Poznan University of Medical Sciences
Poland

JMS Journal of Medical Science

previously Nowiny Lekarskie

Founded in 1889

2019
Vol. 88, No. 1

QUARTERLY

Indexed in:
Polish Medical Bibliography, Index Copernicus,
Ministry of Science and Higher Education, Ebsco, Google Scholar

eISSN 2353-9801
ISSN 2353-9798

www.jms.ump.edu.pl

SECRETARIAT ADDRESS
27/33 Szpitalna Street
60-572 Poznań, Poland
Phone: +48 618491432, fax: +48 618472685
e-mail: jms@ump.edu.pl
www.jms.ump.edu.pl

DISTRIBUTION AND SUBSCRIPTIONS
70 Bukowska Street, 60-812 Poznań, Poland
Phone/fax: +48 618547414
e-mail: sprzedazwydawnictw@ump.edu.pl

PUBLISHER
Poznan University of Medical Sciences

eISSN 2353-9801
ISSN 2353-9798

Publishing Manager: Grażyna Dromirecka
Technical Editor: Bartłomiej Wąsiel

WYDAWNICTWO NAUKOWE
UNIWERSYTETU MEDYCZNEGO
IM. KAROLA MARCINKOWSKIEGO
W POZNANIU

60-812 Poznań, ul. Bukowska 70
tel./fax: +48 61 854 71 51
www.wydawnictwo.ump.edu.pl

Ark. wyd. 7,6. Ark. druk. 8,5.
Zam. nr 115/19.

The Editorial Board kindly informs that since 2014 *Nowiny Lekarskie* has been renamed to *Journal of Medical Science*.

The renaming was caused by using English as the language of publications and by a wide range of other organisational changes. They were necessary to follow dynamic transformations on the publishing market. The Editors also wanted to improve the factual and publishing standard of the journal. We wish to assure our readers that we will continue the good tradition of *Nowiny Lekarskie*.

You are welcome to publish your basic, medical and pharmaceutical science articles in *Journal of Medical Science*.

Ethical guidelines

The Journal of Medical Science applies the ethical principles and procedures recommended by COPE (Committee on Conduct Ethics), contained in the Code of Conduct and Best Practice Guidelines for Journal Editors, Peer Reviewers and Authors available on the COPE website: https://publicationethics.org/resources/guidelines

CONTENTS

ORIGINAL PAPER

REVIEW PAPER

THOUSAND WORDS ABOUT...

THE RATIONALE, DESIGN AND METHODS OF NEW STUDIES

ORIGINAL PAPER

DOI: https://doi.org/10.20883/jms.330

Comparison of beta$_2$-adrenergic and hyperemia-induced arterial vasodilation assessed by digital pulse contour analysis

Andrzej Wykretowicz[a], Karolina Adamska[b], Przemysław Guzik[c], Marcin Zwanzig[d], Mateusz Dziarmaga[e], Tomasz Krauze[f]

Department of Internal Medicine, Division of Cardiology-Intensive Therapy, Poznan University of Medical Sciences, Poland

[a] https://orcid.org/0000-0001-9545-1629
[b] https://orcid.org/0000-0003-1751-5951
[c] https://orcid.org/0000-0001-9052-5027
[d] https://orcid.org/0000-0002-7909-7428
[e] https://orcid.org/0000-0001-5375-770X
[f] https://orcid.org/0000-0002-7675-711X

ABSTRACT

Introduction. The Reflection Index (RIDVP) derived from digital volume pulse (DVP) analysis has proved to be useful in the assessment of endothelium-dependent vasodilation induced by albuterol. Little is known of the effect of shear-stress-induced vasorelaxation on RIDVP.

Material and Methods. Thirty three healthy volunteers (22 females, 11 males, mean age 57 yrs) were recruited. Assessment of endothelium-dependent vasorelaxation was performed by the analysis of digital volume pulse after albuterol challenge or locally-induced hyperemia.

Results. he hyperemia-induced vasodilation led to a significant decrease of RI_{DVP} in comparison with the values obtained at rest (ΔRI_{Hyper} 69 ± 2 % vs 64 ± 2, p < 0.0001). Similarly albuterol administration resulted in a significant drop in RI_{DVP} (ΔRI_{Alb} 71 ± 2 % vs 67 ± 2 %, p < 0.0001). There was no significant difference between ΔRI_{Hyper} and ΔRI_{Alb} (5.2 ± 0.8 % vs 4.6 ± 1.0 %, p = 0.61). We observed a significant correlation between the small vessel reaction in response to albuterol or hyperemia (r = 0.52, p = 0.01).

Conclusions. Our study demonstrated that hyperemia-induced changes in the Reflexion Index derived from the digital volume pulse are similar to those observed after albuterol-challenge and both are correlated.

Keywords: endothelial-dependent vasodilation, digital volume pulse, albuterol.

Introduction

Endothelial dysfunction is an early hallmark of a variety of arterial injuries including those from hypercholesterolemia, smoking, hypertension, diabetes and atherosclerosis [1, 2, 3]. A host of substances, including nitric oxide released by endothelium, are responsible for arterial vasodilation. Therefore assessment of endothelial vasomotor function is one of the most popular ways of obtaining an insight into the "global endothelial health". Endothelial dysfunction is a systemic disorder and can be measured in various vascular beds [4, 5]. Currently, endothelial testing is based on either the administration of vasoactive substances or an increasing shear-stress in the artery, followed by assessment of the subsequent vasodilation [6, 7].

The digital volume pulse (DVP) is obtained by measuring the transmission of infrared light through the finger. Pulse contour analysis of the waveform obtained lead to the establishment of two indices, one corresponding to the large artery stiffness (the SI_{DVP} — stiffness index) and another (the RI_{DVP} — reflexion index) which is related to

the tone of small arteries [8, 9]. Recently, Chowienczyk et al. [10] demonstrated that albuterol (a beta$_2$-adrenergic agonist) affects the RI_{DVP} which is in part, mediated through the nitric oxide pathway. This suggests that measurement of albuterol-induced changes in DVP may be useful in the evaluation of endothelium-dependent arterial vasodilation.

The brief period of arterial ischemia evoked by inflation of the sphygmomanometric cuff and subsequent cuff deflation is followed by shear-stress- induced NO generation and hence endothelium-dependent vasodilation. This flow-mediated vasodilation is frequently used for the assessment of endothelium vasomotor function [11]. We therefore, set out to compare albuterol-induced changes in Reflexion Index of DVP with those induced by local reactive hyperemia.

Material and Methods

Thirty three healthy people were recruited by means of advertising localy. The participants were normotensive and none were taking any medication at the time of the study. The clinical characteristics of the study group are given in **Table 1**. All the volunteers had a normal resting ECG and no cardiovascular and respiratory abnormalities were detected on physical examination. All patients gave their written informed consent before entering the study and the institutional ethics committee approved the study protocol.

Table 1. Clinical characteristics of the study participants

Characteristic	Mean ± SEM
Age (years)	57 ± 2
Female/male	22/11
Smoker/non-smoker	7/26
Body mass index (kg/m^2)	25.6 ± 0.7
Systolic blood pressure (mm Hg)	123 ± 3
Diastolic blood pressure (mm Hg)	76 ± 2
Heart rate (beats/min)	69 ± 2
Total cholesterol (mg/dl)	196 ± 7
Stiffness index (SI_{DVP}) (m/s)	9.3 ± 0.4

Beta$_2$-adrenergic arterial vasodilation and hyperemia-induced arterial vasodilation

Measurement was performed in the supine position, after 10-minutes rest with the use of a photoplethysmograph (Pulse Trace 2000, MicroMedical, UK). The digital volume pulse (DVP) waveforms were recorded over consecutive 10 cardiac cycles and then automatically averaged. The Reflection Index of DVP (RI_{DVP}) was determined as the height of the diastolic component of the DVP, expressed as a percentage of the systolic peak [8, 9]. The RI_{DVP} was considered as a measure of the amount of pulse wave reflection and of the tone of small arteries (**Figure 1**). Arm blood pressure was taken as a mean of three measurements obtained by an oscillometric method (Omron M-5).

In order to demonstrate flow-mediated vasodilation, hyperemia was induced on the arm where the digital volume pulse analysis was performed. After obtaining the baseline resting DVP waveform from a fingertip photoplethysmogram, the sphygmomanometric cuff was placed on the ipsilateral arm and was inflated to 230 mm Hg for

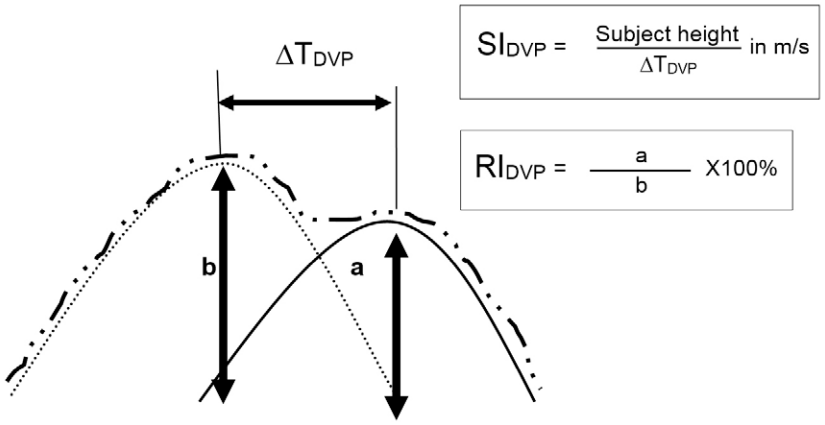


Figure 1. The Stiffness Index (SI_{DVP}) is obtained from subject height divided by the time between the systolic and diastolic peaks of the digital volume pulse (DVP). The Reflection Index (RI_{DVP}) is determined as the height of the peak (a) component of the DVP expressed as a percentage of the systolic peak (b)

5 minutes to induce transient ischemia. Subsequent deflation of the cuff induced a brief state of increased shear stress. The pulse wave was recorded 1, 2 and 3 mins after cuff deflation. Average waveforms were generated after acquisition of 10 sequential pulse wave forms. The response to hyperemia (ΔRI_{Hyper}) was defined as the maximum difference in RI_{DVP} between rest and the post-hyperemia period.

After 20 minutes rest, new baseline resting DVP waveforms were obtained and nitroglycerine (NTG) was then administred sublingually (50 μg) to estimate endothelium-independent arterial vasodilation [10]. Finger pulse waves were obtained after 1, 3 and 5 minutes. The response to NTG-induced vasodilation (ΔRI_{NTG}) was defined as the maximum difference in RI_{DVP} between baseline and the NTG-induced changes. After 20 minutes rest and obtaining a 3-rd baseline resting DVP waveform, albuterol (2 x 200 μg) was given by inhalation, with the use of a spacer, in order to estimate β2-adrenergic-induced vasodilation [10]. Recordings of pulse wave forms were made 5, 10 and 15 minutes after the albuterol inhalation. The response to albuterol (ΔRI_{Alb}) was defined as the maximum difference in ΔRI_{DVP} between rest and the post-albuterol period and was regarded as endothelium-dependent vasodilation [10].

Statistical analysis

The results of continuous variables are expressed as mean values ± SEM. Normal distribution of data was tested by Kolmogorov-Smirnov test. Comparisons between groups were made using the Student t-test and one-way ANOVA. Correlation was evaluated by the Pearson's coefficient test. All tests were two-sided. Statistical significance was set at $p < 0.05$. Statistical analyses were performed using the GraphPad Instat version 3.06 for Windows (GraphPad Software, San Diego, CA, USA)

Results

The effects of hyperemia, albuterol and NTG on the heart rate and blood pressure were similar and did not differ significantly from the values observed at rest (**Table 2**). The hyperemia-induced vasodilation led to a significant reduction in RI_{DVP} in comparison to the background values (69 ± 2 % vs 64 ± 2, $p < 0.0001$, **Table 3**). Similarly, the administration of albuterol or NTG resulted in a significant drop in the RI_{DVP} (71 ± 2 % vs 67 ± 2 %, $p < 0.0001$ and 71 ± 2 % vs 58 ± 2 %, $p < 0.0001$ respectively, **Table 3**). There were no significant differences between ΔRI_{Hyper} and ΔRI_{Alb} (5.2 ± 0.8 % vs 4.6 ± 1.0 %, $p = 0.61$, **Figure 2**). We observed a significant correlation between small vessel reactivity in response to albuterol and hyperemia ($r = 0.52$, $p = 0.01$, **Figure 3**). Neither hyperemia nor albuterol-induced changes in RI_{DVP} correlated with ΔRI_{NTG} ($r = 0.14$, $p = 0.42$ and $r = 0.19$, $p = 0.27$, respectively). RI_{DVP} did not correlate significantly with age or systolic arterial pressure (data not shown).

Table 2. Changes in heart rate and blood pressure after hyperemia, albuterol or nitroglycerine in healthy subjects

Variable	Rest*	Hyperemia	Albuterol	Ntg	P**
Heart rate (beats/min)	67 ± 2	68 ± 2	67 ± 2	67 ± 2	0.7
Systolic blood pressure (mm Hg)	124 ± 2	123 ± 3	124 ± 2	123 ± 2	0.9
Diastolic blood pressure (mm Hg)	77 ± 1	77 ± 2	77 ± 2	76 ± 2	0.9

* The values represent mean of background taken before hyperemia, albuterol and nitroglycerine challenge
** The variation among means was estimated by the one-way ANOVA

Table 3. Changes in RI_{DVP} measurement after hyperemia, albuterol or nitroglycerine

Variable	Background	Hyperemia	Albuterol	NTG	P*
RI_{DVP} (%)	69 ± 2	64 ± 2	–	–	0.0001
	71 ± 2	–	67 ± 2	–	0.0001
	71 ± 2	–	–	58 ± 2	0.0001

* The mean of the differences was assessed by the paired t test

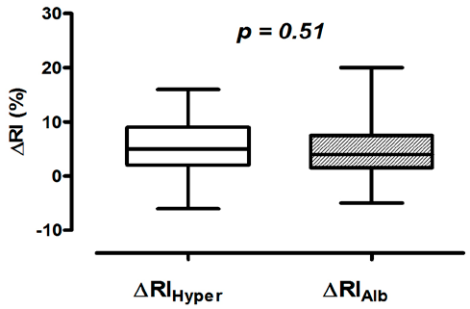


Figure 2. The difference in reflection indices after hyperemia(ΔRI_{Hyper}) or albuterol induced vasodilation (ΔRI_{Alb}). The difference in means was estimated by a paired t-test

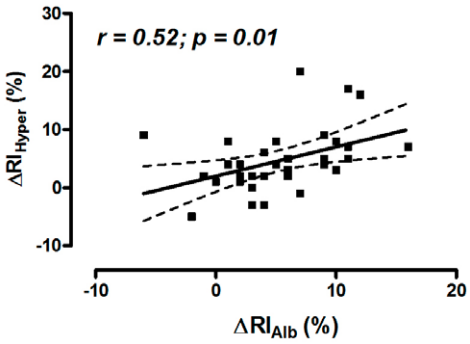


Figure 3. Correlation between hyperemia (ΔRI_{Hyper}) and albuterol induced vasodilation (ΔRI_{Alb})

Discussion

Endothelium plays a pivotal role in regulating vascular function. Endothelial cells lining blood vessels maintain a balance between vasoconstriction and vasodilation, pro and anti-thrombotic factors and pro and anti-inflammatory stimuli [12]. Nitric oxide is an endothelium-derived vasorelaxing substance that plays a central role in maintaining vascular tone and homeostasis [13]. Reduced bio-avaibility of nitric oxide is associated with impaired endothelium-dependent vasodilation [14, 15]. Moreover this diminished vasodilatory response is predictive of cardiovascular complications or of cerebrovascular events in patients with coronary artery disease [16, 17, 18]. The methods currently used for testing endothelium-dependent vasodilation are not easily applied, are time consuming and require considerable skill in order to be adequately performed.

It has been shown recently that β_2-adrenergic receptor stimulation results in the release of nitric oxide from endothelial cells. These receptors are also present in the walls of coronary arteries and are important in maintaining their vascular tone. As demonstrated by Chowienczyk et al. [10] the administration of a β_2-adrenergic agonist (albuterol), both systematically and by inhalation, led to significant changes in the RI_{DVP}. The action of albuterol on RI_{DVP} but not of NTG was attenuated by N^G-monomethyl-L-arginine (NO synthase inhibitor). This led to the conclusion that the effects of albuterol are mediated, in part, through the nitric oxide pathway. Several studies have shown that transient hyperemia, induced by postischemic dilation of vascular beds distal to temporary occlusion, is believed to be mediated by nitric oxide generated from endothelium. In our study the administration of albuterol led to a significant drop in RI_{DVP}. This observation is in accordance with that of others [10, 19]. A similar change in the reflection index was observed in our present study during the postischemic period following the release of temporary occlusion of the brachial artery. Moreover, the RI_{DVP} changes observed after albuterol-induced challenge correlated with the changes in the reflexion index induced by hyperemia. This may suggest that both are mediated, at least partly, by nitric oxide dependent mechanisms. The NTG-induced drop in RI_{DVP} is mediated by an endothelium-independent mechanism and did not correlate with the effects of albuterol (RI_{Alb}) or hyperemia (RI_{Hyper}). RI_{DVP} is affected by several factors such as age, heart rate or blood pressure. Here neither blood pressure nor heart rate were significantly affected by albuterol, hyperemia or NTG and we therefore conclude that the changes in the DVP index was not caused by these hemodynamic factors.

It is also noteworthy that there are some important differences between albuterol-induced vasodilation and the response to locally-produced hyperemia. Albuterol-induced vasorelaxation is caused by reaction evoked in the systemic circulation. Therefore it may be regarded as a measure of "general endothelial health". Reactive hyperemia generated in one arm is also at least partly endothelium — hence NO-dependent, but this reaction has local character, therefore it is more likely to be influenced by the metabolites generated locally in the response to ischemia. These discrepancies may account for the lack of higher than ~50% correlation between both studied methods. However, it is possible that the information gained from the assessment of locally induced hyperemia is to some extent as useful as derived from systemic circulation. For example flow-mediated dilation induced locally in

brachial artery appears to be suitable marker for cardiovascular complication [6, 7].

Limitation of the study

In order to answer the question of to what extent are the effects of hyperemia on RI_{DVP} mediated through L-arginine-NO, it will be necessary to examine this response in the presence and absence of N^G-monomethyl-L-arginine — a nitric oxide synthase inhibitor.

In summary, our study showed that changes in the Reflexion Index caused by locally-induced hyperemia are similar to those observed after albuterol-challenge in the systemic circulation and both are correlated.

Acknowledgements

Conflict of interest statement
The authors declare no conflict of interest.

Funding sources
There are no sources of funding to declare.

References

1. Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circ Res. 2000;87:840–844.
2. Bonetti PO, Lerman LO, Lerman A. Endothelial dysfunction: a marker of atherosclerotic risk. Arterioscler Thromb Vasc Biol. 2003;23:168–175.
3. Gimbrone MA Jr. Vascular endothelium: an integrator of pathophysiologic stimuli in atherosclerosis. Am J Cardiol. 1995;75:67B–70B.
4. Anderson TJ, Gerhard MD, Meredith IT, Charbonneau F, Delagrange D, Creager MA, Selwyn AP, Ganz P. Systemic nature of endothelial dysfunction in atherosclerosis. Am J Cardiol. 1995;75:71B–74B.
5. Verma S, Buchanan MR, Anderson TJ. Endothelial function testing as a biomarker of vascular disease. Circulation. 2003;108:2054–2059.
6. Laurent S, Lacolley P, Brunel P, Laloux B, Pannier B, Safar M. Flow-dependent vasodilation of brachial artery in essential hypertension. Am J Physiol 990;258:H1004–11.
7. Concetta I, Ceravolo R, Notarangelo L, Crescenzo A, Ventura G, Tamburrini O, Perticone F, Gnasso A Comparison of endothelial function evaluated by strain gauge plethysmography and brachial artery ultrasound Atherosclerosis 2001:158;53–59.
8. Millasseau SC, Kelly RP, Ritter JM, CHowienczyk PJ. Determination of age-related increases in large artery stiffness by digital pulse contour analysis Clinical Science 2002:103, 371–377.
9. Millasseau SC, Kelly RP, Ritter JM, Chowienczyk PJ. The vascular impact of aging and vasoactive drugs: comparison of two digital volume pulse measurements. Am J Hypert. 2003;16:467–472.
10. Chowienczyk PJ, Kelly RP, MacCallum H, Millasseau SC, Andersson TLG, Gosling RG, Ritter JM, Anggård EE.Photoplethysmographic assessment of pulse wave reflection: blunted endothelium-dependent response to beta2 adrenergic vasodilation in type II diabetes. J Am Coll Cardiol. 1999;34:2007–2014.
11. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol. 2002;39:257–265.
12. Vallance P, Chan N. Endothelial function and nitric oxide: clinical relevance. Heart. 2001;85:342–350.
13. Ganz P, Vita JA. Testing endothelial vasomotor function: nitric oxide, a multipotent molecule. Circulation. 2003;108:2049–2053.
14. Widlansky ME, Gokce N, Keaney JF Jr, Vita JA. The clinical implications of endothelial dysfunction. J Am Coll Cardiol. 2003;42:1149–1160.
15. Ludmer PL, Selwyn AP, Shook TL, Wayne RR, Mudge GH, Alexander RW, Ganz P. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. N Engl J Med. 1986;315:1046–1051.
16. Schachinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. Circulation. 2000;101:1899–1906.
17. Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR Jr, Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. Circulation. 2000;101:948–954.
18. Targonski PV, Bonetti PO, Pumper GM, Higano ST, Holmes DR Jr, Lerman A. Coronary endothelial dysfunction is associated with an increased risk of cerebrovascular events. Circulation. 2003;107:2805–2809.
19. Kalra L, Rambaran C, Chowienczyk PJ, Goss D, Hambleton I, Ritter J, Shah A, Wilks R, Forrester T. Ethnic differences in arterial responses and inflammatory markers in afro-caribbean and caucasian subjects. Arterioscler Thromb Vasc Biol. 2005 Nov;25(11):2362-7.

Acceptance for editing: 2017-02-15
Acceptance for publication: 2017-03-29

Correspondence address:
Andrzej Wykretowicz
Department of Internal Medicine,
Division of Cardiology-Intensive Therapy
Poznan University of Medical Sciences
49 Przybyszewskiego Street, 60-355 Poznan, Poland
Phone: +48618691391, Fax: +48618691689
email: awykreto@ptkardio.pl

ORIGINAL PAPER

DOI: https://doi.org/10.20883/jms.2019.335

LC-MS/MS based targeted metabolomics method for analysis of serum and cerebrospinal fluid

Szymon Plewa[1, a], Paweł Dereziński[1, b], Jolanta Florczak-Wyspiańska[2, c], Karolina Popławska-Domaszewicz[2, d], Wojciech Kozubski[2, e], Bartosz Sokół[3, f], Roman Jankowski[3, g], Jan Matysiak[1, h], Zenon J. Kokot[1, i]

[1] Department of Inorganic and Analytical Chemistry, Poznan University of Medical Sciences, Poland
[2] Department of Neurology, Poznan University of Medical Sciences, Poland
[3] Department of Neurosurgery, Poznan University of Medical Sciences, Poland

[a] http://orcid.org/0000-0002-9600-3980
[b] http://orcid.org/0000-0002-6066-1260
[c] http://orcid.org/0000-0002-7131-8687
[d] http://orcid.org/0000-0002-9154-389X
[e] http://orcid.org/0000-0003-2777-261X
[f] http://orcid.org/0000-0003-4814-2000
[g] http://orcid.org/0000-0002-2368-4578
[h] http://orcid.org/0000-0002-9993-1504
[i] http://orcid.org/0000-0003-4950-9759

ABSTRACT

Introduction. Recent instrumentation and software advancement enabled to develop new, high-throughput targeted metabolomics methods for in-depth exploration of metabolome in a quantitative manner.
Material and Methods. The presented targeted metabolomics approach allows to analyze both of serum and CSF in the same way, with identical sample preparation procedures. The analyses were carried out using high-performance liquid chromatography system coupled to triple quadrupole tandem mass spectrometer with electrospray ion source (LC-ESI-QqQ-MS/MS).
Results. The applied targeted metabolomics approach enabled to determine a wide panel of metabolites from different chemical classes of compounds including: acylcarnitines, amino acids and biogenic amines, glycerophospholipids, sphingolipids and sum of hexoses. Finally, 148 metabolites in serum and 57 in cerebrospinal fluid were determined.
Conclusions. Here we presented the results of successful implementation of the method of analysis of low-molecular weight compounds in human serum and CSF using targeted metabolomics. The evaluation of selected groups of metabolites resulted in obtaining the mean concentrations of panel of metabolites in serum and CSF, which gives a valuable information about the metabolome of these matrices.

Keywords: liquid chromatography, tandem mass spectrometry, flow injection analysis.

Introduction

Metabolome, as a sum of small molecules which reflects, both of genetic and environmental factors affecting human's health at a given time, seems to be a perfect tool for describing physiological or pathological processes and in the result identifying biomarkers or introducing personalized treatment approach. To reach such important goals, a reliable and reproducible analytical methods are needed [1]. Until today, many different techniques were employed to investigate human metabolome such as separation techniques (gas chromatography (GC), liquid chromatography (LC)) coupled to mass spectrometry (MS) or nuclear magnetic resonance (NMR) spectroscopy [2]. None of these techniques seems to be perfect for reliable analysis of the whole metabolome yet. Thus, researchers' efforts are focused on developing tools which might enable identifying and quantifying as many

metabolites as possible. Until recently, untargeted metabolomics approach was prevailing in discovery-based investigations, but today due to instrumentation and software development we can apply high-throughput targeted metabolomics methods [3] to explore metabolome in a quantitative manner [4]. That approach allows the researchers to skip time consuming step of identification of relevant metabolites — characteristic for untargeted approach, and to focus on data interpretation taking into account clinical needs (e.g. data comparability to reference ranges). The aim of the study was the application of targeted metabolomics method allowing to analyze wide spectrum of metabolites from five different chemical classes of compounds in serum and cerebrospinal fluid (CSF) from human subjects, in the same way.

Material and Methods

Quantitative metabolomics of CSF and serum covers wide spectrum of analytes form different groups of compounds, including: 40 acylcarnitines, 21 biogenic amines, 21 amino acids, 90 glycerophospholipids (14 lysophosphatidylcholines and 76 phosphatidylcholines), 15 sphingolipids and sum of hexoses. It yields the total of 188 metabolites quantified simultaneously (**Figure 1**). The assays were performed using AbsoluteIDQ p180 kit (Biocrates Life Sciences AG, Innsbruck, Austria) according to standard operation procedures. The methodology is based on 2-layers, 96-deepwell plate supplied by manufacturer, with selected internal standards partially integrated with filter layer of the plate.

10 serum and 10 CSF samples were analyzed. All samples were collected from patients who has no diagnosed nervous system disorders. The research was performed in accordance with the Declaration of Helsinki. All study subjects gave written informed consent prior the sample collection. (Bioethics Committee of Poznan University of Medical Sciences — Decision no. 821/16 and 206/17). First step of analytical workflow was to add 10 µL of internal standards mixture containing rest of internal standards on the filter layer. Then phosphate buffered saline, seven calibration standards of different concentration and quality

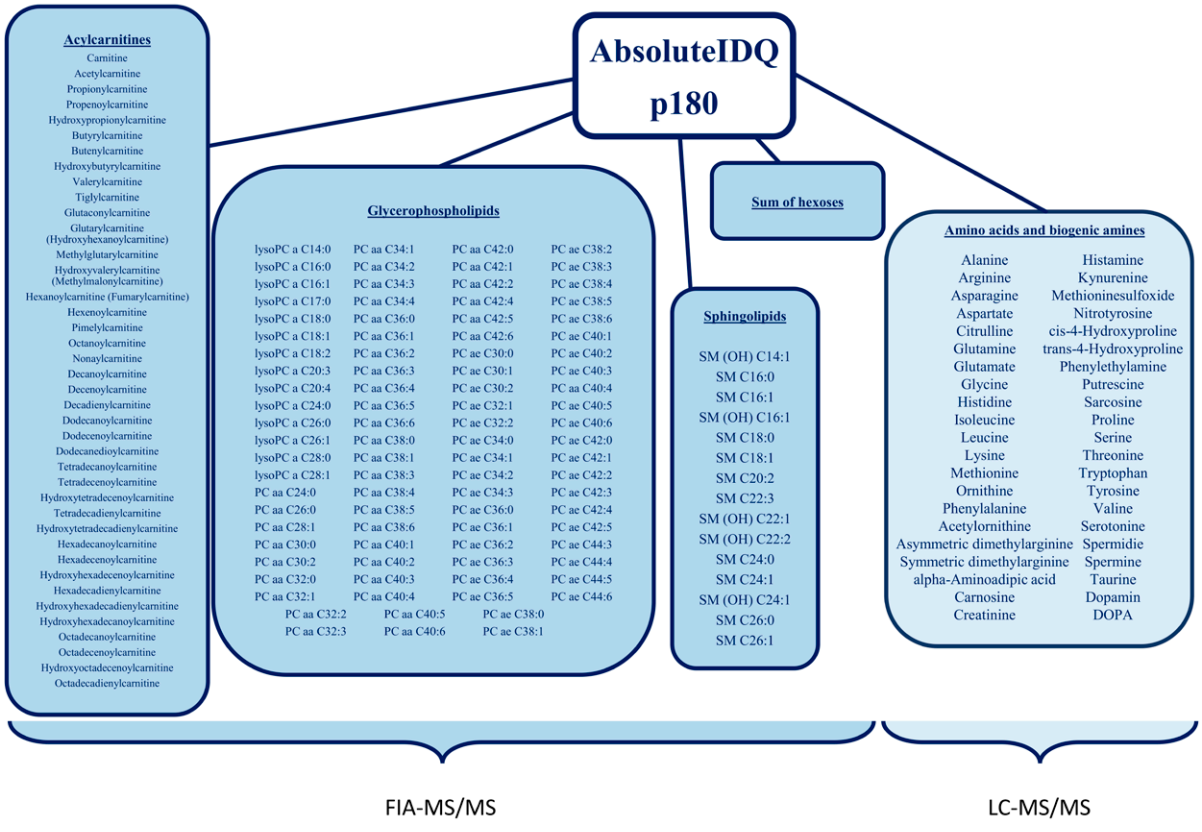


Figure 1. List of all metabolites analyzed using Absolute IDQ p180 methodology in FIA-MS/MS mode and LC-MS/MS mode

controls samples (QC) were put in the respective wells. 3 concentration levels (low, medium, high) of QC samples were analyzed. Biological samples were thawed in room temperature and pipetted on the appropriate spots in amount of 10 µL and 30 µL of serum and CSF, respectively. The plate was then dried under nitrogen flow for 30 minutes. During that time the derivatization agent was prepared by vortexing rigorously 300 µL of phenylisothiocyanate (PITC) with 5700 µL of mixture of pyridine and ethanol in water (1:1:1). After 30-minutes of drying, 50 µL of derivatization solution was put in each well. Covered plate was left for 20-minutes incubation at room-temperature. Next the second round of drying under nitrogen flow was performed for 60 minutes. Then 300 µL of extraction solvent containg 5 mM of ammonium acetate in methanol was pipetted to each well, after which covered plate was shaken at room temperature for 30 minutes using orbital plate shaker at 450rpm. Subsequently sample extracts were passed through the filter layer to the lower capture plate via nitrogen pressure. Finally sample extract was split into two analogous 96-well plates and diluted with 150 µL of water and 400 µL of FIA mobile phase for LC-MS/MS analysis and FIA-MS/MS analysis, respectively. Each plate was sealed with silicone mat to reduce evaporation and placed in autosampler.

The LC run as well as FIA run were carried out using high-performance liquid chromatography system 1260 Infinity (Agilent Technologies, Santa Clara, CA, USA) coupled to triple quadrupole tandem mass spectrometer 4000 QTRAP (Sciex, Framingham, MA, USA). Quantification of amino acids and biogenic amines was preceded by chromatographic separation (LC-MS/MS part) using 0.2% solution of formic acid (phase A) and 0.2% formic acid in acetonitrile (phase B) at the flow rate 0.5 mL/min. Gradient elution was as follows: 0–0.5 min, 100% phase A; 0.5–5.5 min, linear to 5% phase A; 5.5–6.5 min holding at 5% phase A; followed by returning to 100% phase A (6.5–7.0 min); and finally 7.0–9.5 min, 100% phase A. The chromatography was carried out on an ZORBAX Eclipse XDB-C18 (3.0 x 100 mm, 3.5 µm) column (Agilent Technologies, Santa Clara, CA, USA), with a pre-column (C18, 4.0 x 3.0 mm) SecurityGuard (Phenomenex, Torrance, CA, USA). In turn, remaining metabolites were determined by injecting sample into mobile phase, at isocratic flow, directly to mass spectrometer (flow injection analysis, FIA-MS/MS). These compounds were analyzed by mass spectrometer only, bypassing the chromatographic column, according to different m/z ratios.

Data acquisition and quantification were performed under control of Analyst 1.5.2 software (Sciex, Framingham, MA, USA). Sample management and data processing were carried out using MetIDQ version Boron software (Biocrates Life Sciences AG, Innsbruck, Austria). In the next step the raw data (obtained as chromatographic peaks) for QC samples and calibrators were manually reviewed and peak integrations were checked in order to validate the performance of both acquisition and quantitation methods of in-house MS-system. To confirm the reliability of the results, the QC samples evaluation using software build-in tool (MetVAL module, Biocrates Life Sciences AG, Innsbruck, Austria) was performed. Afterwards the concentrations of metaboliteswere calculated (µM) using MetSTAT module (Biocrates Life Sciences AG, Innsbruck, Austria). The metabolites with 50% or more of missing data (concentration below limit of detection) were excluded. Then, the remaining missing data was replaced with half of minimal determined concentration for appropriate metabolite.

Results

Two different matrices: serum and cerebrospinal fluid from human subjects were analyzed using targeted metabolomics approach, according to manufacturer's operation procedures. Quality assessment of the data was passed by QC samples analyzed in LC-MS/MS analysis, as well as QC samples analyzed in FIA-MS/MS analysis (all tested metabolites concentration levels were within reference ranges), indicating satisfactory accuracy and the performance of the method. In subjects' sera 148 metabolites were determined, including: 14 acylcarnitines, 21 amino acids, 13 biogenic amines, 84 glycerophospholipids, 15 sphingolipids and sum of hexoses. Conversely, in CSF 57 metabolites were determined, and they were as follow: 1 acylcarnitine, 18 amino acids, 5 biogenic amines, 23 glycerophospholipids, 9 sphingolipids and sum of hexoses (**Table 1**). It corresponds to 78.72% (serum) and 30.32% (CSF)

Table 1. The number of metabolites determined in serum and cerebrospinal fluid

Groups of metabolites	Metabolites included in the panel (No)	Biological matrix			
		Serum		Cerebrospinal fluid	
		Determined compounds (No)	% of determined metabolites	Determined compounds (No)	% of determined metabolites
Acylcarnitines	40	14	35,00	1	2,50
Aminoacids	21	21	100,00	18	85,71
Biogenic amines	21	13	61,90	5	23,81
Glycerophospholipids	90	84	93,33	23	25,56
Sphingolipids	15	15	100,00	9	60,00
Sum of hexoses	1	1	100,00	1	100,00
Total	188	148	78,72	57	30,32

of the total of 188 metabolites possible to quantitate with applied methodology. The full list of determined metabolites is given in **Table 2**. All possible amino acids and sphingolipids as well as sum of hexoses, were determined in human serum. In the remaining groups of compounds some metabolites were not observed in serum or they did not exceed limit of detection. Nevertheless, abovementioned targeted metabolomics approach enables determining 35.0% acylcarnitines, 61.90% biogenic amines and 93.33% glycerophospholipids amongst wider spectrum of compounds. In case of CSF the applied approach enables determining: sum of hexoses, 85.71%

Table 2. Metabolite concentrations determined in the biological matrices — serum and cerebrospinal fluid (means with standard deviation (SD); minimum and maximum; μM)

Class of compounds	Metabolites	Serum				Cerebrospinal fluid			
		Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
Acylcarnitines	C0	39.860	13.527	15.000	63.900	–	–	–	–
	C2	5.451	2.916	1.390	12.600	0.457	0.208	0.108	0.754
	C3	0.313	0.100	0.167	0.454	–	–	–	–
	C4	0.159	0.049	0.095	0.242	–	–	–	–
	C5	0.103	0.030	0.048	0.150	–	–	–	–
	C10	0.215	0.117	0.084	0.402	–	–	–	–
	C12:1	0.124	0.047	0.049	0.186	–	–	–	–
	C14	0.032	0.016	0.013	0.059	–	–	–	–
	C14:1	0.071	0.039	0.022	0.139	–	–	–	–
	C14:2	0.022	0.012	0.009	0.041	–	–	–	–
	C16	0.112	0.037	0.043	0.159	–	–	–	–
	C18	0.048	0.013	0.028	0.067	–	–	–	–
	C18:1	0.120	0.051	0.039	0.206	–	–	–	–
	C18:2	0.037	0.012	0.012	0.050	–	–	–	–
Amino acids	Ala	396.200	70.345	311.000	516.000	30.040	9.076	21.700	49.000
	Arg	123.280	14.584	97.800	141.000	22.800	2.827	18.800	26.500
	Asn	54.230	8.545	38.700	68.800	6.590	1.146	4.500	8.200
	Asp	28.730	7.001	18.600	38.700	–	–	–	–
	Cit	28.650	6.521	19.900	40.900	2.041	0.561	1.460	3.230
	Gln	676.600	94.663	448.000	789.000	521.700	92.959	437.000	733.000
	Glu	64.510	29.944	31.500	118.000	–	–	–	–
	Gly	340.900	113.998	213.000	625.000	6.047	2.236	2.610	8.970
	His	90.320	20.711	65.200	143.000	15.220	3.409	11.100	20.900
	Ile	73.400	10.456	60.800	89.100	6.633	2.730	3.900	12.400
	Leu	141.700	21.375	118.000	184.000	15.300	5.711	9.270	26.200
	Lys	213.200	25.879	162.000	252.000	34.920	6.411	27.900	46.000
	Met	22.250	2.576	15.800	25.400	3.795	1.290	2.310	6.230

Table 2. Contd.

Class of compounds	Metabolites	Serum				Cerebrospinal fluid			
		Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
Amino acids	Orn	86.430	20.572	57.100	123.000	6.097	1.935	3.970	10.200
	Phe	73.600	7.959	64.200	83.700	10.600	4.005	6.300	19.400
	Pro	212.200	66.758	115.000	314.000	–	–	–	–
	Ser	144.160	26.215	94.600	183.000	28.260	4.085	23.300	36.000
	Thr	122.110	20.364	94.000	153.000	30.600	6.439	21.300	41.300
	Trp	62.260	9.273	49.000	76.800	1.890	0.633	1.160	3.310
	Tyr	61.880	12.507	47.800	89.500	8.932	3.087	5.470	14.400
	Val	207.800	31.815	170.000	254.000	17.930	6.477	11.200	31.500
Biogenic amines	Ac–Orn	0.419	0.385	0.107	1.410	–	–	–	–
	ADMA	0.439	0.086	0.287	0.572	–	–	–	–
	Creatinine	85.120	12.080	69.300	105.000	68.790	13.983	44.300	90.700
	Kynurenine	2.482	0.539	1.810	3.510	–	–	–	–
	Met–SO	0.651	0.261	0.390	1.190	–	–	–	–
	Putrescine	0.106	0.034	0.025	0.143	0.132	0.028	0.090	0.177
	SDMA	0.355	0.224	0.173	0.757	–	–	–	–
	Serotonin	0.662	0.349	0.052	1.170	–	–	–	–
	Spermidine	0.144	0.041	0.086	0.220	–	–	–	–
	Spermine	0.148	0.004	0.141	0.156	–	–	–	–
	t4–OH–Pro	7.959	1.412	6.070	10.300	0.422	0.129	0.266	0.657
	Taurine	128.800	44.465	74.800	199.000	6.260	0.877	4.900	7.430
	total DMA	0.684	0.128	0.499	0.899	0.151	0.070	0.052	0.279
Glycerophospholipids	lysoPC a C16:0	92.670	17.678	68.300	120.000	–	–	–	–
	lysoPC a C16:1	2.586	1.165	1.460	4.760	–	–	–	–
	lysoPC a C17:0	4.299	3.338	1.380	8.670	–	–	–	–
	lysoPC a C18:0	28.300	7.132	20.300	42.600	–	–	–	–
	lysoPC a C18:1	18.160	4.167	12.100	25.000	–	–	–	–
	lysoPC a C18:2	19.790	4.945	12.700	27.100	–	–	–	–
	lysoPC a C20:3	2.165	0.756	1.360	3.630	–	–	–	–
	lysoPC a C20:4	7.167	1.691	5.040	9.610	–	–	–	–
	lysoPC a C26:0	0.312	0.139	0.136	0.611	–	–	–	–
	lysoPC a C26:1	0.186	0.080	0.053	0.309	–	–	–	–
	lysoPC a C28:0	0.346	0.168	0.172	0.716	–	–	–	–
	lysoPC a C28:1	0.474	0.180	0.246	0.845	–	–	–	–
	PC aa C24:0	0.087	0.067	0.039	0.243	–	–	–	–
	PC aa C28:1	2.653	0.899	1.370	4.250	–	–	–	–
	PC aa C30:0	7.266	4.216	3.080	14.200	–	–	–	–
	PC aa C30:2	0.741	0.353	0.402	1.450	0.002	0.001	0.001	0.004
	PC aa C32:0	14.809	3.563	7.690	18.900	0.310	0.119	0.176	0.589
	PC aa C32:1	15.264	7.597	7.170	32.500	0.104	0.030	0.060	0.162
	PC aa C32:2	2.374	1.150	0.681	4.320	0.005	0.003	0.002	0.009
	PC aa C32:3	0.544	0.229	0.281	1.010	–	–	–	–
	PC aa C34:1	209.900	41.065	136.000	280.000	1.658	0.615	0.792	3.020
	PC aa C34:2	303.200	68.618	143.000	397.000	0.120	0.125	0.031	0.367
	PC aa C34:3	12.426	4.076	5.540	17.700	–	–	–	–
	PC aa C34:4	1.624	0.673	0.722	2.890	–	–	–	–
	PC aa C36:0	3.880	1.733	1.950	6.830	–	–	–	–
	PC aa C36:1	50.970	14.887	31.600	73.600	0.225	0.074	0.119	0.374
	PC aa C36:2	182.910	53.762	85.100	287.000	0.202	0.069	0.107	0.318
	PC aa C36:3	120.220	26.770	71.500	156.000	0.089	0.047	0.048	0.208
	PC aa C36:4	187.600	35.991	112.000	233.000	0.210	0.078	0.110	0.366
	PC aa C36:5	23.210	7.030	12.500	32.200	–	–	–	–
	PC aa C36:6	0.965	0.293	0.415	1.340	–	–	–	–
	PC aa C38:0	3.332	0.623	2.310	4.120	–	–	–	–
	PC aa C38:1	1.189	0.508	0.201	2.150	0.006	0.004	0.003	0.013

Class of compounds	Metabolites	Serum				Cerebrospinal fluid			
		Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
Glycerophospholipids	PC aa C38:3	50.940	12.632	35.000	69.000	0.075	0.033	0.040	0.148
	PC aa C38:4	107.970	24.259	69.900	143.000	0.211	0.068	0.119	0.326
	PC aa C38:5	52.010	11.978	34.400	76.100	0.058	0.023	0.037	0.103
	PC aa C38:6	90.610	15.929	71.200	114.000	0.078	0.029	0.041	0.117
	PC aa C40:2	0.302	0.068	0.166	0.382	–	–	–	–
	PC aa C40:3	0.619	0.196	0.405	0.923	–	–	–	–
	PC aa C40:4	3.560	1.032	2.250	5.020	0.017	0.006	0.010	0.024
	PC aa C40:5	9.881	2.927	6.590	15.400	–	–	–	–
	PC aa C40:6	30.280	4.597	23.400	37.000	–	–	–	–
	PC aa C42:0	0.597	0.142	0.401	0.864	–	–	–	–
	PC aa C42:1	0.291	0.062	0.207	0.401	–	–	–	–
	PC aa C42:2	0.241	0.052	0.151	0.326	–	–	–	–
	PC aa C42:4	0.197	0.045	0.136	0.269	–	–	–	–
	PC aa C42:5	0.352	0.100	0.217	0.566	–	–	–	–
	PC aa C42:6	0.461	0.169	0.160	0.738	–	–	–	–
	PC ae C30:0	0.459	0.165	0.282	0.744	–	–	–	–
	PC ae C30:1	0.380	0.203	0.182	0.829	–	–	–	–
	PC ae C32:1	2.458	0.564	1.390	3.590	0.020	0.007	0.011	0.031
	PC ae C32:2	0.810	0.401	0.459	1.520	–	–	–	–
	PC ae C34:0	1.806	0.552	1.120	2.610	–	–	–	–
	PC ae C34:1	9.610	2.304	5.310	13.400	0.072	0.025	0.033	0.115
	PC ae C34:2	9.781	3.198	3.750	15.700	0.053	0.020	0.023	0.084
	PC ae C34:3	7.035	2.620	2.480	11.400	–	–	–	–
	PC ae C36:0	1.024	0.375	0.677	1.760	–	–	–	–
	PC ae C36:1	7.992	1.698	5.590	10.700	–	–	–	–
	PC ae C36:2	11.576	3.178	6.340	18.000	–	–	–	–
	PC ae C36:3	7.687	2.199	3.700	10.200	0.012	0.008	0.002	0.023
	PC ae C36:4	18.869	5.013	8.390	26.300	–	–	–	–
	PC ae C36:5	18.060	8.069	10.600	32.000	0.057	0.050	0.014	0.151
	PC ae C38:0	1.993	0.486	1.350	2.860	–	–	–	–
	PC ae C38:1	0.967	0.449	0.437	1.830	–	–	–	–
	PC ae C38:2	1.616	0.684	0.908	2.730	–	–	–	–
	PC ae C38:3	4.488	1.181	2.970	6.790	–	–	–	–
	PC ae C38:4	14.376	3.235	9.760	20.400	–	–	–	–
	PC ae C38:5	17.869	4.074	8.790	23.400	0.026	0.017	0.010	0.060
	PC ae C38:6	8.255	1.793	4.290	10.600	0.010	0.006	0.004	0.022
	PC ae C40:1	1.307	0.300	0.781	1.690	–	–	–	–
	PC ae C40:2	1.975	0.503	1.390	2.950	–	–	–	–
	PC ae C40:3	1.117	0.292	0.754	1.750	–	–	–	–
	PC ae C40:4	2.308	0.560	1.510	3.550	–	–	–	–
	PC ae C40:5	3.396	0.680	2.170	4.740	–	–	–	–
	PC ae C40:6	5.177	0.882	4.100	6.240	–	–	–	–
	PC ae C42:1	0.308	0.080	0.201	0.440	–	–	–	–
	PC ae C42:2	0.552	0.149	0.302	0.797	–	–	–	–
	PC ae C42:3	0.737	0.146	0.492	0.963	–	–	–	–
	PC ae C42:4	0.684	0.269	0.182	1.070	–	–	–	–
	PC ae C42:5	1.700	0.661	0.955	2.410	–	–	–	–
	PC ae C44:3	0.127	0.033	0.084	0.198	–	–	–	–
	PC ae C44:4	0.426	0.147	0.289	0.798	–	–	–	–
	PC ae C44:5	1.913	0.661	1.150	3.580	–	–	–	–
	PC ae C44:6	1.270	0.427	0.681	2.130	–	–	–	–

Table 2. Contd.

Class of compounds	Metabolites	Serum				Cerebrospinal fluid			
		Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
Sphingolipids	SM (OH) C14:1	4.420	1.879	1.980	7.540	–	–	–	–
	SM (OH) C16:1	3.023	1.016	1.940	4.840	0.007	0.007	0.001	0.021
	SM (OH) C22:1	11.779	3.000	7.100	17.300	0.019	0.006	0.010	0.030
	SM (OH) C22:2	11.338	3.677	7.020	17.900	0.024	0.006	0.016	0.033
	SM (OH) C24:1	1.142	0.332	0.702	1.750	–	–	–	–
	SM C16:0	99.890	22.959	65.900	139.000	0.319	0.140	0.147	0.552
	SM C16:1	13.075	4.911	7.560	23.700	0.034	0.016	0.010	0.062
	SM C18:0	22.480	4.706	19.100	33.100	0.338	0.121	0.185	0.544
	SM C18:1	11.338	3.519	8.040	19.200	0.080	0.028	0.036	0.115
	SM C20:2	0.677	0.493	0.257	1.540	–	–	–	–
	SM C22:3	4.709	5.538	0.443	14.100	–	–	–	–
	SM C24:0	20.330	6.065	12.800	30.200	0.049	0.028	0.028	0.116
	SM C24:1	58.660	13.230	39.600	80.200	0.160	0.072	0.073	0.304
	SM C26:0	0.153	0.059	0.091	0.278	–	–	–	–
	SM C26:1	0.463	0.127	0.331	0.705	–	–	–	–
Sum of hexoses	H1	5972.700	822.089	4480.000	6899.000	3756.500	552.259	2843.000	4497.000

amino acids, 60.00% sphingolipids, 25.56% glycerophospholipids, 23.81% biogenic amines and only 1 acylcarnitine, which corresponds to 2.50% of all acylcarnitines included in panel.

It is easily seen that the quantification of 148 metabolites in serum and 57 in CSF has result in changes of contribution of particular groups of metabolites determined in both fluids compared to their contribution in the whole set of 188 metabolites provided by the applied methodology. **Figure 2** presents the percentage of each of group metabolites in the spectrum of: 188 compounds evaluated in applied methodology (**Figure 2A**); 148 metabolites determined in serum (**Figure 2B**); 57 metabolites determined in CSF (**Figure 2C**). The change in numbers of determined acylcarnitines is the highest. Among 40 acylcarnitines possible to quantitate 14 was determined in serum and only 1 in CSF, which corresponds to 9% of metabolites of this group of compounds quantified in serum and 2% of metabolites quantified in CSF.

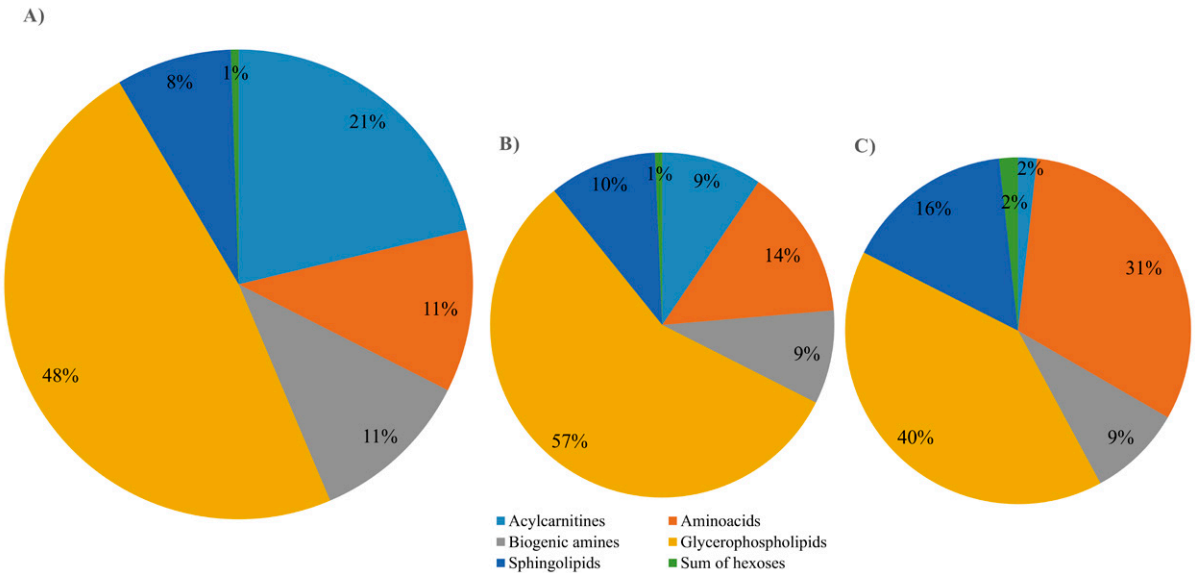


Figure 2. The percentage of each group metabolites in the spectrum of: 188 compounds evaluated in applied methodology (**A**); 148 metabolites determined in serum (**B**); 57 metabolites determined in CSF (**C**)

Discussion

As Aretz and Meierhofer [4] concluded, there is no perfect tool, nor methodology, allowing to reliably measure the entire metabolome at once. Nowadays we distinguish two complementary approaches in metabolomics: targeted metabolomics focused on selected metabolite or groups of metabolites [5, 6] and untargeted metabolomics focused on global profiling of the metabolome [7], very often without quantitative data [8]. Both of them have their own advantages and pitfalls [4].

Here we presented the results of successful implementation of method of the determination of low-molecular weight compounds in human serum and CSF. The assays were performed by using hyphenated mass spectrometric techniques: LC-MS/MS and FIA-MS/MS. This targeted strategy has emerged as a satisfactory compromise between wide spectrum of analyzed compounds (characteristic for untargeted approach) and highly selective and sensitive measurement of selected compounds (targeted approach; MRM mode). What is worth to emphasize, despite different composition of CSF and serum, presented method allows to analyze both matrices in the same way, with identical sample preparation procedures. During the whole analysis the only noteworthy difference between these matrices is an amount of sample pipetted onto the 96-well plate. It gives the invaluable tool for simultaneous analysis CSF and serum that might be crucial for central nervous system (CNS) disorders research. The better understanding of correlation between CSF and serum metabolome as well as the blood-brain barrier permeability for metabolites in different medical conditions could contribute to reducing the number of invasive diagnostic procedures such as lumbar puncture in the future [9]. The limitation of the study is limited number of analyzed samples resulting from invasiveness of CSF collection. That is why we recommend to analyze these data with respect to other metabolomics cohort studies.

Presented results from implementation of the method of evaluation of selected groups of metabolites resulted in obtaining the mean concentrations of panel of metabolites in serum and CSF, which gives a valuable information about metabolome of these matrices for other researchers focused directly on investigations of e.g. central nervous system disorders. This is crucial due to the fact of lacking wide-spectrum targeted metabolomics data of both serum and CSF from human subjects. And secondly, presented results are highly translatable and can be easily compared through the application of widely used methodology with confirmed interlaboratory reproducibility [10] and validation according to European Medicine Agency Guideline [11]. To conclude, we successfully implemented targeted metabolomics method allowing to analyze both of serum and CSF in the same way, and we applied it to analyze serum and CSF from human subjects. Evaluation of 148 metabolites in serum and 57 metabolites in CSF gives a tool for searching for disturbances in metabolism and enables determination of the broad variety of compound classes and confirms usefulness of described methodology for further studies on both serum and CSF metabolome.

Acknowledgements

Conflict of interest statement

The authors declare no conflict of interest.

Funding sources

There are no sources of funding to declare.

References

1. Jacob M, Lopata AL, Dasouki M, Abdel Rahman AM. Metabolomics toward personalized medicine. Mass Spectrom Rev. 2017 Oct 26.
2. Miggiels P, Wouters B, van Westen GJP, Dubbelman A-C, Hankemeier T. Novel technologies for metabolomics: More for less. TrAC Trends Anal Chem.
3. Xia J, Broadhurst DI, Wilson M, Wishart DS. Translational biomarker discovery in clinical metabolomics: an introductory tutorial. Metabolomics. 2013 Apr 4;9(2):280–99.
4. Aretz I, Meierhofer D. Advantages and Pitfalls of Mass Spectrometry Based Metabolome Profiling in Systems Biology. Int J Mol Sci. 2016 Apr 27.
5. Klupczynska A, Plewa S, Dyszkiewicz W, Kasprzyk M, Sytek N, Kokot ZJ. Determination of low-molecular-weight organic acids in non-small cell lung cancer with a new liquid chromatography-tandem mass spectrometry method. J Pharm Biomed Anal. 2016 Sep;129:299–309.
6. Klupczynska A, Plewa S, Sytek N, Sawicki W, Dereziński P, Matysiak J, et al. A study of low-molecular-weight organic acid urinary profiles in prostate cancer by a new liquid chromatography-tandem

mass spectrometry method. J Pharm Biomed Anal. 2018 Sep 10;159:229–36.
7. Klupczynska A, Dereziński P, Garrett TJ, Rubio VY, Dyszkiewicz W, Kasprzyk M, et al. Study of early stage non-small-cell lung cancer using Orbitrap-based global serum metabolomics. J Cancer Res Clin Oncol. 2017 Apr 6;143(4):649–59.
8. Ribbenstedt A, Ziarrusta H, Benskin JP. Development, characterization and comparisons of targeted and non-targeted metabolomics methods. PLoS One. 2018 Nov 15;13(11):e0207082.
9. Engelborghs S, Niemantsverdriet E, Struyfs H, Blennow K, Brouns R, Comabella M, et al. Consensus guidelines for lumbar puncture in patients with neurological diseases. Alzheimer's Dement (Amsterdam, Netherlands). 2017;8:111–26.
10. Siskos AP, Jain P, Römisch-Margl W, Bennett M, Achaintre D, Asad Y, et al. Interlaboratory Reproducibility of a Targeted Metabolomics Platform for Analysis of Human Serum and Plasma. Anal Chem. 2017 Jan 3;89(1):656–65.
11. St John-Williams L, Blach C, Toledo JB, Rotroff DM, Kim S, Klavins K, et al. Targeted metabolomics and medication classification data from participants in the ADNI1 cohort. 2017;4:170140.

Acceptance for editing: 2019-02-08
Acceptance for publication: 2019-03-09

Correspondence address:
Zenon J. Kokot
Department of Inorganic & Analytical Chemistry
Poznan University of Medical Sciences
6 Grunwaldzka Street, 60-780 Poznan, Poland
phone: +48 61 854 66 10
fax: +48 61 854 66 09
e-mail: zkokot@ump.edu.pl

ORIGINAL PAPER

DOI: https://doi.org/10.20883/jms.313

The evaluation of α-tocopherol concentration instead of α-tocopherol:cholesterol ratio in adult cystic fibrosis patients results in the overestimation of vitamin E deficiency

Ewa Sapiejka[1, a], Patrycja Krzyżanowska-Jankowska[2, b], Dariusz Walkowiak[3, c], Szymon Kurek[2, d], Jarosław Walkowiak[2, e]

[1] The Specialist Centre for Medical Care of Mother and Child, Gdansk, Poland
[2] Department of Pediatric Gastroenterology and Metabolic Diseases, Poznan University of Medical Sciences, Poland
[3] Department of Organization and Management in Health Care, Poznan, Poland

[a] https://orcid.org/0000-0003-0867-1717
[b] https://orcid.org/0000-0001-8676-9803
[c] https://orcid.org/0000-0001-8874-2401
[d] https://orcid.org/0000-0002-1409-2933
[e] https://orcid.org/0000-0001-5813-5707

ABSTRACT

Introduction. It has been suggested that serum vitamin E concentrations in CF patients may not properly reflect the body resources of vitamin E. Therefore, we aimed to assess vitamin E status in CF adults relating it to reference values for healthy subjects, also taking into account the α-tocopherol:cholesterol ratio.
Material and Methods. The study group was composed of 33 CF patients – 18 (54.5%) females and 15 (45.5%) males – aged from 18 to 47 years. In all CF patients nutritional status and clinical expression of disease – lung function; *Pseudomonas aeruginosa* colonization; diabetes; cirrhosis; exocrine pancreatic function and vitamin E supplementation were analyzed. Vitamin E (α-tocopherol) concentration was assessed by high-performance liquid chromatography (HPLC).
Resuls. CF adults have lower vitamin E concentrations (median: 830 μg/dl vs. 1132 μg/dl, $p = 0.00174$) and higher vitamin E: cholesterol ratio (median: 7.2 mg/g vs. 6.7 mg/g, $p < 0.00001$) than healthy subjects. The underlying factor that determines this phenomenon is related to low cholesterol levels observed in CF patients. The percentage of low vitamin E concentrations defined in comparison to healthy Polish peers is considerably higher than low vitamin E:cholesterol ratios (39.4% vs. 21.2%, $p = 0.0011$).
Conclusions. The evaluation of α-tocopherol concentration instead of α-tocopherol:cholesterol ratio in CF adults results in the overestimation of vitamin E deficiency. Moreover, there is also potentially a large percentage of individuals with excessive vitamin E body resources. However, this aspect demands further studies.

Keywords: fat-soluble vitamins, gastrointestinal diseases, pancreatitis, high-performance liquid chromatography.

Introduction

It is difficult to determine the optimal markers of vitamin E body resources useful in the clinical care of cystic fibrosis (CF) patients. The vitamin E:total lipid ratio may be preferable, but it is rarely available. Some authors suggested that vitamin E concentration and vitamin E:cholesterol ratio in comparison with the healthy peer group may be the appropriate way to evaluate the vitamin

E status in CF patients [1]. According to the current data, it is unclear whether to assess vitamin E concentration or vitamin E:cholesterol ratio as biomarker for vitamin E status in CF [2]. Moreover, the available evidence shows that age-dependent reference ranges of vitamin E concentrations are very different, which additionally hinders the correct classification of patients with a deficiency or excessive levels of vitamin E [3–6].

Therefore, in the present study, we aimed to assess the body resources of vitamin E in CF adults relating them to reference values for healthy subjects, also taking into account the α-tocopherol:cholesterol ratio.

Material and Methods

Material

The study group was composed of 33 patients with CF — 18 (54.5%) females and 15 (45.5%) males — aged from 18 to 47 years. The diagnosis was based on accepted guidelines [7, 8]. Mutations in one or both alleles of the CFTR gene were found in 31 patients (93.9%). The genotype could not be identified in 2 (6.1%) patients. Eight CF patients were homozygous for the mutation F508del. In the other CF patients the following CFTR gene mutations were identified: F508del/CFTRdele2,3(21kb) (n = 2), F508del/3849+10kbC>T (n = 4), F508del/1717–1G>A (n = 1), F508del/2184insA (n = 1), F508del/3659delC (n = 1), F508del/2183AA>G (n = 1), F508del/3121–2A>G (n = 1), F508del/G551D (n = 1), F508del/R334W (n = 1), F508del/R347P (n = 1), A155P/3171insC (n = 2), 3849+10kbC>T/3600+1G>T (n = 2), 3849+10kbC>T/W1282X (n = 1), N1303K/3849+10kb (n = 1), F508del/- (n = 2), Q1313X/- (n = 1).

In all CF patients nutritional status (standardized body weight and height, serum albumin concentration), clinical expression of the disease and vitamin E supplementation were analyzed. Clinical assessment included: lung function (spirometry), biochemical markers of liver function (aspartate transaminase — AST, alanine transaminase — ALT, gamma-glutamyl transferase — GGT), respiratory tract colonization by *Pseudomonas aeruginosa*, diabetes, liver cirrhosis [9], exocrine pancreatic function (fecal elastase-1 concentration) [10,11] and presented in **Table 1**.

Table 1. Clinical parameters in CF patients

Clinical parameters	Median (1st-3rd quartile)
Age [years]	22.8 (19.7–27.8)
Body weight (Z-score)	-0.65 (-1.00– -0.11)
Body height (Z-score)	-0.24 (-1.00–0.25)
BMI (Z-score)	20.0 (18.3–22.3)
Albumin [g/dl]	3.9 (3.7–4.1)
FEV1 [%]	67.0 (43.0–83.0)
ALT [U/L]	19 (14–30)
AST [U/L]	22 (17–26)
GGT [U/L]	15 (10–22)
Vitamin E dose [mg/day][a]	100.0 (5.0–200.0)

[a] Median and 1st-3rd quartile for vitamin E dose were calculated for all CF patients (receiving and not receiving vitamin E)
FEV1 — forced expiratory volume in 1 second; GGT, gamma-glutamyl transferase; ALT, alanine transaminase; AST, aspartate transaminase

Seventy-nine healthy subjects — 54 (68.4%) females and 25 (31.6%) males — aged 18.5–29 years constituted the comparative group.

Twenty-four (72.7%) CF adults were pancreatic insufficient. Liver cirrhosis was documented in 3 (9.1%) studied patients. *Pseudomonas aeruginosa* had been isolated from the sputum at least once within a 6-month period before the study in 24 (72.7%) patients. Four (12.1%) subjects had diabetes.

Eighteen (54.5%) CF adults were receiving vitamin E according to existing recommendations [2]. The dose ranged from 100.0–400.0 mg per day (mean±SD: 250.2 ± 109.9 mg/day; median: 181.0; 1st-3rd quartile: 181.0–362.0). Seven (21.2%) patients took vitamin E in very low doses (≤15mg/day), not recommended in CF, and 8 (24.3%) patients did not receive any supplementation.

The study was conducted in accordance with the Declaration of Helsinki. Written, informed consent from patients (>16 years old) and patients' parents (for patients under 16 years old) was collected. The project was approved by the Bioethical Committee at Poznan University of Medical Sciences (decisions no. 244/2012 and 200/2018).

Methods

Vitamin E (α-tocopherol) concentration was analyzed by high-performance liquid chromatography (HPLC). Total cholesterol concentration was determined in human serum using the Beckman Coulter AU analyzer.

Normal vitamin E concentrations and α-tocopherol:cholesterol ratios were defined by using 5th to 95th percentile of the studied com-

parative group (healthy adult subjects). These values were used for a comparison with CF subjects. In addition, we used existing reference values of vitamin E concentration for adults in Poland (5.0–20.0 µg/ml / 500–2000 µg/dl) [3] and Great Britain [5].

Statistical analysis

The Mann-Whitney U test was used to assess differences between CF adults and healthy subjects regarding α-tocopherol concentrations, α-tocopherol:total cholesterol ratio and cholesterol levels. The Fisher's exact test was used to estimate the accordance of the distribution of vitamin E and vitamin E:cholesterol ratio. The level of significance was set at $p< 0.05$. Statistical analyses were carried out using StatSoft. Inc (2014) STATISTICA (data analysis software system version 12).

Results

CF adults have lower vitamin E concentrations and higher vitamin E:cholesterol ratio than healthy subjects. The underlying factor that determines this phenomenon is related to low cholesterol levels observed in CF patients (**Table 2**).

The distribution of vitamin E concentrations and of α-tocopherol:cholesterol ratios in CF adults has been presented in **Figure 1**. Depending on the reference values used [3,5, own data from the present study], low vitamin E concentrations were found in 4 (12.1%), 3 (9.1%) and 13 (39.4%) CF

Table 2. Comparison of α-tocopherol and cholesterol concentrations, and α-tocopherol:cholesterol ratio between CF patients and healthy subjects

Median (1st-3rd quartile)	CF adults (N = 33)	Healthy adults (N = 79)	p
α-tocopherol [µg/dl]	830 (640–1300) (274–2570)*	1132 (987–1251) (781–1510)*	0.00174
α-tocopherol:cholesterol [mg/g]	7.2 (5.6–9.7) (2.3–14.4)*	6.7 (6.0–7.3) (5.3–7.9)*	<0.00001
Cholesterol [mg/dl]	128 (114–153)	171 (155–187)	<0.00001

*<5th-95th percentile>

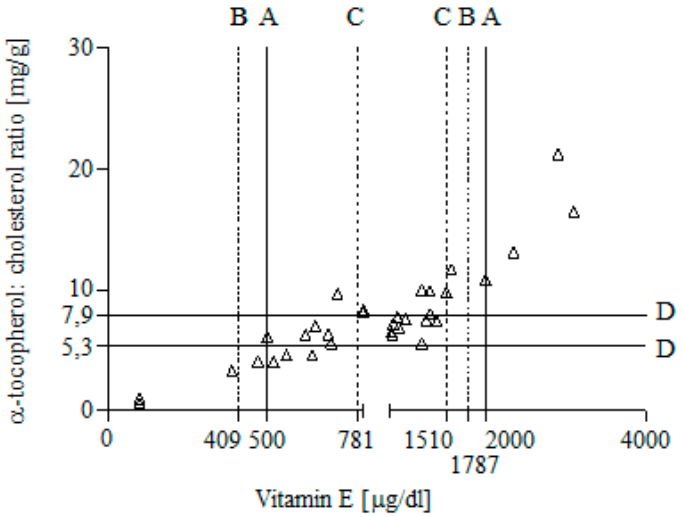


Figure 1. Vitamin E concetrations and α-tocopherol:cholesterol ratios in CF adults. A – Polish reference values of vitamin E concentration for adults in Poland (Prószyńska et al., 1991); B – British reference values (Ford et al., 2006); C, D – normal vitamin E concentrations and normal α-tocopherol:cholesterol ratios defined by using 5th to 95th percentile of the comparative group (healthy adult subjects)

patients, respectively. Similarly, high values were documented in 3 (9.1%), 4 (12.1%) and 5 (15.2%) CF patients, respectively.

What is worth noting, the percentage of low vitamin E concentrations defined in comparison to healthy Polish peers is considerably higher than low vitamin E/cholesterol ratios (39.4% vs. 21.2%, $p = 0.0011$). The distribution of low/normal/high results of both parameters has been presented in **Table 3**. The assessment of vitamin E body resources based upon its concentrations in CF seems to result in the overestimation of occurring deficits. We have found 7 (21.2%) CF adults with low vitamin E concentration and low values of α-tocopherol:cholesterol ratios. However, 6 (18.2%) CF patients with low α-tocopherol levels have normal or high α-tocopherol:cholesterol ratios.

Table 3. The distribution of low/normal/high results of α-tocopherol concentration and α-toco-pherol:cholesterol ratio

		α-tocopherol:cholesterol ratio [mg/g]			p
		Low	Normal	High	
α-tocopherol concentration [μg/dl]	Low	7 (21.2)	5 (15.2)	1 (3.0)	0.00008
	Normal	0 (0)	9 (27.2)	6 (18.2)	
	High	0 (0)	0 (0)	5 (15.2)	

Discussion

In the current study, we documented that the measurement of α-tocopherol, instead of α-tocopherol:cholesterol ratio in adults with CF may overestimate vitamin E deficiency. Vitamin E concentrations were significantly higher in healthy subjects. However, after the correction for cholesterol level this phenomenon appeared to be apparent. In fact vitamin deficiency in CF adults patients was less frequent that one could assess based upon vitamin E levels exclusively.

We intentionally selected patients with varied vitamin E supplementation to have the possibility to assess patients with different vitamin E body resources. In the studied group, there were subjects not receiving vitamin E, and receiving it in non-recommended doses. This study group does not reflect the typical population of CF adults. However, it allowed us to the reach the objective of the study.

According to the current data, the proper way to assess vitamin E status in CF is still uncertain [2]. Available evidence suggests that vitamin E circulates in the blood bound to lipoprotein. Therefore, it seems that vitamin E levels should be estimated using the α-tocopherol to the total lipid (cholesterol, triacylglycerol, phospholipid) ratio [12, 13]. Unfortunately, the ratio α-tocopherol:total lipid has rarely been clinically available [13]. Therefore, instead of α-tocopherol:total lipid it is possible to use α-tocopherol:cholesterol ratio for estimating vitamin E status [4]. The evaluation of α-tocopherol:total lipid ratio may be relevant when serum lipid levels are low (as it is frequently in CF) because of falsely decreased α-tocopherol concentrations [4]. Therefore, we can expect that some patients may have low α-tocopherol concentration and normal vitamin E: lipid ratio or normal vitamin E concentration and high α-tocopherol:lipid ratio. Ford et al. found that 32 (56%) out of 57 subjects studied with vitamin E deficiency had low α-tocopherol concentrations and normal α-tocopherol:cholesterol ratio. They also documented two out of 457 non-CF patients with normal vitamin E levels and low vitamin E:cholesterol ratio in their study. Both of these patients (a 20-week-old child and a 53-year-old male) had cholestasis. In the past, normal vitamin E concentration and low vitamin E:cholesterol ratio were described in patients with chronic cholestasis and neurological symptoms of vitamin E deficiency [14]. In the present study, we have not documented any of CF adults with normal α-tocopherol levels and low α-tocopherol:cholesterol ratios. Another important issue is that normal values of vitamin E concentration differ between countries and publications. According to three different normal ranges considered in the present study, vitamin deficiency could be diagnosed in 12.1%, 9.1% and 39.4%, respectively (**Figure 1**).

The consistency of α-tocopherol concentrations and α-tocopherol:cholesterol ratio in CF is doubtful, as has been documented in the present study. Ford et al. documented high concordance — 92% and 99.5% respectively — of results between α-tocopherol concentrations and α-tocopherol:cholesterol ratio in all subjects aged 11 days to 90 years and participants with normal vitamin E status, respectively. However, the percentage of concordant results in subjects with vitamin E deficiency was only 42.0% [5].

In conclusion, the evaluation of α-tocopherol concentration not α-tocopherol: cholesterol ratio in CF adults results in the overestimation of vitamin E deficiency. Moreover, there is also potentially a large percentage of individuals with excessive vitamin E body resources, when using α-tocopherol:cholesterol ratio for estimating vitamin E status. However, this aspect demands further studies.

Acknowledgements

Conflict of interest statement

The authors declare no conflict of interest.

Funding sources

Supported by a grant from the Poznan University of Medical Sciences (No. 502-0101103115-07588).

Specific author contributions

E.S. designed the study, performed the statistical analysis, analyzed and interpreted data and drafted the manuscript, P.K-J performed the statistical analysis and revised the manuscript, D.W., SZ.K. provided the data and revised the manuscript, J.W. designed the study, coordinated data acquisition, analyzed and interpreted data, drafted and revised the manuscript. All authors read and approved the final manuscript.

References

1. Sokol RJ. Selection bias and vitamin E and cystic fibrosis. J Pediatr. 2007 May;150(5):e85–e86.
2. Turck D, Braegger CP, Colombo C, Declercq D, Morton A, Pancheva R, et al. ESPEN-ESPGHAN-ECFS guidelines on nutrition care for infants, children, and adults with cystic fibrosis. Clin Nutr. 2016 Jun;35(3):557–577.
3. Prószyńska K, Bibik K, Książyk J, Lorenc RS, Socha J. Ocena niedoboru witamin A, E, D u dzieci z chorobami wątroby. Pediatria Polska. 1991;LXVI(9–10):19–25.
4. Huang SH, Schall JI, Zemel BS, Stallings VA. Vitamin E status in children with cystic fibrosis and pancreatic insufficiency. J Pediatr. 2006 Apr;148(4):556–559.
5. Ford L, Farr J, Morris P, Berg J. The value of measuring serum cholesterol-adjusted vitamin E in routine practice. Ann Clin Biochem. 2006 Mar;43(Pt 2):130–134.
6. Rana M, Wong-See D, Katz T, Gaskin K, Whitehead B, Jaffe A, et al. Fat-soluble vitamin deficiency in children and adolescents with cystic fibrosis. J Clin Pathol. 2014 Jul;67(7):605–608.
7. Castellani C, Southern KW, Brownlee K, Dankert Roelse J, Duff A, Farrell M, et al. European best practice guidelines for cystic fibrosis neonatal screening. J Cyst Fibros. 2009 May;8(3):153–173.
8. Farrell PM, White TB, Ren CL, Hempstead SE, Accurso F, Derichs N, et al. Diagnosis of Cystic Fibrosis: Consensus Guidelines from the Cystic Fibrosis Foundation. J Pediatr. 2017 Feb;181S:S4–S15.
9. Debray D, Kelly D, Houwen R, Strandvik B, Colombo C. Best practice guidance for the diagnosis and management of cystic fibrosis-associated liver disease. J Cyst Fibros. 2011 Jun;10(Suppl 2):S29–S36.
10. Walkowiak J. Faecal elastase-1: clinical value in the assessment of exocrine pancreatic function in children. Eur J Pediatr. 2000 Nov;159(11):869–870.
11. Walkowiak J, Nousia-Arvanitakis S, Cade A, Kashirskaya N, Piotrowski R, Strzykala K, et al. Fecal elastase-1 cut-off levels in the assessment of exocrine pancreatic function in cystic fibrosis. J Cyst Fibros. 2002 Dec;1(4):260–264.
12. Morton A. Why bother to take vitamins? J R Soc Med. 2011 Jul;104(Suppl 1):S19–S29.
13. Maqbool A, Stallings VA. Update on fat-soluble vitamins in cystic fibrosis. Curr Opin Pulm Med 2008 Nov;14(6):574–581.
14. Sokol RJ, Heubi JE, Iannaccone ST, Bove KE, Balistreri WF. Vitamin E deficiency with normal serum vitamin E concentrations in children with chronic cholestasis. N Engl J Med. 1984 May;310(19):1209–1212.

Acceptance for editing: 2019-03-12
Acceptance for publication: 2019-03-29

Correspondence address:
Jarosław Walkowiak
Department of Pediatric Gastroenterology and Metabolic Diseases
27/33 Szpitalna Street, 60-572 Poznan, Poland
phone: +48 618491432, fax: +48 618472685
e-mail: jarwalk@ump.edu.pl

ORIGINAL PAPER

DOI: https://doi.org/10.20883/jms.270

The characteristic of dietary supplementation among elderly women

Rafał W. Wójciak[1], Angelika Cisek-Woźniak[2, a], Ewa Tomczak[2]

[1] Department of Clinical Psychology, Poznan University of Medical Sciences, Poland
[2] Chair of Dietetics, Faculty of Physical Culture in Gorzow Wlkp., Poznan University of Physical Education, Poland
[a] https://orcid.org/0000-0002-6345-1795 [a] https://orcid.org/0000-0001-8194-7500

ABSTRACT

Aim. There is a growing awareness in Polish society, that a healthy lifestyle and proper nutrition have positively affected in old age. This effect influences the increasing consumption of dietary supplements to improve the health, however sometimes in an uncontrolled way. Taking above together the aim of this preliminary study was to assess the prevalence of the use of dietary supplements in elderly women.
Material and Methods. The study was conducted on 95 elderly women aged 65 to 89 years. The participants were asked to complete a questionnaire about their physical activity as well as medicines and supplements intake.
Results. Based on the questionnaire, it can be concluded that supplementation was common among the tested group. The most frequently seniors used preparations to assist the circulatory system and diet supplements. They also took this, supporting the work of the intestine, to assist urinary tract and the work of heart. The decision to start of supplementation was most often taken under the influence of television advertisements, pharmacy worker as well as from friends. There were statistically differences in presented results between studying populations according to their age.
Conclusions. Important and essential is education of older people, concerning the appropriate use of dietary supplements, to make supplementation safe and distinct improvement in health.

Keywords: women, elderly, supplementation, physical activity.

Introduction

Dietary supplements are substances that task is to enrich the normal diet with those components to be undernourished. These are concentrated foods which can be a source of vitamins and minerals or other substances, showing the effect of nutrient or other physiological [1, 2]. They are placed on the market in specific doses and in various forms such as tablets, dragees, capsules, drops, sachets of powder and liquid ampoules [1–4]. Dietary supplements are usually low doses of the active substance, what makes their impact on the body is underestimated [3]. Those preparations are usually sells without a prescription.

Seniors have used supplements in order to: improve the nutrition of the body, enhance vitality, improve concentration, condition hair, skin and nails, delay the aging process, reduce the risk of certain chronic diseases and to increase its resistance. The application of vitamin supplementation has a lot of positive reviews in the literature [5–7].

In nutrition practice there are a number of indications for dietary supplementation by persons in old age. The use of supplements is recommended to persons with impaired absorption of nutrients, because of diseases or the use of certain medicines (antibiotics, diuretics) [1, 8]. Dietary supplements are also recommended to persons would

commit in daily practice dietary errors resulting sometimes with economic difficulties and a limited supply of food, but often for the wrong dietary habits or nutritional knowledge is insufficient [1].

There is a common opinion that dietary supplements consumed by seniors have confirmed the beneficial effect on their health and general condition, as well as cognitive status and beauty [6, 9]. There is no unambiguous evidence for this, and the research seems to be unclear and contradictory [10, 11]. The mainly benefits of vitamin and mineral food supplements intake by the elderly are the fortification of the nutritional deficiency, commonly associated with age. On the other hand, it should be noted that there are also dangers arising from uncontrolled use of supplements. Name here you need to egg. interactions between nutrient supplements and drugs, chronic adopted by this group of people, interactions with food and very importantly — the ability to overdose of supplementing substances.

Although some data suggests that the diet supplementation affects more than 50% of adults [8], even 75% [10], there is no data on it in the elderly.

In the presented preliminary study the quality and quantity of medicines and supplements used by elderly women were evaluated, as well as declarative motivation for applying diet supplements and the sources of the information about its. Additionally, the frequency and preferable form of physical activity among seniors in different aging groups were examined, as a marker of general health status of their.

Material and Methods

The study was involved 95 elderly women aged from 65 to 89 years old. The participants were divided into 3 aged depended subgroups: 65–70 years 71–80 years 81–89 years (42, 32, 26%, respectively).

The questionnaire method was applied in the study as well as personal interview. The questionnaire form has contained 16 items (14 closed questions and 2 open questions). In the interview all questions were read to women and interview was also based on the other questions on the age, education, place of residence, marital status and family life. **Table 1** presents the characteristic of studying population. The women participated in the study were mostly in higher education (57%), lived in the cities with more than 50 thousand inhabitants (55%), and live alone more than 5 years (53%). However, the totally all subjects represented apparently good health condition, about half of studying population had some problems. Most often women reported the following disorders: hypertension (60%), hypercholesterolemia and cardiovascular (both 35%), diabetes and arthritis (both 25%), and other (10% — chronic small pain, depression, migraine etc.). The number of reported diseases increases with age. The 25% of the subjects was overweight. All participants were asked about permission to participate to the study and gave it.

To assess the frequency distribution of data between groups according to the age the chi-square test was applied.

Table 1. The characteristic of studying population

	Total (n = 95)	Age groups		
		65–70 (n = 40)	71–80 (n = 30)	81–89 (n = 25)
High education (%)	57	55	50	65
Residents of cities with more than 50 thousand inhabitants	55	45	60	60
Living alone more than 5 years (singles, divorces, widows)	53	55	60	40
Health conditions (%)				
Hypertension	60	50	65	65
Hypercholesterolemia	35	30	35	35
Cardiovascular disease	35	20	30	50
Diabetes	25	25	20	25
Arthritis	25	15	30	30
Other	10	5	10	15
Overweight	25	30	25	20

Results

The results obtained in this study are presented in Tables 2–6. **Table 2** shows the frequency of physical activity among seniors. In the vast majority of respondents (79%) declared one of the forms of physical activity: walking, fitness, swimming, dancing or any other form such as: exercise at the gym, playing team sports games or practicing martial arts. There were observed the statistically significant differences ($p < 0,001$) between women in different age in the frequency of physical activity. About 50% of the oldest ladies declared no activity, thus 20% the youngest. More than twice a week the 70% of the youngest ladies train a sport, thus no the oldest.

When it comes to the kind of physical activity, there were the 5 types of physical activity declared by women. The largest number of seniors has granted to walks — 65% of total active seniors (most popular in groups of women above 71 years old — 40 and 40%, respectively), various kinds of fitness exercise reported 35% of respondents (50% of the youngest ladies), 26% of respondents spoke about the dance, 12% swimming, and 8% had other forms of activity (declared only by youngest women).

Table 3 presents the detail information about what medicaments respondents used chronically. Because of his age and condition of health, respondents have used daily for more than one type of drug. The most commonly used drugs were popular on the pharmaceutical market pain medication (70%). Almost half of the respondents declared antiarrhythmic medicaments used (49%). The drugs using for neutralizing the excess of hydrochloric acid in the stomach were used by 38% of seniors, the antidiabetics drugs by 35%, antihypertensives — 28%, drugs that improve cerebral circulation — 25% and medicines against osteoporosis — 22%. The smallest group of respondents used antidepressants, anxiolytic and hormones (5–7%). There were significant differences between the groups of seniors according to the age. In general, there was an increase in the use of drugs in the oldest group, apart from hormonal drugs (most popular in the youngest).

The total number of medicines and supplements used by studying women is presented in **Table 4**. All participants of the study declared using at least one supplement. The statistically significant differences between age groups were observed ($p < 0,001$). In the youngest ladies, more than 4 preparations were used by 35 and 40% respectively, while in the oldest — 70% of women regularly consumed daily more than 4 preparations.

In accordance with its definition, dietary supplements can be on the market in many forms. **Table 5** shows the preferences of seniors in relation to the form in which most take dietary supplements. It shows that the most frequently adopted the form of the supplements are pills (63%) and liquid form (21%). Less frequently while they reach for effervescent tablets and drops (8%, each). There were no differences between groups according to the age.

Table 2. Physical activity among seniors according to the age

		Total (n = 95)	Age groups (% of total)		
			65–70 (n = 40)	71–80 (n = 30)	81–89 (n = 25)
Frequency of physical activity	No activity	21	20	30	50
	Once a week	47	20	65	15
	Twice a week	16	40	30	30
	More than twice	16	70	30	0
Statistics			$\chi^2 = 49,1$; $p < 0,001$		
		Total (n = 75)	Age groups (% of total)		
			65–70 (n = 36)	71–80 (n = 24)	81–89 (n = 15)
Forms of preferable physical activity	Walk	65	20	40	40
	Fitness	35	50	25	25
	Dance	26	50	40	10
	Swimming	12	40	50	10
	Other	8	100	0	0
Statistics			$\chi^2 = 52,3$; $p < 0,001$		

Table 3. Groups of medicines lengthily applied by seniors (% of population) according to the age

	Parameters	Total (n = 95)	Age groups (% of total)		
			65–70	71–80	81–89
1.	Painkillers	70	25	30	45
2.	Antiarrhythmic	49	20	35	45
3.	Neutralizing the excess of acids	38	30	45	25
4.	Anti-diabetics	35	10	25	65
5.	Anti-hypertensive	28	35	35	30
6.	Improving cerebral circulation	25	35	35	30
7.	Anti-osteoporosis	22	40	35	25
8.	Analgesics and febrifugal	18	30	35	35
9.	Antineoplastic agents	8	5	25	70
10.	Antidepressants	8	10	70	20
11.	Hormones	7	70	20	10
12.	Anxiolytics	7	10	50	40
13.	Other	5	70	15	15
Statistic			$\chi^2 = 128,7; p < 0,001$		

Table 4. The number of regular daily intake of the medicines and supplement according to the age of seniors

	Age groups								
	65–70 (n = 40)			71–80 (n = 30)			81–89 (n = 25)		
Number of preparations	> 4	2–3	1	> 4	2–3	1	> 4	2–3	1
Percentage of population	35	25	40	40	30	30	70	20	10
Statistic	$\chi^2 = 34,3; p < 0,001$								

Table 5 shows also the motivation of seniors for applying diet supplementation and sources of information on its. The five purposes for which older people use supplements in their diet appropriate pharmaceuticals were highlighted. These were: dietary supplementation in minerals and vitamins (27%), complement deficiencies of nutrients caused by chronic use of medications (23%), to improve the health and wellbeing (21%), to improve the external appearance (12%), and general improvement of the good condition (17%). The motivation for applying diet supplementation was significantly different in the aging groups ($p < 0,001$). Although the youngest women also pay attention to such reason as filling the mineral and vitamin deficits or filling up the deficits caused by drugs, it is also important for them to look after their beauty. This reason is poorly represented in the group of the oldest women (5%).

After the analysis of the sources of information about dietary supplements in studying seniors (**Table 5**), it was found that the greatest effectiveness of the information about specific dietary supplements is TV advertisements (30%). The same group of respondents obtain needed information from pharmacists and friends (including) (20%), doctors and press (15%). A worrying situation is the fact that none of the interviewees did not provide that information on the necessary and appropriate for the body's dietary supplements received during consultation with a nutritionist. The oldest ladies as a source of information about supplements declared TV advertisements (45%) and pharmacy workers (30%), while the youngest mostly based on the information from friends (25%). There were statistically significant differences ($p < 0,001$).

Table 6 presents the characteristic of the supplements used by seniors. Seniors most often declared using the preparations to assist the circulatory system (45%, mostly by the oldest women — 45% and 30% the youngest) and diet supplementation (43%, mostly by the youngest women — 50%, while only 20% of the oldest). Almost 20% of seniors declared using the preparations supperting their intestine (mostly women over 71 years old — 80%). There were statistically significant differences between studying group of women depend on their age ($p < 0,001$).

Similar differences between elderly women depend on their age in the most commonly used commertial preparations were observed in this study (p < 0,001). However the youngest women were more likely to buy vitamins an dminerals (70%), pro- and prebiotics (55%), the oldest ladies put on the natural preparations (ginko biloba — 55%, cranberry — 45%).

Table 5. The characteristic of the most popular forms of the supplements, motivation to use and source of information about supplements (% of population)

	Total (n = 95)	Age groups (% of group)		
		65–70	71–80	81–89
Preferences for the form of applicable supplements				
Pills	63	65	60	65
Liquid form	21	18	24	20
Drops	8	10	6	7
Effervescent tablets	8	7	10	8
Statistic			n.S.	
Motivation for applying diet supplementation				
Dietary supplementation in deficits components (minerals and vitamins)	27	30	25	26
Supplementation of drug induced deficiency	23	25	15	30
Improvement in the health and well being	21	5	33	22
Improvement to the external appearance (hair, skin, nails)	12	20	12	5
General improvement of good health and physical activity	17	20	15	17
Statistic		χ^2 = 35,3; p < 0,001		
Sources of the information on dietary supplements				
TV advertisements	30	25	20	45
Pharmacy	20	10	20	30
Friends	20	30	25	5
Doctors	15	15	15	15
Press, internet	15	20	20	5
Statistic		χ^2 = 49,2; p < 0,001		

Table 6.The characteristic of the supplements used by seniors

	Parameters	Total (n = 95)	Age groups (% of total)		
			65–70	71–80	81–89
	The type of supplements				
1.	Preparations to assist the circulatory system	45	30	25	45
2.	Diets supplements	43	50	30	20
3.	Preparations supporting the work of the intestine	20	20	50	30
4.	Preparations to assist urinary tract	18	30	35	35
5.	Preparation to assist the work of heart	15	25	25	50
6.	Preparations supporting the bacterial micro-flora	12	30	40	30
7.	Preparations to assist the memory	10	20	30	50
Statistic			χ^2 = 45,4; p < 0,001		
	The most commonly used commertial preparations				
1.	Diosmin	45	40	35	35
2.	Vitamins and minerals	43	70	15	15
3.	Prebiotics and probiotics	30	55	25	20
4.	Glucosamine	23	35	40	25
5.	Herbs	22	30	35	35
6.	Omega-3 fatty acids	15	40	40	20
7.	White mulberry	15	45	40	15
8.	Cranberry	10	10	45	45
9.	Ginko biloba	10	25	20	55
10.	Lecithin	10	20	55	25
Statistic			χ^2 = 165,9; p < 0,001		

Discussion

In recent times there has been a significant development of civilization and has evolved to medicine. This has contributed to the extension of the life of society [5, 9]. In modern times it is observed considerable diversity of older people in conditions of health, cultural characteristics, socio-demographic and economic [9, 12]. The western society is ageing. The increasing number of the older people in the population is starting to be seen as problematic and has an effect on the occurrence of unfavourable attitudes towards that group. Despite this state of affairs are taken action to change so negative perception of older people. Makes to society, that aging is a natural turn things and is the next phase of life [1, 13].

An aging society requires increase attention to the health problem of elderly people. In modern society more and more popular to use dietary supplements next to recommended drugs is becoming more and more popular among the elderly population. Although there is few data about supplementation of the oldest group of society, some of the authors are in the opinion that 50 — 70% adults take dietary supplements [8, 10]. In this paper we asked almost 100 old ladies about using the dietary supplements, and all of them answer positive. There is similar as reported by other Polish authors that the supplements in Poland are over-consumed [1, 6, 14]. On the other hand Kałużna et al. [6] showed the positive effect on the condition of health of the elderly the vitamin and mineral supplementation. This observation is similar to the those presented by PolSenior population study reported by Bogusz et al [9] who were made a study of physical health of older people. This study shows that the health status of seniors has improved in recent years, especially in seniors between 65 and 79 years old.

Kaczmarczyk and Trafiałek [15] are in the opinion that physical activity of seniors is not greater, although they have more free time than younger. It focuses mainly on helping in the daily chores, children and grandchildren. The process of activation of elderly is slow, but it should be considered to be incremental. This is confirmed by the results presented in this paper. Seniors declared a different forms of physical activity: walking, fitness exercises, swimming, dancing and other activities. Frequency of physical activity declared by the respondents in the vast majority of at least once a week. Just ca. 21% of respondents showed no additional physical activity. Observation in this aspect are very optimistic, however frequency of even small intensive activity was decreased with aged, and more than half of the oldest ladies have not trained, what was not reason of bad health condition. This results are correspond with worldwide general observation about low physical activity of seniors, especially women [12, 15].

The presented study found that supplementation is common among older people. The use of dietary supplements was not dependent on age, education, or place of residents. Brzozowska et al. [1] were found that at the turn of the years in the US has changed only the percentage of people using dietary supplementation in vitamin-mineral substances, but all the time interest in the supplementation exists. Currently, both women and men use dietary supplements.

Interviewed people from a research group not noted for visits to a nutritionist or a doctor to determine the level of vitamins and minerals in their body. Motivation to use a dietary supplement was overwhelmingly catchy TV commercial. This situation is quite dangerous and worrying, since inappropriate use of vitamin-mineral can cause the opposite effect intended and affect the health of the elderly. This can be a reason of increase number of preparations with age. Similarly, Kałuża et al. [6] have established that often use vitamin preparations by the elderly was improper. It was suggested to increase the level of education of older people in terms of the rules of application of supplementation. To similar conclusions reached Sygnowska and Waśkiewicz [7]. Based on this research claimed that dietary supplementation by vitamins and minerals can impact favourably on the body of elderly but without consulting your doctor or nutritionist its activity can cause severe side effects (including overdose of vitamins and mineral substances). More than 70% of European adults use different dietary supplements [10], however this number can be enlarged with age [8, 10, 16]. The using of the medicines are also larger in elderly than in younger population [8, 12, 17] what is associated with chronic disease in this population. In presented study the women aged above 80 years consumed more than four different drugs and

supplements a day, mostly painkillers and minerals, and vitamins. On the other hand it was not observed extremely large number of diseases in this sub-group than in younger women (below 80 years old). It could suggests that the over-consumed of preparations in this age is not caused by currently diseases.

Another problem in this work was to determine the source of the benefit of knowledge about dietary supplements and motivation, which follow the seniors when choosing a specific parapharmacy products. It was found that by far the best source of knowledge about dietary supplements for respondents are TV commercials, followed by pharmacists and the least significant is the press. Very few only sought on this issue applies to a doctor. Confirmation of such results it has been found in the work of Ulatowskiej-Szostak [18]. This author carried out a study on the effect of advertising on purchase of parapharmacy products and vitamin preparations. Confirmed that a significant proportion of patients purchase dietary supplements under the influence of the ads, and only a small group of patients looking for additional information on their purchase. Subsequent researchers Saran and Duda [14] have shown that the main source of knowledge about the dietary supplements are television commercials, and only after them in order: press, doctors, and only at the end of the pharmacists. Wojciak et al. [4] compared the nutritional knowledge of elderly with their nutritional status and depression symptoms. They found that seniors were characterized by low nutritional knowledge what was evidently associated with their health presented by nutritional status (including iron deficiency anemia). The worldwide authors presents similar observations [3, 8, 14]. The aggressive advertisement, TV, press, leaflets, are everywhere and promise good health and long life.

In this work, as the cause of the dietary supplementation was administered a desire to supplement diets in vitamins and minerals, and supplement the shortages incurred as a result of chronic use of drugs. These statements were consistent with the experiences carried out in other universities in Poland. Study of Saran and Duda [2] confirmed obtained in studies tend on the reasons for the use of dietary supplements.

The last evaluation parameter was the impact of dietary supplement on the condition of health of the respondents. According to more than half of the surveyed, supplementation did not bring the economic changes in their well-being. Just about 30% of seniors observed beneficial effects of supplementation on their health. Brzozowska et al. [1] in his work found that the results of research on the use of dietary supplements and their positive or negative action on the body of an older person were ambiguous and, therefore, it was suggested to continue research on this topic among people in this age. As an important note the need for nutrition education of older people, also by professionally dealing with this subject specialists in the field as dietetics and human nutrition. Knowledge about the dietary supplementation and all sorts of nutrients should be updated continuously, and the way in which the older people adjusted adapted to each senior in accordance with his perception [4, 14].

At the end seniors were asked about the impact of used dietary supplements in their current condition of health. It turned out that more than half of the surveyed (51%) did not find any difference in health condition after applying dietary supplement. 38% of respondents noted that the vast health and beneficial effect of supplementation. Other respondents felt that the effect of nutritional supplementation on their condition of health was only halftime (not fully met expectations of tested older person in relation to nutritional supplements). So, what for the elderly people over-consume the supplements, this is the question.

Conclusions

To sum up, the presented work surveys can draw the following conclusions about dietary supplementation among old ladies in Poland. The use of dietary supplements is widespread and all subjects used it, even every day. The process of activation of ageing is slow, but it should be considered for growth. For the vast majority of respondents frequency of different forms of physical activity was not less than 1 time per week. However is independent on the health status. Insufficient knowledge about the actual condition of their own health and the necessity of taking dietary supplements may cause their effects on the body of an elderly person it is imperceptible

for itself. Disturbing is the fact that most elderly people decide to purchase dietary supplements without consulting with professionals, so doctors and nutritionists, and the desire and need to buy a specific parapharmacy products is caused mainly by advertising on television. It should be suggested increasing the education level of older people in the principles of supplementation.

Acknowledgements

Conflict of interest statement

The authors declare no conflict of interest.

Funding sources

There are no sources of funding to declare.

References

1. Brzozowska A. Enriching food and supplementing the diet with nutrients — benefits and risk. Żywność 2001;4(29):16–28.
2. Saran A, Duda G. The influence of selected factors for the purches and use of vitamin and mineral supplements by the elderly. Żywność. Nauka. Technologia. Jakość. 2009;4(65):271–277.
3. Cameron DI, Kurrle SE, Uy C, Lockwood KA, Au L, Schaasfsma FG. Effectiveness of oral nutritional supplementation for older women after a fracture: rationale, design and study of the feasibility of a randomized controlled study. BMC Geriatrics. 2011;11:32–37.
4. Wojciak RW, Mojs E, Staniek H, Marcinek K, Krol E, Sulibursk J, Krejpcio Z. Depression in seniors vs. their nutritional status and nutritional knowledge. J Med Sci. 2016;85(2):83–88.
5. Shibata A, Paganini-Hill A, Ross RK, Henderson BE. Intake of vegetables, fruits, beta-carotene, vitamin C and vitamin supplements and cancer incidence among the elderly: a prospective study. Br J Cancer. 1992;66:673–679.
6. Kałuża J, Bagan A, Brzozowska A. The evaluation of the share of vitamins and minerals from supplements in the diet of older people. Roczn PZH. 2004;55(1): 51–61.
7. Sygnowska E, Waśkiewicz A. The role of supplementation in replenishing the deficiencies of vitamins and minerals in the diet of Poles covered by WOBASZ. Bromat Chem Toksykol. 2008;41(3):389–394.
8. Blumberg JB, Frei B, Fulgoni VL, Weaver CM, Zesel SH. Contribution of dietary supplements to nutritional adequacy in various adult age groups. Nutrients. 2017;9:1325–1334.
9. Bogusz R, Charzyńska-Gula M, Szkuat M, Kocka K. Functional fitness of people over 70 years of age in rural areas and needs for care. MONZ. 2013,19(4):517–522.
10. Burnett AJ, Livingstone KM, Woods JL, McNaughton S. Dietary supplement use among Australian adults: findings from the 2011–2012 National Nutrition and Physical Activity Survey. Nutrients. 2017;9:1248–1259.
11. Locquet M, Honvo G, Rabenda V, van Hees T, Petermans J, Reginster JY, Bruyere O. Adverse health events related to self-medication practices among elderly: a systematic review. Drugs Ageing. 2017;34(5):359–365.
12. Kim J, Lee JS, Shin A, Kang MH, Shin DS, Chung HR, Kim WK. Sociodemographic and lifestyle factors are associated with the use of dietary supplements in a Korean population. J Epidemiol. 2010;20(3):197–203.
13. Marona H, Gunia A, Pękala E. Retinoids — a role in pharmacotherapy in the aspect of the cellular mechanism of action. Farmacja Polska. 2010;66(3):187–192.
14. Saran A, Duda G. The assessment of knowledge of older people regarding vitamins and minerals. Brom Chem Toksykol. 2010;43(1):60–65.
15. Kaczmarczyk M, Trafiałek E. Activation of older people as a chance for successful aging. Gerontologia Polska. 2007;15(4):116–118.
16. Brończyk-Puzoń A, Bieniek J. Nutrition of the elderly on the basis of an amendment to the nutrition standards of the Institute of Food and Nutrition for the Polish population from 2012. Nowa Medycyna. 2013;4:151–255.
17. Park HA. Top 10 dietary supplements of Korean adults from the 4th Korea National Health and Nutrition Examination Survey. Korean J Farm Med. 2011;32:263–266.
18. Ulatowska-Szostak E. The impact of advertising on the purchase of drugs, parapharmaceuticals and vitamin preparations in the opinions of pharmacy customers — comparison of 2002 and 2007. Probl Hig Epidemiol. 2008;89(3):441–444.

Acceptance for editing: 2019-03-13
Acceptance for publication: 2019-03-29

Correspondence address:
Rafał W. Wójciak
Department of Clinical Psychology
Poznan University of Medical Sciences
70 Bukowska Street, 60-812 Poznań, Poland
phone/fax: +48618547274
email: rafwoj@ump.edu.pl

REVIEW PAPER

DOI: https://doi.org/10.20883/jms.2019.333

Posturography examination as a diagnostic tool in children – a review of the literature

Joanna Borek[1, 2, a], Roksana Malak[2, b], Teresa Matthews-Brzozowska[2, c], Włodzimierz Samborski[1, d]

[1] Department of Rheumatology and Rehabilitation, Poznan University of Medical Sciences, Poland
[2] Department and Clinic of Maxillofacial Orthopaedics and Orthodontics, Poznan University of Medical Sciences, Poland

[a] https://orcid.org/0000-0003-3959-7214
[b] https://orcid.org/0000-0003-0521-5249
[c] https://orcid.org/0000-0002-7127-6018
[d] https://orcid.org/0000-0002-0338-894X

ABSTRACT

Introduction. Posturograph tests are used to assess the vestibular reflex, which can be helpful for specialists who analyze body balance disorders. Despite many studies on the stability of body posture in pediatric patients, there is still a lack of reliable analysis.

Aim. Performing a review of the literature to verify the relationship between balance and body posture as well as stomatognathic system as a part of motor system and the craniofacial complex using a posturograph.

Material and Methods. A review of the literature has been carried out for the posturographic examination as a diagnostic tool. The following work is for reference only. A review of the Google Schoolar database and PubMed was made. Keywords used in the search are: (children) and (posturography, posture control, balance, temporomandibular joint). The authors took into account reports published in Polish and English. For the purposes of this review, strict criteria for the inclusion and exclusion of research work have been created.

Results. The authors analyzed 335 research papers, of which 5 articles were qualified after the analysis. Studies show that there is a relationship between posturographic examination and postural disorders in children. However, the results are not conclusive and further research is necessary before these results can be considered as fully generalized.

Summary. It is necessary to standardize the research using a posturograph. There is a need for further research due to the lack of standardized measurements showing norms for individual age groups.

Keywords: children, posturography, postural control, balance, temporomandibular joint.

Introduction

Body balance is defined as maintaining and recovering a stable position. Postural control as a sense of balance is a compilation of variety of mechanisms related to the work of, sensory, muscular, skeletal and nervous systems [1]. Posturographic examination can be used as an easy and quick way to determine if a child has sufficient control of body balance [2]. The cervical spine is closely related to body balance as a part of the body which connects the skull with the masticatory system and the trunk through muscle connections and bones articulations. Many ailments within the facial part of the skull are most often the result of disorders within the temporomandibular joint and masticatory muscles.

In addition, diseases associated with the temporomandibular region reveal a number of clinical symptoms. These are three main disorders of this region called the "triad of dysfunctions", which are the most common ailments such as: myofascial pain, disorders in the temporomandibular joint and changes in the cervical spine [3]. Dysfunctions in body posture in patients with temporomandibular disorders are a controversial issue in the literature. Many researchers point to muscle origin as one of the etiological factors that mainly affects position of the head [4]. The aim of the work is to verify the reports showing the relationship between balance and body posture, and the facial and cranial complex using a posturograph.

Material and Methods

A review of the literature has been carried out in terms of the effectiveness of posturographic examination as a diagnostic tool in children. This work is a review article as an example of metanalysis. The PubMed and Google Schoolar database has been reviewed. Keywords used in the search were: (children) and (posturography, postural control, balance, temporomandibular joint). The study inclusion criteria were:

1. Participants in selected analyzes were children in the age of 2 to 18 years old.
2. The participants of the studies were subjected to the analysis of static bipedal posturography.

Table 1. Characteristics of individual research works

Author	Research group	Age	Type of posturograph	Research goal	Result	Conclusion
Barozzi et al., 2014	173 people	6–14 years	Standard Vestibology Platform	Checking the credibility of the test-retest against postural control measurements to obtain normative values.	Velocity and area decreased significantly with age, which indicates an improvement in attitude control from childhood to adolescence.	The results can be used as a reference point for early detection of postural disorders, to assess dizziness and balance problems.
Roggia et al., 2015	109 people	8–12 years	Software of Posture Analysis - SAPO	Assessment of body posture and balance in patients with open and closed mouth breathing, as well as checking if there is a correlation between the values obtained in this assessment and the values of sensory systems analysis.	Children with open mouth show changes in attitude towards children breathing through the nose, mainly in the positioning of the knee joint. Body balance in the group of children with open mouth showed a greater disorder compared to the group of children breathing through the nose. A correlation was found between the position of the head and various sensory systems.	By posturographic examination, it is possible to detect imbalances earlier.
Lara et al., 2017	80 people	6–7 years	EquiTest System – NeuroCom International	To examine the relationship between posture balance and anthropometric indicators in primary school students.	In some of the subjects, there were connections between the greater body weight and values below the norm, which indicates that anthropometric indicators influenced the balance of posture in children.	The study shows that overweight can have a negative effect stability of the body posture of children.
Mason et al., 2018	41 people	6–12 years	Bertec force plates FP 4060–10	Evaluation of the impact of rapid palatal dilation on posture and gait analysis in people with jaw disorders.	A correlation was found between the occlusion of the teeth and the posture of the body mainly during dynamic analysis.	It was found that changes in the jaws affect the entire body.
Leroux et al., 2018	7 people	15–17 years	Cyber-Sabots, SABOSOFT software	Evaluation of the effect of dental occlusion on the posture of young rowers.	The study showed a negative effect of occlusion disorders on sports performance in young rowers.	Regular tests should be carried out for malocclusion. In cases where defects are detected, an orthodontic treatment plan should be implemented that could improve the performance of athletes.

3. Researchers of particular articles were to show the relationship between the child's body's center of gravity in relation to a given group in relation to age.
4. Original articles in English or Polish were accepted for analysis.

The researchers considered the exclusion criteria as follows:

1. Postural examination of children with hearing or pattern defects.

The search strategy resulted in 335 reports in all databases. In order to obtain the latest research, articles from the last 5 years have been qualified. Referring inclusion and exclusion criteria we took into concideration 5 articles.

Results

In three works, researchers: Barozzi et al., Roggia et al., Lara et al., emphasized the necessity to introduce standardized research using a posturograph for specific age groups in children and adolescents. In addition, Manson et al., As well as Leroux et al., Noted the correlation between dental occlusion and postural disorders.

According to the authors of this work, the presented studies have connections between facial and cranial disorders, balance and changes in body posture. By examining body balance disorders using a posturograph in children, for example with anthropometric disorders, abnormal results were observed, which may be related to the immaturity of postural control or with postural disorders. Abnormalities associated with posture may cause changes in the facial and cranial area. However, the researchers emphasize the lack of sufficient scientific evidence to support a cause-and-effect relationship. Most of the scientific research is carried out without taking into account the control group.

Discussion

Currently, easy access to scientific research allows quick and common access to knowledge. However, the content of the research work should be carefully analyzed to avoid erroneous conclusions. The following discussion concerns selected studies that show the relationship between the facial and cranial complex and the posture of the body. The authors of this work try to present the purpose and usefulness of selected research in clinical practice. The relationship between bite defects and body posture has been discussed in recent decades, but there is still a lack of consensus in the available literature. So far, there have only been references to the correlation between the existing links between postural disorders and malocclusion [5]. Kopczyński et al. emphasized that the practical application of computer dynamic posturography in dental examination in terms of orthodontics is an important topic of clinical research in relation to the obtained correlation between postural defect and malocclusion [6]. The changes occurring within the facial part of the skull have a significant impact on the position of the vertebrae in the cervical region. As a result of disorders in the upper part of the spine, further dysfunctions appear. Complex mechanisms controlled by the senses, namely the somatosensory, visual vestibular system are integrated in the central nervous system. According to Shumway-Cook et al., changes in the sensory system may affect the posture of the body [7]. The area of the facial part of the skull may be related to the posture of the body. Disorders in the facial part of the skull can lead to adaptation in some body structures, where the pain of the patient is minimized, and reconstructs the zones of musculoskeletal stress. These adaptations, if not corrected, may cause deviations in the correct posture [8]. Galasso emphasized the importance of: skulls, teeth and cervical spine, which together form a functional unit inextricably linked to the position of the body. It should therefore be remembered that any oral action can affect the whole body. At the same time, we distinguish three intersections: the cervical spine, the mandible and the hyoid bone, which in turn is closely related to the tongue. Everything that happens in the mouth, through thetemporomandibular joints, thus affects the shoulder girdle, the spine and the feet and vice versa [9]. Barozzi et al. observed that with age, there is an improvement in postural control. It is possible to obtain reliable information on posture stability in children and adolescents using posturographic parameters. These data can be used as a reference point for early detection of abnormal postural development, dizziness and disturbances in children [10]. Roggia

et al. noticed changes in body posture in schoolchildren who breathe open-mouthed compared to breathing people with their mouths closed. Researchers found that there is a correlation between the position of the head and different sensory systems which causes changes in the resting position of the mandible [11]. As reported by Lara et al., overweight can have a negative effect on the stability of the children's attitude. This may be a warning regarding disorders that cause obesity for the child's development [12]. Manson et al. found a relationship between the use of a single-jaw orthodontic braces with body posture. It should be noted that there was a large correlation between the improvement of gait and proper adjustment of the device to the jaw. Therefore, the study shows a detectable relationship between body posture and dental occlusion in the age range of the subjects [13]. Leroux et al., did not observe changes in postural disorders in adolescents who practice rowing. However, the study group showed disorders in temporomandibular joints. It can be assumed that malocclusion affects the body balance only after a longer period of neuronal integration [14]. The assessment of postural disorders in patients with dysfunctions within the facial and cranial complex is of fundamental importance for prophylaxis and control as well as for appropriate treatment. According to Malak et al., posturographic assessment may be helpful for physicians, dentists and physiotherapists, especially during diagnosis, and thus in the selection of the best functional rehabilitation techniques. Early detection of postural disorders makes it possible to prevent the progress of postural defects and their consequences [15].

Conclusions

Scientific literature indicates that there is a relationship between the cervical spine, the facial and cranial complex and the posture of the body in relation to the control of body balance in posturographic examination. However, the results are not conclusive and further research is necessary. There is a need to deepen research and further work on the subject of masticatory system disorders, and the results of posturographic examination. Due to the occurrence of measurement discrepancies, it is necessary to standardize measurements using posturographs.

Acknowledgements

Conflict of interest statement

The authors declare no conflict of interest.

Funding sources

There are no sources of funding to declare.

References

1. Ohlendorf D, Mickel C, Filmann N, Wanke EM, Groneberg DA. Standard values of the upper body posture and postural control: a study protocol. Journal of Occupational Medicine and Toxicology. 2016:11:34.
2. Verbecque E, Vereeck L, Hallemans A. Postural sway in children: A literature review. Gait Posture. 2016;Sep(49):402–410.
3. Rakesh N, Yashoda BK Devi, Patil DJ, Nagi R. Assessment of cervical spine postural disorders in patients with temporomandibular dysfunction: a radiographic evaluation. Oral Radiol. 2014;(30)38–44.
4. Rocha CP, Croci CS, Caria PHF. Is there relationship between temporomandibular disorders and head and cervical posture? A systematic review. Journal of Oral Rehabilitation. 2016:875–881.
5. Parrinia S, Combaa B, Raveraa G, Rossinib G, Cugliarie G. Postural changes in orthodontic patients treated with clear aligners: A rasterstereographic study. Journal of Electromyography and Kinesiology. 2018:44–48.
6. Kopczyński P, Sobkowska Ł, Matthews-Brzozowska T. The role of computerized dynamic posturography in the diagnosis of malocclusion — a literature review. Dental Forum. 2016;1(XLIV).
7. Shumway-Cook A, Woollacott M, Kerns KA, Baldwin M. The effects of two types of cognitive tasks on postural stability in older adults with and without a history of falls. J Gerontol A Biol Sci Med Sci. 1997 Jul;52(4):232–40.
8. Salkar RG, Radke UM, Deshmukh SP, Radke PM. Relationship between temporomandibular joint disorders and body. International Journal of Dental and Health Sciences. 2015;2(6):1523–1530.
9. Galasso M. Posture and dysfunction Skull-Cervix-Mandibular. Frontiera ORL. 2016;2:35–41.
10. Barozzi S, Socci M, Soi D, Di Berardino F, Fabio G, Forti S, Gasbarre AM, Brambilla D, Cesarani A. Reliability of postural control measures in children and young adolescents. European Archives of Oto-Rhino-Laryngology. 2014;271:2069–2077.
11. Roggia B, dos Santos Filha VA, Correa B, Rossi AG. Posture and body balance of schoolchildren aged 8 to 12 years with and without oral breathing. CoDas. 2016;28(4):395–402.

12. Lara S, Graup S, Balk R, Teixeira LP, Farias AD, Alves GD, Leiria VB. Association between postural balance and anthropometric index in elemtarys choolchildren. Rev Paul Pediatr. 2018 36(1):59–65.
13. Mason M, Spolaor F, Guiotto A, De Stefani A, Gracco A, Sawacha Z. Gait and posture analysis in patients with maxillary transverse discrepancy, before and after RPE. International Orthodontics. 2018;16:158–173.
14. Leroux E, Leroux S, Maton F, Ravalec X, Sorel O. Influence of dental occlusion on the athletic performance of young elite rowers: a pilot study. Clinics. 2018;73:453.
15. Malak R, Gajewska E, Samborski W. A new view on the body posture. Pielęg Pol. 2008;2(28):149–151.

Acceptance for editing: 2019-03-13
Acceptance for publication: 2019-03-29

Correspondence address:
Joanna Borek
Department of Rheumatology and Rehabilitation
Poznan University of Medical Sciences, Poland
70 Bukowska Street, 60-812 Poznan, Poland
e-mail: joanna.monika.borek@gmail.com

REVIEW PAPER

DOI: https://doi.org/10.20883/jms.332

Health Care System Structure in the State of Israel

Mohammad Sabbah[a]

Tel Aviv Sourasky Medical Center, Tel Aviv, Israel
[a] https://orcid.org/0000-0002-9451-1893

ABSTRACT

The health care system in the State of Israel consists of two sectors — the public sector, which includes government-owned hospitals and medical institutes. The public health sector includes the community health system, health funds, family medicine, the general care system and the mental health care system. The second sector is the private sector, which includes private hospitals and medical institutes. Both sectors are supervised by the Israeli Ministry of Health, which is the supreme governmental authority through which it implements its policy in the entire health system in Israel. The law provides and guarantees medical insurance for every resident of Israel, the right to receive medical treatment, the prohibition of discrimination, informed consent to medical treatment, the right to receive an additional medical opinion, the dignity and privacy of the patient and the right to attend. Health funds in Israel were established before the State of Israel was established. The ideological concept of the health funds was based on the principle of equality and mutual assistance.

Keywords: Ministry of Health, Patient Rights Law, hospitals, health founds, public health services, private health services.

Introduction

The health system in Israel is very modern and efficient. At the same time, it copes with a large number of challenges and is constantly required to improve and adapt itself to changing conditions and needs. It seems valuable to present the health care system in Israel to enable comparisons with other systems operating throughout the world. It gives an opportunity to find some strong and week sites of compared systems and it enables to improve health care systems in other countries.

The health system in the State of Israel is comprised of several large organizations that operate health services. Mainly the public health system is financed from tax money and from the government budget. All citizens of Israel are granted the right from birth to join one of the four official health services and thus enjoy basic medical coverage. However, coverage can be expanded by purchasing private health insurance. The public health system is intended to provide medical services to the population in a routine manner. For example, preventative health care in clinics, health treatments and institutes, community rehabilitation, and various health promotion activities such as family planning, physical activity, and a healthy lifestyle.

Israel has a large number of hospitals that are scattered throughout the country. According to the distribution of the population in Israel, most of the hospitals are located in the central region. Some hospitals are general — that they are intended to treat a wide range of medical conditions and others are designated for a particular type of medical condition. The Ministry of Health serves as a regulator and service provider and is the governmental body responsible for ensuring the health of the population. Its ministerial duties include supervision and control, licensing, legislation, setting standards, research, training and planning.

The health system in Israel is composed of two sectors: the public sector, which is the central sector, and the private sector. Since health is an essential need and a basic human right [1]. The government of Israel is involved in the national expenditure on health, with 67% of the national expenditure on health financed by it (through the health budget and health tax) and the rest by the hospitals themselves. Most of the general hospitals in Israel (96% of the total number of hospital beds) are public hospitals, some are government hospitals, and some belong to the private sector.

History

The health system in Israel is based on several milestones in its historical development. The health funds in Israel were established before the State of Israel was established. Clalit Health Fund was founded in 1911. The ideological concept of the Clalit. was based on the principle of equality and mutual assistance. These principles have left their mark on the health system in Israel for many years.

The medical services provided to Clalit patients were based on a uniform tax collection, which included membership taxes of the [2]. Histadrut and the sick funds of the health fund.

The Histadrut, founded in 1920, is the largest and oldest workers' organization in Israel. The organization, whose institutions played a leading role in the establishment of the State, continues to play a central role today in protecting the rights of workers and pensioners, with special emphasis on promoting equality and narrowing the gaps in Israeli society.

The Histadrut deals with the professional and economic affairs of the working population: the incorporation of workers and their representation vis-a-vis the employer, the signing of collective agreements for improving conditions and securing employment security, the advancement of pension rights and concern for the future of employees.

The Histadrut promotes its activities throughout the country by means of trade unions, national organizations in various branches of labor, and arrangements in various areas throughout the country. In light of the entry of new sectors into the organized labor world, the momentum of association is also reflected in the establishment of new unions in the Histadrut, such as the Association of Mobile Workers, the Internet and the High-Tech.

Thus, dependence was placed on companies in the Histadrut and the sick fund. Over the years, additional health funds were established, which expressed political ideological identity and constituted an alternative to obtaining medical insurance, without the need for political identification.

In addition to the health funds, the Rothschild and Hadassah Medical Centers in the United States were the main initiators of the development of medical services in Israel prior to the establishment of the State. The activities of the two bodies included the establishment of hospitals, clinics and pharmacies, preventive medical services and operation of hospitals. Hadassah's medical services were characterized by a high level of professionalism and lacked any political or ideological affiliation.

At the end of 1948, the population of the State of Israel numbered 870,000 residents, and only 53% of them were covered by health insurance. Most of the population was insured by Clalit Health Services. Within four years of the establishment of the state, the population of Israel doubled as a result of the large waves of immigrants to the country from European countries and the United States. The increased immigration and morbidity among the immigrants created a deficit in the health system, which the Ministry of Health had no answer to [3].

Clalit Health Services was committed to providing services to the new immigrants and has become the most dominant provider of healthcare services. Later, Clalit worked to integrate the new immigrants as members of the Histadrut and thus constituted the largest medical organization in Israel. The membership of the fund grew, but the control of the Histadrut's income from the Histadrut's money box caused severe financial distress for the health fund. This distress and the political developments in Israel during this period accelerated the need for enactment of the National Health Insurance Law.

Law regulations concerning the health care system in Israel National Health Insurance Law

On January 1, 1995, the National Health Law came into force. The law promised medical insurance to every resident of Israel. This introduced compul-

sory health insurance for every citizen whose collection (taxes) is performed by the National Insurance Institute [4]. The law established a "health basket" — a list of medical services and medicines that each health fund must provide to its members under the conditions prescribed by law. In addition, some services are provided directly by the state, usually through the Ministry of Health. The health funds are forbidden to reject the insured, and the law determines that the health tax will be adjusted to the level of income of the citizen. In addition to compulsory insurance, the health funds are given the option of offering voluntary insurance for services that are not included in the public health services basket. The health funds offer their members an option to purchase supplementary insurance, which is health insurance that includes services that are not included in the health basket in accordance with the law [5]. The collection of health tax through the National Insurance Institute allowed fair competition between the health funds, the free choice of the citizen in his preferred health fund, the deepening of the collection and the guarantee of the fund's income. However, the budget of the Ministry of Health was managed by the Ministry of Finance.

Patient Rights Law 1996

Patient right law enacted in 1996 determines the principles of the court by the laws, the ethical codes of the medical professions and the directives of the Ministry of Health regarding the rights of the patient by lows and principles. The following are the main topics discussed by the law mentioned above:

The right to receive medical treatment

Section 3 (a) of the Law provides that "all those in need of medical treatment are entitled to receive it according to any law and in accordance with the conditions and arrangements that are in effect from time to time in the health system in Israel" [6]. However, "in a medical emergency, a person is entitled to receive urgent medical treatment without conditioning." In other words, the Patient's Rights Law imposes the provision of medical treatments on the existing arrangements in the Israeli health system at the same time, but in a medical emergency, the law stipulates that medical treatment must be given, regardless of whether the payment is covered. Thus, it is an obligation of a medical institution to provide medical treatment, as required, to those who are not insured and to illegal residents, and even when it is known in advance that no one will bear the cost of the treatment.

Prohibition of discrimination

Section 4 (a) of the Law provides that "a caregiver or medical institution shall not discriminate between patients on grounds of religion, race, sex, nationality, country of origin, sexual orientation or any other similar grounds." Section 4 (b) further states that "a caregiver or medical institution shall not discriminate between patients on the grounds of age, but no discrimination shall be considered under this section when the distinction is required for medical reasons" [4].

Informed consent to medical treatment

Section 23 (a) of the National Health Insurance Law states that "medical treatment shall not be given to the patient unless the patient has given informed consent" [4]. The law also provides that in order to obtain the consent of the patient, the caregiver shall provide the patient with the necessary information. More likely to be further details in the law regarding the medical information that will allow him to decide whether to agree to treatment.

The right to receive an additional opinion

Section 1 of the Law provides that "a patient is entitled to obtain on his own initiative an additional opinion regarding the treatment of him" and that "the caregiver and the medical institution shall assist the patient in all that is necessary for the exercise of this right" [4].

Respect for the dignity and privacy of the patient

Section 21 (a) of the Law provides that "a caregiver, any person who works under the supervision of the caregiver and any other employee of the medical institution shall maintain the dignity and privacy of the patient at all stages of medical treatment" [4].

Right to presence accompanied by medical treatment

"A patient is entitled to a person accompanying him, at his choice, be present at the time of

receiving medical treatment, provided that the person does not intervene in the provision of medical treatment [4].

Structure of the health system

Ministry of Health

The Ministry of Health is one of the government ministries in Israel. It is the governmental authority through which the government implements its policy in the entire health system, among other things, through the formulation of the health policy and the setting of priorities and principles of the system's activity. The Ministry of Health, in its ministerial capacity, is responsible for various matters, including legislation, setting standards, licensing, supervision and control, research, training and manpower planning, emergency preparedness and development policy. In addition to these functions, the Ministry of Health has two main functions: the first is the provision of health services and the second is that it is an insurer.

This office is responsible for the issues of health in Israel, including:

- responsibility for the health system in Israel, including hospitals, clinics, and other medical institutions.
- responsibility for all health-related activities in Israel, including preventive medicine, public health, environmental health, student and employee health, and health promotion.
- responsibility for licensing health professions, supervision of health professions in Israel. Doctors, dentists, nurses, midwives, pharmacists, dietitians, physiotherapists, occupational therapists, psychologists, and more.
- responsibility for supervising the activity of health funds under the National Health Insurance Law.
- responsibility for medical research in humans and animals, supervision of Magen David Adom in Israel (ambulances), supervision of production and import of food and medicines [7].

Supply of services

The Ministry of Health is the largest provider of general hospitalization services and owns about half of the total inpatient beds in Israel. In addition, the Ministry of Health is currently the main provider of mental health services and owns most of the largest geriatric centers in Israel. Also, the Ministry of Health operates about half of the family health stations in the community. The Ministry of Health is responsible for ensuring the provision of these services: preventive medical services, including routine examinations of the pregnant woman and her baby, tests for early detection of genetic diseases and metabolic diseases in newborns, vaccinations and preventive treatment, dental health for students, family planning and routine examinations for schoolchildren. Also, geriatric and hospitalization services for the mentally frail, rehabilitation and mobility equipment provided by the Ministry of Health, including hospitalization services, ambulatory mental health services and mental health services.

Public health

Public health includes state-owned hospitals and institutes, and hospitals owned by health funds. This sector is characterized mainly by activity on the basis of deficits that are ultimately covered by the government. The goal is to promote and improve the health of the country's residents over time by providing vaccinations, encouraging a healthy lifestyle and treating patients and victims by providing health services of a reasonable scope and quality based on a medical need and based on principles of equality, justice and mutual responsibility.

Public Health System

The public health system is designed to provide regular medical services to the population by preventive medical services, treatment of ongoing problems in clinics and institutes, rehabilitation, and various health promotion activities such as family health, physical activity, nutrition and a healthy lifestyle.

The health public system is based in Israel mainly on public clinics owned by the health funds and clinics of independent physicians, which relate to various accounting arrangements with the health funds and accordingly, care is provided to the insured by the health funds without any payment from the patients.

The health funds — according to the Bismarck zmodel

Mandatory insurance organizations were first established in Europe at the end of the 19th century thanks to an idea raised by German Chancellor Bismarck. In 1883, Bismarck initiated the establishment of a health and pension insurance system for workers [8].

In 1911 the Clalit Health Fund was established in order to provide medical services for the workers of the [9]. Second Aliyah, it was a small part of the greater emigration of Jews from Eastern Europe to Palestine which lasted from the 1870s until the 1920s, Most of its members were young people inspired by socialist ideals. Many models and components of the rural settlement enterprise came into being at this time, such as "national farms" where rural settlers were trained; the first kibbutz, Degania (1909); and Ha-Shomer, the first Jewish self-defense organization in Palestine. The fund operated according to the Bismarck model. Its ideological concept was based on the principle of equality and mutual aid, which left a mark on the entire health system in Israel [6].

Today there are four health funds operating in Israel. Clalit Health Services, Maccabi Health Services, Leumit Health Fund and Meuhedet Health Fund. Most of whom are insured by Clalit fund, and the rest — in the other three health funds (as shown in the diagram **Figure 1**). The National Health Insurance Law allows each insured to cancel membership in the health fund in which he is registered with and to register as a member of another HMO of his choice. The service should be fully funded by the health tax and the completion of the state [2].

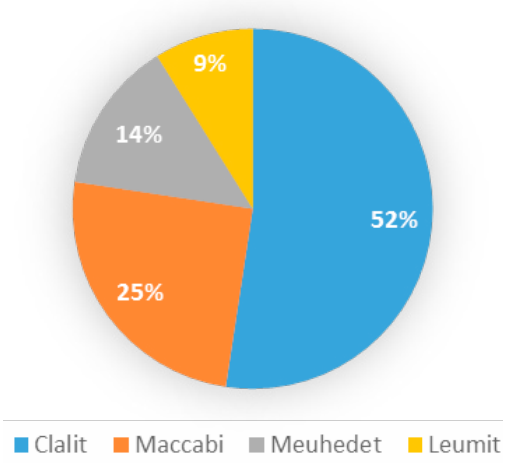


Figure 1. Distribution of insureds by health funds at the end of 2016. Source: Distribution of insureds by health funds, National Insurance Institute, Research and Planning Administration [Internet], State of Israel, 2016. www.btl.gov.il. https://www.btl.gov.il/Publications/survey/Documents/seker289/seker_289.pdf

Family health care

Family health care is the first broad treatment line in the community. The physician that deals with family members is supposed to be the personal physician, a familiar figure to the patient, and the person who sees the whole picture of the patient's health and his family and social environment while maintaining a long-term continuum.

The four health funds in Israel provide family medicine services using 4,627 physicians, 55% of whom are men and 45% are women. 1,485 of these physicians (32%) are specialists in family medicine and 2,053 (44%) are general practitioners, with no specialization whatsoever. The other doctors are specialists in various specialties, about half of whom have expertise in internal medicine. Every physician who practices family medicine handles, on average, 1,780 people [10].

Hospitalization Institutions in Israel

Hospitals in the healthcare system are a major component of the system's costs. Hospitals are divided into three main groups: hospitals for general care, mental health hospitals, and hospitals for chronic diseases and rehabilitation [11].

General Hospitalization

In Israel there are 42 institutions for general care, about 90% of which are government owned, 21 are hospitals. According to the documents of the Knesset Research and Information Centre regarding the general hospitalization system, there is a shortage of general hospital beds in Israel, specifically a shortage of hospital beds in these departments: the internal ward, intensive care, children, surgery, and maternity ward. For many years, various sources, including the State Comptroller (The State Comptroller conducts

external audits of the various activities of government ministries, local government and various public bodies to ensure that their actions are carried out in accordance with the law) have noted the discrepancy between the scope of requirements for the general hospitalization system and the resources in this system, and call for the long-term planning of the general hospitalization system in Israel [9].

The Mental Health Outpatient System

Since the mid-1990s, and many years after other western countries began to act in this direction, Israel has been working to transfer most mental health services from hospitals to community frameworks, in view of the recognition that hospitalization is a therapeutic solution for patients who do not respond to medication or patients who are at an acute stage of their disease. Also, at the stage of the end of treatment, patients should be assisted to resume their routine as much as possible and provide them with various treatment and rehabilitation solutions in the community. The policy that mental patients will be treated in the community and only those with acute illness will be hospitalized is based on the professional medical assumption that this is the optimal treatment for people suffering from mental illness and economic considerations.

Every resident determined to need psychiatric hospitalization is entitled to receive a full state lien through the Ministry of Health. Until 1995, a deductible was collected from the patients and their families, but since the State Insurance Law came into force, the State has borne the full cost [12].

Nursing (institutions) hospitals

In Israel there are over 300 institutions for nursing care, which are hospitals, with more than 17,000 beds for nursing patients. Among these institutions are four government geriatric centers, and many more owned by businesses or owned by non-profit organizations. Residents of Israel are entitled to receive assistance from the Ministry of Health in financing nursing hospitalization. Nursing patients are mainly elderly people who require professional, but not necessarily medical, supervision 24 hours a day. They need daily assistance with simple activities such as eating, bathing, and dressing [3].

Human resources in the health system

Manpower resources are the main input in the health system: about 61% of the national expenditure on health is allocated to the payment of the wages of workers employed in the system. Regarding the lack of tools for planning medical personnel in Israel, Document Research and Information Centre Committee, shows that for more than a decade there has been a discussion in Israel about the issue of emerging shortage of medical manpower. Population growth, aging, changes in morbidity patterns, developments in science and technology along with the increase in the number of doctors' retirees, the sharp decline in the number of doctors emigrating out of Israel and non-increasing scope of frameworks for training doctors in medical schools, created a real fear of a shortage of doctors in Israel and demanded a re-examination of the key data on the rate of physicians — the amount of medical personnel required for the health system.

Doctors

Israel has five medical schools: The Hebrew University of Jerusalem Medical School, the Medical School of Tel Aviv University, the Technion Medical School in Haifa, the Ben-Gurion University Medical School in Be'er Sheva and the Safed Medical School. Medical studies in Israel lasts seven years: during the first six years the studies are conducted by the medical school and in the seventh year an internship is held — a year of practical work in the scope of a full-time job, under the supervision of a licensed physician in a medical institution approved for this purpose. The internship lasts for 12 months and is performed in one of the 21 general hospitals in Israel. Upon completion of the internship period, the graduate receives a license to practice general medicine in Israel. The number of new licenses for general medicine in Israel that are provided each year has been increasing in recent years.

Nurses

Recognition of the professional status of a nurse is determined by the Ministry of Health based on compliance with several criteria, updated from time to time and reflecting the qualifications of a person to engage in the profession. Graduates

of educational programs in Israel who meet all the requirements of the compulsory curriculum are entitled to take the examination of registration Government general test in Nursing. Successful completion of this test is a necessary, but not an individual, requirement for registration in the Register of Brothers and Sisters in Israel. Graduates of study programs abroad will be entitled to be registered in this register after passing a process of professional recognition and having successfully passed the government registration examination [13].

Private Medical Services

Private medical services began working at Hadassah Hospital in Jerusalem in 1945 and in 1975 expanded to other hospitals. Private medicine allows the patient to choose privately a physician who will undergo an examination, diagnosis or perform surgery, when his salary is paid by the patient, using the hospital infrastructure in which the doctor is employed [15]. The private sector includes private hospitals and medical institutes. Competition in the private sector exists mainly between two private networks and a few hospitals. The private sector is characterized by advanced technological capability and high quality of service [2]. Today, private hospitals are not permitted in public hospitals (government hospitals and Clalit Health Services hospitals), and it operates only in private hospitals.

A private system is designed to offer insurance arrangements for financing or supplying arrangements that are not provided within the framework of the public system at all, or to offer insurance arrangements or supply arrangements for improvements to the services provided in the public system.

Private Clinics

Physicians in Israel combine work in various settings: public and private hospitals, health funds, community institutions and private clinics. For example, a wrist surgeon works in the mornings in a public hospital, and at lunch, combines work at the health funds or with a private clinic [14].

In the private services of public hospitals, only physicians whose status and function in the hospital is senior enough and who have established reputations to allow the patients to choose them as their doctor. Most physicians who meet these criteria currently work in private hospitals and have private clinics.

Summary

The health system in the State of Israel is expanding with the percentage of population growth. Although the health system in Israel is advanced and modern, there is still full occupancy in the inpatient departments, a shortage of doctors and nurses, which allows the medical staff to work beyond the required hours of work, with a high density of beds and patients. This is a challenging task for the government and the Israeli Ministry of Health to solve the problem of overcrowding and the number of patients in the wards, recruiting and adding a medical team that includes doctors and nurses and setting up new hospitals throughout the country.

Acknowledgements

Conflict of interest statement

The authors declare no conflict of interest.

Funding sources

There are no sources of funding to declare.

References

1. Shaul Ben Shimol, Sophia Graetz, Yuval Turbati. The health system in Israel — a transition from public medicine to private medicine. Maalot s&p Global rating publishing. 2008 March. 2–3.
2. The Histadrut website on internet www.hahistadrut.org.il. https://www.histadrut.org.il/%D7%90%D7%95%D7%93%D7%95%D7%AA_%D7%94%D7%94%D7%A1%D7%AA%D7%93%D7%A8%D7%95%D7%AA.
3. Noga Boulder. Israel Health System. Management of medical technologies (book). Israel Center for Technology Assessment in Health Services, Gertner Institute. Part A:1–11,2013 May.
4. Shaul Ben Shimol, Sophia Graetz, Yuval Turbati. The health system in Israel — a transition from public medicine to private medicine. Maalot S&P Glopal ratings publishing.2008 March. 2,16–17.
5. National Health Insurance Law. Ministry of Health. State of Israel. www.health.gov.il. https://www.health.gov.il/Subjects/UninsuredRights/HealthInsuranceLaw/Pages/default.aspx.
6. The Patient's Rights Law, 1996. Ministry of Health [internet], State of Israel. www.health.gov.il. https://www.health.gov.il/LegislationLibrary/Zchuyot_01.pdf.

7. Structure of the Ministry of Health, Ministry of Health website. State of Israel [internet]. www.health.gov.il. https://www.health.gov.il/hozer/MK10_2017.pdf.
8. Shifra Schwartz. Politics and Health: The Steps for the Development of the Hebrew Sick Funds in Palestine during the British Mandate Rule. Eyunim Betkumat Israel-Studies in Zionism and the State of Israel. pp.553–582, Ben-Gurion University of the Negev, 2003.
9. Immigration to Israel: The Second Aliya. Jewish Virtual Library. www.jewishhvirtuallibrary.org.il. https://www.jewishvirtuallibrary.org/the-second-aliyah-1904–1914.
10. Amit Sarid, Diana Nakmuli Levi, Amatzia Ginat. Medicine in the public — doctors who practice family medicine in Israel. Ministry of Health publishing, Jerusalem. (conference) 2015 June. 2–9.
11. Hospitalization and Day Care Institutions in Israel, Ministry of Health Website, State of Israel [internet]. www.health.gov.il. https://www.health.gov.il/UnitsOffice/HD/MTI/info/Pages/Inpatient_Institutions.aspx.
12. Shelly Levy. Mental Health Hospitalization in Israel, Knesset, Research and Information Center. Knesset, State of Israel, February 2, 2010. www.knesset.gov.il
13. Key issues in the field of health — the Knesset, Research and Information Center [internet]. State of Israel. www.Knesset.gov.il. https://main.knesset.gov.il/Activity/Info/MMMSummaries19/Health.pdf.
14. Private medical services in Israel [Internet]. TASC Consulting & Capital. www.tasc-consulting.com. www.tascconsulting.com/_ploads/dbsattached-files/tascsharapmail.pdf.

Other sources

15. Fassone E, Rahman S. Complex I deficiency: clinical features, biochemistry and molecular genetics. J Med Genet. 2012 Sep;49(9):578–590.
16. Amit Sarid, Diana Nakmuli Levi, Amatzia Ginat: Medicine in the public — doctors who practice family medicine in Israel, Ministry of Health publishing, Jerusalem (editor). 2005, june. 2–9.
17. Shaul Ben Shimol, Sophia Graetz, Yuval Turbati: The health system in Israel — a transition from public medicine to private medicine, Maalot S&P Global ratings publishing (editor). 2008 March 2,16–17.
18. Noga Boulder. Israel Health System: Management of Medical Technologies, Israel Center for Assessment of Technologies in Health Services, Gertner Institute publishing for Epidemiology and Health Policy Research, Initial release. (book) 2013 May: part A: 1–11.
19. Shifra Schwartz: Politics and Health, The Steps for the Development of the Hebrew Sick Funds in Palestine during the British Mandate Rule, Eyunim Betkumat Israel-Studies in Zionism and the State of Israel, Ben-Gurion University of the Negev. 2003: pp:553–582.
20. Hospitalization and Day Care Institutions in Israel, Ministry of Health Website, State of Israel [internet]. www.health.gov.il. https://www.health.gov.il/UnitsOffice/HD/MTI/info/Pages/Inpatient_Institutions.asx.
21. Key issues in the field of health — the Knesset, Research and Information Center [internet]. State of Israel. www.knesset.gov.il. https://main.knesset.gov.il/Activity/Info/MMMSummaries19/Health.pdf.
22. Shelly Levy. Mental Health Hospitalization in Israel. Research and information center. Knesset, State of Israel, February 2, 2010. www.knesset.gov.il. https://www.knesset.gov.il/mmm/data/pdf/m02428.pdf.
23. Private medical services in Israel [internet]. TASC Consulting & Capital. www.tasc-consulting.com. www.tascconsulting.com/_uploads/dbsattached-files/tascsharapmail.pdf.
24. Structure of the Ministry of Health, Ministry of Health website. State of Israel [internet]. www.health.gov.il. https://www.health.gov.il/hozer/MK10_2017.pdf.
25. The Patient's Rights Law, 1996. Ministry of Health, Knesset, State of Israel [internet]. www.health.gov.il https://www.health.gov.il/LegislationLibrary/Zchuyot_01.pdf.
26. The Histadrut website on internet. www.hahistadrut.org.il. https://www.histadrut.org.il/%D7%90%D7%95%D7%93%D7%95%D7%AA_%D7%94%D7%94%D7%A1%D7%AA%D7%93%D7%A8%D7%95%D7%AA.
27. Immigration to Israel: The Second Aliya. Jewish Virtual Library. www.jewishvirtuallibrary.gov.il. https://www.jewishvirtuallibrary.org/the-second-aliyah-1904–1914.
28. National Health Insurance Law. Ministry of Health. State of Israel. www.health.gov.il. https://www.health.gov.il/Subjects/UninsuredRights/HealthInsuranceLaw/Pages/default.aspx.

Acceptance for editing: 2019-03-27
Acceptance for publication: 2019-03-28

Correspondence address:
Mohammad Sabbah
Tur'an, 16950, Israel
Mailbox number: 610
Phone +972 52-8522280
Home: +972 4-9983053
Fax: +972 4-9983053
email: hamodi_sabbah@yahoo.com

REVIEW PAPER

DOI: https://doi.org/10.20883/jms.218

Some nitroimidazole derivatives as antibacterial and antifungal agents in *in vitro* study

Justyna Żwawiak[a], Dorota Olender[b], Lucjusz Zaprutko[c]

Chair and Department of Organic Chemistry, Pharmaceutical Faculty, Poznan University of Medical Sciences, Poland

[a] https://orcid.org/0000-0002-5420-6499
[b] https://orcid.org/0000-0001-8320-5201
[c] https://orcid.org/0000-0003-1121-6272

ABSTRACT

Nitroimidazoles have wide range of therapeutic uses mainly as anaerobic antibacterials and antiprotozoal agents. Some bicyclic nitroimidazodihydrooxazoles and nitroimidazotetrahydrooxazines are found to be antituberculosis agents. Hence, the biological and chemical properties of mentioned substances are of great interest to scientists. The aim of this review is to show the general knowledge concerning the chemistry and biological activity of some nitroimidazole derivatives, based on experimental studies. The results of biological tests provide many useful information on the effects of particular groups or other structural elements on the level of pharmacological activity. Also, these studies can be helpful in further planning of syntheses of active substances with using nitroimidazole moiety.

Keywords: nitroimidazoles, nitroimidazooxazoles, nitroimidazotetrahydropyrimidines, M. tuberculosis, antifungals.

Introduction

Nitroimidazoles make a group of compounds of great commercial and pharmacological importance [1]. These compounds with a nitro group at position 5 are usually more active than the corresponding 4-nitro-derivatives. However, 4-nitroimidazoles exhibit lesser toxicity than 5-nitro-analogues. These effects are remarkable for 2,4- and 2,5-dinitroimidazoles. Moreover, 2-nitroimidazole derivatives are generally more active as radiosensitizers than metronidazole (5-nitro-derivative) [2]. Introduction of an electron accepting group at position 5 in the 4-nitroimidazole ring causes increase in cytotoxic and radiosensibiliting activity [3]. Nitroimidazoles are vital particularly in the therapy of disorders caused by bacteria and protozoa. The best known — Metronidazole, is effective against *Bacteroides, Fusobacterium, Megasphaera, Clostridium,* sometimes *Peptococcus* and *Helicobacter.* Tinidazole was found to be active against *Gardnella, Propionibacterium, Eubacterium, Campylobacter, Actinomyces* and *Spirochetes*. Moreover, some bicyclic nitroimidazooxazoles show considerable activity against tuberculosis [4, 5]. A series of bicyclic nitroimidazooxazoles, originally investigated as radiosensitizers for use in cancer chemotherapy, have been found to be active against culture replication *M. tuberculosis* [6, 7]. Compounds containing the imidazo[2,1-*b*][1,3]oxazine ring system have been shown to be active against tuberculosis as well. The most promising compound of this series PA-824, has the MIC of 0.06 μg/ml against *M. bovis* BCG and high activity against Mtb H_{37}Rv [5, 8].

The aim of this work is to show our contribution to the current knowledge of antibacterial and antifungal activity of synthesized nitroimidazole derivatives.

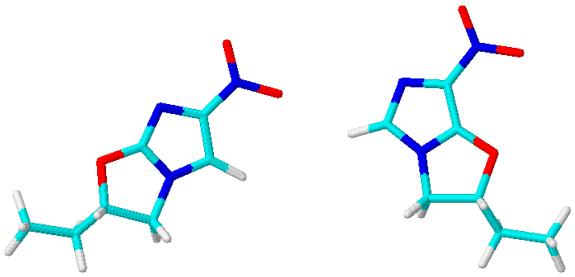
Tuberculostatic activity

In the work on the syntheses of pharmacologically active nitroimidazole derivatives, a series of eight bicyclic 2,3-dihydro-7-nitroimidazo[5,1-*b*] oxazoles were performed [9].

These compounds were obtained in a one-pot reaction by treating 4,5-dinitroimidazole or 2-methyl-4,5-dinitroimidazole with appropriate oxiranes. Selected compounds were tested for antimycobacterial activity *in vitro*, against *M. tuberculosis* $H_{37}R_v$, M. BCG, *M. avium* and two "wild" strains, isolated from the tuberculotic patients. The growth of strains was tested after 21 days. The lowest concentration of the compound investigated, at which no growth of strains was observed, was taken as the MIC. In the concentrations tested, these products showed no activity as well as isoniazid, especially against *Myc.* BCG, *Myc. tbc.* 1676, *Myc. tbc.* 456 and *Myc. avium*. Trying to explain of obtained results, simple analysis of the orientation of molecules of **CGI 17341** and their isomer was performed by PC GAMESS 7.0 program [10] (**Figure 1**). Comparison of these two molecules exhibited similarity in the distances between the oxygen atoms from oxazole rings and the alkyl chains in both of them. However, the distances between the NO_2 groups and the alkyl chains as well as the NO_2 groups and the oxygen atoms from oxazole rings were different and appeared to be an important factor in antitubercular agents. It was possible that the orientation of the isomeric compound and similar products provided too little space for a receptor.

In the last decade, a considerable interest has been dedicated to potential drugs against *M. tuberculosis* [11, 12], including several bicyclic nitroimidazole derivatives characterized by significant MIC values, e.g. mentioned above pretomanid (PA-824) and delamanid (OPC-67683) (**Figure 2**). Nowadays, these compounds are undergoing clinical trials.

Other compounds e.g. TBA-354 emerged as the preferred candidate from a number of analogues that were extensively evaluated for activ-



CGI 17341 CGI isomer

The distances between essential groups or atoms:

	CGI 17341	CGI isomer
NO_2 – O (from oxazole ring)	4.67458 Å	3.28392 Å
NO_2 – CH_3	7.11406 Å	6.05847 Å
O (from oxazole ring) – CH_3	2.98517 Å	2.97649 Å

Figure 1. Analysis of the orientation of molecules of CGI 17341 and their isomer

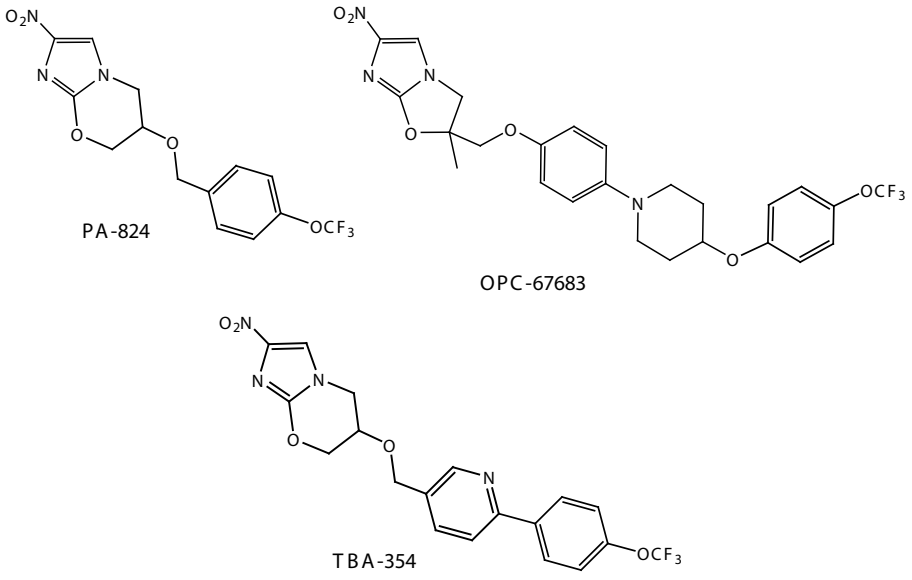


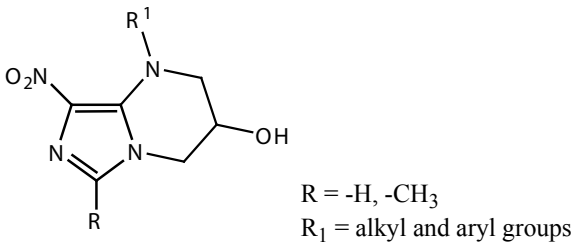
Figure 2. Structures of bicyclic nitroimidazoles as antitubercular drug candidates

ity in mouse models of chronic TB, and for pharmacokinetic, genetic and safety profiling. TBA-354 has recently been approved by the US Federal Drug Administration for clinical trial [13].

The initial Structure — Activity Relationship (SAR) studies have revealed that the replacement of the oxygen atom at C(9) position of tetrahydrooxazine ring from PA-824 with a methylene group results in the loss of antitubercular aerobic and anaerobic activities [14]. However, the C(9) position oxygen of the tetrahydrooxazine ring of PA-824 can be replaced by either nitrogen or sulfur with no significant reduction in MIC value in aerobic conditions, in comparison with MIC of the parent nitroimidazooxazine [15]. These results have encouraged us to synthesise a set of bicyclic, heterocyclic compounds, which are structural isomers of the structure of PA-824.

We decided to prepare 3-hydroxy-8-nitroimi-dazo[5,1-*b*]-1,4,5,6-tetrahydropyrimidine core in which a six-membered ring is connected with N-1 and C-5 atoms of nitroimidazole moiety, but not with N-1 and C-2 atoms [16]. Consequently, the oxazine part of the molecule was replaced with a diazine ring (**Figure 3**).

Moreover, these compounds, in most cases,



1

Figure 3. The structure of 3-hydroxy-8-nitroimidazo[5,1-*b*]-1,4,5,6-tetrahydropyrimidines

exhibited favorable calculated SAR parameters: rather high octanol — water partition coefficient (Log P) and low values (below 140 Å^2) of Polar Surface Area (PSA) that is linked closely to higher bioavailability. This fact strongly prompted us to study 3-hydroxy-8-nitroimidazo[5,1-*b*]-1,4,5,6-tetrahydropyrimidines as potential new tuberculostatic substances. Some of these products were tested against *M. tuberculosis* 2441, 9656, 14023 — SM (streptomycin), INH (isoniazid), RFP (rifampicin) resistant and *M. tuberculosis* 5318 — SM, INH, RFP, EMB (ethambutol) resistant in *in vitro* assays. Finally, biological assays *in vitro* were performed to determine the effects of chosen products on *M. tuberculosis* species. These new compounds were tested against four drug resistant strains: *M. tuberculosis* 5318 (res. SM, INH, RMP, EMB), *M. tuberculosis* 2441, 9656 and 14023 (res. SM, INH, RMP) using *M. tuberculosis* $H_{37}R_v$ as a control to validate the assay results. The measured MIC's showed no inhibition activity even at the highest concentrations. For all products tested the MIC values were higher then 25 µg/mL.

While PA-824 and 3-hydroxy-8-nitroimi-dazo[5,1-*b*]-1,4,5,6-tetrahydropyrimidine core have structural similarities, their bioactivities against *M. tuberculosis* are completely different. As proved, binding a six-membered ring to the N-1 and C-5 atoms of nitroimidazole moiety, in contrast to N-1 and C-2 substitution, totally abrogated antitubercular activity. The lack of inhibition is probably connected with the specific orientation of this bicyclic molecule that may interfere with formation of e.g. hydrogen bonds with active sites of receptors. Moreover, they have different stereochemistry than PA-824 [17].

Antifungal properties

In our earlier works [16,18], substitution of the halogen atom in the –CH_2X group in 2-chloromethyl-7-nitroimidazo[5,1-*b*]-2,3-dihydrooxazole system with phenols [18] and primary amines [16] has been described. The main feature of these syntheses is dihydrooxazole ring opening reaction. This mechanism has been used for forming new nitroimidazole derivatives with thiophenol and secondary amine moieties as a result of nucleophilic substitution reaction in 2-chloromethyl-7-nitroimidazo[5,1-*b*]-2,3-dihydrooxazole (**2**) and 2-chloromethyl-5-methyl-7-nitroimidazo[5,1-*b*]-2,3-dihydrooxazole (**3**) [19] (**Figure 4**).

Treatment of the nitroimidazodihydrooxazole with equimolar amount of amine or thiophenol furnished the products with one amino or thiopheno- group substituted at C-5 position of nitroimidazole ring. Increasing this ratio to 4 equivalents of nucleophile lead to form respective derivatives with two newly introduced cyclic moieties — the first one at C-5 position and the second one — at N-1 alkyl chain, as a result of

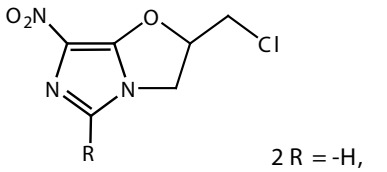


Figure 4. The structure of 7-nitroimidazo[5,1-*b*]-2,3-dihydrooxazoles

nucleophilic substitution of chlorine atom. It was found that some of these compounds are highly active against *Trichoderma viride*. Antifungal activity of these compounds was observed even after 21 days of the study. Additionally, moderate anti- *Trichoderma viride* activity was observed for few products. One compound (**4**) (**Figure 5**) showed the high efficiency relative to *Aspergillus niger*, *Penicillium funiculosum* and *Paecilomyces variotti* after 4 days of exposure. The results of visual assessment on 7th and 14th day showed that fungistatic properties decreased significantly. This fact can suggest diffusion of drug into malt agar. Among products tested, compound **4** was the most effective nitroimidazole derivative against mixture of fungi. Higher effectiveness probably was induced by the presence of methyl group at C-2 position of imidazole ring.

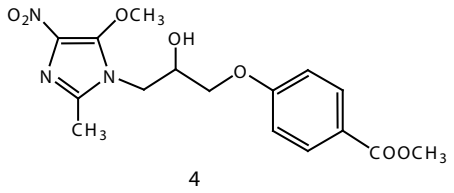


Figure 5. The structure of nitroimidazole derivative with the highest antifungal activity

Some N-substituted nitroimidazole derivatives containing phenacyl group were tested for their antifungal activity. In the studies were used the standard nutrient method against *Scleropho-ma pityophila*. The results of tests were expressed as the ED_{50} (substances concentrations retarding the fungal growth rate by 50 percent in comparison with plates where the agent studied was absent), and the effective dose ED_{100} (substances concentrations retarding the fungal growth rate by 100 percent in comparison with plates where the agent studied was absent. As it was shown in the citated work [20], all compounds are weakly active against the fungi used. On the other hand, N-phenacyl derivatives 5 and 6 (**Figure 6**) showed high fungistatic activity (ED's < 25) against *S. pityophila*. High effectiveness was induced by the displacement of nitro group at 4-position on the imidazole ring to morpholine or piperidine rest. Also, the presence of a chlorine atom at 4-position on the phenyl ring on the N-phenacyl moiety influences the increase in antifungal activity.

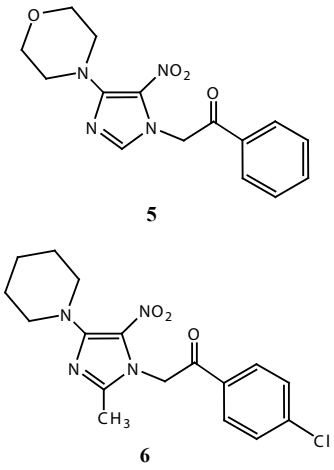


Figure 6. The structures of N-phenacyl nitroimidazole derivatives with high fungistatic activity

Also, some derivatives of 1,3-bis-(1-imida-zoyl)-2-hydroxy- (or acetoxy-) propane, for which synthesis was described in one of earlier works [21], now favorably undergoing antifungal test. The most effective among them is unsymmetrical acetoxy- derivative **7** (**Figure 7**).

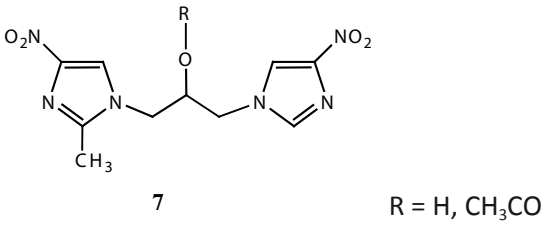


Figure 7. The structure of derivatives of 1,3-bis-(1-imidazoyl)--2-acetoxypropane

Conclusions

As shown results of our different chemical and biological studies, nitroimidazole derivatives are important group of bioactive compounds. Sev-

eral substituted nitroimidazoles are of considerable pharmacological significance, particularly as antifungal agents. Our results provide important information on the effects of certain substituents and structural elements on the occurrence of certain activity or lack thereof. Moreover these studies can be helpful in future planning of syntheses of active drugs with using nitroimidazole scaffold.

Acknowledgements

Conflict of interest statement

The authors declare no conflict of interest.

Funding sources

There are no sources of funding to declare.

References

1. Mital A. Synthetic Nitroimidazoles: Biological Activities and Mutagenicity Relationships. Sci Pharm. 2009 Aug;77:497–520.
2. Mukherjee T, Boshoff H. Nitroimidazoles for the treatment of TB: past, present and future. Future Med Chem. 2011 Nov;3(11):1427–1454.
3. Boyer JH. Nitroazoles. VCH Publishers Inc, Florida. 1986,165–166.
4. Clifton EB, Boshoff HIM, Dowd CF. Prospects for clinical introduction of nitroimidazole antibiotics for the treatment of tuberculosis. Curr Pharm Design. 2004 Oct;10:3239–3262.
5. Adib M, Sheibani E, Mostofi M, Ghanbary K, Bijanzadeh HR. Efficient highly diastereoselective synthesis of 1,8a-dihydro-7H-imidazo[2,1-b][1,3]oxazines. Tetrahedron. 2006 Apr; 62:3435–3438.
6. Agrawal KC, Bears K, Sehgal RK, Brown JN, Rist PE, Rupp WD. Potential radiosensitizing agents. Nitroimidazoles. J Med Chem. 1979;22:583–586.
7. Nagarajan K, Shankar R, Rajappa S, Shenoy ST, Costa-Pereira R. Nitroimidazoles XXI. 2,3-dihydro-6-nitroimidazo[2,1-b]oxazoles with antitubercular activity. Eur J Med Chem. 1989;24:631–633.
8. Otera J, Orita A. US Patent No. 7, 115, 736 B2 (2006).
9. Żwawiak J, Olender D, Zwolska Z, Augustynowicz-Kopeć E, Zaprutko L. Synthesis of 2,3-dihydro-7-nitroimidazo[5,1-b]oxazoles as potential tuberculostatic agents. Acta Pol Pharm. 2008 Apr;65:229–233.
10. Nemukhin AV, Grigorenko BI, Granovsky AA. Molecular modeling by using the PC GAMESS program: From diatomic molecules to enzymes. Moscow University Chemistry Bulletin. 2004;45,75–102.
11. Thompson AM, Blaser A, Anderson RF, Shinde SS, Franzblau SG, Ma Z et al. Synthesis, reduction potentials, and antitubercular activity of ring A/B analogues of the bioreductive drug (6S)-2-nitro-6-{[4-(trifluoromethoxy)benzyl]oxy}-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine (PA-824). J Med Chem. 2009 Feb;52:637–645.
12. Bhaumik K, Akamanchi KG. 2,4-Dinitroimidazole: Microwave assisted synthesis and use in synthesis of 2,3-dihydro-6-nitroimidazo[2,1-b]oxazole analogues with antimycobacterial activity. J Heterocyclic Chem. 2004 Jan;41:51–55.
13. Denny WA. TBA-354: A new drug for the treatment of persistent tuberculosis. Chemistry in New Zealand 2015;January:18–22.
14. Kim P, Zhang L, Manjunatha UH, Singh R, Patel S, Jiricek J et al. Structure-activity relationships of antitubercular nitroimidazoles. 1. Structural features associated with aerobic and anaerobic activities of 4- and 5-nitroimidazoles. J Med Chem. 2009 Mar;52:1317–1328.
15. Kim P, Kang S, Boshoff HI, Jiricek J, Collins M, Singh R et al. Structure-activity relationships of antitubercular nitroimidazoles. 2. Determinants of aerobic activity and quantitative structure-activity relationships. J Med Chem. 2009 Mar;52:1329–1344.
16. Zaprutko L, Żwawiak J, Zwolska Z, Augustynowicz-Kopeć E, Bartoszak-Adamska E, Nowicki W. Synthesis, structure and biological evaluation of novel bicyclic nitroimidazole derivatives. Arch Pharm Chem Life Sci. 2012 Dec;345:463–467.
17. Żwawiak J, Kujawski J, Zaprutko L. Structure — tuberculostatic activity evaluation among isomeric bicyclic nitroimidazoles. Acta Pol Pharm. 2017; in press.
18. Żwawiak J, Gzella A, Zaprutko L. Reactions of nitroimidazodihydrooxazole with alcohols and phenols in the presence of potassium carbonate. Pol J Chem. 2009 Mar;83:1309–1315.
19. Żwawiak J, Perdoch W, Mazela B, Zaprutko L. Synthesis and antifungal properties of some nitroimidazole derivatives. Int J Med Pharm Res. 2014 Aug;2:622–633.
20. Olender D, Żwawiak J, Lukianchuk V, Lesyk R, Kropacz A, Fojutowski A, Zaprutko L. Synthesis of some N-substituted nitroimidazole derivatives as potential antioxidant and antifungal agents. Eur J Med Chem. 2009 May;44:645–652.
21. Tułecki J, Zaprutko L. Synteza związków pochodnych 2-propanolu z układem nitroimidazolu. Acta Pol Pharm. 1984;3:281–292.

Acceptance for editing: 2019-03-13
Acceptance for publication: 2019-03-29

Correspondence address:
Justyna Żwawiak
Department of Organic Chemistry,
Pharmaceutical Faculty,
Poznan University of Medical Sciences, Poland
6 Grunwaldzka Street, 60-780 Poznań, Poland
phone: +48 618546678, fax: +48 618546680
e-mail: jzwawiak@ump.edu.pl

DOI: https://doi.org/10.20883/jms.338

A thousand words about the link between red blood cell distribution width and heart failure

Pawel Nowinka[1, a], Eduoard Korab-Karpinski[2], Przemyslaw Guzik[2, b]

[1] Department of Cardiology-Pulmonology, Heliodor Swiecicki University Hospital, Poznan University of Medical Sciences, Poland
[2] Department of Cardiology-Intensive Therapy, Poznan University of Medical Sciences, Poland
[a] https://orcid.org/0000-0002-2161-2693 [b] http://orcid.org/0000-0001-9052-5027

ABSTRACT

A link between the red blood cell distribution width (RDW) and clinical outcomes in heart failure (HF) was reported for the first time in 2007. Since then, many studies have shown that an increased RDW is an independent and strong predictor of mortality and morbidity in patients with acute, decompensated or chronic HF. The evidence for such a link comes from dozens of prospective and retrospective studies in which clinical data from hundreds or even thousands of patients were examined. Although many processes such as nutritional deficiencies (e.g. iron, folate, vitamin B12), inflammation (interleukin 6, tumour necrosis factor), malnutrition, renal failure or tissue and organ hypoxia have been proposed, no clear explanation exists or is commonly accepted. This mini-review summarises the clinical evidence on the increased RDW as a predictor of adverse clinical outcomes in HF patients, and hypothetical mechanisms that might be responsible for this interesting clinical observation.

Keywords: adverse clinical outcomes; heart failure; mortality, red blood cell distribution width.

Dyspnoea and progressive poor exercise tolerance with early tiredness are typical symptoms of heart failure (HF), which is a consequence of structural remodelling and/or functional impairment of the heart. In HF, cardiac output is reduced due to the compromised contractile ventricular function or generated at the cost of elevated intracardiac pressures owing to impaired diastolic function. The amount of blood pumped by the heart is not sufficient to meet all metabolic body demands for oxygen and nutrients, so all tissues and organs are affected by this disease [1].

Recently, an increased red blood cell distribution width (RDW) has been reported to predict mortality and other major adverse clinical events in HF (**Table 1**) [2, 3, 4, 5, 6]. In 2007, Felker et al. were first who found that an increase in RDW associates with a higher risk for the cardiovascular death or hospitalization in HF patients enrolled to the CHARM (Candesartan in Heart Failure: Assessment of Reduction in Mortality and Morbidity) program [7]. This observation was confirmed in an independent the Duke Databank — RDW was a strong predictor of mortality [7]. Since then, several other studies showed a similar linkage between elevated RDW and a poor outcome in patients with either acute or chronic HF (**Table 1**).

The RDW is routinely measured by automated haematology analysers as a part of red blood cell count analysis and reported as a component of the complete blood count. Usually, RDW is quantified in two ways. First, as the standard deviation of the size of red blood cells (RDW_{SD}) with the nor-

Table 1. Examples of clinical studies showing an association between RDW and adverse clinical outcomes, including mortality, in patients with heart failure

Study	Study design	Patients	Main findings
the CHARM program [7]	Retrospective	2679 CHF pts	RDW identified as a novel and important predictor of morbidity and mortality in CHF patients
the Duke Databank [7]	Retrospective	2140 CHF pts	Confirmatory analysis of the CHARM cohort — RDW is a predictor of total mortality
Pascual-Figal et al. [22]	Prospective	628 AHF	Increased RDW predicts long-term survival, regardless of haemoglobin concentration and the presence of anaemia
van Kimmenade et al. [23]	Post-hoc analysis	205 AHF	RDW is a one-year mortality predictor after hospital discharge. Prognostic value of RDW is additive to NT-proBNP
STAMINA-HFP Registry [24]	Prospective	1012 CHF	RDW is an independent and strong predictor of both mortality and hospitalisation, even after adjustment for a variety of other clinical and laboratory variables
UNITE-HF Biomarker Registry [24]	Prospective	235	
Makhoula et al. [25]	Prospective	614 AHF pts	RDW independently predicts morbidity and mortality. An increase of RDW in time is also a predictor of mortality.
Muhlestein et al. [26]	Prospective	6414 AHF pts	Elevated RDW and ΔRDW during HF hospitalisation were associated with 30-day mortality
Vizzardi et al. [16]	Prospective	232 CHF pts	RDW predicts better adverse outcomes than echocardiographic parameters
Förhécz et al. [15]	Prospective	195 CHF pts	RDW is a strong, independent predictor of morbidity and mortality
Wasilewski et al. [27]	Retrospective	1734 CHF pts	The highest RDW tertile associates with increased long-term mortality

Abbreviations: AHF — acute heart failure; CHARM — Candesartan in Heart Failure: Assessment of Reduction in Mortality and Morbidity; CHF — chronic heart failure; STAMINA-HFP — Study of Anemia in a Heart Failure Population; UNITE-HF Biomarker Registry — United Investigators to Evaluate Heart Failure.

mal range between 39 and 47 fL. Second, as the ratio of RDW_{SD} to the mean corpuscular volume of red blood cells, i.e., the coefficient of variation (RDW_{CV}) — this is a unitless parameter with the normal range between 11.5–14.5% [8, 9] (**Figure 1**). The direct interpretation of increased measures of RDW is as follows: the distribution of the red blood cells width is wider than it should be (see the comparison of panels **A** and **B** in **Figure 1**).

Traditionally, an increased RDW is a quantitative measure of anisocytosis, and it is found in various diseases and pathologies of erythrocytes leading to ineffective production or increased destruction of these cells. Some examples of ineffective erythrocyte production are iron, folate or vitamin B12 deficiency, haemoglobinopathies (e.g., sickle cell anaemia, thalassaemia). Increased or accelerated erythrocyte destruction is observed in acute or chronic haemolytic anaemias. For example, in the course of some infections, chronic diseases of other organs (liver or kidneys), toxic or allergic reactions to drugs, venoms, certain foods, autoimmune diseases, hypersplenism or owing to some mechanical narrowing in the blood flow such as severe aortic stenosis or presence of mechanical prosthetic heart valves. Finally, RDW increases after transfusion of red blood cells [8, 10].

In general, an increased RDW suggests that some of the conditions during erythrocyte development were not optimal and mature erythrocytes getting into the circulation had a wide range of different sizes, from small to large. If we consider the usual lifespan of red blood cell of 100–120 days, then an increased RDW means that a substantial part of all circulating red blood cells did not have proper conditions during their development in the bone marrow.

As already mentioned, chronic haemodynamic disarrangements in HF affect all tissues and cells. Co-existence of renal failure, cognitive decline or impairment of the alimentary tract in HF are some examples of how left ventricular dysfunction affects other, remote organs and systems [11, 12, 13]. Thus it is not surprising that the bone marrow function might be modified in HF as well resulting in the altered development of red blood cells.

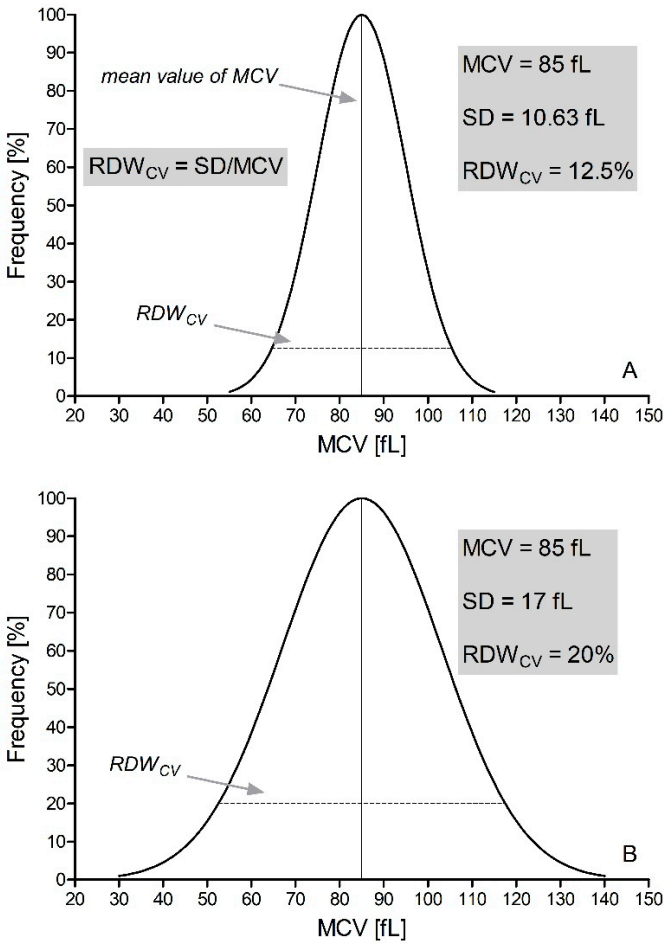


Figure 1. Two examples of theoretical distributions of red blood cell sizes represented by the mean corpuscular volume. Panel A shows normal distribution of the sizes of erythrocytes with RDWcv in the normal range (12.5%). Panel B presents a wider distribution of the sizes of red blood cells with an increased value of RDWcv (20%). Of note, the averaged value of MCV for each distribution of red blood cells is taken as the reference value represented as 100% of the frequency

Potential mechanisms linking HF with RDW

Mechanisms linking RDW and worse clinical outcomes in HF patients are not well understood. Most probably, the anisocytosis observed in HF is a net effect of many different processes and conditions (**Table 2** and **Figure 2**). HF is a complex disease of many causes and consequences. There are nutritional deficiencies due to the dysfunction of the alimentary tract and impaired absorption of various nutrients, including iron, folate and vitamin B12 [14]. Congestion of blood, if right ventricular failure is present, and reduced tissue perfusion, if both left and right ventricles are involved, are typical for HF and may cause dysfunction of kidneys, liver, worse oxygen and carbon dioxide exchange in the lungs. Chronic inflammation is also common in HF patients and may lead to the development of malnutrition and cardiac cachexia. Inflammatory cytokines may alter the function of bone marrow and iron metabolism, including its incorporation to red blood cells. Förhécz et al. have described that markers of inflammation such as cytokines, soluble cytokine receptors, acute phase reactant, were associated with high RDW volume in HF patients [15]. We have observed (**Figure 2**) a significant correlation between the concentration of C-reactive protein and RDW$_{cv}$ in HF patients, both in those who died or survived the three-year follow-up. Reduced production and release of erythropoietin or resistance of bone marrow cells to this hormone observed in HF might contribute as well. [15, 16]. Some cytokines regulating the inflammation may directly inhibit erythropoietin-induced maturation of red blood cells reflected by the elevated worth of RDW [17, 18]. One of

Table 2. Summary of potential mechanisms linking heart failure with increased values of RDW

Mechanism	Explanation
Ineffective Red cell Production [7]	RDW is typically elevated in conditions of ineffective erythropoiesis such as iron deficiency, B12 or folate deficiency. These conditions are not rare in HF patients.
Inflammatory Cytokines, IL-1, IL-6. [28]	Inflammatory cytokines have shown to partially contribute to the pathology of HF. Associations between inflammatory cytokines and RDW have been reported. Chronic inflammation may aggravate HF, and impair iron incorporation to red blood cells in the bone.
Tumour Necrosis Factor Alpha (TNF-a) [24, 29]	TNF-a induces B-adrenergic receptor uncoupling, which then increases reacting oxygen species (ROS) formation and increases Nitric Oxide Synthases (INOS) activity leading to high output NO formation. These mechanisms contribute to HF development and progression. TNF-a interacts with most of the cells in the human body, including bone marrow cells and might influence the development and maturation of red blood cells.
Reticuloendothelial block [24, 31]	Impaired mobilisation and ineffective usage of existing iron stores, even in adequate quantities of iron within the body, is mediated by overexpression of hepcidin, which is synthesised and secreted by the liver and regulates iron metabolism. Hepcidin decreases cell surface expression of ferroportin thus decreasing iron absorption from the intestine and iron release from the reticuloendothelial stores, contributing to an increased RDW and anaemia of chronic disease.
Hypoxia-related aetiologies [32]	Acute or chronic hypoxia stimulates a large increase in serum erythropoietin which then induces the formation of enlarged erythrocytes and increased RDW. Similar RDW increases are seen in multiple acute diseases with the risk of hypoxia such as HF, pneumonia, atelectasis or sepsis.

Abbreviations: HF — heart failure; NO — nitric oxide; TNF — tumor necrosis factor.

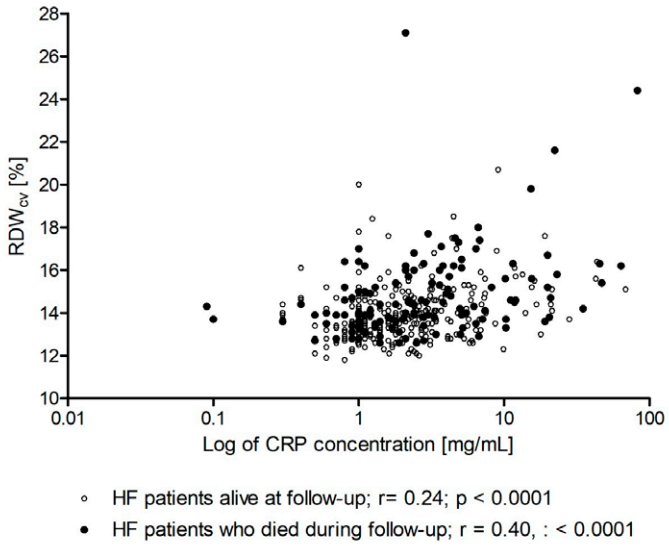


Figure 2. A sample correlation (Pearson) between the concentration of CRP and the value of RDWcv in a group of 419 ambulatory patients with chronic heart failure, reduced ejection fraction < 50% and followed-up up to 3 years. One hundred and four patients died and 295 survived during the 3-year follow-up (unpublished data from the project [33])

the most interesting concepts that might help to link increased RDW with HF is proposed by Yčas et al. who analysed hospital database with hundreds of thousands of patients with different clinical conditions and results of RDW analysis. They have suggested that an increased RDW is a marker of bone marrow hypoxia, which seems to be common in HF patients, particularly those with a more severe form of this disease.

After the initial publication by Felker et al., other studies have shown the association between increased RDW and adverse clinical outcomes in many other non-hematologic disorders like coronary artery disease [3], respiratory diseases [4, 19], stroke [5], critical illness [20], including sepsis [6], or renal failure [21].

Conclusion

There is a solid clinical evidence that an increased RDW is an independent and strong risk

factor for higher mortality and morbidity in HF patients. However, no simple explanation exists for this interesting association between RDW and HF course. Many interesting hypotheses try to explain this association, but so far the mechanisms linking RDW and clinical outcomes in HF patients remain unsolved.

Acknowledgements

Conflict of interest statement
The authors declare no conflict of interest.

Funding sources
This work is a part of the project "Predicting adverse clinical outcomes in patients with implanted defibrillating devices", which was supported by the Foundation for Polish Science—TEAM program co-financed by the European Union within the European Regional Development Fund. Project numer: TEAM/2009–4/4; Principal Investigator — PG.

References

1. Task Force Members, McMurray JJ, Adamopoulos S, Anker SD, Auricchio A, Böhm M, Dickstein K, et al. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC. Eur J Heart Fail. 2012;14:803–69.
2. Perlstein TS, Weuve J, Pfeffer MA, Beckman JA. Red blood cell distribution width and mortality risk in a community-based prospective cohort. Arch Intern Med. 2009;169:588–94.
3. Tsuboi S, Miyauchi K, Kasai T, Ogita M, Dohi T, Miyazaki T, et al. Impact of red blood cell distribution width on long-term mortality in diabetic patients after percutaneous coronary intervention. Circ J. 2013;77:456–461. http://www.ncbi.nlm.nih.gov/pubmed/23075764. Accessed January 31, 2019.
4. Nathan SD, Reffett T, Brown AW, Fischer CP, Shlobin OA, Ahmad S, et al. The red cell distribution width as a prognostic indicator in idiopathic pulmonary fibrosis. Chest. 2013;143:1692–8.
5. Kim J, Kim YD, Song TJ, Park JH, Lee HS, Nam CM, et a. Red blood cell distribution width is associated with poor clinical outcome in acute cerebral infarction. Thromb Haemost. 2012;108:349–56.
6. Wang F, Pan W, Pan S, Ge J, Wang S, Chen M. Red cell distribution width as a novel predictor of mortality in ICU patients. Ann Med. 2011;43:40–6.
7. Felker GM, Allen LA, Pocock SJ, Shaw LK, McMurray JJ, Pfeffer MA, et al. Red cell distribution width as a novel prognostic marker in heart failure: data from the CHARM Program and the Duke Databank. J Am Coll Cardiol. 2007;50:40–7.
8. Evans TC, Jehle D. The red blood cell distribution width. J Emerg Med. 1991;9:71–4.
9. Salvagno GL, Sanchis-Gomar F, Picanza A, Lippi G. Red blood cell distribution width: A simple parameter with multiple clinical applications. Crit Rev Clin Lab Sci. 2015;52:86–105.
10. Bessman JD, Gilmer PR, Gardner FH. Improved classification of anemias by MCV and RDW. Am J Clin Pathol. 1983;80:322–6
11. Alves TCTF, Rays J, Fráguas R, et al. Localized Cerebral Blood Flow Reductions in Patients With Heart Failure: A Study Using 99mTc-HMPAO SPECT. J Neuroimaging. 2005;15:150–156.
12. Bongartz LG, Cramer MJ, Doevendans PA, Joles JA, Braam B. The severe cardiorenal syndrome: "Guyton revisited". Eur Heart J. 2005;26:11–17.
13. Roman DD, Kubo SH, Ormaza S, Francis GS, Bank AJ, Shumway SJ. Memory improvement following cardiac transplantation. J Clin Exp Neuropsychol. 1997;19:692–697.
14. Sciatti E, Lombardi C, Ravera A, et al. Nutritional Deficiency in Patients with Heart Failure. Nutrients. 2016;8:442.
15. Förhécz Z, Gombos T, Borgulya G, Pozsonyi Z, Prohászka Z, Jánoskuti L. Red cell distribution width in heart failure: Prediction of clinical events and relationship with markers of ineffective erythropoiesis, inflammation, renal function, and nutritional state. Am Heart J. 2009;158:659–666.
16. Vizzardi E, Sciatti E, Bonadei I, Pezzali NL, Lombardi CM, Metra M. Red cell distribution width and chronic heart failure: prognostic role beyond echocardiographic parameters. Monaldi Arch Chest Dis. 2016;84:59.
17. Chiari MM, Bagnoli R, De Luca P, Monti M, Rampoldi E, Cunietti E. Influence of Acute Inflammation on Iron and Nutritional Status Indexes in Older Inpatients. J Am Geriatr Soc. 1995;43:767–71
18. Pierce CN, Larson DF. Inflammatory cytokine inhibition of erythropoiesis in patients implanted with a mechanical circulatory assist device. Perfusion. 2005 2005;20:83–90.
19. Kalemci S, Akin F, Sarihan A, Sahin C, Zeybek A, Yilmaz N. The relationship between hematological parameters and the severity level of chronic obstructive lung disease. Polish Arch Intern Med. 2018;128:171–177.
20. Meynaar IA, Knook AH, Coolen S, Le H, Bos MM, Van Der Dijs F, et al. Red cell distribution width as predictor for mortality in critically ill patients. Neth J Med. 2013;71:488–93.
21. Lu YA, Fan PC, Lee CC, Wu VC, Tian YC, Yang CW, et al. Red cell distribution width associated with adverse cardiovascular outcomes in patients with chronic kidney disease. BMC Nephrol. 2017;18:361.
22. Pascual-Figal DA, Bonaque JC, Redondo B, Caro C, Manzano-Fernandez S, Sánchez-Mas J, et al. Red blood cell distribution width predicts long-term outcome regardless of anaemia status in acute heart failure patients. Eur J Heart Fail. 2009; 11:840–6.
23. van Kimmenade RR, Mohammed AA, Uthamalingam S, van der Meer P, Felker GM, Januzzi Jr JL. Red blood cell distribution width and 1-year mortality in acute heart failure. Eur J Heart Fail. 2010;12:129–36.

24. Allen LA, Felker GM, Mehra MR, Chiong JR, Dunlap SH, Ghali JK, et al. Validation and potential mechanisms of red cell distribution width as a prognostic marker in heart failure. J Card Fail. 2010;16:230–8.
25. Makhoul BF, Khourieh A, Kaplan M, Bahouth F, Aronson D, Azzam ZS. Relation between changes in red cell distribution width and clinical outcomes in acute decompensated heart failure. Int J Cardiol. 2013;167:1412–6.
26. Muhlestein JB, Lappe DL, Anderson JL, Muhlestein JB, Budge D, May HT, et al. Both initial red cell distribution width (RDW) and change in RDW during heart failure hospitalization are associated with length of hospital stay and 30-day outcomes. Int J Lab Hematol. 2016;38:328–337.
27. Wasilewski J, Pyka Ł, Hawranek M, Tajstra M, Skrzypek M, Wasiak M, Suliga K, et al. Prognostic value of red blood cell distribution width in patients with left ventricular systolic dysfunction: Insights from the COMMIT-HF registry. Cardiol J. 2018;25:377–385.
28. Deswal A, Petersen NJ, Feldman AM, Young JB, White BG, Mann DL. Cytokines and cytokine receptors in advanced heart failure: an analysis of the cytokine database from the Vesnarinone trial (VEST). Circulation. 2001;103:2055–9.
29. Sinagra E, Perricone G, Romano C, Cottone M. Heart failure and anti tumor necrosis factor-alpha in systemic chronic inflammatory diseases. Eur J Intern Med. 2013;24:385–92.
30. Feldman AM, Combes A, Wagner D, Kadakomi T, Kubota T, Li YY, et al. The role of tumor necrosis factor in the pathophysiology of heart failure. J Am Coll Cardiol. 2000;35:537–44.
31. Divakaran V, Mehta S, Yao D, Hassan S, Simpson S, Wiegerinck E, Swinkels DW, Mann DL, Afshar-Kharghan V. Hepcidin in anemia of chronic heart failure. Am J Hematol. 2011;86:107–9.
32. Yčas JW, Horrow JC, Horne BD. Persistent increase in red cell size distribution width after acute diseases: A biomarker of hypoxemia? Clinica Chimica Acta. 2015;448:107–17.
33. Guzik P, Piskorski J, Wysocki H, Wykrętowicz A. Prospective observational study on predicting adverse clinical outcomes in patients with implanted defibrillating devices–a study rationale, design and principal methods. J Med Sci 2016; 83: 84–88.

Acceptance for editing: 2019-03-13
Acceptance for publication: 2019-03-29

Correspondence address:
Przemyslaw Guzik, MD, PhD, FESC, ISHNE Fellow
Department of Cardiology-Intensive Therapy
Poznan University of Medical Sciences, Poland
49 Przybyszewskiego Street, 60-355 Poznań, Poland
phone: +48618691391; fax: +48618691689
e-mail: pguzik@ptkardio.pl

THOUSAND WORDS ABOUT...

DOI: https://doi.org/10.20883/jms.290

CYP3A drug metabolism in the developmental age: recent advances

Jan Krzysztof Nowak[1, a], Bartłomiej Bancerz[1], Alicja Bartkowska-Śniatkowska[2, b]

[1] Department of Pediatric Gastroenterology and Metabolic Diseases, Poznan University of Medical Sciences, Poland
[2] Department of Pediatric Anaesthesiology and Intensive Therapy, Poznan University of Medical Sciences, Poland

[a] https://orcid.org/0000-0003-0953-2188 [b] https://orcid.org/0000-0003-4950-2346

ABSTRACT

Introduction. The 3A subfamily of cytochrome P450 (CYP3A) accomplishes phase I metabolism for approximately half of the available medications. We aimed to review the recent advances in our understanding of CYP3A activity, which could apply to infants and toddlers.
Material and Methods. A literature review.
Results. The reviewed recent data cover: CYP3A7 expression and functions, changes of CYP3A4 function in the first two years of life, CYP3A intestinal metabolism and zonation, CYP3A metabolic programming, pediatric CYP3A pharmacogenetics, the impact of critical illness on CYP3A, phenotyping, and other clinical implications of a better comprehension of CYP3A biology.
Conclusions. Although the knowledge of CYP3A enzymes has already changed pediatric practice, much more is to be expected in the upcoming years. The areas to watch include: endogenous markers for phenotyping, new CYP3A7 substrates and products, pharmacogenetic interactions with transporter genes for non-immunomodulatory drugs, as well as interactions with microbiota and specific bioactive foodstuffs.

Keywords: CYP3A5, pharmacokinetics, children, pediatric, midazolam, omeprazole.

The 3A subfamily of cytochrome P450 (CYP3A) includes four monooxydases: CYP3A4, CYP3A5, CYP3A7, and CYP3A43. The first two of these enzymes are the most highly expressed of the P450 proteins and accomplish phase I metabolism for approximately half of the available medications. CYP3A activity is subject to ample interindividual variability and may also be affected by a wide range of factors including pharmaceuticals, xenobiotics, foodstuffs, and diseases. We aimed to review the recent advances in our understanding of CYP3A drug metabolism, which could apply to infants and toddlers.

CYP3A7

CYP3A7 constitutes 30–50% of P450 molecules in the fetal liver and may account for over 80% of CYP3A drug metabolism in term neonates [1]. Liver transcriptome analyses revealed that in infants CYP3A7 expression is 3-fold greater than in children aged 1–6 years and over 15-fold greater than in adolescents [2]. Curiously, CYP3A7 might be expressed in adults, but this usually is accompanied by very low CYP3A5 activity [3].

CYP3A7 has a known contribution to sex hormone metabolism, which probably underlies its

link with birthweight [4]. CYP3A7 metabolizes cyclosporine, dronedarone, tacrolimus, and to a small extent sildenafil. Yet, the list of CYP3A7 substrates is far from complete. Overall, CYP3A7 is a crucial, but unexplored contributor to P450 function in the first months of life.

CYP3A4 and CYP3A5

CYP3A4 and CYP3A5 activity profiles at the ages of 1 month–2 years were determined by Emoto et al. [5]. While CYP3A4 activity increases after birth, CYP3A5 is already at the target level, although subject to strong interindividual variation. Other studies suggested that CYP3A4 metabolic capacity continuously rises from birth to 16th month of life, when it reaches adult levels [6]. On the other hand, fentanyl CYP3A4 metabolism is subject to most considerable variation: the reported half-time in neonates may range from 6 (like in adults) to over 20 hours [7]. The elimination of another opioid, buprenorphine, which requires CYP3A4 and UGT1A1, seems to correspond to adult levels shortly after birth [8]. Sirolimus clearance increases with age, along CYP3A activity; in the first year the former augments two-fold [9], but varies greatly from patient to patient. The above examples demonstrate that it is challenging to predict the metabolism of CYP3A4 substrates.

Brussee et al. who investigated midazolam pharmacokinetics in 37 preterm neonates found that the oral bioavailability of midazolam was 92% as opposed to 30% in adults [10]. They concluded that CYP3A activity was poor not only in the liver, but also in the intestine. Currently we do not know when CYP3A4 commences intestinal metabolism. Experiments in a murine model revealed that CYP3A zonation — a selective increase of cytochrome expression in parts of liver exposed to xenobiotics to a greater degree — does not occur until the pre-weaning period. This raises questions: does zonation appear only after CYP3A7 slows down and if it is coupled with changes of CYP3A intestinal activity [11].

Interestingly, there is evidence for metabolic programming with regard to CYP3A. In a murine model provision of high doses of phenobarbital early in life produced an effect of Cyp3a induction that persisted into the adulthood [12]. Exposure of rats with low birth weight to high-fat and high-energy diet led to a longer-term increase in CYP3A1 mRNA expression [13]. Moreover, in rats CYP3A activity shortly after birth might be induced by malnutrition during pregnancy [14]. It might be that programming underlies the large interindividual differences observed in the clinical setting.

Pediatric CYP3A pharmacogenetics

In the last five years a series of articles regarding tacrolimus metabolism in transplant recipients have been published with very similar results. For instance, in a study of over 100 pediatric liver transplant recipients, carriers of CYP3A5*1 allele (yielding large amounts of full-length enzyme), who received liver from CYP3A5*1-positive donors, required over 70% larger doses of tacrolimus [15]. In the work by Uesugi et al. CYP3A5*1 genotype of the transplanted liver associated with a relative risk of acute cellular rejection equaling 2.6 [16]. Moreover, steroid-free kidney transplant recipients who did not carry CYP3A5*1 had higher tacrolimus concentrations [17].

Pharmacogenetic analyses regarding other CYP3A substrates in infants or children are less abundant. In one such study, the lack of CYP3A4*1B (normal AA genotype of rs2740574) associated with the resistance to antiepileptic medication [18]. In various groups, CYP3A5*3 inconsistently associated with vincristine neurotoxicity. CYP3A5 and CYP3A7 polymorphisms seemed to modify the relationship between *in utero* mercury exposure and neurodevelopment [19].

Clinical context

Midazolam pharmacokinetics in infants indicated that CYP3A activity was reduced by two-thirds when CRP values reached 300 mg/L and by one-third when insufficiency of three organs was stated (as compared with one) [20]. Critical illness was found to be the most significant factor to affect CYP3A4 and CYP3A5 function in a group of children aged from 1 month to 17 years [21]. Such severe conditions are one of many examples for how drug metabolism may be altered, further rising uncertainty regarding CYP3A function in individuals.

The possible answer to large variation could be CYP3A phenotyping. However, the use of an endogenous marker would be more convenient than the application of midazolam, alfentanil or buspirone [22]. One such compound could be 4β-hydroxycholesterol [23]; however, its specificity is contested by data, which suggest that its levels depend to a large degree on other factors. Introduction of CYP3A phenotyping into pediatric practice would augment the availability of some classes of drugs, for instance, prokinetic agents, such as domperidone [24].

An example of the impact of the new knowledge of CYP3A4 interactions was provided by Bernard et al. who used lacidipine as a calcium inhibitor with no effect on CYP3A4 in pediatric oncohematology patients receiving other medication e.g., cyclosporine [25]. As was recently noted, CYP3A inhibitors might pose a risk of patent ductus arteriosus in critically ill neonates [26]. A recently published case report highlighted the potential of dexmedetomidine to inhibit tacrolimus metabolism by 75% [27]. CYP3A4 immaturity was hypothesized to be the cause of transient neutropenia in rifabutin-treated children with concomitant human immunodeficiency virus infection and tuberculosis [28]. Finally, CYP3A4 seems to be an alternative route for the inactivation of metabolites of vitamin D: its induction with rifampin in children with infantile hypercalcemia was successful [29]. Our growing comprehension of CYP3A roles and interactions will likely provide further vital data, similar to the above.

Conclusion

Although the knowledge of CYP3A enzymes has already changed pediatric practice, much more is to be expected in the upcoming years. The areas to watch include: endogenous markers for phenotyping, new CYP3A7 substrates and products, pharmacogenetic interactions with transporter genes for non-immunomodulatory drugs, as well as interactions with microbiota and specific bioactive foodstuffs.

Acknowledgements

Conflict of interest statement
The authors declare no conflict of interest.

Funding sources
There are no sources of funding to declare.

Author contributions:
JKN — study design, information acquisition and analysis, manuscript drafting, final approval and agreement to be accountable.
BB — interpretation of data for the work, information acquisition, revising manuscript for important content, final approval and agreement to be accountable.
ABŚ — study concept, design, and supervision; information analysis and interpretation, revising manuscript for important content, final approval and agreement to be accountable.

References

1. Strougo A, Yassen A, Monnereau C, Danhof M, Freijer J. Predicting the „First dose in children" of CYP3A-metabolized drugs: Evaluation of scaling approaches and insights into the CYP3A7-CYP3A4 switch at young ages. J Clin Pharmacol. 2014;54:1006–15.
2. Meier R, Bi C, Gaedigk R, Heruth DP, Ye SQ, Leeder JS, et al. Ontogeny-related pharmacogene changes in the pediatric liver transcriptome. Pharmacogenet Genomics. 2018;28:86–94.
3. Creemer OJ, Ansari-Pour N, Ekong R, Tarekegn A, Plaster C, Bains RK, et al. Contrasting exome constancy and regulatory region variation in the gene encoding CYP3A4: an examination of the extent and potential implications. Pharmacogenet Genomics. 2016;26:255–70.
4. Beaumont RN, Warrington NM, Cavadino A, Tyrrell J, Nodzenski M, Horikoshi M, et al. Genome-wide association study of offspring birth weight in 86 577 women identifies five novel loci and highlights maternal genetic effects that are independent of fetal genetics. Hum Mol Genet. 2018;27:742–56.
5. Emoto C, Fukuda T, Johnson TN, Adams DM, Vinks AA. Development of a Pediatric Physiologically Based Pharmacokinetic Model for Sirolimus: Applying Principles of Growth and Maturation in Neonates and Infants. CPT Pharmacomet Syst Pharmacol. 2015;4:e17.
6. Salem F, Johnson TN, Abduljalil K, Tucker GT, Rostami-Hodjegan A. A re-evaluation and validation of ontogeny functions for cytochrome P450 1A2 and 3A4 based on in vivo data. Clin Pharmacokinet. 2014;53:625–36.
7. Pacifici GM. Clinical pharmacology of fentanyl in preterm infants. A review. Pediatr Neonatol. 2015;56:143–8.
8. Johnson TN, Jamei M, Rowland-Yeo K. How Does In Vivo Biliary Elimination of Drugs Change with Age? Evidence from In Vitro and Clinical Data Using a Systems Pharmacology Approach. Drug Metab Dispos Biol Fate Chem. 2016;44:1090–8.
9. Emoto C, Fukuda T, Mizuno T, Schniedewind B, Christians U, Adams DM, et al. Characterizing the Developmental Trajectory of Sirolimus Clearance in Neonates and Infants. CPT Pharmacomet Syst Pharmacol. 2016;5:411–7.
10. Brussee JM, Yu H, Krekels EHJ, de Roos B, Brill MJE, van den Anker JN, et al. First-Pass CYP3A-Mediated

Metabolism of Midazolam in the Gut Wall and Liver in Preterm Neonates. CPT Pharmacomet Syst Pharmacol. 2018.
11. Kitaoka S, Hatogai J, Ochiai W, Sugiyama K. Zonation of the drug-metabolizing enzyme cytochrome P450 3A in infant mice begins in pre-weaning period. J Toxicol Sci. 2018;43:223–7.
12. Tien Y-C, Liu K, Pope C, Wang P, Ma X, Zhong X. Dose of Phenobarbital and Age of Treatment at Early Life are Two Key Factors for the Persistent Induction of Cytochrome P450 Enzymes in Adult Mouse Liver. Drug Metab Dispos. 2015;43:1938–45.
13. Ni S-Q, Lou Y, Wang X-M, Shen Z, Wang J, Zhao Z-Y, et al. A high-fat high-energy diet influences hepatic CYP3A expression and activity in low-birth-weight developing female rats. World J Pediatr. 2016;12:489–97.
14. Zhu Z-W, Ni S-Q, Wang X-M, Wang J, Zeng S, Zhao Z-Y. Hepatic CYP3A expression and activity in low birth weight developing female rats. World J Pediatr. 2013;9:266–72.
15. Yang T-H, Chen Y-K, Xue F, Han L-Z, Shen C-H, Zhou T, et al. Influence of CYP3A5 genotypes on tacrolimus dose requirement: age and its pharmacological interaction with ABCB1 genetics in the Chinese paediatric liver transplantation. Int J Clin Pract Suppl. 2015;53–62.
16. Uesugi M, Kikuchi M, Shinke H, Omura T, Yonezawa A, Matsubara K, et al. Impact of cytochrome P450 3A5 polymorphism in graft livers on the frequency of acute cellular rejection in living-donor liver transplantation. Pharmacogenet Genomics. 2014;24:356–66.
17. Madsen MJ, Bergmann TK, Brøsen K, Thiesson HC. The Pharmacogenetics of Tacrolimus in Corticosteroid-Sparse Pediatric and Adult Kidney Transplant Recipients. Drugs RD. 2017;17:279–86.
18. López-García MA, Feria-Romero IA, Serrano H, Rayo-Mares D, Fagiolino P, Vázquez M, et al. Influence of genetic variants of CYP2D6, CYP2C9, CYP2C19 and CYP3A4 on antiepileptic drug metabolism in pediatric patients with refractory epilepsy. Pharmacol Rep. 2017;69:504–11.
19. Llop S, Tran V, Ballester F, Barbone F, Sofianou-Katsoulis A, Sunyer J, et al. CYP3A genes and the association between prenatal methylmercury exposure and neurodevelopment. Environ Int. 2017;105:34–42.
20. Vet NJ, Brussee JM, de Hoog M, Mooij MG, Verlaat CWM, Jerchel IS, et al. Inflammation and Organ Failure Severely Affect Midazolam Clearance in Critically Ill Children. Am J Respir Crit Care Med. 2016;194:58–66.
21. Ince I, de Wildt SN, Peeters MYM, Murry DJ, Tibboel D, Danhof M, et al. Critical illness is a major determinant of midazolam clearance in children aged 1 month to 17 years. Ther Drug Monit. 2012;34:381–9.
22. Hohmann N, Haefeli WE, Mikus G. CYP3A activity: towards dose adaptation to the individual. Expert Opin Drug Metab Toxicol. 2016;12:479–97.
23. Aubry A-F, Dean B, Diczfalusy U, Goodenough A, Iffland A, McLeod J, et al. Recommendations on the Development of a Bioanalytical Assay for 4β-Hydroxycholesterol, an Emerging Endogenous Biomarker of CYP3A Activity. AAPS J. 2016;18:1056–66.
24. Doggrell SA, Hancox JC. Cardiac safety concerns for domperidone, an antiemetic and prokinetic, and galactogogue medicine. Expert Opin Drug Saf. 2014;13:131–8.
25. Bernard E, Mialou V, Dony A, Garnier N, Renard C, Bleyzac N. [Lacidipine efficacy and safety for high blood pressure treatment in pediatric oncohematology]. Arch Pediatr Organe Off Soc Francaise Pediatr. 2014;21:1101–5.
26. Cotton RB, Shah LP, Poole SD, Ehinger NJ, Brown N, Shelton EL, et al. Cimetidine-associated patent ductus arteriosus is mediated via a cytochrome P450 mechanism independent of H2 receptor antagonism. J Mol Cell Cardiol. 2013;59:86–94.
27. Stiehl SR, Squires JE, Bucuvalas JC, Hemmelgarn TS. Tacrolimus interaction with dexmedetomidine--a case report. Pediatr Transplant. 2016;20:155–7.
28. Moultrie H, McIlleron H, Sawry S, Kellermann T, Wiesner L, Kindra G, et al. Pharmacokinetics and safety of rifabutin in young HIV-infected children receiving rifabutin and lopinavir/ritonavir. J Antimicrob Chemother. 2015;70:543–9.
29. Hawkes CP, Li D, Hakonarson H, Meyers KE, Thummel KE, Levine MA. CYP3A4 Induction by Rifampin: An Alternative Pathway for Vitamin D Inactivation in Patients With CYP24A1 Mutations. J Clin Endocrinol Metab. 2017;102:1440–6.

Acceptance for editing: 2019-03-13
Acceptance for publication: 2019-03-29

Correspondence address:
Jan Krzysztof Nowak
Department of Pediatric Gastroenterology and Metabolic Diseases
Poznan University of Medical Sciences
ul. Szpitalna 27/33, 60-574 Poznań, Poland
phone: +48 618491432; fax: +48 618472685
e-mail: jan.nowak@ump.edu.pl

THE RATIONALE, DESIGN AND METHODS OF NEW STUDIES

DOI: https://doi.org/10.20883/jms.317

Study of serum metabolic profiles of patients with non-small cell lung cancer with special emphasis on the smoking status of patients

Agnieszka Klupczynska[1, a], Mariusz Kasprzyk[2, b], Wojciech Dyszkiewicz[2, c], Marcin Grabicki[3, d], Halina Batura-Gabryel[3, e], Zenon J. Kokot[1, f], Jan Matysiak[1, g]

[1] Department of Inorganic and Analytical Chemistry, Poznan University of Medical Sciences, Poland
[2] Department of Thoracic Surgery, Poznan University of Medical Sciences, Poland
[3] Department of Pulmonology, Allergology and Respiratory Oncology, Poznan University of Medical Sciences, Poland

[a] https://orcid.org/0000-0002-5028-1408
[b] https://orcid.org/0000-0001-6553-7675
[c] https://orcid.org/0000-0001-6259-7381
[d] https://orcid.org/0000-0002-6469-9469
[e] https://orcid.org/0000-0003-4555-7905
[f] https://orcid.org/0000-0003-4950-9759
[g] https://orcid.org/0000-0002-9993-1504

ABSTRACT

The project entitled "Study of serum metabolic profiles of patients with non-small cell lung cancer with special emphasis on the smoking status of patients" is a study based on metabolomics, which is the latest of the "omics" technologies and involves a comprehensive analysis of small molecule metabolites of a specific biological sample. High-throughput and sensitive analytical techniques used in metabolomic investigations are powerful tools in the field of oncology and aids understanding what is happening in cancer cells and searching for new cancer markers. The aim of the project is to determine whether lung cancer patients have a distinct serum metabolic profile and whether this profile is associated with patients' smoking status. The application of liquid chromatography-high-resolution mass spectrometry-based methodology along with advanced statistical methods will enable to select potential molecules that can be useful in early lung cancer detection..

Keywords: lung cancer, metabolomics, chronic-obstructive pulmonary disease, mass spectrometry.

General information

The project entitled "Study of serum metabolic profiles of patients with non-small cell lung cancer with special emphasis on the smoking status of patients" was founded by the National Science Centre, Poland within MINIATURA1 competition (grant number 2017/01/X/NZ7/02064). The duration of the grant is 12 months. The project is planned for 12 months, and it is run by the Department of Inorganic and Analytical Chemistry, Poznan University of Medical Sciences, Poland in the cooperation with Department of Thoracic Surgery as well as Department of Pulmonology, Allergology and Respiratory Oncology, Poznan University of Medical Sciences, Poland. The principal investigator is Agnieszka Klupczyńska, Ph.D. and the total grant value is 49467 PLN. The project includes an internship of the principal investigator in Integrative Molecular Phenotyping Laboratory, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden. The study was approved by the ethics

committee at the Poznan University of Medical Sciences (Decision No. 746/17 and 314/18).

Research Project Objectives

The aim of the study is to perform serum untargeted metabolic profiling of lung cancer patients and patients with chronic obstructive pulmonary disease (COPD) as a control, non-cancer group. A novelty is the application of patient categorization based on the smoking status, which allows for distinguishing molecular markers associated with lung cancer from changes in the metabolic profile resulting from tobacco smoking. Lung cancer remains one of the major challenges in contemporary oncology. Although its incidence rate is similar to other malignant tumors, such as prostate cancer and breast cancer, lung cancer is characterized by 4–5 times higher death rate and it has been the main cause of malignant tumor-related deaths for years [1, 2]. Due to the high morbidity and mortality of lung cancer, there is a high demand for identification of cancer biomarkers that can contribute clinically relevant information. Tobacco smoking is the main factor in the development of lung cancer, and therefore the effect of this factor on the metabolic profiles of the patients should be taken into account in metabolomic experiments. So far the issue of the influence of smoking on the metabolic profiles of patients has been neglected in lung cancer biomarker studies and only a few of them present patient stratification by smoking status [3, 4]. Previous research of serum metabolome of lung cancer patients indicated alterations in many distinct groups of metabolites, such as amino acids, organic acids and acylcarnitines [5–7]. However, little is known about the differences between the levels of angiogenic markers between smoking and not smoking patients. Therefore, it should be investigated whether the observed differences in the metabolome result from the development of lung cancer or if they are the result of chronic smoking. The answer to the above research question can be obtained by profiling a broad spectrum of metabolites in serum samples taken from patients with lung cancer and a control group. Endogenous metabolites are at the end of a series of processes beginning with the genome followed by the transcriptome and proteome. Therefore, metabolome fills the gap between a genotype and phenotype. Components of metabolic profiles include such compound classes as amino acids, amines, organic acids, fatty acids, steroids, and sugars [8, 9].

Research Plan and Basic Concept

The project includes untargeted mass spectrometry-based metabolic profiling, which represents a promising and valuable tool in the analysis of complex cancer-associated metabolic changes [8, 10]. The study is conducted in serum samples derived from individuals with untreated non-small cell lung cancer and a matched non-cancer group (individuals with COPD). The resulting metabolite profiles will be subjected to univariate and multivariate statistical tests, and significant features will be identified using different databases (**Figure 1**).

The research plan includes the following steps:

1. Sample collection
   Collection of serum specimens from patients with newly diagnosed lung cancer and individuals with COPD (non-cancer group). Conducting a questionnaire survey among all individuals who donated blood samples. Selection of the samples of the study group (people with lung cancer) based on histopathological examination of tissue samples.
2. Untargeted metabolic profiling of serum samples using high-resolution mass spectrometry.
   Performing serum sample extraction and metabolite profiling using quadrupole-time-of-flight (Q-TOF) mass spectrometer coupled to a liquid chromatograph. Analysis of quality control samples.
3. Data analysis and identification of metabolite markers
   Univariate and multivariate statistical analyses performed on the obtained bioanalytic data together with clinical data. Significant feature identification. Characterization of correlations between the endogenous metabolites and the individual's health status (presence or absence of lung cancer). Estimation of the correlation of the obtained results (metabolite profiles) with the smoking status of patients. Estimation of the diagnostic value of the selected endogenous compounds.

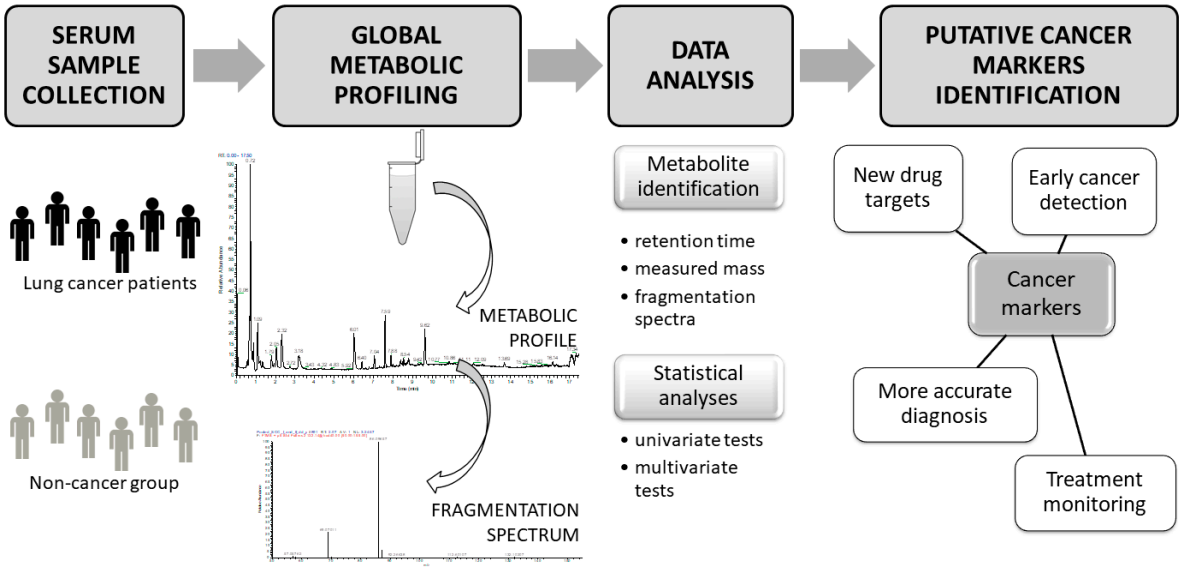


Figure 1. Basic concept of metabolomic studies in cancer research

Research Methodology

Patients and samples

Serum samples are collected from 2 groups of patients divided into subgroups based on smoking status: current smokers and ex-smokers (**Table 1**). Subgroups of smokers consist of patients smoking at least ten pack-years and a minimum of 10 cigarettes/day the past 6 months. In case of the ex-smokers criterion of minimum 2 years since smoking cessation is applied. Study participants are recruited in the Department of Thoracic Surgery, Poznan University of Medical Sciences and Department of Pulmonology, Allergology and Respiratory Oncology, Poznan University of Medical Sciences. Lung cancer diagnosis is performed by the histopathological examination of tissues. Blood samples are collected before the initiation of any anti-cancer treatment.

Table 1. Classification of patients enrolled to the study

Lung cancer patients (n = 40)		COPD patients (n = 40)	
Current smokers[1] (n = 20)	Ex-smokers[2] (n = 20)	Current smokers[1] (n = 20)	Ex-smokers[2] (n = 20)

Analytical methodology

The goal of the project will be reached with the use of untargeted metabolic profiling and by the application of high-resolution mass spectrometry. In untargeted metabolomic experiment all detectable metabolites in a specimen are analyzed, and as a result, a unique, global metabolic profile of samples is obtained. One of the most commonly used analytical platforms in global metabolomics is Q-TOF mass spectrometer coupled to liquid chromatograph and equipped with electrospray ionization source. Since Q-ToF instrument is not available at Poznan University of Medical Sciences, the assays will be performed in Integrative Molecular Phenotyping Laboratory, Division of Physiological Chemistry II, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden. The project includes an internship of the principal investigator in that foreign research centre. Integrative Molecular Phenotyping Laboratory is one of the leading metabolomic centres in Europe and has experience in metabolomic studies of respiratory diseases [11–13].

Data analysis

Due to its comprehensive nature, the untargeted metabolomics produces large dataset, which requires extensive data processing as well as advanced statistical methods for data analysis. Data processing consists of several steps, i.a. smoothing, chromatogram deconvolution, peak alignment, duplicate peak removing, peak filtering. For statistical analyses of data arising from high-throughput metabolomics specialized software such as MetaboAnalyst is used [14]. The performed tests will allow for select features that are significantly different between the two analyzed

groups (biomarker discovery). The next crucial and also challenging step in data analysis of global metabolic profiles is the reliable identification of detected significant signals. Metabolite identification is performed by matching accurate mass, retention time and tandem mass spectrometry fragmentation patterns to different chemical reference databases, i..a., the in-house library of standards acquired previously using the same instrument, Human Metabolome Database and others.

Measurable Effects and Expected Results

The proposed metabolomic research will broaden our pathophysiological understanding of cancer and will be used as a source of new potential cancer-associated biomarkers. The study will allow to better understand the effect of tobacco smoking on the complex and multidimensional pathogenesis of lung cancer. The obtained results will enable the design of further studies focused on a specific group of metabolites to better estimate their potential as lung cancer markers. The further plans involve the determination of a panel of identified metabolic markers using a targeted approach with the application of triple quadrupole mass spectrometry to obtain quantitative data and prove the clinical usefulness of the selected molecules.

Acknowledgements

Conflict of interest statement
The authors declare no conflict of interest.

Funding sources
The paper is a part of the project supported by the National Science Centre, Poland (grant number: 2017/01/X/NZ7/02064).

References

1. Subramaniam S, Thakur RK, Yadav VK, Nanda R, Chowdhury S, Agrawal A. Lung cancer biomarkers: State of the art. J Carcinog. 2013 Feb;12:3.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin. 2018 Jan;68(1):7–30.
3. Musharraf SG, Mazhar S, Choudhary MI, Rizi N, Atta-ur-Rahman. Plasma metabolite profiling and chemometric analyses of lung cancer along with three controls through gas chromatography-mass spectrometry. Sci Rep. 2015 Feb;5:8607.
4. Pamungkas AD, Park C, Lee S, Jee SH, Park YH. High resolution metabolomics to discriminate compounds in serum of male lung cancer patients in South Korea. Respir Res. 2016 Aug;17(1):100.
5. Klupczynska A, Dereziński P, Dyszkiewicz W, Pawlak K, Kasprzyk M, Kokot ZJ. Evaluation of serum amino acid profiles' utility in non-small cell lung cancer detection in Polish population. Lung Cancer. 2016 Oct;100:71–76.
6. Klupczynska A, Plewa S, Dyszkiewicz W, Kasprzyk M, Sytek N, Kokot ZJ. Determination of low-molecular-weight organic acids in non-small cell lung cancer with a new liquid chromatography–tandem mass spectrometry method. J Pharm Biomed Anal. 2016 Sep;129:299–309.
7. Klupczynska A, Dereziński P, Garrett TJ, Rubio VY, Dyszkiewicz W, Kasprzyk M, et al. Study of early stage non-small-cell lung cancer using Orbitrap-based global serum metabolomics. J Cancer Res Clin Oncol. 2017 Apr;143(4):649–659.
8. Klupczynska A, Derezinski P, Kokot ZJ. Metabolomics in medical sciences — Trends, challenges and perspectives. Acta Pol Pharm. 2015 Jul-Aug;72(4):629–41.
9. Bu Q, Huang Y, Yan G, Cen X, Zhao YL. Metabolomics: a revolution for novel cancer marker identification. Comb Chem High Throughput Screen. 2012 Mar;15(3):266–75.
10. Armitage EG, Barbas C. Metabolomics in cancer biomarker discovery: Current trends and future perspectives. J Pharm Biomed Anal. 2014 Jan;87:1–11.
11. Naz S, Kolmert J, Yang M, Reinke SN, Kamleh MA, Snowden S, et al. Metabolomics analysis identifies sex-associated metabotypes of oxidative stress and the autotaxin-lysoPA axis in COPD. Eur Respir J. 2017 Jun;49(6).
12. Reinke SN, Gallart-Ayala H, Gómez C, Checa A, Fauland A, Naz S, et al. Metabolomics analysis identifies different metabotypes of asthma severity. Eur Respir J. 2017 Mar;49(3).
13. Wheelock CE, Goss VM, Balgoma D, Nicholas B, Brandsma J, Skipp PJ, et al. Application of 'omics technologies to biomarker discovery in inflammatory lung diseases. Eur Respir J. 2013 Sep;42(3):802–25.
14. Chong J, Soufan O, Li C, Caraus I, Li S, Bourque G, et al. MetaboAnalyst 4.0: Towards more transparent and integrative metabolomics analysis. Nucleic Acids Res. 2018 Jul;46(W1):W486-W494.

Acceptance for editing: 2019-03-13
Acceptance for publication: 2019-03-29

Correspondence address:
Agnieszka Klupczynska
Department of Inorganic and Analytical Chemistry
Poznan University of Medical Sciences
6 Grunwaldzka Street, 60-780 Poznań, Poland
phone: +48 618546616, fax: +48 618546609
e-mail: aklupczynska@ump.edu.pl

Journal of Medical Science
Instructions for Authors

Journal of Medical Science (JMS) is a PEER-REVIEWED, OPEN ACCESS journal that publishes original research articles and reviews which cover all aspects of clinical and basic science research. The journal particularly encourages submissions on the latest achievements of world medicine and related disciplines. JMS is published quarterly by Poznan University of Medical Sciences.

ONLINE SUBMISSION:
Manuscripts should be submitted to the Editorial Office by an e-mail attachment: nowinylekarskie@ump.edu.pl. You do not need to mail any paper copies of your manuscript.

All submissions should be prepared with the following files:
- Cover Letter
- Manuscript
- Tables
- Figures
- Supplementary Online Material

COVER LETTER: *Manuscripts* must be accompanied by a *cover letter* from the author who will be responsible for correspondence regarding the manuscript as well as for communications among authors regarding revisions and approval of proofs. The cover letter should contain the following elements: (1) the full title of the manuscript, (2) the category of the manuscript being submitted (e.g. Original Article, Brief Report), (3) the statement that the manuscript has not been published and is not under consideration for publication in any other journal, (4) the statement that all authors approved the manuscript and its submission to the journal, and (5) a list of at least two referees.

MANUSCRIPT: Journal of Medical Science publishes Original Articles, Brief Reports, Review articles, Mini-Reviews, Images in Clinical Medicine and The Rationale and Design and Methods of New Studies. From 2014, only articles in English will be considered for publication. They should be organized as follows: Title page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgments, Conflict of Interest, References and Figure Legends. All manuscripts should be typed in Arial or Times New Roman font and double spaced with a 2,5 cm (1 inch) margin on all sides. They should be saved in DOC, DOCX, ODT, RTF or TXT format. Pages should be numbered consecutively, beginning with the title page.

Ethical Guidelines
Authors should follow the principles outlined in the Declaration of Helsinki of the World Medical Association (www.wma.net). The manuscript should contain a statement that the work has been approved by the relevant institutional review boards or ethics committees and that all human participants gave informed consent to the work. This statement should appear in the Material and Methods section. Identifying information, including patients' names, initials, or hospital numbers, should not be published in written descriptions, illustrations, and pedigrees. Studies involving experiments with animals must be conducted with approval by the local animal care committee and state that their care was in accordance with institution and international guidelines.

Authorship:
According to the International Committee on Medical Journal Ethics (ICMJE), an author is defined as one who has made substantial contributions to the conception and development of a manuscript. Authorship should be based on all of the following: 1) substantial contributions to conception and design, data analysis and interpretation; 2) article drafting or critical advice for important intellectual content; and 3) final approval of the version to be published. All other contributors should be listed as acknowledgments. All submissions are expected to comply with the above definition.

Conflict of Interest
The manuscript should contain a conflict of interest statement from each author. Authors should disclose all financial and personal relationships that could influence their work or declare the absence of any conflict of interest. Author's conflict of interest should be included under Acknowledgements section.

Abbreviations
Abbreviations should be defined at first mention, by putting abbreviation between brackets after the full text. Ensure consistency of abbreviations throughout the article. Avoid using them in the title and abstract. Abbreviations may be used in tables and figures if they are defined in the table footnotes and figure legends.

Trade names
For products used in experiments or methods (particularly those referred to by a trade name), give the manufacturer's full name and location (in parentheses). When possible, use generic names of drugs.

Title page
The first page of the manuscript should contain the title of the article, authors' full names without degrees or titles, authors' institutional affiliations including city and country and a running title, not exceeding 40 letters and spaces. The first page should also include the full postal address, e-mail address, and telephone and fax numbers of the corresponding author.

Abstract
The abstract should not exceed 250 words and should be structured into separate sections: Background, Methods, Results and Conclusions. It should concisely state the significant findings without reference to the rest of the paper. The abstract should be followed by a list of 3 to 6 Key words. They should reflect the central topic of the article (avoid words already used in the title).

The following categories of articles can be proposed to the Journal of Medical Science:

ORIGINAL RESEARCH

Original articles: Manuscripts in this category describe the results of original research conducted in the broad area of life science and medicine. The manuscript should be presented in the format of Abstract (250-word limit), Keywords, Introduction, Material and Methods, Results, Discussion, Perspectives, Acknowledgments and References. In the Discussion section, statements regarding the importance and *novelty of the study* should be presented. In addition, the limitations of the study should be articulated. The abstract must be structured and include: Objectives, Material and Methods, Results and Conclusions. Manuscripts cannot exceed 3500 words in length (excluding title page, abstract and references) and contain no more than a combination of 8 tables and/or figures. The number of references should not exceed 45.

Brief Reports: Manuscripts in this category may present results of studies involving small sample sizes, introduce new methodologies, describe preliminary findings or replication studies. The manuscript must follow the same format requirements as full length manuscripts. Brief reports should be up to 2000 words (excluding title page, abstract and references) and can include up to 3 tables and/or figures. The number of references should not exceed 25.

REVIEW ARTICLES

Review articles: These articles should describe recent advances in areas within the Journal's scope. Review articles cannot exceed 5000 words length (excluding title page, abstract and references) and contain no more than a combination of 10 tables and/or figures. Authors are encouraged to restrict figures and tables to essential data that cannot be described in the text. The number of references should not exceed 80.

A THOUSAND WORDS ABOUT... is a form of Mini-Reviews. Manuscripts in this category should focus on *latest achievements of life science and medicine.* Manuscripts should be up to 1000 words in length (excluding title page, abstract and references) and contain up to 5 tables and/or figures and up to 25 most relevant references. The number of authors is limited to no more than 3.

OTHER SUBMISSIONS

Invited Editorials: Editorials are authoritative commentaries on topics of current interest or that relate to articles published in the same issue. Manuscripts should be up to 1500 words in length. The number of references should not exceed 10. The number of authors is limited to no more than 2.

Images in Clinical Medicine: Manuscripts in this category should contain one distinct image from life science or medicine. Only original and high-quality images are considered for publication. The description of the image (up to 250 words) should present relevant information like short description of the patient's history, clinical findings and course, imaging techniques or molecular biology techniques (e.g. blotting techniques or immunostaining). All labeled structures in the image should be described and explained in the legend. The number of references should not exceed 5. The number of authors is limited to no more than 5.

The Rationale, Design and Methods of New Studies: Manuscripts in this category should provide information regarding the grants awarded by different founding agencies, e.g. National Health Institute, European Union, National Science Center or National Center for Research and Development. The manuscript should be presented in the format of Research Project Objectives, Research Plan and Basic Concept, Research Methodology, Measurable Effects and Expected Results. The article should also contain general information about the grant: grant title, keywords (up to five), name of the principal investigator and co-investigators, founding source with the grant number, *Ethical Committee permission number,* code in clinical trials (if applicable). Only grant projects in the amount over 100,000 Euro can be presented. Manuscripts should be up to 2000 words in length (excluding references) and can include up to 5 tables and/or figures. The abstract should not exceed 150 words. The number of authors is limited to the Principal Investigator and Co-investigators.

Acknowledgements

Under acknowledgements please specify contributors to the article other than the authors accredited. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.). Also acknowledge all sources of support (grants from government agencies, private foundations, etc.). The names of funding organizations should be written in full.

References

All manuscripts should use the 'Vancouver' style for references. References should be numbered consecutively in the order in which they appear in the text **and listed at the end of the paper.** References cited only in Figures/Tables should be listed in the end. Reference citations in the text should be identified by Arabic numbers in square brackets. Some examples:

This result was later contradicted by Smith and Murray [3].
Smith [8] has argued that...
Multiple clinical trials [4–6, 9] show...

List all authors if there are six or fewer; if there are seven or more, list first six follower by "et al.". Journal names should be abbreviated according to Index Medicus.

Some examples

Standard journal articles

1. Fassone E, Rahman S. Complex I deficiency: clinical features, biochemistry and molecular genetics. J Med Genet. 2012 Sep;49(9):578–590.

2. Pugh TJ, Morozova O, Attiyeh EF, Asgharzadeh S, Wei JS, Auclair D et al. The genetic landscape of high-risk neuroblastoma. Nat Genet. 2013 Mar;45(3):279–284.

Books

Personal author(s)

1. Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology. 5th ed. Edinburgh: Churchill Livingstone; 2003.

Editor(s) or compiler(s) as authors

2. Beers MH, Porter RS, Jones TV, Kaplan JL, Berkwits M (editors). The Merck manual of diagnosis and therapy. 18th ed. Whitehouse Station (NJ): Merck Research Laboratories; 2006.

Chapter in the book

1. Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. p. 465–478.

TABLES: Tables should be typed on sheets separate from the text (each table on a separate sheet). They should be numbered consecutively with Arabic numerals. Tables should always be cited in text (e.g. table 2) in consecutive numerical order. Each table should include a compulsory, concise explanatory title and an explanatory legend. Footnotes to tables should be typed below the table body and referred to by superscript lowercase letters. No vertical rules should be used. Tables should not duplicate results presented elsewhere in the manuscript (e.g. in figures).

FIGURES: All illustrations, graphs, drawings, or photographs are referred to as figures and must be uploaded as separate files when submitting a manuscript. Figures should be numbered in sequence with Arabic numerals. They should always be cited in text (e.g. figure 3) in consecutive numerical order. Figures for publication must only be submitted in high-resolution TIFF or EPS format (*minimum 300 dpi resolution).* Each figure should be self-explanatory without reference to the text and have a concise but descriptive legend. All symbols and abbreviations used in the figure must be defined, unless they are common abbreviations or have already been defined in the text. Figure Legends must be included after the reference section of the Main Text.

Color figures: Figures and photographs will be reproduced in full colour in the online edition of the journal. In the paper edition, all figures and photographs will be reproduced as black-and-white.

SUPPLEMENTARY ONLINE MATERIAL: Authors may submit supplementary material for their articles to be posted in the electronic version of the journal. To be accepted for posting, supplementary materials must be essential to the scientific integrity and excellence of the paper. The supplementary material is subject to the same editorial standards and peer-review procedures as the print publication.

Review Process

All manuscripts are reviewed by the Editor-in-Chief or one of the members of the Editorial Board, who may decide to reject the paper or send it for external peer review. Manuscripts accepted for peer review will be blind reviewed by at least two experts in the field. After peer review, the Editor-in-Chief will study the paper together with reviewer comments to make one of the following decisions: accept, accept pending minor revision, accept pending major revision, or reject. Authors will receive comments on the manuscript regardless of the decision. In the event that a manuscript is accepted pending revision, the author will be responsible for completing the revision within 60 days.

Copyright