homeostasis and/or redox metabolism [17]. If it is possible to delay these dysregulations at an early stage it will be possible to a) apply interventions to correct existing problem and b) control its efficacy and modify treatment if needed, for example as demonstrated in real clinical situation using interleukin-6 (IL-6) as inflammatory biomarker[18]. Therefore, dissecting these psycho-somatic interactions and identifying early changes in patients is expected to be key not only in more accurate diagnostics but also in search of interventions capable to arrest disease (PTSD) progression and prevent the development of complication and to find the way to bring patients back to normal physiological regulation in a safe way [14].

At present time there is growing interest to apply instrumental methods to obtain robust information about neurological dysregulations. The recent literature consistently indicates that decreased Heart Rate Variability (HRV) is a characteristic feature of PTSD [14,19,20,21]. Our preliminary pilot study showed significantly reduced HRV, particularly high frequency (HF) power in war veterans, who are treated and undergoing rehabilitation in our Military Hospital compared with an age and gender matched control group. It was suggested that reduced HRV is an important part of PTSD, and its determination could be a very informative diagnostic tool [22,23]. Moreover, an improvement of HRV may indicate progress of treatment and could be very sensitive marker [24]. At the same time, lower initial parasympathetic modulation of HRV is associated with increased susceptibility to PTSD as a short and long-term outcome [20].

Recent findings consistently indicate that decreased HRV is a characteristic feature of PTSD [12-15]. Typical patterns include reduced overall variability and time-domain parameters (SDNN and RMSSD), as well as decrease in total spectral power, mainly due high-frequency (HF) and to some extent a low-frequency (LF) oscillations decrease [16,17]. Our preliminary pilot study shows significantly reduced HRV, particularly HF power in Veterans, who are treated and undergoing rehabilitation in our Military Hospital compared to the age and gender matched control group. It was stressed that reduced HRV is an important part of PTSD and that its determination could be a useful diagnostic tool [16,18]. Moreover,

an improvement of HRV indicates progress of treatment and could be very sensitive marker [19]. At the same time, lower initial parasympathetic modulation of HRV is associated with increased susceptibility to PTSD as a short and long-term outcome [20].

Probably most important properties of the HRV approach are its accuracy in assessing the function ("golden standard") of the autonomic nervous system (ANS) and that changes appear very early, when they are still reversible. We showed in our previous studies that HRV correlates with glycated hemoglobin and other glucose metabolism parameters in sedentary young men [25]. Later, we demonstrated that Helicobacter pylori positivity in asymptomatic apparently healthy subjects increases sympathetic activity and heart rate in parallel increased insulin resistance [26]. However, we were unable to demonstrate clinically meaningful differences in hormonal panel, immunological panel (IL-6, IL-10, TNF-α, hsCRP) and oxidative stress biomarkers such as urine levels of iso-prostaglandin F2-α and 1,4-dihydroxynonane mercapturic acid [27].

Telomere instability due to telomere shortening is a recognized risk factor for cancer development and a potential biomarker due to cumulative effects of environmental exposure and life experience, such as stress. A number of other studies indicate that depression and PTSD lead to short leukocyte telomere length (LTL) [28,29]. Recent studies on war veterans and ex-prisoners of war (ex-POWs) found shortening in PBMC telomere lengths [30]. However, earlier studies suffer from methodological limitations due to small sample size and long time-intervals between examination periods.

Another relevant topic which will be addressed covers long term effects which are causally related to damage of genetic material. It is known that DNA instability leads to cancer, degenerative diseases, reduced life span and infertility. A number of earlier studies indicate that depression and other psychiatric diseases lead to damage of genetic material [20,24,25]. However, all earlier studies focused on the detection of DNA breaks which can be repaired and on oxidative stress [24,26]. No information is currently available concerning chromosomal damage (which will be monitored in the present study) a reliable marker of increased cancer risk [27,31,32].

Preliminary data: HRV in veterans with PTSD

Preliminary HRV data based on the ECG records obtained in a small pilot study by the colleagues with a group of 30 male participants with PTSD (military veterans participating in the war since 2014) and compared with 30 healthy male subjects (own historical data from previous studies) is presented in Tables 1 to 4. As it can be seen in the Table 1 participants with PTSD have significantly increased heart rate (HR) and decreased majority of HRV indices. This is consistent with our expectations and confirms our predictions. Despite limitations due to low sample size and imperfect match in terms of age and background health state (participants with PTSD were hospitalized due to other reasons) the pattern of HRV changes clearly indicates suppression of parasympathetic activity, low overall spectral power and dominance of sympathetic branch of autonomic nervous system in participants with PTSD.

As it was shown in the Table 1 HR is significantly higher in PTSD patients and SDNN, RMSSD,

Table 1

Time domain characteristics of HRV in supine position in participants with PTSD and healthy volunteers (data presented as Mean ± Standard Deviation)[1]

	PTSD, n=30	Control, n=30	p-value
HR, bpm	80.9±15.0	61.9±8.0	<0.001
SDNN, ms	31.9±13.6	59.4±34.9	<0.001
RMSSD, ms	24.0±16.4	54.6±46.4	0.001
pNN50, %	5.7±10.7	24.3±21.6	<0.001
CV, %	4.1±1.6	6.0±3.6	<0.001

[1]Abbereviations: HR – heart rate, SDNN – standard deviation of normal RR intervals; RMSSD – the square root of mean squared differences of successive RR interval; pNN50 – the percentage of differences between adjacent normal RR intervals exceeding 50 milliseconds, CV – coefficient of variability.

pNN50 and CV are higher in controls. pNN50 is particularly sensitive marker of parasympathetic activity which clearly is suppressed in PTSD patients.

In healthy subjects average ratio of VLF, LF and HF approximately 1:1:1, but in participants with PTSD clear reduction of HF and total spectral power (TP), signs of reduced parasympathetic activity was demonstrated (Table 2).

During orthostatic test time-domain characteristics in participants with PTSD are generally reduced, particularly pNN50 that is close to 0 that indicates low parasympathetic tone. In healthy subjects (control group) parasympathetic tone is substantially higher. This indicates reduced adaptation capacity in participants with PTSD.

Changes in spectral characteristics of HRV in orthostatic test also reflects the pattern of the changes in the supine position: TP is significantly lower in PTSD group, and, as demonstrated by HF higher parasympathetic tone in the healthy subjects' group. These results,

Table 2

Frequency-domain characteristics of HRV in supine position in participants with PTSD and healthy volunteers (data presented as Mean ± Standard Deviation)[1]

	PTSD, n=30	Control, n=30	p-value
TP, ms^2	1246.0±795.3	3248.2±2251.2	<0.001
VLF, ms^2	478.0±302.1	1052.7±576.1	<0.001
LF, ms^2	479.6±413.2	1089.4±905.4	<0.001
HF, ms^2	288.5±316.8	1106.1±1220.7	<0.001
LF/HF	2.9±3.1	1.8±1.5	<0.001

Abbreviations: TP - total power (0.01 to 0.40 Hz), HF – high frequency power (0.15 to 0.40 Hz), LF – low frequency power (0.04 to 0.15 Hz), and VLF – very low frequency power (0.01 to 0.04 Hz), LF/HF – the ratio of low frequency and high frequency spectral power.

Table 3

Time domain characteristics of HRV during orthostatic tests in patients with PTSD and healthy volunteers (data presented as Mean ± Standard Deviation)[1]

	PTSD, n=30	Control, n=30	p-value
HR, bpm	104.5±19.1	76.8±15.7	<0.001
SDNN, ms	31.5±24.7	52.9±15.8	0.02
RMSSD, ms	17.9±31.3	23.5±10.2	<0.001
pNN50, %	0.5±1.2	5.8±7.0	<0.001
CV, %	5.2±4.0	6.8±1.6	<0.001

[1]Abbereviations: HR – heart rate, SDNN – standard deviation of normal RR intervals; RMSSD – the square root of mean squared differences of successive RR interval; pNN50 – the percentage of differences between adjacent normal RR intervals exceeding 50 milliseconds, CV – coefficient of variability.

Table 4

Frequency-domain characteristics of HRV in supine position in patients with PTSD and healthy volunteers (data presented as Mean ± Standard Deviation)[1]

	PTSD, n=30	Control, n=30	p-value
TP, ms^2	974.3±625.7	3506.0±2086.6	<0.001
VLF, ms^2	495.1±400.4	1597.4±1527.0	<0.001
LF, ms^2	403.6±311.7	1584.8±970.6	<0.001
HF, ms^2	75.5±70.5	323.8±248.4	<0.001
LF/HF	8.0±6.4	7.4±5.7	0.6

[1]Abbreviations: TP - total power (0.01 to 0.40 Hz), HF – high frequency power (0.15 to 0.40 Hz), LF – low frequency power (0.04 to 0.15 Hz), and VLF – very low frequency power (0.01 to 0.04 Hz), LF/HF – the ratio of low frequency and high frequency spectral power.

however, should be regarded caution, as PTSD and healthy control groups were not perfectly matched by age and baseline characteristics. In present study HRV parameters will be evaluated thoroughly with appropriate selection of participants into PTSD and control group and both groups will be matched.

Scientific novelty and hypothesis

There are two aspects regarding scientific novelty of the study. First, a complex approach to PTSD as a multisystemic disease with its neurological, immunological, and metabolic components that will be addressed by state-of-the-art functional measurements such as HRV reflecting ANS function, continuous glucose monitoring (CGM) functional measure of metabolic regulation and a complex of highly informative immunological parameters in order to identify associations between them that may be relevant for understanding neuro-psycho-somatic interactions in the context of PTSD as well as other related disorders (Figure 1). To our best knowledge, this will be the first attempt to characterize thoroughly complexity of PTSD and its neuro-psycho-somatic manifestations.

Second, the search for a pathophysiologic neuro-immuno-metabolic pattern is expected to allow the identification of potential targets for intervention. In case of ANS dysfunction as a leading pathophysiological mechanism – behavioral (for example breathing biofeedback therapy/training) and pharmacological approaches may be applicable. Metabolic correction (in case found to be dominant) may include lifestyle modifications – exercise therapy, healthy individualized nutritional recommendations as well as possible pharmacological correction (sodium-glucose transporter 2 inhibitors, glucagon-like peptide-1 receptor agonists). Therefore, we will be able to go beyond static approach as in the prototype metabolomic study [13] considering highly dynamic changes in metabolites levels, particularly glucose [17]. Immunological aspects are missing in other neuro-psycho-metabolic studies. Therefore, balanced coverage of all three vectors in pathogenesis of PTSD and development of its long-term complications makes our approach novel (regulatory triangle neuro-immuno-endocrinology, Fig. 1).

Our scientific hypothesis is novel on the assumption of reduced HRV indices association with deteriorations/shifts in metabolic, immunologic parameters, telomere length and biomarkers of DNA damage. The extend and

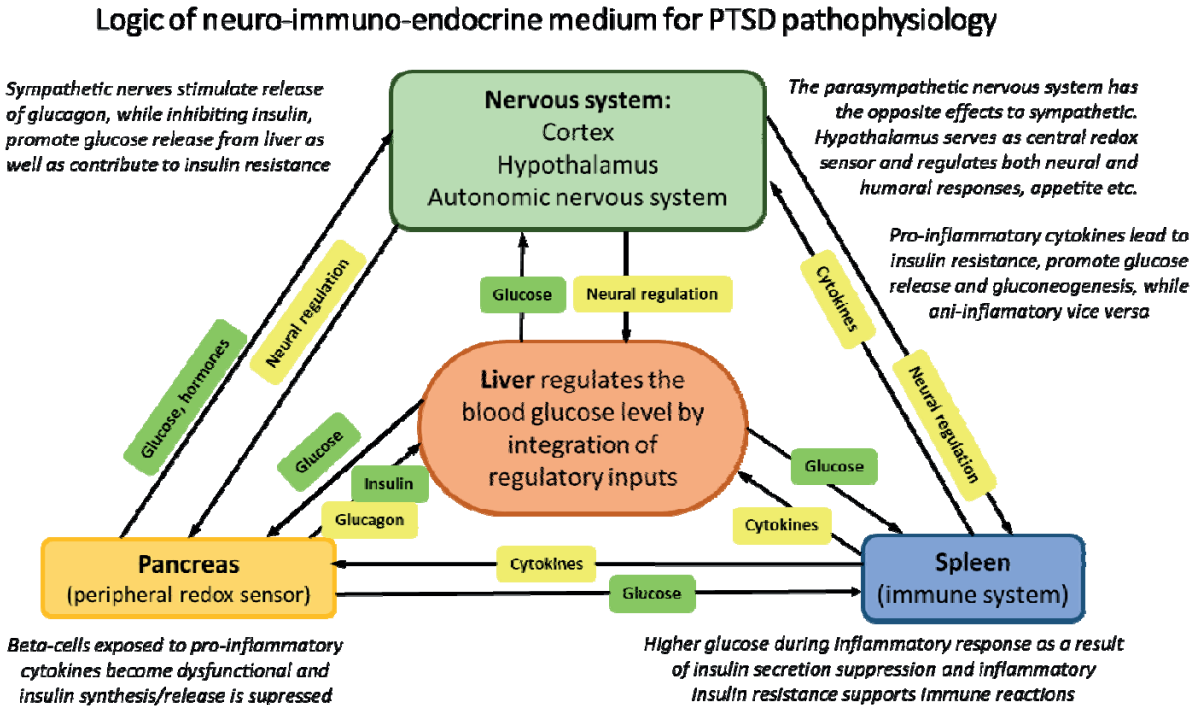

Figure 1. Nervous, immune and endocrine systems regulate metabolism with multiple feedback loops (adapted from [17])

directions of these relationships will be established in the study.

Methodology and design of the study

The study will be realized in agreement with the standards of Good Clinical Practice (GCP) and Good Laboratory Practice (GLP)[33]. The protocol of the study, subjects informed consent form was approved by ethical committee of Lviv National Medical University (protocol № 9 of 22.11.2021).

At the time of manuscript drafting the preparation for the start of recruitment of patients (Military Medical Clinical Center of the Western region, Lviv, Ukraine) and matched healthy volunteers took place under difficult circumstances. The following enrichment strategies are implemented to enroll a homogenous groups of participants with PTSD and healthy volunteers: a) only males with age interval 18-50 years; b) BMI index under 19,0 and above 30,0 will be excluded; c) any heart rhythm disorders, clinically significant heart disease, diabetes mellitus type 1 and 2, chronic organ failure (respiratory, heart, kidney, liver) will be excluded; d) screened patients will undergo detailed psychiatric examinations in order to identify patients with overt PTSD and patients with no signs of PTSD in order to be attributed to respective study group.

After a subject has signed the ICF and meets the eligibility criteria, he will undergo diagnostic and laboratory set of tests, CGM, HRV immunological/inflammatory panel. Data will be collected as one timepoint only f. Statistical analysis plan includes basic descriptive statistics, correlation matrix, parametric (t-test) and nonparametric statistics (Mann-Whitney U-test, $\chi 2$-test or Fisher´s test) depending on nature of variables and normal/non-normal distribution (based on Shapiro-Wilk test). Calculated sample size sufficient to provide the needed power (>80% for exploratory study) is approximately 40 subjects per group. Therefore, it is planned to include 45±5 subjects/group that will fit well with ELISA kits (96 wells designed for 90 measurements and 6 for standard curve) and is consistent with the design of previous similar studies[13].

PTSD diagnosis will be confirmed by a professional certified psychiatrist according to Diagnostic and Statistical Manual for Mental Disorders, 5th Edition (DSM5), International Disease Classification- 10th edition (ICD-10) and the Mississippi scale for evaluation of posttraumatic reactions. Short time ECG records (5 minutes in supine position and 6 minutes in orthostatic test) recorded by "CARDIOLAB" (Kharkiv, Ukraine) digital ECG device with software to analyze HRV will be used to calculate time-domain and frequency-domain parameters of HRV as described here [25,26].

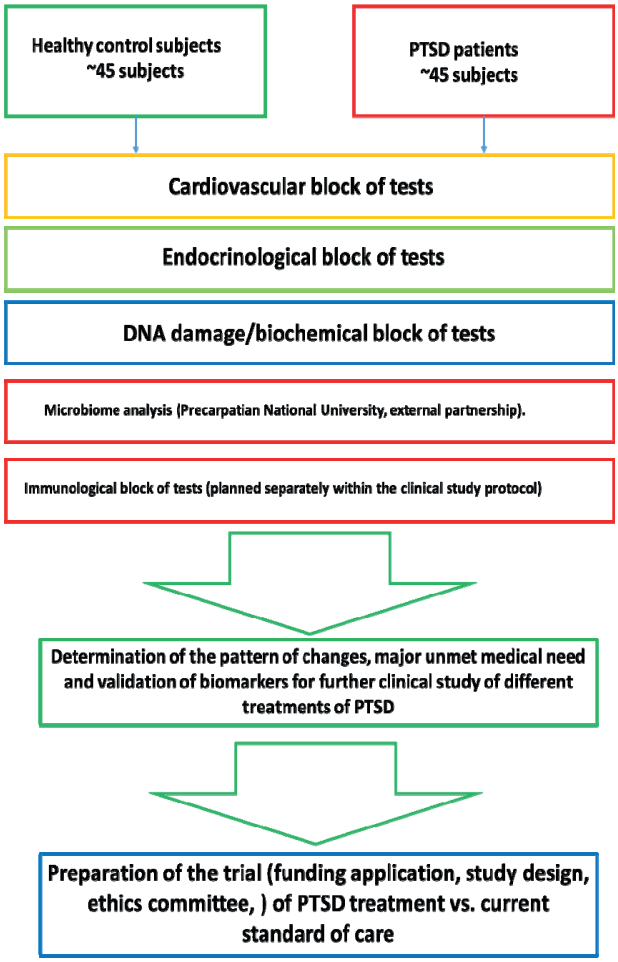

Figure 2. The exploratory study of reliable physiological and biochemical biomarkers of PTSD beyond psychiatric/psychological changes will enable better understanding of somatic changes occurring in these patients as well as will build up a robust diagnostic platform for further clinical trials to test applicability and efficacy of different treatments of PTSD

ELISA-based studies will be performed by an experienced clinical laboratory expert. The list of parameters include hsCRP (high sensitivity C-reactive protein), a panel of pro-inflam-

matory and anti-inflammatory cytokines/interleukins (ILs) IL-1-beta, IL-6, IL-10, Tumor Necrosis Factor-α (TNF- α), interferons -α and -γ, C-peptide. A two-week continuous glucose monitoring (CGM) will be performed with the use of Free Style Libre 3 CGM system (Abbot Diabetes Care, Alameda, CA, USA).

To study structural and numerical chromosomal aberrations, a specific technique will be employed in which micro-nuclei (DNA containing extranuclear bodies) will be scored in exfoliated cells obtained from oral cavity. In addition, also other nuclear anomalies will be recorded which are indicative impaired cell division and acute cytotoxicity [34-35]. This approach has been used successfully in the past to assess health risks (cancer risks) caused by environmental and occupational exposures [32,36-38]. Study of telomere length of bulk PMNC is a specific qPCR technique applied on DNA isolated from blood cells. The method is standardized and has been used successfully to assess tissue and blood cells [39-40].

Discussion

Since there is no effective standard treatment of PTSD available for clinicians, the establishment of the pattern of clinical and laboratory changes in PTSD patients is important as allows to assess interactions within the pathophysiological regulatory triangle (Figure 3). This will enable the identification of the most sensitive objective biomarkers characterizing the severity of the clinical condition of the patients and prediction of short- and long-term complications of the disease. This in turn will help to identify most significant health risks and unmet needs to address it in the clinical trials of treatment interventions that will be elaborated during this project depending on the evidence that will be generated. An ultimate long-term goal to bring new effective (and possibly personalized) therapies to patients particularly to those who sacrificed their health to protect us from insidious enemy.

The realization of the project will provide reliable information concerning the detection of increased health risks in PTSD patients which are related to dysregulation of immune system and metabolism changes. This is important as such alterations are related to long-term adverse health effects have not been studied. The results will also provide insights if the current treatment strategies are relevant in regard to the improvement of the health conditions of PTSD patients (Figure 3).

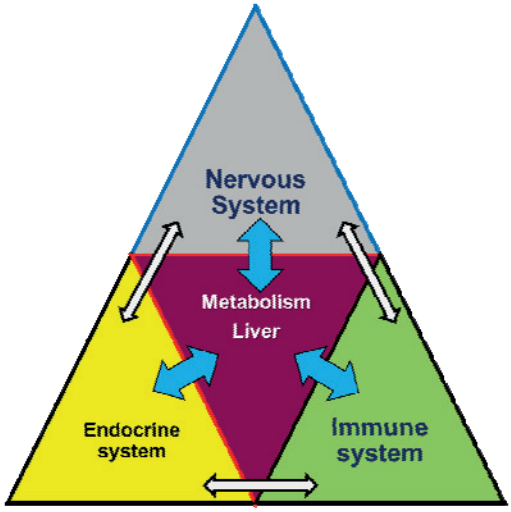

Figure 3. Schematic diagram showing the integration of regulatory interactions between nervous, endocrine, immune systems and metabolism [41]. Arrows show bidirectional influences between integral parts of regulatory circuits. Damage of one compartment leads to adjacent changes or adaptations in all others. The variety of multi-level regulatory mechanisms ensures stability of the system under extreme circumstances

Another practical aspect of the study is HRV, and CGM in this group of patients. Neither of these highly informative functional methods is used as a standard of care in current clinical practice by psychiatrist and military physician in Ukraine.

To our best knowledge no similar studies are performed and/or planned in Ukraine. We will be able to apply dynamic approach by using HRV and CGM to monitor glucose in contrast to static approach used in a recent metabolomic study [13] considering highly dynamic changes in metabolites levels, particularly glucose [17]. This will be first study of this kind and it will provide important information for planning studies of clinical interventions in future.

In conclusions: The medical, social and economic burden of PTSD in Ukraine is massive due to the war with Russia, and it is also a global problem in a post-COVID-19 world [3]. This medical problem has no effective treatment so far and the best clinical practices

rarely reach efficacy of 50%. The realization of the exploratory clinical study described in this paper will provide insights in the mechanisms underlying systemic consequences of PTSD and will build the evidence enabling clinical trial(s) for studying treatment intervention for patients with PTSD.

Acknowledgments
Funding: The study was supported by the Bilateral Austrian-Ukrainian project No. UA 08/2020 of the Austrian Federal Ministry of Education, Science and Research „Exploratory study of health related physiological and biochemical parameters to identify simple, robust and relevant biomarkers for therapeutic interventions for PTSD patients ".

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee of Danylo Halytskyi National Medical University (protocol № 9, 22.11.2021).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

References

1. Fink, D. S. et al. Subthreshold PTSD and PTSD in a prospective-longitudinal cohort of military personnel: Potential targets for preventive interventions. Depress Anxiety 35, 1048-1055, doi:10.1002/da.22819 (2018).
2. Богомолець О, Пінчук І, Ладик-Бризгалова А. Поширеність та структура посттравматичних психічних порушень в учасників бойових дій. Архів психіатрії 2016:11-5.
3. Rogers, J. P. et al. Psychiatric and neuropsychiatric presentations associated with severe coronavirus infections: a systematic review and meta-analysis with comparison to the COVID-19 pandemic. Lancet Psychiatry, doi:10.1016/S2215-0366(20)30203-0 (2020).
4. Marazziti, D. et al. Impact of economic crisis on mental health: a 10-year challenge. CNS Spectr, 1-7, doi:10.1017/S1092852920000140 (2020).
5. Hruska, B., Pacella, M. L., George, R. L. & Delahanty, D. L. The Association Between Daily PTSD Symptom Severity and Alcohol-Related Outcomes in Recent Traumatic Injury Victims. Psychol Addict Behav, doi:10.1037/adb0000262 (2017).
6. Shah, A. J. et al. Posttraumatic stress disorder and impaired autonomic modulation in male twins. Biol Psychiatry 73, 1103-1110, doi:10.1016/j.biopsych.2013.01.019 (2013).
7. Chalmers, J. A., Quintana, D. S., Abbott, M. J. & Kemp, A. H. Anxiety Disorders are Associated with Reduced Heart Rate Variability: A Meta-Analysis. Front Psychiatry 5, 80, doi:10.3389/fpsyt.2014.00080 (2014).
8. Somvanshi, P. R. et al. Mechanistic inferences on metabolic dysfunction in posttraumatic stress disorder from an integrated model and multiomic analysis: role of glucocorticoid receptor sensitivity. Am J Physiol Endocrinol Metab 317, E879-E898, doi:10.1152/ajpendo.00065.2019 (2019).
9. Wang, W. et al. Characteristics of pro- and anti-inflammatory cytokines alteration in PTSD patients exposed to a deadly earthquake. J Affect Disord 248, 52-58, doi:10.1016/j.jad.2019.01.029 (2019).
10. Kim, T. D., Lee, S. & Yoon, S. Inflammation in Post-Traumatic Stress Disorder (PTSD): A Review of Potential Correlates of PTSD with a Neurological Perspective. Antioxidants (Basel) 9, doi:10.3390/antiox9020107 (2020).
11. Vogt, D. et al. Consequences of PTSD for the work and family quality of life of female and male U.S. Afghanistan and Iraq War veterans. Soc Psychiatry Psychiatr Epidemiol 52, 341-352, doi:10.1007/s00127-016-1321-5 (2017).
12. Bersani, F. S. et al. Novel Pharmacological Targets for Combat PTSD-Metabolism, Inflammation, The Gut Microbiome, and Mitochondrial Dysfunction. Mil Med 185, 311-318, doi:10.1093/milmed/usz260 (2020).
13. Mellon, S. H. et al. Metabolomic analysis of male combat veterans with post traumatic stress disorder. PLoS One 14, e0213839, doi:10.1371/journal.pone.0213839 (2019).
14. Thome, J., Densmore, M., Frewen, P. A., McKinnon, M. C., Théberge, J., Nicholson, A. A., Koenig, J., Thayer, J. F., & Lanius, R. A. (2017). Desynchronization of autonomic response and central autonomic network connectivity in posttraumatic stress disorder: CAN Connectivity and HRV in PTSD. Human Brain Mapping, 38(1), 27–40. https://doi.org/10.1002/hbm.23340
15. Dibaba, D. T. et al. Association between obesity and biomarkers of inflammation and metabolism with cancer mortality in a prospective cohort study. Metabolism 94, 69-76, doi:10.1016/j.metabol.2019.01.007 (2019).
16. Wang, A., Luan, H. H. & Medzhitov, R. An evolutionary perspective on immunometabolism. Science 363, doi:10.1126/science.aar3932 (2019).
17. Cherkas, A., Holota, S., Mdzinarashvili, T., Gabbianelli, R. & Zarkovic, N. Glucose as a Major Antioxidant: When, What for and Why It Fails? Antioxidants (Basel) 9, doi:10.3390/antiox9020140 (2020).

18. Amitai, M. et al. Increased circulatory IL-6 during 8-week fluoxetine treatment is a risk factor for suicidal behaviors in youth. Brain Behav Immun, doi:10.1016/j.bbi.2019.12.017 (2019).
19. Dennis, P. A. et al. Trauma and Autonomic Dysregulation: Episodic - Versus Systemic - Negative Affect Underlying Cardiovascular Risk in Posttraumatic Stress Disorder. Psychosom Med, doi:10.1097/PSY.0000000000000438 (2016).
20. Shaikh al arab, A., Guédon-Moreau, L., Ducrocq, F., Molenda, S., Duhem, S., Salleron, J., Chaudieu, I., Bert, D., Libersa, C., & Vaiva, G. (2012). Temporal analysis of heart rate variability as a predictor of post traumatic stress disorder in road traffic accidents survivors. Journal of Psychiatric Research, 46(6), 790–796. https://doi.org/10.1016/j.jpsychires.2012.02.006
21. Minassian, A. et al. Heart rate variability characteristics in a large group of active-duty marines and relationship to posttraumatic stress. Psychosom Med 76, 292-301, doi:10.1097/PSY.0000000000000056 (2014).
22. Chang, H. A. et al. Decreased cardiac vagal control in drug-naive patients with posttraumatic stress disorder. Psychiatry Investig 10, 121-130, doi:10.4306/pi.2013.10.2.121 (2013).
23. Cohen, H. & Benjamin, J. Power spectrum analysis and cardiovascular morbidity in anxiety disorders. Auton Neurosci 128, 1-8, doi:10.1016/j.autneu.2005.06.007 (2006).
24. Cohen, H., Kotler, M., Matar, M. & Kaplan, Z. Normalization of heart rate variability in post-traumatic stress disorder patients following fluoxetine treatment: preliminary results. Isr Med Assoc J 2, 296-301 (2000).
25. Cherkas, A. et al. The correlations of glycated hemoglobin and carbohydrate metabolism parameters with heart rate variability in apparently healthy sedentary young male subjects. Redox Biol 5, 301-307, doi:10.1016/j.redox.2015.05.007 (2015).
26. Cherkas, A. et al. Helicobacter pylori in sedentary men is linked to higher heart rate, sympathetic activity, and insulin resistance but not inflammation or oxidative stress. Croat Med J 57, 141-149, doi:10.3325/cmj.2016.57.141 (2016).
27. Cherkas, A. et al. A Helicobacter pylori-associated insulin resistance in asymptomatic sedentary young men does not correlate with inflammatory markers and urine levels of 8-iso-PGF2-alpha or 1,4-dihydroxynonane mercapturic acid. Arch Physiol Biochem 124, 275-285, doi:10.1080/13813455.2017.1396346 (2018).
28. O'Donovan, A., Epel, E., Lin, J., Wolkowitz, O., Cohen, B., Maguen, S., Metzler, T., Lenoci, M., Blackburn, E., & Neylan, T. C. (2011). Childhood trauma associated with short leukocyte telomere length in posttraumatic stress disorder. Biological Psychiatry, 70(5), 465–471. https://doi.org/10.1016/j.biopsych.2011.01.03
29. Lindqvist, D., Epel, E. S., Mellon, S. H., Penninx, B. W., Révész, D., Verhoeven, J. E., Reus, V. I., Lin, J., Mahan, L., Hough, C. M., Rosser, R., Bersani, F. S., Blackburn, E. H., & Wolkowitz, O. M. Psychiatric disorders and leukocyte telomere length: Underlying mechanisms linking mental illness with cellular aging. Neuroscience and Biobehavioral Reviews, 55, 333–364. https://doi.org/10.1016/j.neubiorev.2015.05.007 (2015).
30. Jergović, M., Tomičević, M., Vidović, A., Bendelja, K., Savić, A., Vojvoda, V., Rac, D., Lovrić-Čavar, D., Rabatić, S., Jovanovic, T., & Sabioncello, A. Telomere shortening and immune activity in war veterans with posttraumatic stress disorder. Progress in Neuro-Psychopharmacology & Biological Psychiatry, 54, 275–283. https://doi.org/10.1016/j.pnpbp.2014.06.010 (2014).
31. Bonassi, S., Norppa, H., Ceppi, M., Strömberg, U., Vermeulen, R., Znaor, A., Cebulska-Wasilewska, A., Fabianova, E., Fucic, A., Gundy, S., Hansteen, I-L., Knudsen, L.E., Lazutka, J., Rossner, P., Sram, R.J., Boffetta, P. Chromosomal aberration frequency in lymphocytes predicts the risk of cancer: results from a pooled cohort study of 22 358 subjects in 11 countries, *Carcinogenesis* 29, Issue ,1178–1183, https://doi.org/10.1093/carcin/bgn075 (2008)
32. Wultsch, G., Nersesyan, A., Kundi, M., Al-Serori, H., & Knasmüller, S. Induction of chromosomal damage in exfoliated buccal and nasal cells of road markers. Journal of Toxicology and Environmental Health. Part A, 82(17), 969–976. https://doi.org/10.1080/15287394.2019.1673578 (2019).
33. Cherkas, A. Research involving human subjects in Ukraine: How to leverage arising opportunities? Proceedings of the Shevchenko Scientific Society. Medical Sciences, 64(1). https://doi.org/10.25040/ntsh2021.01.02 (2021).
34. Thomas, P., & Fenech, M. (2011). Buccal micronucleus cytome assay. Methods in Molecular Biology (Clifton, N.J.), 682, 235–248. https://doi.org/10.1007/978-1-60327-409-8_17
35. Thomas, P., Holland, N., Bolognesi, C., Kirsch-Volders, M., Bonassi, S., Zeiger, E., Knasmueller, S., & Fenech, M. (2009). Buccal micronucleus cytome assay. Nature Protocols, 4(6), 825–837. https://doi.org/10.1038/nprot.2009.53
36. Bonassi, S., Milić, M., & Neri, M. (2016). Frequency of micronuclei and other biomarkers of DNA damage in populations exposed to dusts, asbestos and other fibers. A systematic review. Mutation Research. Reviews in Mutation Research, 770(Pt A), 106–118. https://doi.org/10.1016/j.mrrev.2016.05.004

37. Menicagli, R., Marotta, O., & Serra, R. (2020). Micronucleus test for diagnosing uncertain cases (BI-RADS 3) in breast cancer screening: A review and preliminary results. The Gulf Journal of Oncology, 1(32), 45–50. https://europepmc.org/article/med/32342918
38. Wultsch, G., Nersesyan, A., Kundi, M., Wagner, K-H., Ferk, F., Jakse, R., Knasmueller, S. Impact of exposure to wood dust on genotoxicity and cytotoxicity in exfoliated buccal and nasal cells, Mutagenesis, Volume 30, Issue 5, September 2015, Pages 701–709, https://doi.org/10.1093/mutage/gev034
39. Wultsch, G., Setayesh, T., Kundi, M., Al-Serori, H., Kreilmeier-Berger, T., Ropek, N., Holzmann, K., Nersesyan, A., & Knasmüller, S. (2018). Impact of extended working periods on genomic and telomeric DNA and on inflammatory markers: Results of an intervention study with office workers and carpenters. Mutation Research. Genetic Toxicology and Environmental Mutagenesis, 836(Pt A), 78–81. https://doi.org/10.1016/j.mrgentox.2018.05.004
40. Sampl, S., Pramhas, S., Stern, C., Preusser, M., Marosi, C., & Holzmann, K. (2012). Expression of telomeres in astrocytoma WHO grade 2 to 4: TERRA level correlates with telomere length, telomerase activity, and advanced clinical grade. Translational Oncology, 5(1), 56–65. https://doi.org/10.1593/tlo.11202
41. Lushchak, O., Strilbytska, O., Koliada, A., & Storey, K. An orchestrating role of mitochondria in the origin and development of post-traumatic stress disorder. Frontiers Physiology, 13, 1094076. doi: 10.3389/fphys.2022.1094076 (2022).