



# Wiadomości Lekarskie

Official journal of the Polish Medical Association



Memory of  
dr Władysław  
Biegański

VOLUME LXXIV, ISSUE 3 PART 2, MARCH 2021

Since 1928

---



ALUNA Publishing House

Wiadomości Lekarskie is abstracted and indexed in: PUBMED/MEDLINE, SCOPUS, EMBASE, INDEX COPERNICUS, POLISH MINISTRY OF SCIENCE AND HIGHER EDUCATION, POLISH MEDICAL BIBLIOGRAPHY

Copyright: © ALUNA Publishing House.

Articles published on-line and available in open access are published under Creative Commons Attribution-Non Commercial-No Derivatives 4.0 International (CC BY-NC-ND 4.0) allowing to download articles and share them with others as long as they credit the authors and the publisher, but without permission to change them in any way or use them commercially.

## **Wiadomości Lekarskie monthly journal**

**You can order the subscription for the journal from Wydawnictwo Aluna by:**

prenumerata@wydawnictwo-aluna.pl  
Wydawnictwo Aluna  
Z.M. Przesmyckiego 29  
05-510 Konstancin-Jeziorna  
Poland

Place a written order first.

If you need, ask for an invoice.

Payment should be done to the following account of the Publisher:

**account number for Polish customers (PLN):**

82 1940 1076 3010 7407 0000 0000

Credit Agricole Bank Polska S. A., SWIFT: AGRIPLP

**account number for foreign customers (EURO):**

57 2490 0005 0000 4600 7604 3035

Alior Bank S. A.: SWIFT: ALBPPLPW

Subscription of twelve consecutive issues (1-12):

Customers in Poland: 360 PLN/year

Customers from other countries: 320 EURO/year



# Wiadomości Lekarskie

**Editor in-Chief:**

Prof. Władysław Pierzchała

**Deputy Editor in-Chief:**

Prof. Aleksander Sieroń

**Editors of the issue:**

Prof. Tetiana Gruziewa, D. Med. Sci.

Liudmyla Haliienko, D. Med. Sci.

Hanna Inshakova, PhD.

**Statistical Editor:**

Dr Lesia Rudenko

**Managing Editor:**

Agnieszka Rosa – amarosa@wp.pl

**International Editorial Office:**

Lesia Rudenko (editor) – l.rudenko@wydawnictwo-aluna.pl

Nina Radchenko (editor's assistant)

– n.radchenko@wydawnictwo-aluna.pl

**Polish Medical Association (Polskie Towarzystwo Lekarskie):**

Prof. Waldemar Kostewicz – President PTL

Prof. Jerzy Woy-Wojciechowski – Honorary President PTL

Prof. Tadeusz Petelenz

---

**International Editorial Board – in-Chief:**

Marek Rudnicki

Chicago, USA

**International Editorial Board – Members:**

Kris Bankiewicz	San Francisco, USA	George Krol	New York, USA
Christopher Bara	Hannover, Germany	Krzysztof Łabuzek	Katowice, Poland
Krzysztof Bielecki	Warsaw, Poland	Henryk Majchrzak	Katowice, Poland
Zana Bumbuliene	Vilnius, Lithuania	Ewa Małecka-Tendera	Katowice, Poland
Ryszarda Chazan	Warsaw, Poland	Stella Nowicki	Memphis, USA
Stanislav Czudek	Ostrava, Czech Republic	Alfred Patyk	Gottingen, Germany
Jacek Dubiel	Cracow, Poland	Palmira Petrova	Yakutsk, Russia
Zbigniew Gasior	Katowice, Poland	Krystyna Pierzchała	Katowice, Poland
Andrzej Gładysz	Wroclaw, Poland	Tadeusz Płusa	Warsaw, Poland
Nataliya Gutorova	Kharkiv, Ukraine	Waldemar Priebe	Houston, USA
Marek Hartleb	Katowice, Poland	Maria Siemionow	Chicago, USA
Roman Jaeschke	Hamilton, Canada	Vladyslav Smiiianov	Sumy, Ukraine
Andrzej Jakubowiak	Chicago, USA	Tomasz Szczepański	Katowice, Poland
Oleksandr Katrushov	Poltava, Ukraine	Andrzej Witek	Katowice, Poland
Peter Konturek	Saalfeld, Germany	Zbigniew Wszolek	Jacksonville, USA
Jerzy Korewicki	Warsaw, Poland	Vyacheslav Zhdan	Poltava, Ukraine
Jan Kotarski	Lublin, Poland	Jan Zejda	Katowice, Poland

---

**Distribution and Subscriptions:**

Bartosz Guterman prenumerata@wydawnictwo-aluna.pl

**Graphic design / production:**

Grzegorz Sztank

www.red-studio.eu

**Publisher:**

ALUNA Publishing House

ul. Przesmyckiego 29,

05-510 Konstancin – Jeziorna

www.wydawnictwo-aluna.pl

www.wiadomoscilekarskie.pl

www.wiadlek.pl

## FOR AUTHORS

- The monthly "Wiadomości Lekarskie" Journal is the official journal of the Polish Medical Association. Original studies, review papers as well as case reports are published.
- The publication of the manuscript in "Wiadomości Lekarskie" is paid. The cost of publishing the manuscript is PLN 1,000 plus 23% VAT (for foreign authors 250 Euro). If the first author of the manuscript is a member of the Editorial Board or a team of journal reviewers, we do not charge a fee for printing the manuscript, and if she or he is the next co-author – the fee is PLN 500 plus 23% VAT. The publisher issues invoices. The fee should be paid after receiving positive reviews, and before publishing the manuscript. Membership of the Polish Medical Association with documented paid membership fees for the last 3 years is also the exempt from publication fee.
- Only papers in English are accepted for publication. The editors can help in finding the right person for translation or proofreading.
- Papers should be sent to the editor via the editorial panel (Editorial System), available on the journal's website at <https://www.wiadlek.pl>. In order to submit an article, free registration in the system is necessary. After registration, the author should follow the instructions on the computer screen.
- All editorial work is under control and using the editorial panel. This applies in particular to sending manuscripts, correspondence between the editor and author and the review process. In special cases, the editor may agree to contact outside the panel, especially in case of technical problems.
- Acceptable formats for individual elements of the article are as follows:
  - Content of the article – doc, docx, rtf, odt.
  - Tables – doc, docx, rtf, odt
  - Figures – JPG, GIF, TIF, PNG with a resolution of at least 300 dpi
  - Captions for figures and tables.
 These elements are sent to the editor separately using the editorial panel. References and article metadata such as titles, keywords, abstracts etc. are supplemented by the author manually in the editorial panel in appropriate places.
- The volume of original papers – including figures and references – must not exceed 21,600 characters (12 pages of typescript), and review papers – up to 28,800 characters (16 pages).
- The original manuscript should have the following structure: Introduction, Aims, Material and methods, Results, Discussion and Conclusions which cannot be a summary of the manuscript.
- When using abbreviations, it is necessary to provide the full wording at the first time they are used.
- In experimental manuscripts in which studies on humans or animals have been carried out, as well as in clinical studies, information about obtaining the consent of the Ethics Committee should be included.
- The Editorial Board follow the principles contained in the Helsinki Declaration as well as in the Interdisciplinary Principles and Guidelines for the Use of Animals in Research, Testing and Education, published by the New York Academy of Sciences Ad Hoc Committee on Animal Research. All papers relating to animals or humans must comply with ethical principles set out by the Ethics Committee.
- The abstract should contain 150–250 words. Abstracts of original, both clinical and experimental, papers should have the following structure: Aims, Material and methods, Results, Conclusions. Do not use abbreviations in the title or the abstract. The abstract is pasted or rewritten by the authors into the appropriate field in the application form in the editorial panel.
- Keywords (3–5) should be given according to MeSH (Medical Subject Headings Index Medicus catalogs – <http://www.nlm.nih.gov/mesh/MBrowser.html>). Keywords cannot be a repetition of the title of the manuscript.
- Illustrative material may be black and white or color photographs, clearly contrasting or drawings carefully made on a white background. With the exception of selected issues, the Journal is printed in shades of gray (black and white illustrations).
- The content of the figures, if present (e.g. on the charts), should also be in English
- Links to all tables and figures (round brackets) as well as references (square brackets) the author must place in the text of the article.
- Only references to which the author refers in the text should be included in the list of references ordered by citation. There should be no more than 30 items in original papers and no more than 40 items in review papers. Each item should contain: last names of all authors, first letters of first names, the title of the manuscript, the abbreviation of the journal title (according to Index Medicus), year, number, start and end page. For book items, please provide: author's (authors') last name, first letter of the first name, chapter title, book title, publisher, place and year of publication. It is allowed to cite websites with the URL and date of use of the article, and if possible the last names of the authors. Each literature item should have a reference in the text of the manuscript placed in square brackets, e.g. [1], [3–6]. Items should be organized as presented in Annex 1 to these Regulations.
- When submitting the article to the editor, the authors enclose a statement that the work was not published or submitted for publication in another journal and that they take full responsibility for its content, and the information that may indicate a conflict of interest, such as:
  - financial dependencies (employment, paid expertise, consulting, ownership of shares, fees),
  - personal dependencies,
  - academic and other competition that may affect the substantive side of the work,
  - sponsorship of all or part of the research at the stage of design, collection, analysis and interpretation of data, or report writing.
- The authors in the editorial panel define their contribution to the formation of scientific work according to the following key:
  - Work concept and design
  - Data collection and analysis
  - Responsibility for statistical analysis
  - Writing the article
  - Critical review
  - Final approval of the article.
- In the editorial panel along with the affiliation, the author also gives her or his ORCID number.
- The Journal is reviewed in double, blind review mode. The submitted papers are evaluated by two independent reviewers and then qualified for publishing by the Editor-in-Chief. Reviews are anonymous. The authors receive critical reviews with a request to correct the manuscript or with a decision not to qualify it for publishing. The procedure for reviewing articles is in line with the recommendations of the Ministry of Science and Higher Education contained in the paper "Good practices in review procedures in science" (Warsaw 2011). Detailed rules for dealing with improper publishing practices are in line with COPE guidelines. The publishing review rules are in the Review Rules section.
- Each manuscript is subject to verification in the anti-plagiarism system.
- Manuscripts are sent for the author's approval. The author's corrections should be sent within the time limit indicated in the system. No response within the given deadline is tantamount to the author's acceptance of the submitted material. In special cases, it is possible to set dates individually.
- Acceptance of the manuscript for publishing means the transfer of copyright to the Aluna Publishing House (Aluna Anna Łuczynska, NIP 5251624918).
- Articles published on-line and available in open access are published under Creative Commons Attribution-Non Commercial-No Derivatives 4.0 International (CC BY-NC-ND 4.0) allowing to download articles and share them with others as long as they credit the authors and the publisher, but without permission to change them in any way or use them commercially.
- The authors receive a free PDF of the issue in which their manuscript is enclosed, and on request – a printed copy. The printed copy is sent to the address indicated by the authors as the correspondence address.
- Manuscripts not concordant with the above instructions will be returned to be corrected.
- The editors do not return papers which have not been commissioned.
- The editors take no responsibility for the contents of the advertisements.

## CONTENTS

### ORIGINAL ARTICLES

- Liubov M. Zakhartseva, Mariia A. Yanovytska  
PROGNOSTIC VALUE OF TUMOR STROMA RATIO IN TRIPLE NEGATIVE BREAST CANCER 565
- Svitlana P. Koshova, Zoriana V. Hbur, Andriy V. Kolomoyets  
RESEARCH OF PSYCHOLOGICAL READINESS OF DOCTORS TO ADAPTIVE TRAINING 572
- Alina E. Baylo, Tetiana O. Maksymets, Vadym P. Shypulin, Volodymyr V. Chernyavskiy, Luiza M. Parunian  
HEMOSTATIC POTENTIAL ASSESSMENT OF PATIENTS WITH LIVER CIRRHOSIS AND ATRIAL FIBRILLATION BY LOW-FREQUENCY PIEZOELECTRIC THROMBOELASTOGRAPHY 578
- Valery N. Lekhan, Liudmyla O. Hrytsenko  
SUBSTANTIATION OF COMPONENTS OF AVAILABILITY AND INTEGRATION OF PRIMARY HEALTH CARE ASSOCIATED WITH AMBULATORY CARE SENSITIVE CONDITIONS IN UKRAINE 584
- Natalia V. Stuchynska, Igor V. Belous, Pavlo V. Mykytenko  
USE OF MODERN CLOUD SERVICES IN RADIOLOGICAL DIAGNOSTICS TRAINING 589
- Tetiana I. Domanchuk, Zhanetta A. Chornenka, Mariana I. Hrytsiuk  
COMPARATIVE ANALYSIS OF INCIDENCE AND MORTALITY FROM GASTRIC CANCER AMONG THE POPULATION OF EUROPE AND UKRAINE 596
- Dmytro D. Dyachuk, Volodymyr A. Gandzyuk, Oleg L. Zyukov  
ANALYSIS OF THE EFFECTIVENESS OF THE FUNCTIONAL ORGANIZATIONAL MODEL FOR KNOWN PROPHYLAXIS OF CHRONIC NONINFECTIOUS DISEASES BASED ON THE ASSESSMENT OF THE QUALITY OF LIFE OF PATIENTS AT THE MEDICAL INSTITUTION 603
- Iryna I. Shaposhnikova, Svitlana M. Korsun, Larysa P. Arefieva, Olga V. Kostikova, Volodymyr M. Serhiienko, Svitlana A. Korol, Viktor G. Riabchenko  
ANALYSIS OF STUDENTS' SOMATIC HEALTH AND EMOTIONAL STATE DURING SPORTS GAMES CLASSES 608
- Olga M. Ostash, Liudmyla E. Grygorenko, Oksana V. Shvager, Svetlana V. Stepanchuk, Nina V. Balenko, IGOR O. CHERNYCHENKO  
THE MODIFYING ROLE OF TOXIC SUBSTANCES ON GENOTOXIC EFFECT IN THE BODY DURING COMBINED ADMINISTRATION WITH CARCINOGEN (BENZO[A]PYRENE) 613
- Vitalii I. Tsybaliuk, Sergii S. Strafun, Ihor B. Tretyak, Iaroslav V. Tsybaliuk, Alexander A. Gatskiy, Yuliia V. Tsybaliuk, Mykhailo M. Tatarchuk  
SURGICAL TREATMENT OF PERIPHERAL NERVES COMBAT WOUNDS OF THE EXTREMITIES 619
- Ievgenii V. Andriev, Yulia M. Makukha, Anatolii M. Kravchenko, Ludmila V. Gayova  
BIOCHEMICAL MARKERS OF ENDOTHELIAL DYSFUNCTION, THEIR CHANGES UNDER THE INFLUENCE OF TREATMENT WITH VARIOUS BETA-ADRENOBLOCKERS IN YOUNG MEN WITH MYOCARDIAL INFARCTION 625
- Kostyantyn V. Balashov, Gennady O. Slabkiy, Olesya P. Hulchii, Nadiia M. Zakharova, Solomiya M. Turianytsia  
RELATIONSHIPS BETWEEN THE SOURCE OF HEALTH INFORMATION AND THE BEHAVIOR OF MOTHERS OF CHILDREN UNDER 5 YEARS OLD: CROSS-SECTIONAL STUDY ANALYSIS 630
- Iryna M. Kremsar, Victoria I. Klymenko  
THE INFLUENCE OF THE EXTERNAL AND INTERNAL ENVIRONMENT OF PRIMARY CARE FACILITIES ON THE PREVENTION OF DISEASES OF THE CIRCULATORY SYSTEM 636
- Grygorii P. Griban, Olena T. Kuznietsova, Natalia A. Lyakhova, Volodymyr M. Prystynskiy, Dmytro G. Oleniev, Olena V. Otravenko, Olena O. Pantus  
DYNAMICS OF STUDENTS' FITNESS LEVEL WHILE DIFFERENTIATING PHYSICAL EDUCATION CLASSES IN ACCORDANCE WITH THEIR HEALTH AND NOSOLOGY OF DISEASES 641
- Olena S. Shcherbinska, Hennadiy O. Slabkiy  
THE PRIORITY DIRECTIONS OF INTEGRATION OF OBSTETRICAL AND GYNECOLOGICAL CARE TO THE FEMALE POPULATION OF UKRAINE AT THE PRIMARY LEVEL 647
- Zinaida V. Lashkul, Dmytro A. Lashkul  
MEDICO – SOCIAL SUBSTANTIATION OF THE CONCEPT OF PERSONALIZED MEDICINE IN THE PREVENTION OF ARTERIAL HYPERTENSION AMONG THE ADULT POPULATION AT THE REGIONAL LEVEL
- Tetiana S. Gruzieva, Mykhailo D. Diachuk, Hanna V. Inshakova, Ivan M. Soroka, Vasyl A. Dufynets  
HEALTH OF THE ELDERLY PEOPLE AS THE BASIS FOR FORMATION OF MEDICAL AND SOCIAL NEEDS 658
- Liudmyla I. Haliienko, Viktoriia B. Zamkevych, Oleksandr V. Zholobko, Nataliia M. Mykytenko, Nataliia V. Velikaia  
MODERN EPIDEMIOLOGICAL ASPECTS OF THE PROBLEM OF UROGENITAL MYCOPLASMIS AMONG THE FEMALE POPULATION OF UKRAINE 665
- Mariia G. Dolynska, Gennadii A. Dolynskiy, Tetiana V. Duhlii, Vasyl I. Petrenko  
MOLECULAR EPIDEMIOLOGY APPROACH TO TACKLE TREATMENT FAILURE IN DRUG RESISTANT TUBERCULOSIS (DR-TB) 669
- Anatoly G. Krut, Viktoriia V. Horachuk  
PATIENTS' SATISFACTION WITH DENTAL CARE (ON THE RESULTS OF SOCIOLOGICAL RESEARCH) 674

Tatiana A. Vezhnovets, Vitaliy G. Gurianov, Natalia V. Prus, Oleksandr V. Korotkyi, Olena Y. Antonyuk HEALTH CARE EXPENDITURES OF 179 COUNTRIES WITH DIFFERENT GNI PER CAPITA IN 2018	678
Yurii N. Deputat, Olesia M. Ivanko, Valerii L. Savitskyi, Anatolii P. Kazmyrchuk, Maria P. Gyluch, Borys I. Palamar RESEARCH OF THE ACTUAL ENERGY CONSUMPTION OF THE MILITARY PERSONNEL OF THE ARMED FORCES OF UKRAINE TO SUBSTANTIATE THE CORRECTION OF THEIR DAILY DIET	684
Larysa V. Harashchenko, Svitlana G. Kondratiuk, Svitlana P. Palamar, Halyna O. Vaskivska, Liudmyla L. Nezhyva VALUE ATTITUDE TO HEALTH AS THE BASIS OF AN ACTIVE LIFE POSITION OF AN INDIVIDUAL	690
Diana I. Sobko, Tetiana O. Ilashchuk, Ihor V. Navchuk MELATONIN AND GRHELIN AS "EARLY" PROGNOSIS MARKERS OF PROGRESSION OF ARTERIAL HYPERTENSION AND OSTEOARTHRITIS IN THE CASE OF THEIR COMORBIDITY	697
Galyna F. Biloklytska, Svitlana Yu. Viala RESOURCES TO IMPROVE THE EFFECTIVENESS OF PERIODONTAL TREATMENT IN PATIENTS WITH DIABETES MELLITUS	702
Tetiana P. Yurochko, Maryna V. Shevchenko, Anthony Hassan Jr. Wenom EATING BEHAVIOUR OF INTERNATIONAL STUDENTS IN UKRAINE	708
Tetiana S. Gruzieva, Nataliia V. Hrechyshkina, Olena Ya. Antonyuk, Vasyl A. Dufynets, Serhii E. Konovalov SELF-ASSESSMENT OF THE CONTENT OF THE PUBLIC HEALTH MASTER'S EDUCATIONAL PROGRAM FOR COMPLIANCE WITH THE EUROPEAN PUBLIC HEALTH CORE COMPETENCES PROGRAMME	713
Ivanna V. Sakhandia, Rimma L. Skrypnyk, Kostyantyn L. Kosyachenko, Oleh M. Vlasenko, Anatolii P. Kazmirchuk ANALYSIS OF MEDICINAL PROVISION OF PATIENTS WITH ARTERIAL HYPERTENSION IN HOSPITAL CONDITIONS	718
Nataliia I. Zhachko, Tamara S. Nespriado-Monborgne, Iryna L. Skrypnyk, Maksym S. Zhachko IMPROVING DENTAL HEALTH – IS IMPROVING QUALITY OF LIFE	722
Andrii A. Borysenko, Anna M. Antonenko, Sergii T. Omelchuk, Vasyl G. Bardov, Olena P. Vavrinevych COMPARATIVE HYGIENIC ASSESSMENT OF WORKING CONDITIONS AND OCCUPATIONAL RISK IN THE APPLICATION OF PESTICIDES (ON THE EXAMPLE OF FUNGICIDE AMISTAR EXTRA 280, SC) USING DIFFERENT TYPES OF SPRAYERS	726
Iryna V. Vasylieva, Kateryna O. Hololobova, Ruslan S. Symbaliuk, Olha V. Nechushkina, Viacheslav V. Kobrzhytskyi, Serhii V. Kiriienko, Anna V. Laputko ARTIFICIAL TERMINATION OF PREGNANCY IN UKRAINE: BIOETHICAL, PHILOSOPHICAL AND RELIGIOUS ASPECTS	731
Volodymyr O. Korobchansky, Yuliia O. Oliinyk, Valentyna G. Nesterenko, Vladyslava V. Sarkis-Ivanova, Olena V. Hryhorian HYGIENIC ASPECTS OF LIFESTYLE OF KHARKIV NATIONAL MEDICAL UNIVERSITY JUNIOR STUDENTS IN THE CONDITIONS OF QUARANTINE	736
Dmytro D. Dyachuk, Oleg L. Zyukov, Olena O. Oshyvalova, Yuriy B. Yashchenko, Olena M. Lishchysyna APPROACHES TO DEVELOPING AND IMPLEMENTING CLINICAL AND ORGANIZATIONAL REGULATIONS ENSURING INFECTIOUS SAFETY AND EPIDEMIOLOGICAL RESPONSE IN THE WORK PROCESS (THE CASE OF COVID-19)	741
Oleksandr A. Melnychenko, Viktor A. Ognev, Dmytro I. Marakushin, Inna M. Isaieva, Liliya V. Batyuk, Ganna O. Chovpan, Vladyslav O. Melnychenko STUDENTS' HEALTH STATUS AND THEIR ABILITY TO ADAPT TO A MULTINATIONAL UNIVERSITY ENVIRONMENT	746
<b>REVIEW ARTICLES</b>	
Taras G. Gutor, Natalia I. Zarembo, Oksana R. Kovalska, Dzvenyslava Je. Moskvial-Lesniak, Iryna M. Gerasymovych, Oleh Ja. Kobyletskyi, Yaryna V. Nahurna COMPARATIVE ANALYSIS OF THE MAIN SOCIAL HEALTH DETERMINANTS OF LIFE EXPECTANCY AND INFANT MORTALITY IN UKRAINE AND POLAND	750
Mykola P. Stovban, Vasyl M. Mykhalchuk, Alexander K. Tolstanov, Vira V. Maglona INTERACTION LINKS OF HEALTHCARE INSTITUTIONS WITHIN ONE HOSPITAL DISTRICT	756
Raisa O. Moiseienko, Nina G. Gojda, Olena O. Dudina, Nataliya M. Bodnaruk DEVELOPMENT OF PERINATAL MEDICINE IN UKRAINE IN THE CONTEXT OF INTERNATIONAL APPROACHES	761
Tetiana M. Komarova, Oksana P. Vitovska, Julia I. Komisarenko, Vita M. Kohan AGE-RELATED MACULAR DEGENERATION – CURRENT STATE OF THE PROBLEM AND PROPHYLAXIS METHODS	767
Olena M. Dubovyk, Violetta Y. Dubovyk HEALTH OF THE TEACHER OF HIGHER EDUCATION INSTITUTIONS (EFFICIENCY – DEVELOPMENT)	773
Vladyslav O. Berestoviy, Ahmad A. Mahmood, Oleg O. Berestoviy, Valentyna G. Ginzburg, Dmytro O. Govsieiev AN OVERVIEW OF AUTOIMMUNITY IN IMPLANTATION FAILURE: A LITERATURE REVIEW	777
Natalia V. Stytsiuk, Zhanna M. Zolotarova, Iryna V. Stovban, Halyna Y. Yukish WOMEN'S MODERNISM IN MEDICAL SCIENCE OF WESTERN UKRAINE – SOFIA OKUNEVSKA-MORACHEVSKA, SOFIA PARFANOVYCH AND VOLODYMYRA KRUSHELNYTSKA	784
ABSTRACT BOOK INTERNATIONAL SCIENTIFIC AND PRACTICAL CONFERENCE, DEDICATED TO THE WORLD HEALTH DAY 2021 APRIL 2, 2021, BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE	789

## ORIGINAL ARTICLE

# MOLECULAR EPIDEMIOLOGY APPROACH TO TACKLE TREATMENT FAILURE IN DRUG RESISTANT TUBERCULOSIS (DR-TB)

DOI: 10.36740/WLek202103219

**Mariia G. Dolynska, Gennadii A. Dolynskiy, Tetiana V. Duhlii, Vasyl I. Petrenko**

BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE

**ABSTRACT**

**The aim:** To analyze molecular epidemiology features of *M. tuberculosis* in Kyiv oblast; and to identify the safest mode of TB treatment, which will allow clinicians to minimize the risk of drug-resistant strains nosocomial transmission.

**Materials and methods:** 55 isolates of *M. tuberculosis* were collected in January–April, 2018 from 31 patients with new cases and 24 patients with re-treatment cases of sputum culture-positive pulmonary TB, in Kyiv oblast, Ukraine. DNA samples extracted from all the isolates were used for 15-loci MIRU-VNTR molecular typing with further *M. tuberculosis* strains comparison by means of MIRU-VNTRplus web tool (<http://www.miru-vntrplus.org>).

**Results:** Phylogenetic tree that reflects strains interrelationship reveals four main clusters, the largest of which spans 34 isolates. The presence of two big subclusters with 10 and 7 identical genotypes inside the largest cluster strongly suggests their tight epidemiologic relationship. Smaller clusters consist of five (Harlem), three (URAL), and two unidentified isolates. 10 singletons were detected, among which LAM, URAL and Cameroon lineages were identified; in these cases, epidemiological connection was presumably ruled out.

**Conclusions:** High clustering level of isolates of *M. tuberculosis* suggests possible contact between patients from whom these isolates were obtained. Predominance of Beijing family in the clusters is associated with high DR-TB level, at least in eastern European countries. Similarity of the clusters isolated from different patients in a household or a hospital suggests high probability of recent disease transmission. Clustering genotypes from households and hospital wards can be a surrogate criterion of infection control effectiveness.

**KEY WORDS:** *Mycobacterium tuberculosis*, drug resistance, MIRU-VNTR, tuberculosis

Wiad Lek. 2021;74(3 p.II):669-673

**INTRODUCTION**

Drug resistance critically jeopardizes outcomes of tuberculosis (TB) treatment. As of 2020, a treatment success rate in drug-resistant tuberculosis cases (DR-TB) was 57% globally and 59% in the European region. TB is the 10th leading cause of death worldwide and, since 2007, it has been the leading cause of death from a single infectious agent, ranking above HIV/AIDS cases. Among 30 countries of high tuberculosis burden, which account 87% of global DR-TB cases, nine are located in eastern European region, and Ukraine is among them. Among 11,210 patients who started treatment for DR-TB globally, and were also resistant to fluoroquinolones, only 47% completed treatment successfully and 24% died; treatment failed for 11%, and 18% were lost to follow-up or their treatment outcome was not evaluated. India, the Russian Federation and Ukraine accounted for 73% of this cohort of patients [1]. Thus, DR-TB is a significant public health threat that is of particular importance in Ukraine.

Currently outpatient TB treatment is recognized as a preferred model, which is the safest and the most patient-oriented [2]. Long hospital stay does not improve treatment success [3], but does increase the potential for exposure to unrecognized or inadequately-treated DR-TB. From 2014 to 2018, 14 of the 15 eastern European

and central Asia (EECA) countries reduced the number of bed days per patient. The relative size of the reduction (which is influenced by both the percentage of TB patients hospitalized and the average length of stay if hospitalized) ranged from 11% in Romania to 75% (54 to 14 days) in Armenia. Ukraine is an exception with the average number of days in hospital increase by 12% in 2017, remained stable in 2018 [4]. As of 2020, Ukraine reported an average of 85.6 days per patient [5].

This risk is especially high in the cases when *M. tuberculosis* belongs to Beijing genotype, which is known to be associated with a higher transmission rate [6]. The molecular epidemiology data on *M. tuberculosis* strains circulating in Ukraine are scarce. Studies performed in southern and eastern Ukraine suggest an increasing prevalence of Beijing family, which was identified in 30.5% and 54.8% of new and retreatment cases respectively in a sample from Odesa and Mykolaiv oblast in 2007 [7], and in 81% of the cases from Kharkiv oblast in 2018 [8]. Whereas the abovementioned regions are located at the transport routes connecting Asia and Europe that can partly explain high prevalence of an Asia-originated Beijing strain [9], TB molecular epidemiology in the central and western parts of Ukraine remains unclear.



**Fig. 1.** Dendrogram of analysed DNA samples interrelationship according to MIRU-VNTR typing

**THE AIM**

We hypothesized that patients in hospital for TB treatment are being exposed to and infected with DR-TB. Given that the city of Kyiv as a capital of Ukraine, and Kyiv oblast are experiencing the most active migration in the country, we presumed that Beijing family prevalence in this region can be also high, and therefore

the risk of nosocomial TB and DR-TB transmission is significant.

The study aim was (i) to preliminary analyze molecular epidemiology features of *M. tuberculosis* in Kyiv oblast; and (ii) to identify the safest mode of TB treatment, which will allow clinicians to minimize the risk of drug-resistant strains nosocomial transmission.



**Table I.** General statistic results of bioinformatics analysis for MIRU-VNTR imprinting

Samples number		55							
Clustering level		38%							
Alleles diversity									
0424	0,48	0802	0,38	1955	0,61	2401	0,31	3690	0,31
0577	0,40	0960	0,47	2163b	0,59	2996	0,55	4052	0,72
0580	0,02	1644	0,25	2165	0,54	3192	0,61	4156	0,29
Assignment of identified isolates									
Beijing	Unknown	Haarlem	URAL	LAM					
61,82%	25,45%	7,27%	3,64%	1,82%					

## MATERIALS AND METHODS

55 isolates of *M. tuberculosis* were collected in January-April, 2018 from 31 patients with new cases and 24 patients with re-treatment cases of sputum culture-positive pulmonary tuberculosis. All patients were the residents of Kyiv oblast. 45 (84%) of the patients had DR-TB. All patients had a history of hospitalisation in TB clinic of at least 3 weeks, and sputum to isolate DNA was collected during the treatment monitoring procedures, normally after one month of treatment for DR-TB casves and after two months for drug-susceptible cases. Patients' age ranged from 17 to 79 years old (median 47), with 84% male and 16% female.

*M. tuberculosis* genome typing technique. Molecular epidemiology monitoring was performed using Mycobacterial Interspersed Repetitive Unit-Variable Number of Tandem Repeat (MIRU-VNTR) typing. The method distinguishes the *M. tuberculosis* strains by the difference in the number of copies of tandem repeats at specific regions of the *M. tuberculosis* genome and so enables fast and high-resolution genotyping of *M. tuberculosis* isolates [10]. Polymerase chain reaction (PCR) utilizing forward and reverse primers tailored for each MIRU-VNTR loci results in production of corresponding PCR-amplicons. As the size of a DNA base pairs of each PCR-amplicon is the sum of the size of the tandem repeat plus the offsets at both ends, the number of copies of tandem repeats can be calculated according to the following formula:

$$\text{Amplicon Size} = \text{Offset Size} + (\text{Copynumber} \times \text{Repeat Size})$$

$$\text{Copynumber} = (\text{Amplicon Size} - \text{Offset Size}) / \text{Repeat Size}$$

*M. tuberculosis* DNA sampling and handling. DNA samples were isolated by CTAB-technique [11] from solid media cultures of mycobacteria obtained from TB patients and stored under refrigeration at 21°C until genotyping procedure, which was performed at GenoScreen Innovative Genomics research centre (GenoScreen Corporation, Lille, France). For PCR-amplification core set of 15 VNTRs was selected (MIRU-VNTR loci: 0424, 0577, 0580, 0802, 0960, 1644, 1955, 2163b, 2165, 2401, 2996, 3192, 3690, 4052, 4156) that are both sufficiently and complementary discriminatory [12]. All samples were treated to amplify these 15 markers using 3 quadruplex and 3 simplex PCRs with fluorescent primers specific for the flanking regions of the targeted loci. Amplified fragments were separated by capillary electrophoresis to determine the PCR product sizes.

In case of failure or double alleles, analysis was repeated for corresponding markers using simplex PCRs. As the length of the repeat units is known, sizes reflect the numbers of repeated sequences in the amplified loci. The final result is a portable numerical genotype, corresponding to the repeat number in each locus.

*Genotyping datamation.* Further bioinformatics analysis was performed by means of MIRU-VNTRplus web tool (<http://www.miru-vntrplus.org>) for polyphasic genotyping of *M. tuberculosis* complex bacteria, which comprise a reference database containing genotyping data of 186 strains representing the major MTBC lineages [13]. The comparison with the reference database allows identification of a phylogenetic lineage of the analysed strains.

## RESULTS AND DISCUSSION

Statistical data analysis for 55 processed DNA samples presented in Table I.

Phylogenetic tree that reflects strains interrelationship reveals four main clusters, the largest of which spans 34 isolates (Fig. 1). The presence of two big subclusters with 10 and 7 identical genotypes inside the largest cluster strongly suggests tight epidemiologic relationship. Smaller clusters consisted of five (Harlem), three (URAL), and two unidentified isolates. A total of 10 singletons were detected, among which LAM, URAL and Cameroon lineages were identified; in these cases, epidemiological relationship was presumably ruled out.

Among 55 patients whose genotypes were analysed, 34 (62%), were identified as Beijing cases and had close epidemiological relations, more likely due to contracting the infection from one source. This suggests that Beijing family is a dominating genotype of *M. tuberculosis* in Kyiv oblast among new and re-treatment cases. As a result, high risk of tuberculosis transmission in hospitals and households arises. In addition, studies from eastern Europe present high association of Beijing genotype and risk of DR-TB [14] in contrast with some studies from Asia [15]. Therefore, prevention interventions become crucial in Ukraine due to high DR-TB burden and risk for contact persons to contract a DR-TB strain, which requires longer and more toxic treatment with poorer results.

This risk increases a need for meticulous contact tracing of each bacteriologically confirmed TB patient, which

optimally should cover not only household contacts, but also close contacts, i.e. those who share an enclosed space with the index case, such as a place of social gathering, workplace or facility, for extended time periods within the day during 3 months before commencement of the current treatment episode [16].

TB hospitals are the possible place of the DR-TB strains exchange. Given that the majority of TB facilities in Ukraine were built in 1960s and do not meet current infection control standards, this risk is significant. Molecular epidemiology study performed in Latvia where TB hospitals are of the similar time of construction and layouts, demonstrated high association of DR-TB with Beijing genotype (OR 41.67), previous hospitalisation (OR 18.33) and previous TB treatment (OR 17.68). Another finding of this study is that direct epidemiological links in hospitals were found for almost one third (32%) of DR-TB Beijing cases [14].

Thus, Ukraine urgently needs the paradigm shift with the priority to outpatient treatment in line with the current WHO recommendations. This is the only way to prevent TB and DR-TB transmissions in hospitals. New all-oral DR-TB treatment regimens, which WHO introduced in 2020 [2], enable convenient outpatient treatment without injections for both drug-susceptible and DR-TB cases from the first days of commencement. As it is known that patients who receive TB treatment regimen, which is tailored according to the individual drug resistance patterns, stop spreading the disease after 2-3 weeks of treatment [17], outpatient TB treatment is safe. However, patients with treatment failure can spread the disease and be a source of infections in hospitals and households. This is the reason why early treatment start and meticulous laboratory monitoring are regarded as infection control measures and the prerequisite of a safe treatment mode [18].

MIRU-VNTR typing as a relatively cheap and easy-to-perform method can be discriminative enough surrogate of whole genome sequencing (WGS) to follow the disease transmission in households and hospitals, as WGS requires much more investments and experienced personnel [19, 20]. Therefore, freezing of a culture at the treatment start to ensure the possibility of MIRU-VNTR genotyping in a case of poor treatment response might be a solution to differentiate a superinfection with other genotype of *M. tuberculosis* during the treatment course, from a case of treatment failure. Comparison of the MIRU-VNTR results for the initial and the most recent strain can confirm superinfection if the genotypes are different, and treatment failure in a case of the genetic similarity. In addition, clustering of genotypes obtained from patients with confirmed superinfection who have stayed in same wards is a sign of poor infection control and nosocomial transmission.

## CONCLUSIONS

High clustering level of isolates of *M. tuberculosis* suggests possible close contact among patients from whom

these isolates were obtained. Predominance of Beijing family in the clusters is associated with high DR-TB level, at least in eastern European countries. Similarity of the clusters isolated from different patients in a household or a hospital suggests high probability of recent disease transmission. Clustering genotypes from households and hospital wards can be a surrogate criterion of infection control effectiveness.

## REFERENCES

1. WHO. Global tuberculosis report 2020. (WHO/HTM/TB/2020).
2. WHO consolidated guidelines on tuberculosis: Module 4: Treatment: Drug-resistant tuberculosis treatment. WHO. 2020.
3. Tuberculoz v Ukraini. Analitichno-statystychni dovidnyk za 2019 r. Centr Hromadskogo zdorov'a MOZ Ukrainy. 2020 (in Ukrainian). [https://phc.org.ua/sites/default/files/users/user90/TB\\_surveillance\\_statistical-information\\_2019\\_dovidnyk.pdf](https://phc.org.ua/sites/default/files/users/user90/TB_surveillance_statistical-information_2019_dovidnyk.pdf).
4. WHO. Global tuberculosis report 2019 (WHO/HTM/TB/2019).
5. WHO. Global tuberculosis report 2018 (WHO/HTM/TB/2018).
6. Liu Y., Zhang X., Zhang Y. et al. Characterization of Mycobacterium tuberculosis strains in Beijing, China: drug susceptibility phenotypes and Beijing genotype family transmission. BMC Infect Dis. 2018;18:658. doi: 10.1186/s12879-018-3578-7.
7. Nikolayevskyy V.V., Brown T.J., Bazhora Y.I. et al. Molecular epidemiology and prevalence of mutations conferring rifampicin and isoniazid resistance in Mycobacterium tuberculosis strains from the southern Ukraine. Clin Microbiol Infect. 2007;13(2):129–138. doi: 10.1111/j.1469-0691.2006.01583.x.
8. Daum L.T., Konstantynovska O.S., Solodiankin O.S. et al. Next-generation sequencing for characterizing drug resistance-conferring Mycobacterium tuberculosis genes from clinical isolates in the Ukraine. J Clin Microbiol. 2018;56(6):e00009–18. doi: 10.1128/JCM.00009–18.
9. Pichat C., Couvin D., Carret G. et al. Combined genotypic, phylogenetic, and epidemiologic analyses of Mycobacterium tuberculosis genetic diversity in the Rhône Alpes region, France. PLoS One. 2016;11(4):e0153580. doi: 10.1371/journal.pone.015358029-138.
10. Skenders G., van Soolingen D. Revised by Niemann S. and Nikolayevskyy V. Molecular typing of Mycobacterium tuberculosis complex isolates. In: European Centre for Disease Prevention and Control. Handbook on TB laboratory diagnostic methods for the European Union, Stockholm: ECDC. 2016: 83–91.
11. van Soolingen D., de Haas P.E., Hermans P.W., van Embden J.D. DNA fingerprinting of Mycobacterium tuberculosis. Methods Enzymol. 1994;235:196–205. doi: 10.1016/0076-6879(94)35141-4.
12. Supply P., Allix C., Lesjean S., et al. Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of Mycobacterium tuberculosis. J Clin Microbiol. 2006;44(12):4498–4510. doi: 10.1128/JCM.01392-06.
13. Weniger T., Krawczyk J., Supply P. et al. MIRU-VNTRplus: a web tool for polyphasic genotyping of Mycobacterium tuberculosis complex bacteria. Nucleic Acids Res. 2010;38:326–331. doi: 10.1093/nar/gkq351.
14. Nodieva A., Jansone I., Broka L. et al. Recent nosocomial transmission and genotypes of multidrug-resistant Mycobacterium tuberculosis. Int J Tuberc Lung Dis. 2010;14(4):427–33.
15. Liu Y., Jiang X., Li W. et al. The study on the association between Beijing genotype family and drug susceptibility phenotypes of Mycobacterium tuberculosis in Beijing. Sci Rep. 2017;7:15076. doi: 10.1038/s41598-017-14119-z.

16. Fair E., Miller C.R., Ottmani S.E. et al. Tuberculosis contact investigation in low- and middle-income countries: standardized definitions and indicators. *Int J Tuberc Lung Dis.* 2015;19(3):269–72. doi: 10.5588/ijtld.14.0512.
17. Dharmadhikari A.S., Mphahlele M., Venter K. et al. Rapid impact of effective treatment on transmission of multidrug-resistant tuberculosis. *Int J Tuberc Lung Dis.* 2014;18(9):1019–1025. doi: 10.5588/ijtld.13.0834.
18. Van Cutsem G., Isaakidis P., Farley J. et al. Infection control for drug-resistant tuberculosis: early diagnosis and treatment is the key: Table 1. *Clin Infect Dis.* 2016;62:238–243. doi: 10.1093/cid/ciw012.
19. Rasoahantrisoa R., Rakotosamimanana N., Stucki D. et al. Evaluation of spoligotyping, SNPs and customised MIRU-VNTR combination for genotyping *Mycobacterium tuberculosis* clinical isolates in Madagascar. *PLoS One.* 2017;12(10):e0186088. doi: 10.1371/journal.pone.0186088.
20. Liu Y., Zhang X., Zhang Y. et al. Characterization of *Mycobacterium tuberculosis* strains in Beijing, China: drug susceptibility phenotypes and Beijing genotype family transmission. *BMC Infect Dis.* 2018;18(1):658. doi: 10.1186/s12879-018-3578-7.

*Research programme “Use of novel technologies for tuberculosis diagnosis and treatment” of Ministry of health of Ukraine (2018-2020, № state registration 0118U001213).*

#### **ORCID and contributionship:**

*Mariia G. Dolynska: 0000-0001-8862-1554* <sup>A, B, D</sup>

*Gennadii A. Dolynskyi: 0000-0001-9942-293X* <sup>B, C, D</sup>

*Tetiana V. Duhlii: 0000-0003-2071-584X* <sup>B</sup>

*Vasyl I. Petrenko: 0000-0002-5450-308X* <sup>A, E, F</sup>

#### **Conflict of interest:**

*The Authors declare no conflict of interest.*

---

#### **CORRESPONDING AUTHOR**

##### **Maria Dolynska**

Bogomolets National Medical University  
68 Avtozavodska st., 02000 Kyiv, Ukraine  
tel: +380500759513  
e-mail: dolynskamaria@gmail.com

**Received:** 18.11.2020

**Accepted:** 09.03.2021

---

**A** – Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article