ISSN 2537-6373 (Print)
ISSN 2537-6381 (Online)

The Moldovan Medical Journal

The Publication of the Scientific Medical Association of Moldova

Frequency – 4 per year
Category – B+

Vol. 63, No 3
September 2020

This issue is dedicated to the 75th anniversary of *Nicolae Testemitanu* State University of Medicine and Pharmacy of the Republic of Moldova (1945-2020)

https://congres.usmf.md

HR EXCELLENCE IN RESEARCH

Welcome to the Moldovan Medical Journal!

The Moldovan Medical Journal is an international scientific double-blind peer reviewed periodical edition, 4 per year, of the Scientific Medical Association of the Republic of Moldova designed for specialists in the areas of medicine, dentistry, pharmacy, social medicine and public health. From its debut the journal has striven to support the interests of Moldovan medicine concerning the new concepts of its development.

The Editorial Board warmly welcomes both the readers of and the authors for the journal, all those who are enthusiastic in searching new and more effective ways of solving numerous medicine problems. We hope that those who want to make their contribution to the science of medicine will find our journal helpful and encouraging.

Editorial Board

INDEXING AND ABSTRACTING

1. DOAJ Aworded the Seal, Directory of Open Access Journals (www.doaj.org)
2. Google Scholar (https://scholar.google.com/citations?hl=en&user=weWUEMAAAAAJ).
3. VINITI, All-Russian Scientific and Technical Information Institute (www.viniti.ru)
4. Ulrichsweb Global Serials Directory (www.ulrichsweb.serialssolutions.com)
5. Directory of Open Access Scholarly Resources (https://portal.issn.org/resource/ISSN/2537-6381#)
6. Zenodo (https://www.zenodo.org/search?page=1&size=20&q=The%20Moldovan%20Medical%20Journal)
7. eLIBRARY.RU – Scientific Electronic Library (https://elibrary.ru/contents.asp?titleid=65889)
8. Sherpa Romeo, open access policies analyzer (https://v2.sherpa.ac.uk/id/publication/35606)
9. EuroPub, Directory of Academic and Scientific Journals (https://europub.co.uk/journals/19267)
10. Collective Catalogue of Moldovan University Libraries Consortium (www.primo.libuniv.md)
11. National Bibliometric Instrumnt (https://ibn.idsi.md/ro/vizualizare_revista/moldmedjournal)
12. Institutional Repository in Medical Sciences (http://library.usmf.md:8080/jspui/handle/123456789/5643)
13. WorldCat (https://www.worldcat.org/title/moldovan-medical-journal/oclc/1029854308&referer=brief_results)
14. NLM LocatorPlus (https://locatorplus.gov/cgi-bin/Pwebrecon.cgi)
15. BASE, Bielefeld Academic Search Engine (https://www.base-search.net/Search/Results?lookfor=moldovan+medical+journal&name=&oaboost=1&newsearch=1&refid=dcbasen)
16. Integrated Information System of Moldova's Libraries (SIBIMOL) (http://cc.sibimol.bnrm.md/opac/bibliographic_view/573823?pn=opac%2FSearch&q=moldovan+medical+journal#level=all&location=0&ob=asc&q=moldovan+medical+journal&sb=relevance&start=0&view=CONTENT)
17. CiteFactor Academic Scientific Journals (https://www.citefactor.org/journal/index/23553#.XAjn89szbIU)
18. Central and East European Index (https://www.ceendx.eu/?page_id=/93/moldovan-medical-journal/)
19. Electronic Journals Library University of Regensburg (http://rzblx1.uni-regensburg.de/detail.phtml?bibid=AAAAA&colors=7&lang=en&jour_id=431225)
20. Journal Factor (http://www.journalfactor.org/Journal.php?JOURNAL=JF3174&NAME=Moldovan_Medical_Journal)
21. CYBERLENINKA (https://cyberleninka.ru/journal/n/the-moldovan-medical-journal)
22. Index Copernicus International (https://journals.indexcopernicus.com/search/journal/issue?issueId=all&journalId=51450)
23. JIFACTOR, Global Society for Scientific Research (http://www.jifactor.org/journal_view.php?journal_id=4696)
24. Central Scientific Medical Library of Sechenov First Moscow State Medical University (www.scsml.rssi.ru)
25. East View Information Services (https://shop.eastview.com/results/item?SKU=5140480P)
26. Atlant Clinical Inc (www.atlantclinical.com)
27. Information Society Development Institute (https://idsi.md/en/home)
28. Open Academic Journal Index (http://oaji.net/journal-detail.html?number=7489).

9 772537 637004 >

Index for subscription – 32130

Editorial Staff

Anatol Calistru, MD, PhD, Head of the Editorial Office: +373 22 205 209
Ludmila Martinenko, English Corrector, telephone: +373 22 205 209
Irina Litvinenko, Editorial Secretary, telephone: +373 32205877

Address of the Editorial Office
192, Stefan cel Mare Avenue, Chisinau, the Republic of Moldova
Phone: +373 22 205 209, Office: +373 79 429 274 mobile
www.moldmedjournal.md editor@moldmedjournal.md

Publisher

Revista Curier Medical
192, Stefan cel Mare Avenue
Chisinau, the Republic of Moldova

Printing House Tipografia Sirius
2, A. Lapusneanu str., Chisinau, MD-2004
the Republic of Moldova
www. sirius.md

TABLE OF CONTENTS

ORIGINAL RESEARCHES

PAGES OF HISTORY

ORIGINAL RESEARCHES

DOI: 10.5281/zenodo.3958423
UDC: 616.613-003.7-089.878

Percutaneous nephrolithotomy – effective method in contemporary treatment of renal lithiasis. Clinical implementation experience

*[1]Pavel Banov, [1]Andrei Galescu, [2]Ilie Ceban, [1]Emil Ceban

[1]Department of Urology and Surgical Nephrology, *Nicolae Testemitanu* State University of Medicine and Pharmacy
[2]Section of Urology, *Timofei Mosneaga* Republican Clinical Hospital
Chisinau, the Republic of Moldova

Authors' ORCID iDs, academic degrees and contributions are available at the end of the article

*Corresponding author: pavel.banov@usmf.md
Manuscript received May 25, 2020; revised manuscript July 23, 2020; published online August 26, 2020

Abstract

Background: Percutaneous nephrolithotomy (PNL) is the minimally invasive treatment elected for large kidney calculi (>2cm), staghorn calculi, inferior calyceal lithiasis, hard consistency calculi (calcium oxalate monohydrate or cystine) and lithiasis at the level of a malformed kidney. The aim was to analyse the results, which were obtained in the clinic for one year from the implementation of the method in patients with urolithiasis treated by PNL.
Material and methods: The transversal descriptive study was performed in the group of 43 patients with urolithiasis, subjected to PNL treatment, throughout 2019.
Results: The mean age of kidney stone patients treated by PNL was 55±7 years, among them 29 (67.5%) women and 14 (32.5%) men. The anatomical distribution of nephrolithiasis was: right kidney – 21(48.9%) patients, left kidney –22 (51.1%) patients. The post-operative period in most cases was 5 days. Most of the operated calculi had the following dimensions: 2-2.5 cm in 18 (41.86%) patients, 2.6-4 cm in 16 (37.2%) patients and over 4.5 cm in 9 (20.93%) patients. Postoperative complications were detected and distributed according to Clavien-Dindo scale (CDS). The majority of detected complications were minor (CDS grade 1) in 31 (72%) patients, CDS 2 in 7 (14%) patients and CDS 3b in 6 (14%) patients. Patients with CDS complications grade 4 and 5 were not detected. In 3 (7%) patients the procedure was of "tube-less" type and in 1 (2.3%) patient with double-pointed canal.
Conclusions: The success of PNL intervention depends on multiple factors, such as calculus composition, dimension and location in the urinary tract, the patient's body mass index, as well as collecting system anatomy. PNL is a safe and effective procedure with a 90-100% stone-free rate and minor complications.
Key words: percutaneous nephrolithotomy, renal lithiasis, complications, Clavien-Dindo scale.

Cite this article
Banov P, Galescu A, Ceban I, Ceban E. Percutaneous nephrolithiasis – effective method in contemporary treatment of renal lthiasis. Clinical implementation experience. *Mold Med J.* 2020;63(3):5-11. doi: 10.5281/zenodo.3958423.

Introduction

Renal lithiasis is a complex pathology based on: disorder of metabolism in the human body, a series of lithogenic substances, disorder of their transportation through intestine, kidneys and urinary tract, pathological changes in the physical-chemical and biological characteristics of urine, creation of favourable conditions for the formation of crystals and kidney calculi [1, 2, 3].

The renal lithiasis occupies one of the leading places in the structure of urological diseases in all regions of the world and is found in about 1-12% of the general population. It is well known that urolithiasis mainly affects people of productive age, being very rare in the elderly and children, with a frequency of over 70% in patients aged 20-50 years, which leads to the loss of work capacity. The 8.9% of men and 3.2% of women suffer from urolithiasis during lifetime according to some authors' data [4, 5, 6].

Currently, in highly developed countries 400 thousand people out of 10 million suffer from urolithiasis. There are 85000 of new cases annually, out of which 62000 are recurrent [6]. The global prevalence is estimated between 1-5%, in developed countries 2-13% (with a very large variation from country to country), in developing countries 0.5-1% [7]. The general probability of the population to develop calculi differs in different parts of the world: 1-5% in Asia, 5-9% in Europe, 13% in North America. The annual incidence of urolithiasis is about 0.1-0.4% of the population (Romania, the Republic of Moldova) [8, 9, 10].

Lithiasis reduces the average life expectancy from 5 to 20% of patients, but recurrences are detected in 50-67% of cases [4].

The implementation of percutaneous nephrolithotomy (PNL) in the treatment of renal lithiasis reduced the need

for open surgery, hospitalization time, postoperative pain and its complications, as well as convalescence period minimization [11, 12, 13].

Percutaneous nephrolithotomy is a minimally invasive surgery that involves the extraction of renal calculi through a minimal incision, about 2 cm on the skin, with direct kidney approach. PNL is the elective treatment for various forms of renal lithiasis, including staghorn calculi, inferior calyceal lithiasis, hard consistency calculi (calcium oxalate, monohydrate or cystine). This type of intervention offers the benefit of avoiding a large skin incision compared to open surgery, which involves multiple postoperative complications, difficult physical recovery, and also skin scars [14].

Brief history. In the last 3 decades of the last century, the treatment of reno-ureteral lithiasis was the subject of special transformations, introduction of minimally invasive endoscopic methods: percutaneous nephrolithotomy (PNL) and retrograde ureteroscopy (URS) with rigid or flexible instruments. In 1941, an obstructive renal calculus was extracted through classic nephrostomy tract by Rupel and Brown [15]. Goodwin and Casey communicated the first time about the performance of percutaneous pyelostomy via extrarenal pelvic percutaneous puncture in a pregnant woman with hydronephrosis, in 1955 [16]. More than 20 years passed from this audacious intervention until the first percutaneous nephrostomy (PCN) for kidney stone percutaneous extraction was performed by Fernstrom and Johanson in 1976. In 1979, Marberger and Alken introduced ultrasonic lithotripsy in the endoscopic treatment of renal lithiasis, the method was afterwards popularized in the USA by Segura and Clayman. Marberger and Miller operated percutaneously on a congenital hydronephrosis, in 1983, introducing Sachse urethrotome through the previously made percutaneous nephrostomy and incised endoscopically the stenotic pyelo-ureteral junction (PUJ), an operation called "pyelolysis", later popularized by A. D. Smith in the USA, under the name of "endopyelotomy" [17].

The onset in 1980 and extracorporeal shock wave lithotripsy (ESWL) unanimous acceptance shortly after PNL reduced its therapeutic indications. However, PNL maintains its election indication for voluminous pelvic or calyceal calculi, staghorn calculi of any type, or those located in pyelocaliceal cavities, where they cannot be eliminated spontaneously or after ESWL (calyx stem stenoses, calyceal diverticula, stenoses or vicious insertions of JPU etc.) [18].

The first percutaneous nephrolithotomy in Romania in the treatment of renal lithiasis was implemented by Prof. Dr. Radu Boja on January 26, 1986. He practiced it successfully, having accumulated until 2015 over 7.000 cases, thus becoming one of the European leaders in the field [19]. In the Republic of Moldova this method was introduced in 2017.

The aim of the paper is to analyse the results, which were obtained in the clinic for the period of one year from the implementation of the method in patients with urolithiasis treated by PNL.

Material and methods

The transversal descriptive study was performed within the Department of Urology and Surgical Nephrology of *Nicolae Testemitanu* State University of Medicine and Pharmacy, in *Timofei Mosneaga* Republican Clinical Hospital, in the group of 43 patients diagnosed with urolithiasis, which were subjected to PNL intervention in 2019. Patients underwent a transversal retrospective study (extraction of data from patients' medical records). The patients included in the study were divided into groups, according to age, sex, location and size of the calculi. Prior to the intervention, all the patients underwent a complex clinical evaluation, which included: laboratory examination, imaging examination (computed tomography (CT), ultrasonography (USG)).

In our clinic the indications for PNL were: calyceal calculi with calyceal infundibular stenosis impossible to handle by ESWL or ureteroscopy (URS), renal lithiasis with congenital or acquired stenosis of pyelo-ureteral junction, bulky pelvic calculus (over 2 cm) or multiple lithiasis, staghorn calculus as monotherapy or in combination with ESWL, the extraction of residual stones after open surgery or ESWL, stones that are difficult to disintegrate by ESWL, stone embedded in the pyelo-ureteral junction and stationed over 4 weeks, with hypofunctional kidney, but with acceptable parenchymal index on ultrasound examination.

Descriptive statistics was applied. The results of the study are presented in the form of absolute and relative values. Epi InfoTM version 7 software (Centers for Disease Control and Prevention, Atlanta, GA, United States of America) was used for statistical processing.

Results

The demographic data of the patients with urolithiasis are presented in table 1. The mean age of the patients with urolithiasis treated by PNL was 55.3±7 years, 29 (67.5%) women and 14 (32.5%) men.

Renal calculi anatomical distribution: right kidney – 19 (44.2%) patients, left kidney – 12 (27.9%) patients, bilateral lithiasis was detected in 12 (27.9%) patients. There were according to localization of calculi in the kidney: in renal pelvis – 18 (41.8%) patients, in renal calyx – 16 (37.2%) patients, staghorn calculi – in 9 (21%) patients.

The size of the renal calculi varied from 2 cm up to massive staghorn calculi (> 4.5 cm). Most of the operated calculi had the following dimensions: 2-2.5 cm in 18 (41.86%) patients, 2.6-4 cm in 16 (37.2%) patients and over 4.5 cm in 9 (20.93 %) patients.

The hospitalization period of patients with urolithiasis treated by PNL averaged 5.6 days. The post-operative period in most cases constituted 5 days.

The postoperative complications in patients after PNL are shown in Figure 1. Postoperative complications were stratified according to Clavien-Dindo scale (CDS) [20]. The majority of detected complications were minor (CDS grade 1) in 31 (72%) patients, CDS 2 in 7 (14%) patients and CDS 3b in 6 (14%) patients. Patients with complications of 4-5

Table 1

Demographic data of the patients with urolithiasis

Index	Patients (n=43)
Gender:	
Men, n (%)	14 (32.5%)
Women, n (%)	29 (67.5%)
Age, years (CI 95%)	55.3 (25-78)
Age group:	
18-30 years, n (%)	5 (11.6%)
31-60 years, n (%)	30 (69.8%)
60 years and more, n (%)	8 (18.6%)
Concomitant pathologies:	
Diabetes mellitus, n (%)	5 (11.6%)
Obesity (BMI>30), n (%)	9 (20.9%)
CVS pathologies, n (%)	14 (32.6%)
Urologic status:	
Urinary infection/ chronic pyelonephritis, n (%)	41 (95.3%)
Staghorn lithiasis, n (%)	32 (74.4%)
Multiple lithiasis, n (%)	35 (81.4)
Calculus localization:	
Unilateral, n (%)	31 (72.1%)
On the left, n (%)	12 (27.9%)
On the right, n (%)	19 (44.2%)
Bilateral, n (%)	12 (27.9%)
Calculi dimensions	
2-2.5 cm, n (%)	18 (41.9%)
2.6-4.5 cm, n (%)	16 (37.2%)
> 4.5 cm	9 (20.9%)
Antecedent urolithiasis treatment:	
ESWL, n (%)	7 (16.3%)
Pyelolithotomy, n (%)	9 (20.9%)

Note: CI – Confidence Interval, ESWL – Extracorporeal Shock Wave Lithotripsy, BMI – Body Mass Index, CVS – cardio-vascular system.

grade CDS were not detected. In 3 (7%) patients the procedure was of "tube-less" type and in 1 (2.3%) patient with double tract kidney access.

Clinical case. Abdominal CT of the female patient subsequently operated on by PNL is shown in figure 2. The stages of the intervention are shown in figure 3. The first stage of PNL procedure is cystoscopy, ureter catheterization by a simple ureteral catheter through which contrast substance was injected which delimited the inferior calyceal area and allowed correct puncture of the chosen calyx under imaging control (fig. 3d. *Puncture of the inferior calyx, imaging visualization*). Once the posterior-inferior calyx punctured, the verification of the puncture correctness is confirmed by the leakage of urine from the puncture needle (fig. 3c. *Placement of the puncture needle, control of urine eliminated through the needle*). The puncture of the pyelocaliceal system can also be done ultrasound-guided, after which the contrast substance can be injected and pyelocaliceal system visualized (fig. 3a. *US-guided kidney puncture*, fig. 3b, *renal USG, staghorn calculus, right kidney, US-guided caliceal puncture trajectory).* In this situation the procedure is much easier if the pyelocaliceal system is dilated. A hydrophilic guide is inserted through the puncture needle which must be flexible enough to allow it to advance to the level of the ureter. The guide is placed and the puncture needle is removed. Alken telescopic metal dilators (dilation path) are successively introduced on the guide, Amplaz sheath is positioned on the last dilator, a working sheath that will ensure the access of the nephroscope in the renal cavities (fig. 3e). After the formation of the path, the nephroscope is inserted and forceps are introduced through working canal with the help of which calculi are extracted as a single piece or calculi fragments (fig. 3f. *Litextraction of calculus fragments*). Calculi fragmentation is done with the help of ballistic type lithotripter. At the end of the procedure, the nephrostomy is mounted with the tip in the pelvis, anchored to the skin

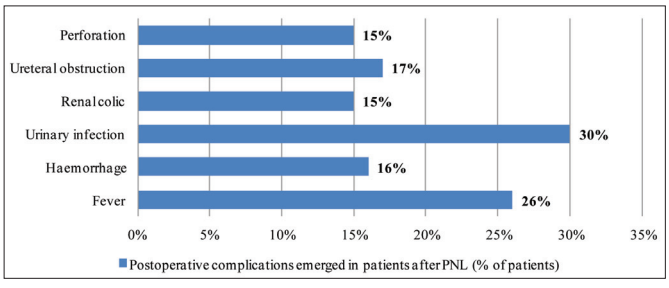

Fig. 1. Postoperative complications emerged in patients after PNL

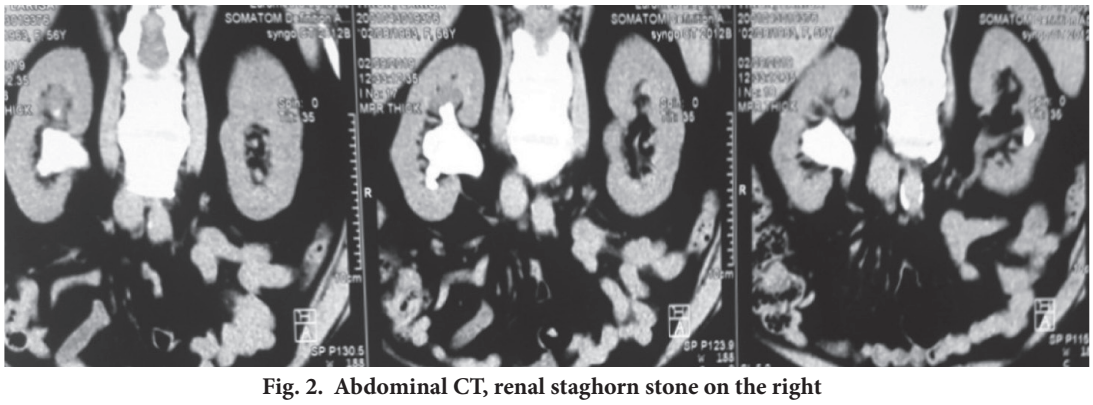

Fig. 2. Abdominal CT, renal staghorn stone on the right

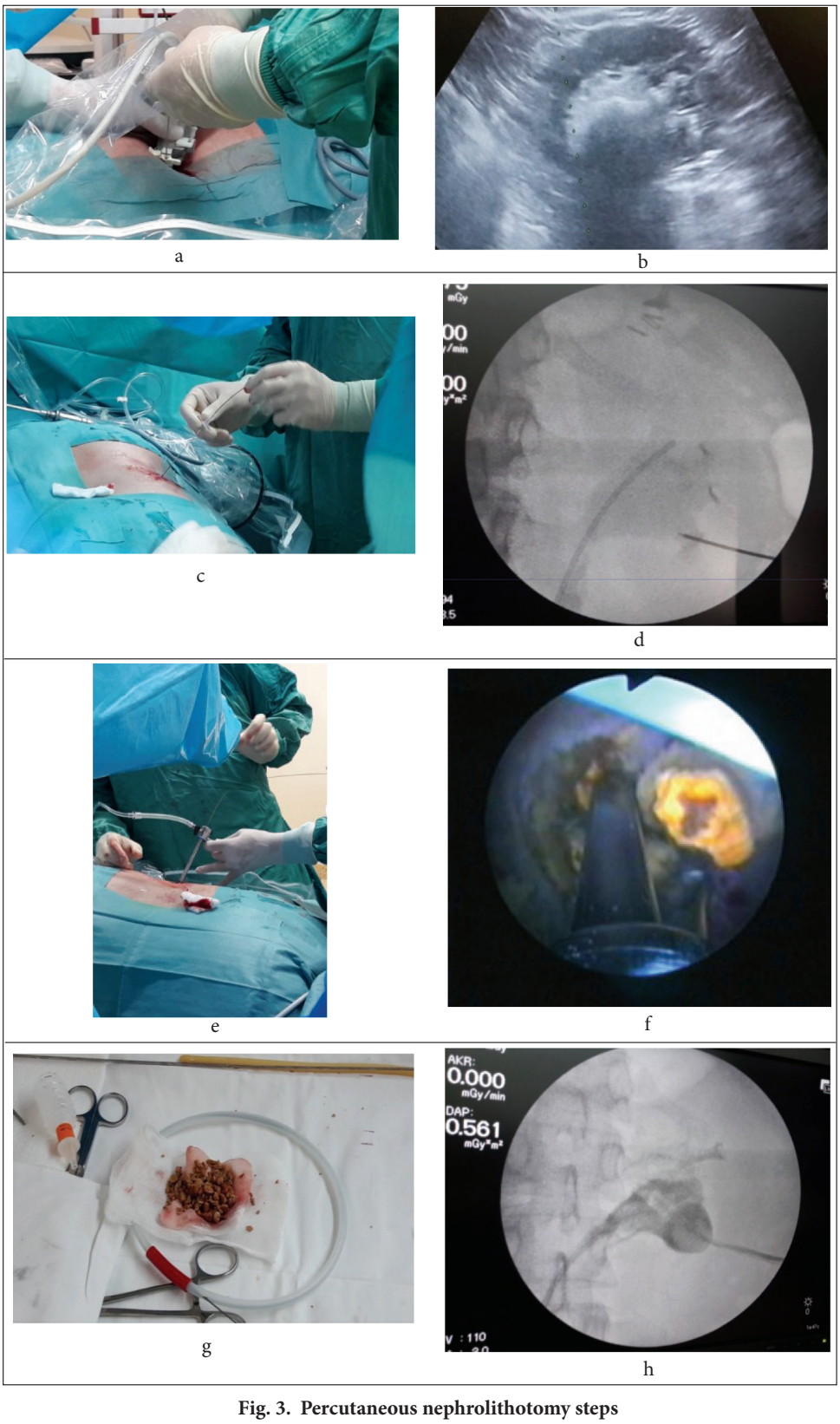

Fig. 3. Percutaneous nephrolithotomy steps

a. Echo-guided kidney puncture.
b. Renal USG, right kidney staghorn calculus, echo-guided calyceal puncture trajectory.
c. Puncture needle placement, control of urine excretion through needle.
d. Inferior calyceal puncture, imaging visualization.
e. Path dilation with the help of Alken or Amplatz types of telescopic dilators, nephroscope placement.
f. Lithextraction of stone fragments.
g. Fragments of extracted stone.
h. Placement of the nephrostomy tube in the pyelocaliceal system.

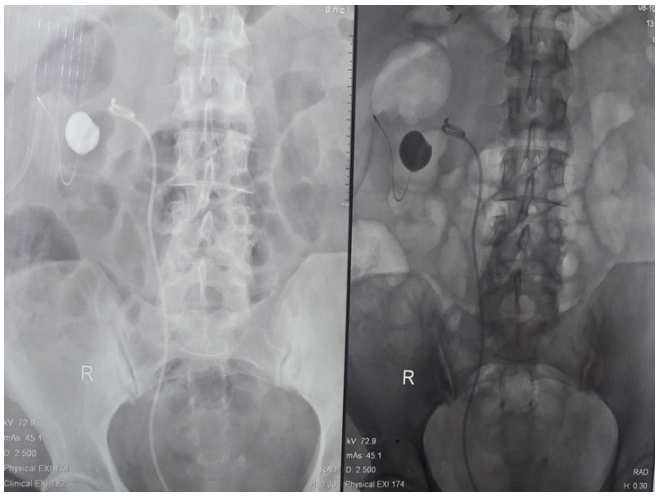

Fig. 4. KUB X-ray, postoperative control highlights the absence of suspected opacities in the kidney

and adapted to a collecting bag (fig. 3h. *The placement of the nephrostomy tube in the pyelocaliceal system*).

The kidney-ureter-bladder abdominal roentgenography (KUB) was performed first day postoperatively to confirm the complete calculi fragments elimination (fig. 4) – "total stone-free". Postoperative evolution was favorable. Foley tube was removed on the 2nd postoperative day, but the nephrostomy tube on the 4th postoperative day after a day of nephrostomy tube clamping, lack of urine extravasation and other signs of urinary passage disorder.

The patient was discharged on the 5th day postoperatively. The ureteral stent was removed one month postoperatively in outpatient conditions.

Discussions

The obtained data demonstrate that percutaneous nephrolithotomy is effective in the treatment of renal lithiasis, with a comparable efficacy index. The surgical treatment by PNL offers a high rate of "stone free", and realizes such objectives as complete removal of calculi and renal parenchyma preservation. However, analyzing the results of the method in terms of postoperative complications and recovery time, compared to similar parameters obtained in case of open surgery, we find out that the indications for nephrolithiasis open surgery decreased significantly and are currently practiced in a limited number of cases [21].

PNL replaced open surgery performed in case of large and complex stones, inclusively in children. Although PNL is considered a relatively invasive technique compared to other minimally invasive techniques currently available, the evidence shows that its use in recent years is increasing [22, 23].

Among the factors that increase PNL introduction in practice are: the increasing incidence of nephrolithiasis, the high degree of safety and efficiency of the method. The argument is valid, especially, for calculi larger than 2 cm, staghorn calculi, which are resistant to fragmentation and the calculi from the kidneys with pathological anatomy [24].

Stress and systemic inflammatory syndrome persist both in the patients treated by PNL and in those treated by open surgery, but without any obvious impact on renal function. However, the extent of stress and systemic inflammatory syndrome is lower in the patients treated with PNL, demonstrating the benefits of the method. Postoperative recovery in the PNL group is faster than in the open surgery group [25]. Therefore, PNL is a safe and feasible procedure with a lesser effect on renal function [26].

The results of the study evaluated PNL effectiveness compared to open surgery in the treatment of complex staghorn calculi. Thus, we found out that PNL is a valuable treatment option for this type of calculi, with a "stone-free" condition rate similar to that of open surgery. PNL has the advantages of reduced morbidity, shorter operating time, shorter hospitalization period and faster return to work [27].

Post-operative complications are an important indicator for determining the success and quality of surgical outcomes. The European Association of Urology recommends the use of Clavien-Dindo classification stratifying complications after urological procedures [28, 29].

The reason for using this standardized and well-defined classification is to eliminate the subjective interpretation of serious adverse events and to underestimate their severity, which leads to complications. For these reasons, we also used this classification. Thus, in our study, the rates of found complications were minimal [30].

At the same time, the obtained results are similar to the researches results of specialists in the field, according to them the implementation, development and improvement of equipment and endourological methods have an important role in improving treatment outcomes, reducing intra- and postoperative complications in patients with renal lithiasis [20].

Conclusions

Percutaneous nephrolithotomy is the first-choice treatment method. The indications for other methods are more limited, usually addressed to "non-standard" patients. PNL is used in the treatment of staghorn lithiasis as an effective, less aggressive method compared to anatrophic nephrolithotomy. Parenchymal loss is less significant than after classic open surgery. The advantages of the method consist in the minimum traumatism, morbidity and hospitalization duration decrease, postoperative complications rate decrease. PNL is also very useful in urological emergencies because there are certain groups of patients who need drainage of the kidney collecting system (obstructive anuria with urosepsis and severe biomolecular imbalance), but the momentary resolution of the calculus is not indicated. The success of PNL depends on many factors, such as calculi composition, size, number, location, body mass index and the anatomy of the kidney collection system. All the abovementioned variables were carefully taken into consideration in order to maximize the success of PNL intervention. The total stone-free success rate is about 90% after primary PNL and 90-100% after a secondary procedure such as ESWL.

References

1. Ceban E. Particularitățile managementului contemporan la pacienții cu pielonefrită cronică calculoasă [Contemporary management features in patients with chronic calculous pyelonephritis]. Curierul medical (Chisinau). 2013;56(1):12-19. Romanian.
2. Tiktinskii OL, Aleksandrov VP. Mochekamennaia bolezn' [Urolithiasis]. Saint Petersburg: Piter; 2000. 384 p. [Russian].
3. Moe O. Kidney stones: pathophysiology and medical management. Lancet. 2006;367(9507):333-344. doi: 10.1016/S0140-6736(06)68071-9.
4. Ceban E. Tratamentul multimodal al nefrolitiazei complicate [Multimodal treatment of complicated nephrolithiasis]. Chișinău: Medicina; 2013. 206 p. Romanian.
5. Banov P, Ceban E. The efficacy of metaphylaxis in the treatment of recurrent urolithiasis. J Med Life. 2017;10(3):188-193.
6. Turk C, Knoll T, Petrik A, et al. Guidelines on urolithiasis. Arnhem: European Association of Urology; 2015. 102 p.
7. Morris D, Wei J, Taub D, et al. Temporal trends in the use of percutaneous nephrolithotomy. J Urol. 2006;175(5):1731-1736. doi: 10.1016/S0022-5347(05)00994-8.
8. [Ministry of Health of the Republic of Moldova]. Urolitiaza la adult: Protocol clinic național [Urolithiasis in adults: National clinical protocol]. Chișinău: The Ministry; 2011. 56 p. (PCN-88). Romanian.
9. Geavlete P, Georgescu D, Mulțescu R. Litiaza urinară: Noțiuni generale [Urinary stones: General notions]. In: Sinescu I, Gluck G, editors. Tratat de Urologie [Treatise on Urology]. Bucharest: Editura Medicală; 2009. p. 1025-1089. Romanian.
10. Stamatelou K, Francis M, Jones C, et al. Time trends in reported prevalence of kidney stones in the United States: 1976-1994. Kidney Int. 2003;63(5):1817-1823. doi: 10.1046/j.1523-1755.2003.00917.x.11. Bumbu G, Bumbu GA, Berechet M, et al. Urologie: ghid ilustrat de tehnici chirurgicale [Urology: illustrated guide to surgical techniques]. Vol. 1, 2. Bucharest: Editura Medicală; 2013. Romanian.
12. Preminger G, Assimos D, Lingeman J, et al. AUA guideline on management of staghorn calculi: diagnosis and treatment recommendations. J Urol. 2005;173(6):1991-2000. doi: 10.1097/01.ju.0000161171.67806.2a.
13. Falahatkar S, Khosropanah I, Roshani A, et al. Tubeless percutaneous nephrolithotomy for staghorn stones. J Endourol. 2008;22(7):1447-1451. doi: 10.1089/end.2007.0285.
14. Guven S, Istanbulluoglu O, Gul U, et al. Successful percutaneous nephrolithotomy in children: multicenter study on current status of its use, efficacy and complications using Clavien classification. J Urol. 2011;185(4):1419-1424. doi: 10.1016/j.juro.2010.11.055.
15. Rupel E, Brown R. Nephroscopy with removal of stone following nephrostomy for obstructive calculous anuria. J Urol. 1941;46(2):177-182.
16. Goodwin WE, Casey WC, Wolf W. Percutaneous trocar (needle) nephrostomy in hydronephrosis. J Am Med Assoc. 1955;157(11):891-894.
17. Stoller M, Meng M. Urinary stone disease. Totowa: Humana Press Inc; 2007. 694 p.
18. Srisubat A, Potisat S, Lojanapiwat B, et al. Extracorporeal shock wave lithotripsy (ESWL) vs percutaneous nephrolithotomyn (PCNL) or retrograde intrarenal surgery (RIRS) for kidney stones. Cochrane Database Syst Rev. 2009;(4):CD007044. doi: 10.1002/14651858.CD007044.pub2.
19. Boja R. Chirurgia percutanată reno-ureterală [Percutaneous reno-ureteral surgery]. Constanța: Leda, Muntenia; 2000. 277 p. Romanian.
20. Yoon P, Chalasani V, Woo H. Use of Clavien-Dindo classification in reporting and grading complications after urological surgical procedures: analysis of 2010 to 2012. J Urol. 2013;190(4):1271-1274. doi: 10.1016/j.juro.2013.04.025.
21. Honeck P, Wendt-Nordahl G, Krombach P, et al. Does open stone surgery still play a role in the treatment of urolithiasis? Data of a primary urolithiasis center. J Endourol. 2009;23(7):1209-1212. doi: 10.1089/end.2009.0027.
22. Skolarikos A, de la Rosette J. Prevention and treatment of complications following percutaneous nephrolithotomy. Curr Opin Urol. 2008;18(2):229-234. doi: 10.1097/MOU.0b013e3282f46afc.
23. Bayrak O, Seckiner I, Erturhan S, Duzgun I, et al. Comparative analyses of percutaneous nephrolithotomy versus open surgery in pediatric urinary stone disease. Pediatr Surg Int. 2012;28(10):1025-1029. doi: 10.1007/s00383-012-3130-6.
24. Skolarikos A, Alivizatos G, de la Rosette J. Percutaneous nephrolithotomy and its legacy. Eur Urol. 2005;47(1):22-28. doi: 10.1016/j.eururo.2004.08.009.
25. Ceban E, Banov P, Galescu A, Botnari V. Oxidative stress and antioxidant status in patients with complicated urolithiasis. J Med Life. 2016;9(3):259-262.
26. Shen P, Wei W, Yang X, et al. The influence of percutaneous nephrolithotomy on human systemic stress response, SIRS and renal function. Urol Res. 2010;38(5):403-408. doi: 10.1007/s00240-010-0259-4.
27. Basiri A, Karrami H, Moghaddam S, et al. Percutaneous nephrolithotomy in patients with or without a history of open nephrolithotomy. J Endourol. 2003;17(4):213-216. doi: 10.1089/089277903765444320.
28. Resorlu B, Unsal A, Ziypak T, et al. Comparison of retrograde intrarenal surgery, shockwave lithotripsy, and percutaneous nephrolithotomy for treatment of medium-sized radiolucent renal stones. World J Urol. 2013;31(6):1581-1586. doi:10.1007/s00345-012-0991-1.
29. Mitropoulos D, Artibani W, Graefen M, et al. Reporting and grading of complications after urologic surgical procedures: an ad hoc EAU guidelines panel assessment and recommendations. Actas Urol Esp. 2013;37(1):1-11. doi: 10.1016/j.acuro.2012.02.002.
30. Dindo D, Demartines N, Clavien P. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. Ann Surg. 2004;240(2):205-213. doi: 10.1097/01.sla.0000133083.54934.ae.

Authors' ORCID iDs and academic degrees

Pavel Banov, MD, PhD, Assistant Professor – https://orcid.org/0000-0002-6904-0743.
Andrei Galescu, MD, PhD, Assistant Professor – https://orcid.org/0000-0002-7953-2450.
Ilie Ceban, MD, Undergraduate – https://orcid.org/0000-0002-4875-1239.
Emil Ceban, MD, PhD, Professor – https://orcid.org/0000-0002-1583-2884.

Authors' contribution

PB and IC acquired, interpreted the data, drafted the first manuscript, AG performed most of the analyzed interventions, EC designed the trial and revised the manuscript critically. All the authors revised and approved the final version of the manuscript.

Funding

The study was supported by *Nicolae Testemitanu* State University of Medicine and Pharmacy. The authors are independent and take responsibility for the integrity of the data and accuracy of the data analysis.

Ethics approval and consent to participate

The protocol No 6 (of March 3, 2020) was approved by the Research Ethic Board of *Nicolae Testemitanu* State University of Medicine and Pharmacy. Written informed consent was obtained from all participants of the study.

Conflict of Interests

The authors have no conflict of interests to declare.

DOI: 10.5281/zenodo.3958429
UDC: 616.284-002-089.168.1-07-053.2

Post-surgical noninvasive monitoring of middle ear in otitis media prolonged forms

Svetlana Diacova

Department of Otorhinolaryngology, *Nicolae Testemitanu* State University of Medicine and Pharmacy Chisinau, the Republic of Moldova

Author's ORCID iD, academic degrees and contributions are available at the end of the article

Corresponding author: svetlana.diacova@usmf.md
Manuscript received June 15, 2020; revised manuscript August 14, 2020; published online August 26, 2020

Abstract

Background: Prolonged forms of otitis media (OM) lead to chronic hearing loss and disability from childhood. Wide spectrum of therapeutic approaches is used in management of OM in children. Objective evaluation of the middle ear after different curative modalities will help in analysis of treatment feasibility. The objective of this article was to compare the results of middle ear noninvasive monitoring after different surgical procedures in order to select the most effective one in prolonged otitis media forms.
Material and methods: Patients represent 150 children with prolonged OM. Analyzed treatment modalities: I – myringotomy, II – classical tympanostomy, III – modified tympanostomy. Middle ear monitoring included otoscopy and audiometry what was repeated 4 times during 2 years, otomicroscopy in 1 and 2 years and impedance audiometry in 2 years after surgery. The quality of life and general health scores were analyzed before surgery and in 1 and 2 years after surgery.
Results: Otoscopic and audiometric data showed stable improvement in 32% of children after myringotomy, 90% of children after classical tympanostomy and 97% of children after modified tympanostomy. Impedance audiometry in 2 years after surgery demonstrated complete restoration of middle ear function in 32% of children after myringotomy, in 78% of children after classical tympanostomy, and in 94% of children after modified tympanostomy.
Conclusions: Post-surgical noninvasive monitoring demonstrated advantages of tympanostomy and especially in modified version: improvement and stability of middle ear function and low rate of otitis media persistence or recurrence.
Key words: noninvasive monitoring, otitis media prolonged forms, types of surgery, modified tympanostomy.

Cite this article
Diacova S. Post-surgical noninvasive monitoring of middle ear in otitis media prolonged forms. *Mold Med J.* 2020;63(3):11-15. doi: 10.5281/zenodo.3958429.

Introduction

Otitis media (OM) represents the group of middle ear (ME) diseases, which affect 90% of small children. Primary forms of OM in childhood are classified by pediatric otology on acute otitis media (AOM) and otitis media with effusion (OME) [1-3]. Symptoms and signs are different, but the common characteristic is hearing loss, what is difficult to suspect in a small child without special clinical diagnostic methods [1-8]. The duration of primary forms is limited: maximum 6 weeks in AOM and 12 weeks in OME. Further evolution of these two forms may be different, including transformation from AOM into OME and vice versa, but in 15-25% of cases they both progress into the prolonged forms such as recurrent acute otitis media (RAOM) and persistent otitis media with effusion (POME) [1]. Prolonged OM forms are characterized functionally by chronic hearing loss what corresponds to chronic pathological changes in the middle ear. The prolonged OM affects child's quality of life and general health [1-8]. Prognostics and detection of prolonged OM in small children and intensive treatment including surgical one have to prevent from the progressive disease evolution [1-14].

Importance of hearing organ function, necessity of early and precise diagnostics and unsatisfactory results of different therapy types facilitated development of objective diagnostic tools – electro-acoustical and electrophysiological methods, as: impedance audiometry (IA), Brainstem Evoked Response Audiometry (BERA) and registration of otoacoustical emissions (OAE) for early detection of ME pathology [1-6]. Are they useful in prognosis of OM negative evolution?

Management of OM prolonged forms such as POME and RAOM, consists of different approaches and methods. Traditional modality includes diagnostic tests for children with evident OM manifestations. Treatment approach is composed by adenoidectomy with/or without physiotherapy as a first, sometimes single, stage. Myringotomy with tympanostomy tube (TT) insertion or tympanostomy (TS) is performed rarely, as a second procedure in a few months or years on a later stage. The effectiveness of this classical intervention is not clear [3-8].

Another therapeutic approach to POME and RAOM treatment is presented in Protocols of OM management from USA, Finland, Australia, Japan, etc. [1-6].

Comprehensive standards are based on early objective

detection of ME pathology in small children with risk factors (RF), "watchful waiting" period of 3 months and relatively early surgical intervention – TS as a first and single procedure. Adenoidectomy, one of the classical "treatment" of OM, is recommended rarely, only in cases of evident and "proven true" hypertrophy of adenoids or in case of further OM progression or recurrence, in complex with second TS-surgery [1-4]. Physiotherapy in OM is not recommended by contemporary Otorhinolaryngology. This modality evidently reduces the OM recurrence, chronicity and complication rates. High prevalence of prolonged OM in the world and standardized surgical approach increases the number of ear surgery and makes TS the most frequently surgery performed in children in USA, Finland, Australia, etc. [1-5].

Chronic suppurative OM, otogenic complications and chronic hearing loss rates still are relatively high, including in Moldova, what stimulates OM researches [9-11].

We conducted several projects in collaboration with Mayo Foundation, Mayo Clinic, Rochester, MN, USA and Society "Pediatricians due Monde", Clermont-Ferrand (France) in order to create a system of OM management in Moldova. The goal of OM management is the ME physical and acoustical restoration and prevention of OM recurrence [10-14].

Our approach includes several steps: 1) evaluating of evident risk factors (RF) for prolonged OM in every child, 2) monitoring of ME status in children with high score of RF, 3) medical treatment of basic pathology, 4) surgical treatment including TS and adenoidectomy, 5) monitoring of ME in post-surgical period.

We formulated the diagnostic algorithm to highlight the somatic conditions and diseases, which predispose to OM prolonged forms. Medical therapy of identified pathology in complex with further ME monitoring helped in differentiation between the symptomatic (treatable) and independent prolonged OM [2, 10-12]. Our surgical complex included tympanostomy in our modification and adenoidectomy. Modified tympanostomy (MTS) is designed in order to improve cleaning and visualization of the tympanic cavity [15]. Monitoring of the otoscopical, physical and acoustical characteristics, dynamics of quality of life (QL) and general health (GH) is necessary for estimation of the feasibility of any therapeutic activities conducted in prolonged OM [1-14, 16-18].

The purpose of this article is to compare outcomes of prolonged OM in function of applied treatment modalities on the base of noninvasive monitoring.

Material and methods

Material. Patients' selection: Children at 1-7 years of age with OM and predisposing factors to recurrence and chronicity in the number of 950 were included in the group for observation. Monitoring of ME included screening-impedance audiometry and screening-otoscopy four times during 1 year (once in 3 months). Additional diagnostic methods and medical treatment of pathological conditions in children with OM improved ME status in the majority of cases. The presence of pathological changes during at least 3 months we classified as persistent otitis media with effusion (POME). Recurrence of OM signs 3 times during 6 months or 4 times during 12 months we classified as recurrent acute otitis media (RAOM) [1-8, 10-14]. As a result of patients' selection we included in this study 150 patients with prolonged OM.

Methods. Monitoring methods included screening otoscopy and otomicroscopy, audiometry, impedance audiometry, quality of life (QL) and general health (GH) characteristics before and after the treatment. Monitoring was performed for 24 months period.

Otomicroscopy test. Were used 2 otoscopy sets: "Carl Storz" set and Otomicroscope "Zeiss". We created the database of every child's otoscopical profile, which includes pre-surgical otomicroscopic data, surgical findings and otoscopical monitoring after surgery. Unification and statistical analysis of otoscopic data were implemented on the base of profile numerical assessment in accordance with elaborated scale. Our scale provides gradation of all TM changes. Normal tympanic membrane appearance was graded 0 points, doubtful otoscopic shifts – 1 point, minimal pathologic changes – 2-5 points, evident inflammatory signs – 6-8 points, manifested otoscopic signs – 9-10 points and formation of chronic purulent OM – 11-13 points.

Electro-acoustical tests. Two sets were used: a) impedance audiometer for screening MT-10, b) clinical impedance audiometer. Both instruments have the probe tone frequency of 226 Hz and a positive and negative pressure sweep between +200 and -400 daPa and sweep speed – 600 daPa/s, near the tympanogram peak – 200 daPa/s, and the compliance range – 0.1-0.6 ml. We analyzed and monitored before surgery and after surgery in 24 months the tympanogram type, compliance, absolute gradient, relative gradient, width and pressure at tympanogram pick.

Audiometry. General hearing assessment, evaluation of hearing thresholds level and differentiation between conductive, sensorineural and mixed hearing loss were done using the conventional audiometry. The objective examination such as registration of otoacoustical emissions (OAE) or brainstem electrical response audiometry (BERA) were performed for children with communication difficulties. We analyzed and monitored before surgery and in 12 and 24 months after surgery hearing thresholds level and pick latency.

General health evaluation. The general health in the group of children with prolonged OM was analyzed during 2 years: before surgery, and in 1 and 2 years after surgery. We evaluated some indexes: the number of days off, clinic visits, antibiotic courses, and OM recurrence rate. The total general health deterioration score was calculated as a sum of these indexes data divided by the number of indexes [10, 13, 14].

Quality of life evaluation. Using elaborated questionnaire we analyzed quality of life of children with prolonged OM. According to the questionnaire parents assessed quali-

ty of life indexes: physical suffering, hearing loss, speech impairment, emotional distress, activity limitations and others by the scale of 7 points (from "very bad" to "very good"). Quality of life deterioration score was calculated by totaling each item's score and dividing the sum by the number of indexes [10, 13, 14, 17-19].

Quality of life and general health deterioration scores of children were calculated for every child before, and in 1 and 2 years after surgical treatment. The data were recorded in database. Statistical analyses: Student-test, Fisher-statistics and discriminate analysis were performed.

Results and discussion

Characteristics of patients. Clinically, prolonged forms of OM had relatively silent course and became evident for child's parents at the age of 3-4 years. Electroacoustical monitoring of small children with RF demonstrated that prolonged OM background developed during 1-3 years. Otoscopical and especially otomicroscopical profile demonstrated prolonged changes of TM and TC, with medium score 5.5 (SD 1.4). Audiometry revealed mild to moderate conductive hearing loss, which correlated to ME pathology independently of OM form. Impedance audiometry examination revealed type B of tympanogram in 73% of patients and stable abnormal type C with low characteristics of curve in 27% of cases. All children with prolonged OM in median had more than 71 days of illness, 8 clinic visits, received 6 courses of antibiotics, including for 4.5 cases of OM. The General health (GH) deterioration mean score in three groups of children was 6.63 (SD 0.3). In all patients with prolonged OM Quality of life (QL) deterioration mean score was 5.5 (SD 0.5).

Surgical treatment modalities. Complex of surgical treatment for the children with POME and RAOM included ear surgery and adenoidectomy. According to ear surgery patients were divided in 3 groups: M – myringotomy, CTS – classical tympanostomy, MTS – modified tympanostomy. Each group of patients consisted of 50 children with prolonged OM.

Myringotomy is a microsurgical procedure, schematically consists of two steps: the incision of the tympanic membrane (TM) in posterior-inferior quadrant and aspiration of the pathological content from tympanic cavity (TC) through this perforation. Classical tympanostomy provides insertion of tympanostomy tube (TT) in the place of incision [3, 4, 14].

We analyzed and compared the advantages and disadvantages of these classical surgery types. The myringotomy incision is enough big in order to eliminate the pathological secret from TC, but in small children the incision closes itself in a few days after the surgery what limits drainage and aeration time. Classical tympanostomy (CTS) differs from classical myringotomy by some important characteristics: 1) the location of incision has to be changed to anterior quadrants of the TM, where tympanostomy tube will not provoke any damages; 2) the size of incision has to be smaller, in correspondence to the small size of tympanostomy tube (TT); 3) the insertion of TT provides longer aeration of TC, but complicates complete drainage. As a result, otorrhea and recurrence of OM are still registered in 18-33% of cases [2-8].

The most important disadvantage of tympanostomy consists in impossibility to solve the 2 different tasks of the surgery by one incision: complete and long evacuation of pathological content and TT fixation. The incision size has to be very small in correspondence to TT size. But effusion thickness and volume in TC make cleaning incomplete through this tiny incision. Remnants of viscous, mucous or purulent liquid are present in TC after surgery. As a result, otorrhea develops in post-surgical period in 11–32%. Additionally, otomicroscopic exploration of the TC through this small incision is practically impossible. Another cause of OM recurrence may be connected to infection from rhinopharynx. Absence of adenoidectomy in a complex surgical procedure may explain relatively high recurrence rate [2, 3, 6, 7].

We elaborated modified tympanostomy in order to avoid these disadvantages [15]. We introduced the additional incision in posterior quadrants for complete evacuation of pathological content from the TC. Second incision opens TC larger than incision for TT what improves evacuation of pathological material from TC and increases the visibility of TC changes. The additional incision closes itself during a few days with no any negative further influence on ME function and/or otoscopical appearance, according to our previous results [10-14].

Results of monitoring. Otoscopic and otomicroscopic data showed an improvement in 61% of children after myringotomy (M group), 90% of children after classical tympanostomy (CTS group) and 97% of children after modified tympanostomy (MTS group). During 2-year follow-up worsening of otoscopic view was characteristic for 42% of patients from M group, 20% of patients from CTS group and 7% of patients from MTS group.

Hearing of patients in MTS group was normalized (from 36 dB to 20 dB in median) during first 1-3 months after the surgery and was stable during 24 months after surgery in 97% of cases (tab.1).

Table 1

Results of hearing examination before and after surgery in 3 groups of childre

Groups	Hearing thresholds level (dB)							
	Before surgery	Time of examination after surgery (months)						
		1	3	6	9	12	18	24
M	36	20	25	30	33	34	35	35
CTS	37	23	24	26	26	26	26	26
MTS	37	20	20	21	20	21	22	22

M – myringotomy, CTS – classical tympanostomy, MTS – modified tympanostomy.

During 2 years we noted a temporary (1–2 weeks) worsening till 25 dB in 1% – 3% of cases. Dynamics of hearing

in patients from CTS group was positive in the majority of cases. Normalization of hearing level (20 dB) during first 1-3 months after surgery was characteristic for 86% of the patients, significant improvement (till 25 dB) was noted in 14% of cases. During next 24 months we registered periodic worsening of hearing level in 28% of patients. Hearing loss till 25 dB was characteristic for 19% of patients from CTS group, lower than 25 dB hearing loss – for 9%.

In M group of patients, hearing dynamics had an undulating character: an improvement of hearing in the majority of patients – 95 % during first 1–3 months after surgery and gradual reduction of hearing level during first 3-6 months in 68 % of cases. Totally, 32 % of children from M group had normal hearing during 2 years of follow up. These results significantly differ from the data of MTS and CTS groups ($p<0.01$).

Impedance audiometry performed in 2 years after surgery (tab. 2) demonstrated complete restoration of middle ear function (type A of tympanogram with normal characteristics of the curve) in 32% of children from M group, in 78% of children from CTS group, and in 94% of children from MTS group. Type B of tympanogram was registered in 42% of cases from M group, 10% of cases from CTS group and 2% of cases from MTS group. Type C of tympanogram was characteristic for 26% of patients from M group, 12% of patients from CTS group and 4% of patients from MTS group ($p<0.01$).

Table 2

Results of impedance audiometry before and after surgery in 3 groups of children

Groups	Ears with tympanogram type (%)					
	Before surgery			24 months after surgery		
	Type A	Type B	Type C	Type A	Type B	Type C
M	0	73	27	32	42	26
CTS	0	72	28	78	10	12
MTS	0	74	26	94	2	4

M – myringotomy, CTS – classical tympanostomy, MTS – modified tympanostomy.

General health. During one year of observation after surgical treatment otitis media recurrence rate in MTS group was 2%, what is significantly lower in comparison with 38% in M subgroup and 16% in CTS group, $p<0.001$ (tab. 3). During the follow up after surgery, we have registered a statistically significant reduction in the number of days off, clinic visits number, antibiotic courses and presence of OM in MTS group.

These children after surgery had less than 10 days of illness in average, 2 clinic visits, received 0.1 courses of antibiotics, including for 0.08 cases of OM. The GH deterioration score in MTS group after surgery was 0.84 (SD 0.3) what differs from the same data before surgery – 6.75 (SD 0.4), $p<0.01$ (tab. 3). The GH deterioration score in CTS group after surgery was 1.99 (SD 0.3), what differs from the same data before surgery – 6.68 (SD 0.4), $p<0.01$. This GH score for children from M group after surgery was 3.34 (SD 0.6), what differs from the data before surgery – 6.62 (SD 0.4), $p<0.05$.

Quality of life. The comparison of QL deterioration scores in 3 subgroups before and after surgery confirmed the most significant shift in MTS group: before surgery – 5.6 (SD 0.5), after surgery – 1.3 (SD 0.5), $p<0.01$. Approximately the same dynamics was found in CTS group, where QL deterioration score improved from 5.5 (SD 0.4) to 2.0 (SD 0.7), $p <0.01$. In group M an improvement of the children's QL index has been less significant. For M subgroup this score before surgery was – 5.5 (SD 0.5), after surgery – 2.83 (SD 1.2), $p<0.05$.

According to our research, post-surgical electro-acoustical monitoring in combination with evaluation of general health and quality of life indexes gives the important information of middle ear status and function and is useful in prognosis of otitis media evolution.

We consider that our medical treatment of predisposing inflammatory diseases in children with prolonged OM permitted to resolve OM cases, which depended on this pathology. This treatment also represented the process of differentiation of true prolonged OM, which is necessary to treat by ear surgery. We think that it determinates lower rate of surgical ear intervention. Classical tympanostomy in our research was performed in complex with adenoidectomy what was the reason for better results in comparison with other researches data [2-8, 11-17]. The monitoring demonstrated the advantages of complex surgical tympanostomy especially in modified version. Better results of modified tympanostomy in comparison with results after classical tympanostomy in our study are explicated by additional incision, which increases cleaning options of surgery. Surgical treatment in our modification is helpful in restoration of the

Table 3

Dynamics of recurrence rate, GH and QL indexes before and after surgery in 3 groups of children

Groups	Recurrence rate (%)	Scores mean (SD)					
		General health			Quality of life		
		Before surgery	12 months	24 months	Before surgery	12 months	24 months
M	38	6.62 (0.4)	3.30 (0.4)	3.34 (0.6)	5.5 (0.5)	2.6 (1.5)	2.8 (1.2)
CTS	16	6.68 (0.4)	1.93 (0.5)	1.99 (0.3)	5.5 (0.4)	1.8 (0.6)	2.0 (0.7)
MTS	2	6.75 (0.4)	0.87 (0.3)	0.84 (0.2)	5.6 (0.5)	1.3 (0.5)	1.3 (0.5)

M – myringotomy, CTS – classical tympanostomy, MTS – modified tympanostomy.

ME anatomy and function and prevention of OM persistence or recurrence.

Conclusions

Post-surgical electro-acoustical monitoring with GH and QL evaluation demonstrated different outcome of existed surgical techniques of prolonged OM. According to the monitoring results, tympanostomy, especially its modified version, is more effective than myringotomy in prevention of OM recurrence, hearing restoration and normalization of GH and QL indexes.

References

1. Rosenfield R, Shin J, Schwartz S, et al. Clinical practice guideline: otitis media with effusion (Update). Otolaryngol Head Neck Surg. 2016;154(1 Suppl):S1-S41. doi: 10.1177/0194599815623467.
2. Venekamp R, Burton M, van Dongen T, et al. Antibiotics for otitis media with effusion in children. Cochrane Database Syst Rev. 2016;(6):CD009163. doi: 10.1002/14651858.CD009163.pub3.
3. Popova D, Varbanova S, Popov T. Comparison between myringotomy and tympanostomy tubes in combination with adenoidectomy in 3-7-year-old children with otitis media with effusion. Int J Pediatr Otorhinolaryngol. 2010;74(7):777-80. doi: 10.1016/j.ijporl.2010.03.054.
4. Browning G, Rovers M, Williamson I, Lous J, Burton MJ. Grommets (ventilation tubes) for hearing loss associated with otitis media with effusion in children. Cochrane Database Syst Rev. 2010;(10):CD001801. doi:10.1002/14651858.CD001801.pub3.
5. *Venekamp R, Mick P, Schilder A, Nunez D. Grommets (ventilation tubes) for recurrent acute otitis media in children. Cochrane Database Syst Rev. 2018;5(5):CD012017. doi: 10.1002/14651858.CD012017.pub2.*
6. *Lous J, Ryborg C, Thomsen J. A systematic review of the effect of tympanostomy tube in children with recurrent acute otitis media. Int J Pediatr Otorhinolaryngol. 2011;75(9):1058-61. doi: 10.1016/j.ijporl.2011.05.009.*
7. Steele D, Adam G, Di M, et al. Effectiveness of tympanostomy tubes for otitis media: a meta-analysis. Pediatrics. 2017;139(6):e20170125. doi: 10.1542/peds.2017-0125.
8. Rajamani SK, Choudhary VC, Mogre DA. Tympanometric screening for Otitis media of paediatric patients with respiratory tract infection in rural setting a prospective observational study. Trop J Ophthalmol Otolaryngol. 2019;4(2):100-108. doi: 10.17511/ jooo.2019.i2.06.
9. Vetricean S. Maladia urechii operate [Operated ear disease]. Chişinău; 2018. 224 p. Romanian.
10. Diacova S. Electrophysiology characteristics of middle ear in understanding of otitis media development in early childhood. In: 2017 E-Health and Bioengineering Conference (EHB 2017); 2017 June 22-24; Sinaia, Romania. Piscataway, NJ: IEEE; 2017. p. 377-381.
11. Diacova S, McDonald T, Ababii I. Clinical, functional, and surgical findings in chronic bilateral otitis media with effusion in childhood. Ear Nose Throat J. 2016;95(8):E31-E37.
12. Diacova S, McDonald T. A comparison of outcomes following tympanostomy tube placement or conservative measures for management of otitis media with effusion. Ear Nose Throat J. 2007,86(9):552-554. https://doi.org/10.1177/014556130708600909
13. Diacova S. Effectiveness of modified miniinvasive otomicrosurgery in recurrent and persistent otitis media in children. In: 2017 E-Health and Bioengineering Conference (EHB 2017); 2017 June 22-24; Sinaia, Romania. Piscataway, NJ: IEEE; 2017. p. 386-390.
14. Diacova S. Electro-acoustical examination in noninvasive monitoring as a basis for treatment selection. In: Tiginyanu I, Sontea V, Railean S, editors. 4th International Conference on Nanotechnologies and Biomedical Engineering; 2019 Sep 18-21; Chisinau, Moldova. Cham: Springer; 2020. p. 627-631. (IFMBE Proceedings; vol. 77, Series Online ISSN 14433-9277). doi: 10.1007/978-3-030-31866-6_111.
15. Diacova S, Ababii I. Metoda de tratament al otitei medii exudative la copii [Method for treating exudative otitis media in children]. Republic of Moldova patent MD 674Y. 2013 March 7. [Official Bulletin of Industrial Property] (Chisinau). 2013;(9):24-25.
16. Kujala T, Alho O, Luotonen J, et al. Tympanostomy with and without adenoidectomy for the prevention of recurrences of acute otitis media. Pediatr Infect Dis J. 2012;31(6):565-569. doi: 10.1097/INF.0b013e318255ddde.
17. Rosenfeld R, Bhaya M, Bower C, et al. Impact of tympanostomy tubes on child's quality of life. Arch Otolaryngol Head Neck Surg. 2000;126(5):585-592. doi:10.1001/archotol.126.5.585.
18. Brouwer C, Maille' A, Rovers M, et al. Health-related quality of life in children with otitis media. Int J Pediatr Otorhinolaryngol. 2005;69(8):1031-1041. doi: 10.1016/j.ijporl.2005.03.013.
19. Brouwer C, Rovers M, Maillé A, et al. The impact of recurrent acute otitis media on the quality of life of children and their caregivers. Clin Otolaryngol. 2005; 30(3):258-265. doi: 10.1111/j.1365-2273.2005.00995.x.

Author's ORCID iD and academic degrees
Svetlana Diacova, MD, PhD, Associate Professor – https://orcid.org/0000-000301224-6481.

Author's contribution
SD conceptualized the idea, designed the trial and conducted the study, interpreted the data, wrote the manuscript, revised and approved the final text.

Funding
This study was supported by *Nicolae Testemitanu* State University of Medicine and Pharmacy. The trial was the author's initiative. The author is independent and takes responsibility for the integrity of the data and accuracy of the data analysis.

Ethics approval and consent to participate
The research was performed as part of research project "Optimization of otoprotective pharmacotherapy and diagnosis of auditory analyzer disorders". The project was approved by Ethics Committee of Research of *Nicolae Testemitanu* State University of Medicine and Pharmacy (Protocol No 26, of 14.11.2016). It was obtained an informed consent from all participants in the study.

Conflict of Interests
No competing interests were disclosed.

Acknowledgment
The author expresses the appreciation for the support, received from Academician Ion Ababii and from the staff and associates in the Department of Otorhinolaryngology at *Nicolae Testemitanu* State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova.
We also acknowledge the contributions of the Mayo Clinic staff (USA), especially from Dr. Thomas McDonald and from the Society Pediatricians du Monde in Clermont-Ferrand (France), Dr. Desvignes Veronique.

DOI: 10.5281/zenodo.3958433
UDC: 582.998:615.322.07(478)

Comparative microscopic study of *Solidago* species from the Republic of Moldova flora

[*1,2]Cornelia Fursenco, [1]Tatiana Calalb, [2]Livia Uncu

[1]Department of Pharmacognosy and Pharmaceutical Botany, [2]Scientific Center for Drug Research
Nicolae Testemitanu State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova

Authors' ORCID iDs, academic degrees and contributions are available at the end of the article

[*]Corresponding author**:** cornelia.fursenco@usmf.md
Manuscript received June 21, 2020; revised manuscript July 30, 2020; published online August 26, 2020

Abstract

Background: Genus *Solidago* is represented in the Republic of Moldova flora by two species: *S. virgaurea* – spontaneous and *S. canadensis* – cultivated, known as medicinal plants in the world, also being studied in our country. The objective of this paper was the comparative microscopic study of *Solidago* species from the Republic of Moldova flora.
Material and methods: Superficial preparations and sections were made from vegetal products of *S. virgaurea* and *S. canadensis* species and studied in the *Micros* microscope coupled with digital camera.
Results: The main specific structural indicators with diagnostic role in the identification of g. *Solidago* species from the flora of the Republic of Moldova were highlighted: the presence of secretory channels in rhizome and stem (both species); the contour of stem in cross-section (polygonal outline – *S. virgaurea*, circular outline – *S. canadensis*); the mesophyll structure of leaf (dorsoventral mesophyll – *S. virgaurea*, equifacial mesophyll – *S. canadensis*); anomocytic type of stomata (both species); multicellular protective conical trichomes and flabelliform ones (both species); secretory trichomes (with unicellular foot – *S. virgaurea*, and with multicellular one – *S. canadensis*); abundant pappus composed of multiseriate bristles and spherical pollen grains (both species).
Conclusions: *S. virgaurea* and *S. canadensis* species from the Republic of the Moldova flora could be identified by specific structural indices that have an important diagnostic role.
Key words: genus *Solidago*, microscopy, structural indices.

Cite this article
Fursenco C, Calalb T, Uncu L. Comparative microscopic study of *Solidago* species from the Republic of Moldova flora. *Mold Med J.* 2020;63(3):16-21. doi: 10.5281/zenodo.3958433.

Introduction

The genus *Solidago* L. is one of the most complex genera of higher plants, including about 120 species, most of which are native to North America, less to Eurasia, being spread throughout the continents. In the Central Europe, there are 5 species of g. *Solidago*: 4 of them being of American origin – *S. canadensis* L., *S. gigantea* Aiton., *S. altissima* L. and *S. graminifolia* (L.) Elliot, and only one species – *S. virgaurea* L. is native to Europe [1, 2]. In the flora of the Republic of Moldova g. *Solidago* is represented by 2 species: the native one, spontaneously spread *S. virgaurea* and the cultivated species *S. canadensis*, known as adventive one [3, 4]. Species *S. virgaurea* with the common name European goldenrod is known for Romanian population as "*vargă-de-aur*", "*splinuță*", "*floare-boierească*", "*mănunchi*", "*splinăriță*", "*smeoaică*". The aerial parts of European goldenrod have been used for centuries as diuretic and anti-inflammatory agents in traditional medicine for the treatment of several urological symptoms, such as urolithiasis, kidney and bladder inflammation, cystitis, etc. [5-7].

On the other hand, Canada goldenrod (*S. canadensis*), known as popular Romanian common names*: "sălcioară", "sânziană-de-grădină",* is included in the uncultivated synanthropic flora of the Republic of Moldova, being specific for degraded ecosystems and rural areas, particularly in ruderal and human-created habitats, as well, being cultivated as an ornamental plant [3, 4]. Also, it has been used in the European phytotherapy for 700 years for the treatment of chronic nephritis, cystitis, urolithiasis, rheumatism and as an antiphlogistic drug [8-11]. The multi-pharmacological properties of g. *Solidago* species are due to the presence of many bioactive compounds: flavonoids, phenolic compounds, triterpene saponins, carotenoids, chlorophylls and essential oils [1, 5-17].

The increased interest for *Solidago* species as medicinal plants and the fact that *S. virgaurea* and *S. canadensis* species from the Republic of Moldova flora have not been practically researched, motivated us to carry out this comparative microscopic study.

Material and methods

Plant material. The different plant materials (rhizomes and roots – *rhizomata et radices*, aerial parts – *herba,* leaves – *folia,* and flowers – *flores*) were harvested in the middle of July, beginning of August (2019), during the flowering phase. Vegetal products of *S. virgaurea* species have been collected from "The Trebujeni landscape reservation", Orhei district, and vegetal products of *S. canadensis* species – from the collection of the Scientific Centre for Medicinal Plants Cultivation of *Nicolae Testemitanu* State University of Medicine and Pharmacy. The plant material was dried in natural conditions, in dark, dry rooms, ventilated, thus preserving the color of the flowers and avoiding the molding of the biological material.

Microscopic method. The microscopic study was performed on clarified (with chloralhydrate or 3% NaOH) superficial preparations of the leaf, flowers, and on cross-sections through leaf lamina, stem, and rhizome according to the classical techniques [18]. The anatomical study was performed at the Department of pharmacognosy and pharmaceutical botany, *Nicolae Testemitanu* State University of Medicine and Pharmacy, using the binocular optical microscope *Micros* (Austria), with a digital camera, coupled to the computer, at 4x, 10x and 40x objective magnification.

The results of the microscopic determinations were statistically processed using the GraphPad Prism 7.0 program.

Results and discussion

Microscopy of S. virgaurea rhizome. In cross-section the rhizome has a cylindrical contour, with the following histological zones: periderm, cortex and the central cylinder. The covering tissue is represented by a multilayered periderm, consisting of thickened polygonal cells. The secretory channels are distributed in the endoderm region of the cortex. The central cylinder occupies most of the rhizome, which consists of a system of collateral-open vascular bundles, arranged in circle. Above the phloem there are thick-walled lignified cells – the pericyclic sclerenchyma (fig. 1 A, B).

Microscopy of S. canadensis rhizome. The structure is, in general, similar to that of the European goldenrod, with the same cylindrical contour of the rhizome. Inside, the collateral-open vascular bundles are located radially and the secretory channels are distributed in the endoderm region. In contrast to the European goldenrod rhizome, medullary parenchyma cells contain idioblasts with raphides (fig. 2 A, B).

Microscopy of S. virgaurea stem. According to the microscopic images, stem in cross-section has a polygonal contour. The epidermis is a single-layer, composed of cutinized, well-packed, polygonal cells. Below the epidermis, we mention the angular collenchyma with cell thickening at intercellular contact points, followed by the cortical parenchyma, comprising several layers of large, oval-spherical cells. At the border between cortical parenchyma and endo-

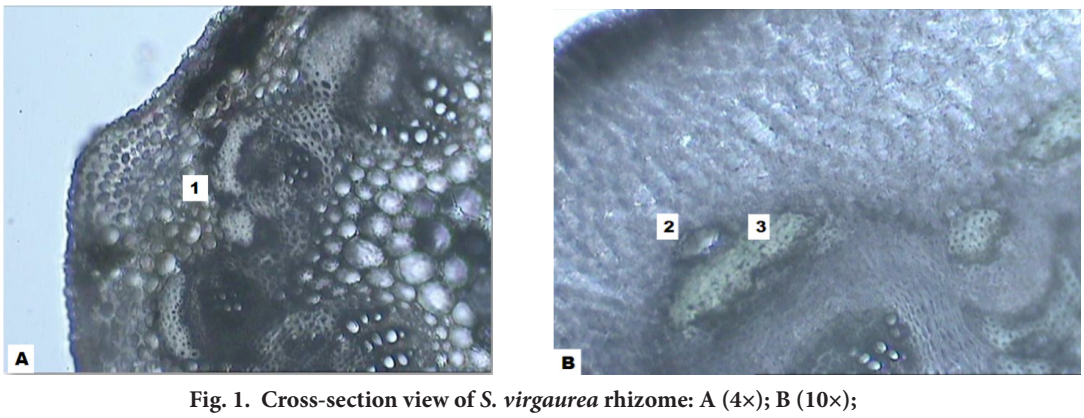

Fig. 1. Cross-section view of *S. virgaurea* rhizome: A (4×); B (10×);
1 – collateral-open vascular bundles, 2 – secretory channel, 3 – pericyclic sclerenchyma

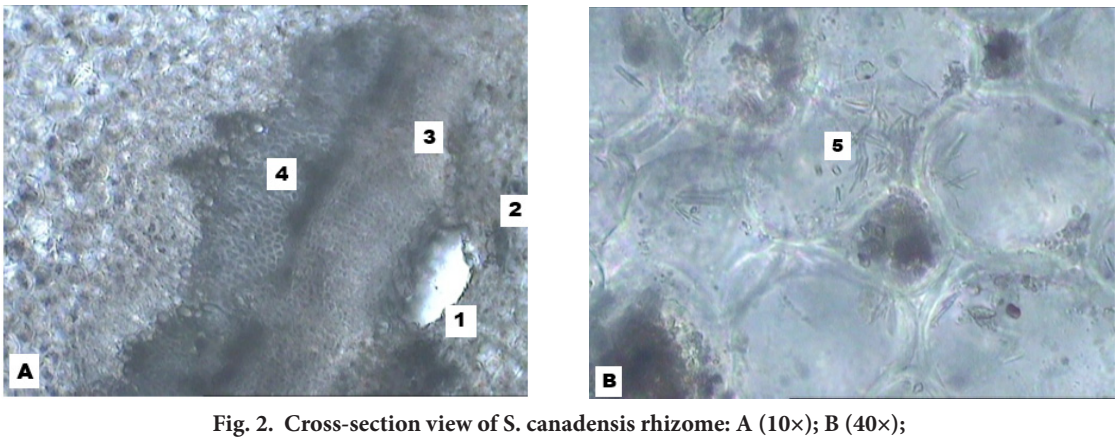

Fig. 2. Cross-section view of S. canadensis rhizome: A (10×); B (40×);
1 – secretory channel, 2 – cortical parenchyma, 3 – phloem, 4 – xylem, 5 – idioblasts with raphides

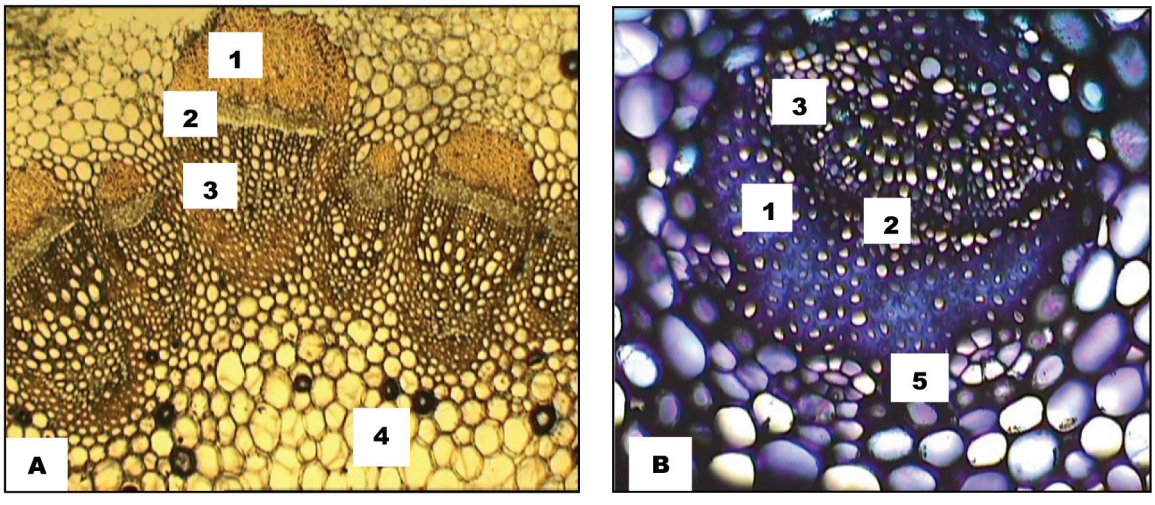

Fig. 3. Cross-section view of *S. virgaurea* stem: A (10×), Lugol solution; B (40×), Methylene blue solution; 1 – phloem, 2 – cambium, 3 – xylem, 4 – medullary parenchyma, 5 – secretory channel

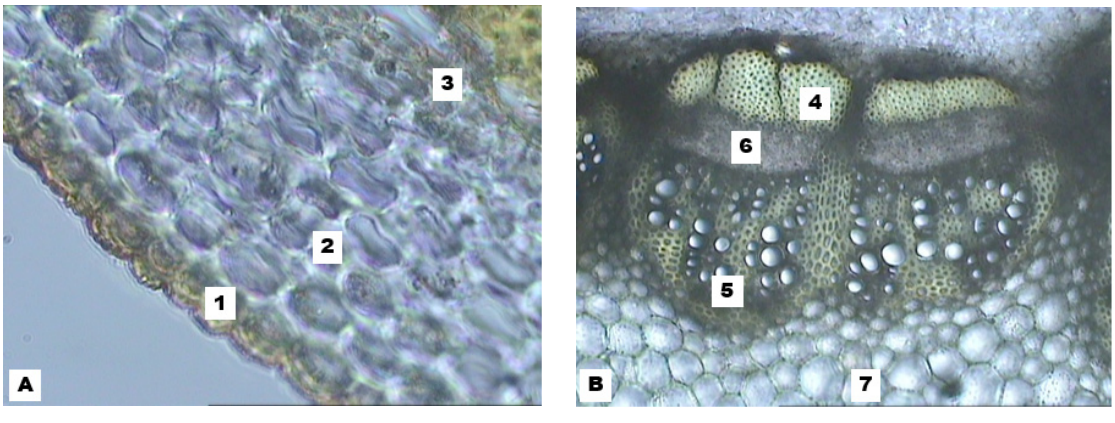

Fig. 4. Cross-section view of *S. canadensis* stem: A (40×); B (10×); 1 – epidermis, 2 – cortical parenchyma, 3 – sclerenchyma, 4 – phloem, 5 – xylem, 6 – cambium, 7 – medullary parenchyma

dermis, secretory channels are present. The vascular cylinder is made up of roundly arranged collateral-open vascular bundles, situated very close to each other (fig. 3 A, B).

Microscopy of S. canadensis stem. In comparison with European goldennrod stem, the microscopic analysis of Canada goldenrod reveals that stem in cross-section has a circular outline. The epidermis is unilayered and consists of well-packed oval cells. Similar to European goldenrod, the cortex contains secretory channels, and the endoderm is differentiated on the outside of the phloem tissue of the vascular bundle. The central cylinder consists of collateral-open vascular bundles, arranged radially, being located very close to each other (fig. 4 A, B).

Microscopy of S. virgaurea and S. canadensis leaf. The analysis of superficial preparations of leaves from both *Solidago* species, reveals similar structures of epidermis, which is single-layered and composed of well-wrapped cells. The cells of the upper epidermis of the leaf are polygonal, isodiametric, with slightly sinuous and uniformly thickened external walls. Stomata are present on both epidermises (amphistomatic leaf), but numerically, more on the abaxial surface. For the upper epidermis, it is specific the anomocytic type of stomata: the guard cells are surrounded by 4-5 subsidiary cells of the same shape as other epidermal cells. For the lower epidermis, it is specific the anomotetracytic type of stomata: the guard cells are surrounded by rule of 3-5 cells, moreover there are 4 subsidiary cells.

According to our analysis of epidermis and the previous one [19], for both *Solidago* species, there were found the following specific trichomes, which presented only some numerical variations:

1. Multicellular protective trichomes describing a conical shape, consisting of a single row of 2-7 cells; the cell walls are thin, with a smooth or slightly wavy surface. They are frequent on the both surfaces of the leaf blade, mainly on the veins and leaf margins. Often, the apical cell of the trichome is curved or may easily break, only the basal cells of the trichomes remaining on the leaves (fig. 5 A). Usually, around the trichomes, the epidermal cells are radially disposed, forming a basal rosette. The basal cells of the trichomes are filled with a brownish-red content (fig. 5 A). This type of trichomes is found in abundance on the leaves of both *Solidago* species (fig. 5 A, D)
2. Uniseriate multicellular trichomes consisting of 2-3

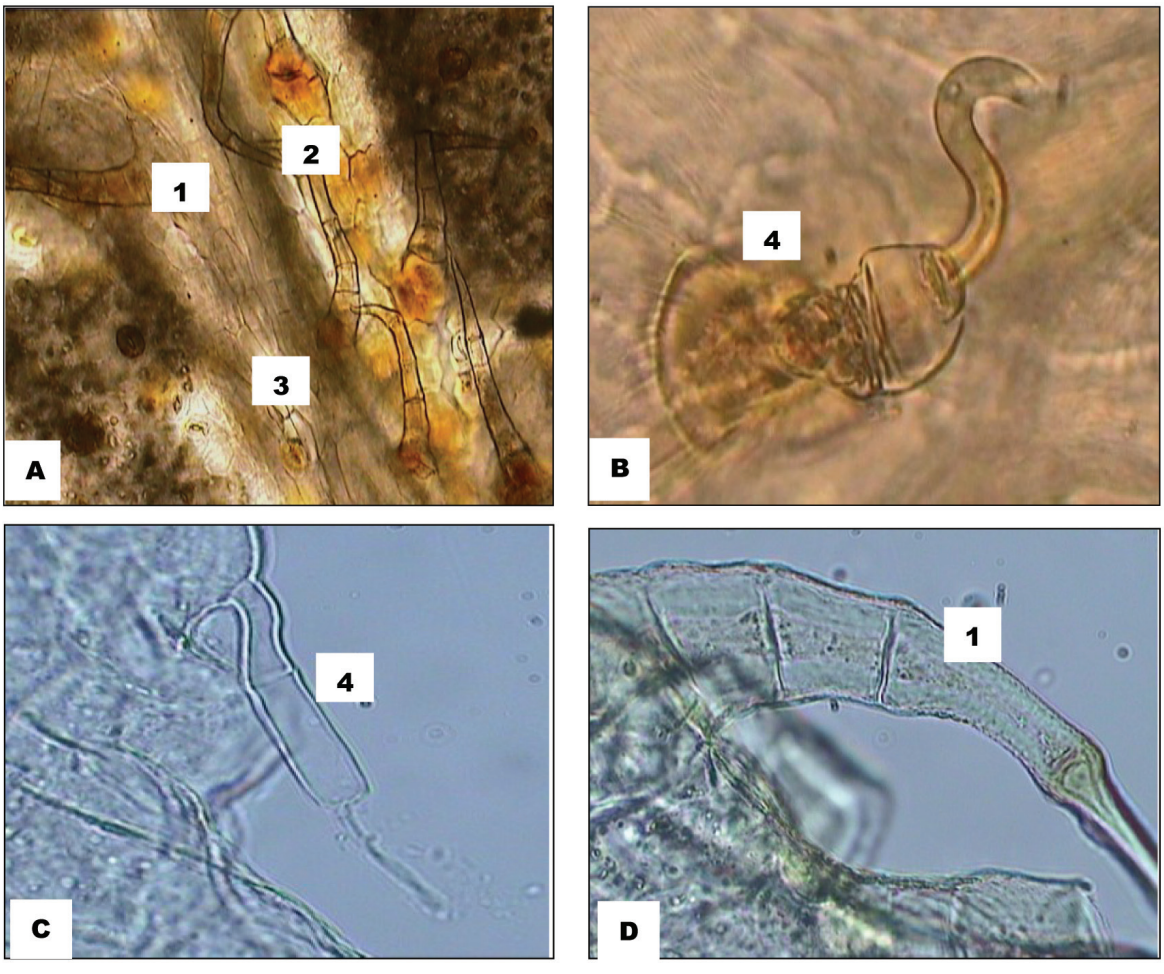

Fig. 5. Superficial view of *Solidago* species leaf: *S. virgaurea* – A (10×); B (40×); *S. canadensis* – C (40×); D (40×); 1 – multicellular protective trichomes, 2 – protective trichome with the broken apical cell, 3 – basal cells of the trichome filled with a brownish-red content, 4 – flabelliform trichome

cells, the apical one can be elongated to the filiform, because of this, these trichomes are also found in the literature review [20, 21] as flabelliform, moniliform or flagellate trichomes. This type of trichomes is more common on the lower epidermis of *S. virgaurea* middle leaves (fig. 5 B). There are fewer such flabelliform trichomes only on *S. canadensis* leaves (fig. 5 C).

3. Secretory trichomes, being short, with a stalk formed of 1-2 cells and a unicellular head, representing the apical secretory cell. The unicellular foot secretory trichomes were found more on leaves of *S. virgaurea*, and the multicellular (2 cells) foot secretory trichomes – on leaves of *S. canadensis*. The secretory trichomes are less numerous compared to the covering ones for both *Solidago* species.

The comparative microscopic study of *S. virgaurea and S. canadensis* leaf in cross-section view demonstrates that leaf of *S. virgaurea* has a dorsoventral (bifacial) structure, but leaf of *S. canadensis* – equifacial structure. For *S. virgaurea* leaf it was distinguished the upper and lower epidermis, including well developed mesophyll, differentiated into palisade parenchyma – occurred in two rows in the upper part of the leaf blade, and spongy parenchyma – located in the lower part of the leaf blade (fig. 6). The mesophyll structure of *S. canadensis* differed from that of *S. virgaurea*: the palisade cells occurred in two layers: upper (near adaxial epidermis), composed of two rows, and lower, with one row of palisade cells. Spongy cells formed a clearly differentiated thin layer between the palisade layers.

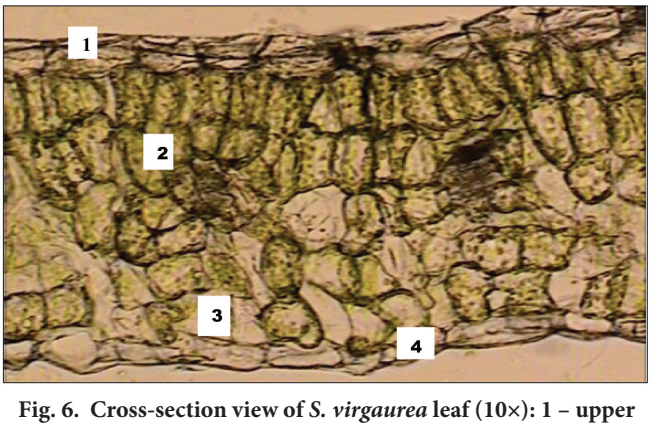

Fig. 6. Cross-section view of *S. virgaurea* leaf (10×): 1 – upper epidermis, 2 – palisade parenchyma, 3 – spongy parenchyma, 4 – lower epidermis

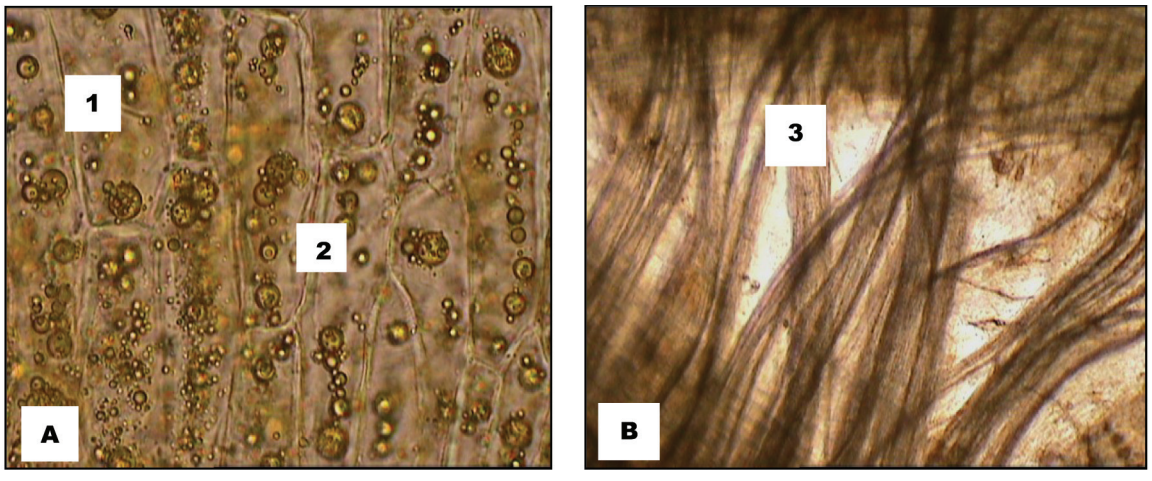

Fig. 7. Superficial view of *S. virgaurea* florets: A – ligulate floret (40x); B – tubular floret (4x); 1 – spherical chromoplasts, 2 – ligulate floret epidermis cells, 3 – pappus at the base of tubular floret

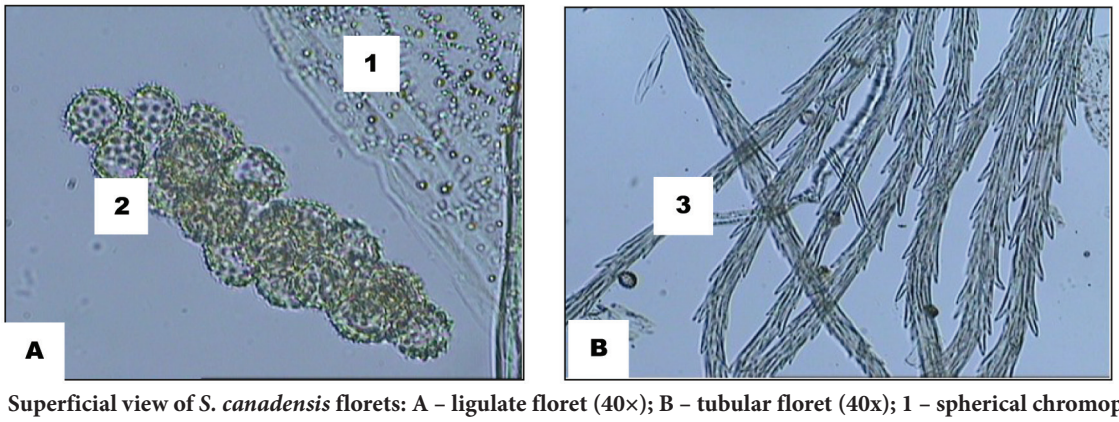

Fig. 8. Superficial view of *S. canadensis* florets: A – ligulate floret (40×); B – tubular floret (40x); 1 – spherical chromoplasts, 2 – spherical pollen grains with irregular surface, 3 – multiserriate pappus bristles, branched on the outside

Microscopy of S. virgaurea and S. canadensis flower (superficial view). The anatomical structure of the ligulate and tubular florets has a lot of common features for *Solidago* species, with only a few small differences. For the ligulate ray florets of *Solidago* species, it is characteristic the epidermis consisting of rectangular-shaped cells with thin cell walls without intercellular spaces. The cells are rich in spherical, globular chromoplasts (fig. 7 A). The epidermis of tubular floret is formed from cells of the same shape as those of the ray floret epidermis, although, we mention the elongation of the cells and thickening of their walls as well as the formation of cuticle strips. Chromoplasts are present in abundance and usually form conglomerates (fig. 7 A, fig. 8 A).

On the stigma of the tubular floret, there are numerous pollen grains of spherical shape with irregular surface, a common feature for plant species from Asteraceae family (fig. 8 A). At the base of the floret there is attached the pappus, made up of numerous bristles. The pappus is abundant, being composed of multiseriate bristles (fig. 7 B). The pappus bristles from *S. canadensis* florets are much more branched on the outside, compared to those from *S. virgaurea* (fig. 8 B).

Conclusions

The main anatomical indices with diagnostic role for *S. virgaurea* and *S. canadensis* species from the flora of the Republic of Moldova were highlighted: the presence of secretory channels in rhizome and stem (both species); the contour of stem in cross-section (polygonal outline – *S. virgaurea*, circular outline – *S. canadensis*); the mesophyll structure of leaf (dorsoventral mesophyll for *S. virgaurea* and equifacial mesophyll – *S. Canadensis)*; anomocytic type of stomata (both species); multicellular protective conical trichomes and flabelliform ones (both species); secretory trichomes (with unicellular foot for *S. virgaurea,* and with multicellular one – *S. canadensis*); abundant pappus composed of multiseriate bristles (both species, but with some specifications for *S. canadensis* – pappus bristles from florets are much more branched on the outside); numerous pollen grains of spherical shape with irregular surface (both species).

As a result of this anatomical study, there have been determined specific structural indices that have an important diagnostic role for the identification of *S. virgaurea* and *S. canadensis* species from the Republic of Moldova flora.

References

1. Toiu A, Vlase L, Vodnar DC, Gheldiu AM, Oniga I. *Solidago graminifolia* L. Salisb. (Asteraceae) as a valuable source of bioactive polyphenols: HPLC profile, *in vitro* antioxidant and antimicrobial potential. Molecules. 2019;24(14):2666. doi:10.3390/molecules24142666.
2. Tutin TG, Heywood VH, Burges NA, et al. Flora Europaea. Vol. 4. Plantaginaceae to Compositae (and Rubiaceae). Cambridge: Cambridge University Press; 1976. 505 p. doi:10.5281/zenodo.293764.
3. Negru A. Determinator de plante din flora Republicii Moldova [Determinant of plants from the flora of the Republic of Moldova]. Chișinău: Universul; 2007. 391 p. Romanian.
4. Marza M, Negru A, Mamai I. Flora sinantropă necultivată a Republicii Moldova [Uncultivated synanthropic flora of the Republic of Moldova]. Studia Universitatis Moldaviae. Revista Științifică a Universității de Stat din Moldova. 2013;6(66):154-168. Romanian.
5. Frumenzio E, Maglia D, Salvini E, et al. Role of phytotherapy associated with antibiotic prophylaxis in female patients with recurrent urinary tract infections. Arch Ital Urol Androl. 2013;85(4):197-199. doi:10.4081/aiua.2013.4.197.
6. Yarnell E. Botanical medicines for the urinary tract. World J Urol. 2002;20(5):285-293. doi:10.1007/s00345-002-0293-0.
7. Apáti P, Szentmihályi K, Kristó ST, et al. Herbal remedies of *Solidago* – correlation of phytochemical characteristics and antioxidative properties. J Pharm Biomed Anal. 2003;32(4-5):1045-1053. doi:10.1016/s0731-7085(03)00207-3.
8. Radušiene J, Marksa M, Ivanauskas L, et al. Assessment of phenolic compound accumulation in two widespread Goldenrods. Ind Crops Prod. 2015;63:158-166. doi:10.1016/j.indcrop.2014.10.015.
9. Marksa M, Zymone K, Ivanauskas L, et al. Antioxidant profiles of leaves and inflorescences of native, invasive and hybrid *Solidago* species. Ind Crops Prod. 2020;145:1-9. doi:10.1016/j.indcrop.2020.112123.
10. Kołodziej B, Kowalski R. Antibacterial and antimutagenic activity of extracts aboveground parts of three *Solidago* species: *Solidago virgaurea* L., *Solidago canadensis* L. and *Solidago gigantea* Ait. J Med Plants Res. 2011;5(31):6770-6779. doi:10.5897/JMPR.1098
11. Wozniak D, Slusarczyk S, Domaradzi K, et al. Comparison of polyphenol profile and antimutagenic and antioxidant activities in two species used as source of *Solidaginis herba* – goldenrod. Chem Biodivers. 2018;15(4):1-16. doi:10.1002/cbdv.201800023.
12. Bradette-Hébert ME, Legault J, Lavoie S, Pichette A. A new labdane diterpene from the flowers of *Solidago canadensis*. Chem Pharm Bull (Tokyo). 2008;56(1):82-84. doi:10.1248/cpb.56.82.
13. Apáti P, Houghton PJ, Kite G, Steventon GB, Kéry A. *In vitro* effect of flavonoids from *Solidago canadensis* extract on glutathione S-transferase. J Pharm Pharmacol. 2006;58(2):251-256. doi:10.1211/jpp.58.2.0013.
14. Jaiswal R, Kiprotich J, Kuhnert N. Determination of the hydroxycinnamate profile of 12 members of the Asteraceae family. Phytochemistry. 2011;72(8):781-790. doi:10.1016/j.phytochem.2011.02.027.
15. Kalemba D, Thiem B. Constituents of the essential oils of four micropropagated *Solidago* species. Flavour Fragr J. 2004;19(1):40-43. doi:10.1002/ffj.1271.
16. Abdel Motaal A, Ezzat SM, Tadros MG, El-Askary HI. *In vivo* anti-inflammatory activity of caffeoylquinic acid derivatives from *Solidago virgaurea* in rats. Pharm Biol. 2016;54(12):2864-2870. doi:10.1080/13880209.2016.1190381.
17. Dobjanschi L, Paltinean R, Vlase L, et al. Comparative phytochemical research of *Solidago* genus: *S. graminifolia*. Note I. Flavonoids. Acta Biologica Marisiensis. 2018;1(1):18-26. doi:10.2478/abmj-2018-0003.
18. Saukel J, Ginko E. Microscopic analysis of plants. In: Meyers RA, editor. Encyclopedia of analytical chemistry. Hoboken: Wiley Online Library; 2014. doi:10.1002/9780470027318.a9906.
19. Calalb T, Fursenco C, Ionita O, Ghendov V. The morpho-anatomical study of *Solidago virgaurea* L. species from the flora of the Republic of Moldova. East Eur Sci J. 2018;2(30):4-13.
20. Souza DMF, Sá RD, Araújo EL, Randau KP. Anatomical, phytochemical and histochemical study of *Solidago chilensis* Meyen. An Acad Bras Cienc. 2018;90(2 suppl 1):2107-2120. doi:10.1590/0001-3765201720160280.
21. Szymura M, Wolski K. Leaf epidermis traits as tools to identify *Solidago* L. taxa in Poland. Acta Biol Cracov Ser Bot. 2011;53(1):38-46. doi:10.2478/v10182-011-0006-3.

Authors' ORCID iDs and academic degrees
Cornelia Fursenco, MD, PharmD, PhD Applicant – https://orcid.org/0000-0003-0692-6819.
Tatiana Calalb, MD, Biology PhD, Professor of Biology – https://orcid.org/0000-0002-8303-3670.
Livia Uncu, MD, Pharm PhD, Associate Professor of Pharmacy – https://orcid.org/0000-0003-3453-2243.

Authors' contribution
CF collected and processed the plant material, performed the microscopic study, drafted the first manuscript; TC designed the study, conducted the laboratory work, interpreted the data, revised the manuscript; LU conducted the laboratory work, revised the manuscript. All the authors revised and approved the final version of the manuscript.

Funding
This study was supported by *Nicolae Testemitanu* State University of Medicine and Pharmacy. The trial was the authors' initiative. The authors are independent and take responsibility for the integrity of the data and accuracy of the data analysis.

Ethics approval and consent to participate
No approval was required for this study.

Conflict of Interests
No competing interests were disclosed.

DOI: 10.5281/zenodo.3958435
UDC: 616.831-005.1+616.831.37+616.831.71

White matter hyper-intensity patterns in patients with amyloid angiopathy and cerebellum involvement

***[1]Pavel Gavriliuc, [1]Mihail Gavriliuc, [2]Stanislav Groppa, [3]Ronen Leker**

[1]Department of Neurology No 1, [2]Department of Neurology No 2
Nicolae Testemitanu State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova
[3] Department of Neurology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

Authors' ORCID iDs, academic degrees and contribution are available at the end of the article

*Corresponding author: gavriliucpavel@gmail.com

Manuscript received July 20, 2020; revised manuscript August 14, 2020; published online August 26, 2020

Abstract

Background: Pathological changes in the cerebral white matter can be determined both in small vessel disease and in cerebral amyloid angiopathy. The pattern of involvement may be different depending on the etiology and severity of the process. Objective of the study: Determination and analysis of the pattern of cerebral white matter changes in patients with amyloid angiopathy and involvement of the cerebellum.
Material and methods: Patients with intracerebral hemorrhages who were examined by magnetic resonance imaging were prospectively analyzed. Patients were diagnosed with cerebral amyloid angiopathy (CAA) according to Boston criteria. Changes in white matter were interpreted using the Fazekas scale and compared for patients with CAA and patients with CAA and cerebellar involvement. Of the 614 patients with intracerebral hemorrhage, 96 were examined by cerebral magnetic resonance imaging. Of these, 41 patients were diagnosed with amyloid angiopathy, 19 patients with possible amyloid angiopathy, 21 patients – probable and 1 case with defined amyloid angiopathy.
Results: Cerebellar involvement was determined in 34% (14/41) of cases. Severe changes in white matter (Fazekas 2-3) were seen in patients with cerebellar involvement (12/14; 86% versus 8/27 and 30% p = 0.002).
Conclusions: Involvement of the white matter in the pathological process is more significant in patients with amyloid angiopathy and the involvement of the cerebellum, even after adjusting for risk factors. Patients with cerebellar haemorrhage and severe white matter should be screened for amyloid angiopathy.
Key words: amyloid, angiopathy, cerebral, white matter.

Cite this article
Gavriliuc P, Gavriliuc M, Groppa S, Leker R. White matter hyper-intensity patterns in patients with amyloid angiopathy and cerebellum involvement. *Mold Med J.* 2020;63(3):22-25. doi: 10.5281/zenodo.3958435.

Introduction

Cerebral amyloid angiopathy (CAA) is characterized by β-amyloid deposition in the media and adventitia of small and medium vessels of the cerebral cortex, subcortex, and leptomeninges [1-3]. CAA is a major cause of spontaneous lobar intracerebral hemorrhage (ICH) in normotensive elderly [1-3]. Hereditary and sporadic forms may occur, and the latter increases with age [2, 4-8]. ICH can take the form of macro-hematoma (MH) or micro-bleedings (MB) with various clinical presentations [9]. CAA frequently involves the occipital lobes, followed by the frontal, temporal, or parietal lobes, respectively [5, 9], but involvement of the cerebellum in CAA remains uncertain [5, 10]. Micro- or macro-hemorrhages in the deep brain structures or brainstem are an exclusion criterion for CAA.

The objectives of the current study were to assess the presence of cerebellar involvement in patients with CAA according to the modified Boston criteria [5] and to study the changes in the white matter.

Patients with spontaneous ICH were collected in two academic tertiary care centers and were included in a continuous database. The institutional IRB (Hadassah Medical Organization) approved the anonymous collection of data in this database and waived the need for informed consent.

For the current analysis, we retroactively analyzed the data accumulated in the period 2009-2015. The diagnosis of spontaneous ICH was confirmed in all patients using non-contrast computed tomography (CT). Patients with ICH secondary to trauma, vascular malformations, or tumors were excluded.

Material and methods

All 614 included patients underwent a standardized MRI protocol that included susceptibility weighted imaging or T2 star imaging (SWI or T2 * respectively) as soon as possible after the hemorrhage. Recommendations for performing post-ICH MRI were left to the discretion of participating neurologists and were based on clinical suspicion of CAA or atypical presentation or localization of the bleeding on CT. From the group of patients who had MRI, we identified patients who met the diagnostic criteria for CAA, according

to the modified Boston criteria [5]. Patients with non-lobar ICH and systolic blood pressure over 150 mmHg were considered to have a hypertensive ICH.

In patients who have undergone surgical procedures to remove the hematoma or decompressive craniectomy, the biopsy material has been studied for the presence of vascular amyloid deposits. Hematoma types, location and volumes were accumulated along with the vascular risk factor being noted. Bleedings were classified as MH or MB [5, 14] by experienced vascular neurologists and the absolute number of MB in the cerebral cortex were counted. The SWI or T2 * sequences were used to identify MB, while the other sequences were used to exclude MB mimicry, according to STRIVE criteria [15]. The presence of superficial cortical siderosis (CSS) has been documented and CSS has been classified as disseminated if it involved more than three grooves according to current recommendations [16-18]. Hyperintensities of deep white matter were measured on T2 FLAIR according to STRIVE methods [15] and according to the Fazekas scale [19] (0 – none, 1 – dotted scattered, 2 – starting to be confluent and 3 – confluent) and dichotomized in severe (grade 2-3) or non-severe (grade 1-2).

Patients with CAA and cerebellar involvement were compared to patients with CAA without any cerebellar involvement. Cerebellar MB locations were studied and divided into superficial cortical versus deep, involving the nuclei [11, 20].

Neurological deficit was analyzed using the National Institute of Health Stroke Scale (NIHSS) at admission and discharge. Disability was studied with the modified Rankin scale (mRS) at discharge and day 90 post-ICH.

Statistical analysis was performed using SPSS software. The Student's t-test was used to compare continuous variables and the chi-square test was used to compare nonparametric variables.

A multivariate regression analysis model was used to determine the factors that are associated with cerebellar involvement in patients with CAA. This model controlled for age, the severity of superficial siderosis, the existence of previous of ICH, the severity of the stroke at presentation, the volume of ICH, and the number of cortical micro-hemorrhages counted.

Results

Overall, 614 patients with spontaneous ICH were included in our database (343 at one center and 241 at the other) and 85 (54 and 31, respectively) had a post-ICH MRI (14%). Of the 85 patients, 41 (48%) were diagnosed with CAA according to the modified Boston criteria [5] (19 possible CAAs, 21 probable CAAs, and 1 defined CAA).

MRI patients were significantly younger (67.3 ± 12.2 versus 72.4 ± 12.9; p = 0.005) and had significantly lower hemorrhage volumes (13.2 ± 20.9 vs. 36 .0 ± 57.2 cc; p = 0.003) compared to non-NMR group. Hypertension was significantly more common in patients who did not have MRI (67% versus 13%; p = 0.034), but other risk factors did not differ between groups.

In the total group of 614 patients, 64 (10%) presented with cerebellar MH, and 10 of them had MRI. Most of the 54 patients who presented with cerebellar MH who did not have an MRI (95% and 72% of the patients in the participating centers) had hypertensive ICH.

In the CAA-related hemorrhage group, cerebellar participation was present in 34% (14 of 41 patients). Most cerebellar lesions were MB (fig. 1) and most patients had multiple cerebellar lesions (mean 8.4 ± 13.3). Cerebellar MB was more frequently superficial (11/14 patients involved superficial cerebellar, 2/14 had deep cerebral MB and 1/14 combined deep and superficial MB). A more severe degree of white matter hyperintensity (Fazekas 2–3) was more common in patients with cerebellar involvement (12/14; 86% vs. 8/27; 30% p = 0.002).

Clinical variables including age, sex, risk factor profile, stroke severity, 90-day mRS scores, or survival were not different between CAA patients with and without cerebellar involvement (tab. 1).

Patients with CAA-related cerebellar involvement had a significantly higher number of lobular cortical MB compared

Table 1

Baseline characteristics of patients with CAA

	CAA without cerebellar hemorrhage (n = 27)	CAA with cerebellar hemorrhage (n = 14)	P value
Age (mean ± SD)	70.5 ± 9.4	69.8 ± 8.2	0.82
Gender male (%)	13 (48%)	7 (50%)	0.910
Hypertension (%)	14 (52%)	8 (57%)	0.747
Diabetes (%)	3 (11%)	4 (29%)	0.159
Previous stroke (%)	3 (11%)	4 (29%)	0.159
Previous ICH (%)	3 (11%)	3 (21%)	0.375
Smoking (%)	3 (11%)	2 (14%)	0.768
Family history of ICH (%)	0 (0)	1 (7%)	0.160
Vitamin K antagonists (%)	2 (7%)	2 (14%)	0.482
Antiplatelet (%)	10 (37%)	8 (57%)	0.219
NOACs (%)	0 (0)	1 (7%)	0.457
History of dementia (%)	1 (7%)	3 (21%)	0.070
Hematoma size (ml mean ± SD)	26 ± 29.5	17.3 ± 21.1	0.315
Ventricular extension (%)	1 (7)	0 (0)	0.457
Spot sign (%)	1 (7)	0 (0)	0.248
Admission NIHSS (mean ± SD)	5.4 ± 5.5	4.0 ± 4.0	0.418
Total cortical microbleeds (mean ± SD)	2.8 ± 8.5	37.9 ± 39.6	< 0.00001
Confluent white matter hyperintensities (%)	8 (30)	12 (86)	0.002
Disseminated cortical superficial siderosis (%)	1 (3.7)	3 (21.4)	0.107
Modified Rankin score≤2 at day 90 (%)	13 (48)	6 (43)	0.747
Mortality (%)	2 (7)	0 (0)	0.296

ICH – Intracerebral hemorrhage, NOAC – Non-Vitamin K oral anticoagulants, NIHSS – National Institute of Health Stroke Scale. *Fazekas score 2–3.

to patients without cerebellar involvement (37.8 ± 39.5 vs. 2.8 ± 8.5; p <0.00001). CSS was present in 11 patients who had MRI and was classified as disseminated in 3. However, the absolute combined number of macro-hemorrhages (old + new) or the presence of disseminated CSS (> 3 grooves) did not differ significantly (7.3 vs. 2.4%; p = 0.07).

In the multivariate analysis that controls age, stroke severity, previous ICH episodes, number of micro-bleeds, age, and severity of superficial siderosis, the only variable that was significantly correlated with cerebellar involvement in CAA patients was the number of cortical hemorrhages (OR 1.045, 95% CI 1.005-11.087) (tab. 2). Interestingly, the severity of stroke was inversely correlated with cerebellar involvement in patients with CAA (OR 0.814 95% CI 0.664–0.997).

Table 2

Multivariate analysis for cerebellar CAA presence

Variable	OR	P	95% C.I.
Age (yr)	1.018	0.604	0.952 - 1.089
Previous ICH	1.729	0.564	0.269 - 11.135
Hematoma size (ml)	1.016	0.353	0.982 - 1.051
Degree of superficial siderosis	1.518	0.052	0.997 - 2.311
Number of MB	1.045	0.025	1.005 - 1.087
Admission NIHSS	0.814	0.047	0.664 - 0.997

ICH – intracerebral hemorrhage, MB – micro bleeds,
NIHSS – National Institute of Health Stroke Scale.

Discussion

The current study shows that cerebellar involvement is not uncommon in CAA-related ICH. Most patients have multiple cerebellar MBs, but some have only had MH involving the cerebellum in combination with MB in the cerebral cortex. Clinical features do not differ between CAA patients with or without cerebellar involvement, but patients with cerebellar involvement have a higher number of MB cortical lobar and also more often tend to have more intensity of white matter hyper-intensity, measured with the Fazekas score.

In both patients with CAA and patients with small vessel disease (SVD), white matter hyperintensities (WHM) are seen, but the patterns of subcortical WMH are different. In our study age did not prove to be associated with the severity of white matter involvement, or with the number of microbleeds seen in the cerebellum. However, the finding that WMH are more severe in patients with cerebellar involvement may be used a marker of CAA-related leukoaraiosis or of cerebrovascular amyloid load.

Our findings show that cerebellar involvement was observed in 43% of patients diagnosed with CAA based on the modified Boston criteria [5]. Both MB and MH were observed in our patients, most of the bleeding being of the MB type. Also, most patients with cerebellar involvement appear to have numerous lobar MBs (mean value of 37.8 ± 39.5) and a more severe degree of white matter hyper-intensities, which may involve a more severe or prolonged course of the disease.

The presence and number of lobar MBs as well as white matter burden have been associated with cognitive decline in patients with CAA [23]. Cognitive testing was not typically performed upon admission to our data sets, and therefore we cannot confirm this application. However, mRS scores at 90 days or survival do not differ between those with or without cerebellar involvement.

It should be noted that previous studies examining the pathological and radiological findings of CAA, as well as small vessels disease, have not examined the involvement of cerebellums [14, 16, 23, 24]. Brain MBs are divided into MBs in the lobar, which are largely secondary to CAA, and deep MBs, which are largely secondary to hypertension [3, 14, 23-25]. Because the cerebellum is a relatively common site for hypertensive ICH, it could be speculated that cerebellar MB, together with WMH, may also be linked to chronic hypertension [11]. However, in the light of findings of the current study, cerebellar MB may be correlated with the advance of CAA, could support the hypothesis that WMH in CAA has a distinct pattern that may be a marker of amyloid load and be included in the future diagnostic criteria for CAA.

For unknown reasons, CAA tends to involve the posterior lobes areas that are provided by the posterior circulation. Because the cerebellum is also provided by posterior circulation, similar mechanisms that are currently unknown may also be responsible for the involvement of the cerebellum in CAA.

Our study has significant limitations. First, although the study was based on potentially accumulated data, not all patients with ICH underwent MRI and the decision to perform or not MRI was left to the discretion of the neurologist who might be prejudiced. However, the indications and rate for MRI were similar between centers, as were all other patients' characteristics. Although this can only reflect national preferences, in reality it reflects daily practices in academic centers where not all patients with ICH, especially those with suspected hypertensive ICH, are subjected to magnetic imaging.

Second, the diagnosis of CAA according to the modified Boston criteria is based on the presence of MRI markers. These criteria have high specificity, but lower sensitivity, because CAA can be underdiagnosed in cases where MRI is not performed. Third, as a registry-based cohort study, variations in data acquisition over time or between centers are possible. Finally, long-term cognitive data as well as data on ICH recurrence rates were not available and could have added value to our study, given that cerebellar involvement was more common in patients with a greater number of cortical MB.

Conclusions

Our data shows that cerebellar involvement may be quite common in patients with CAA, especially when CAA has been more advanced, as noted by the association with higher numbers of lobar CMB, CSS, and more severe white matter hyper-intensities. Cerebellar involvement may be present as cerebellar MH or more frequently as cerebellar MB in patients with lobar MH, and tends to be

more frequently superficial, i.e. not involving the cerebellar nuclei, and more often associated with a more severe form of white matter abnormality. Clinical presentation of patients with cerebellar involvement in CAA and without cerebellar involvement does not appear to differ. Severe white matter changes seen on non-contrast CT in patients with lobar or cerebellar hemorrhage should prompt the treating physician to perform MRI and to look for CAA.

References

1. Alafuzoff I, Thal DR, Arzberger T, et al. Assessment of beta-amyloid deposits in human brain: a study of the BrainNet Europe consortium. Acta Neuropathol. 2009;117(3):309-320. doi: 10.1007/s00401-009-0485-4.
2. Charidimou A, Boulouis G, Gurol ME, et al. Emerging concepts in sporadic cerebral amyloid angiopathy. Brain. 2017;140(7):1829-1850. doi: 10.1093/ brain/awx047.
3. Charidimou A, Martinez-Ramirez S, Reijmer YD, et al. Total magnetic resonance imaging burden of small vessel disease in cerebral amyloid angiopathy: an imaging pathologic study of concept validation. JAMA Neurol. 2016;73(8):994-1001. doi: 10.1001/jamaneurol.2016.0832.
4. Fukutani Y, Cairns NJ, Rossor MN, et al. Cerebellar pathology in sporadic and familial Alzheimer's disease including APP 717 (Val— > Ile) mutation cases: a morphometric investigation. J Neurol Sci. 1997;149(2):177-184. doi: 10.1016/s0022-510x(97)05399-9.
5. Linn J, Halpin A, Demaerel P, et al. Prevalence of superficial siderosis in patients with cerebral amyloid angiopathy. Neurology. 2010;74(17):1346-1350. doi: 10.1212/WNL.0b013e3181dad605.
6. Nishitsuji K, Tomiyama T, Ishibashi K, et al. Cerebral vascular accumulation of Dutch-type Abeta42, but not wild-type Abeta42, in hereditary cerebral hemorrhage with amyloidosis, Dutch type. J Neurosci Res. 2007;85(13):2917-2923. doi: 10.1002/jnr.21413.
7. Thal DR, Ghebremedhin E, Orantes M, et al. Vascular pathology in Alzheimer's disease: correlation of cerebral amyloid angiopathy and arteriosclerosis/lipohyalinosis with cognitive decline. J Neuropathol Exp Neurol. 2003;62(12):1287-1301. doi: 10.1093/jnen/62.12.1287.
8. Tian J, Shi J, Bailey K, et al. Association between apolipoprotein E e4 allele and arteriosclerosis, cerebral amyloid angiopathy, and cerebral white matter damage in Alzheimer's disease. J Neurol Neurosurg Psychiatry. 2004;75(5):696-699. doi: 10.1136/jnnp.2003.012096.
9. Knudsen KA, Rosand J, Karluk D, et al. Clinical diagnosis of cerebral amyloid angiopathy: validation of the Boston criteria. Neurology. 2001;56(4):537-539. doi: 10.1212/wnl.56.4.537.
10. De Reuck JL, Deramecourt V, Auger F, et al. The significance of cortical cerebellar microbleeds and microinfarcts in neurodegenerative and cerebrovascular diseases. A post-mortem 7.0-tesla magnetic resonance study with neuropathological correlates. Cerebrovasc Dis. 2015;39(2):138-143. doi: 10.1159/ 000371488.
11. Pasi M, Marini S, Morotti A, et al. Cerebellar hematoma location: implications for the underlying microangiopathy. Stroke. 2018;49(1):207-210. doi: 10.1161/STROKEAHA.117.019286.
12. Eichel R, Khoury ST, Ben-Hur T, et al. Prior use of statins and outcome in patients with intracerebral haemorrhage. Eur J Neurol. 2010;17(1):78-83. doi: 10.1111/j.1468-1331.2009.02747.x.
13. Wada R, Aviv RI, Fox AJ, et al. CT angiography "spot sign" predicts hematoma expansion in acute intracerebral hemorrhage. Stroke. 2007;38(4):1257-1262. doi: 10.1161/01.STR.0000259633.59404.f3.
14. Greenberg SM, Vernooij MW, Cordonnier C, et al. Cerebral microbleeds: a guide to detection and interpretation. Lancet Neurol. 2009;8(2):165-174. doi: 10.1016/S1474-4422(09)70013-4.
15. Wardlaw JM, Smith EE, Biessels GJ, et al. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. Lancet Neurol. 2013;12(8):822-838. doi: 10.1016/S1474-4422(13)70124-8.
16. Charidimou A. Cortical superficial siderosis presumed due to cerebral amyloid angiopathy: minimum standards for rating and reporting. Am J Neuroradiol. 2016;37(5):E43-E44. doi: 10.3174/ajnr.A4748.
17. Charidimou A, Linn J, Vernooij MW, et al. Cortical superficial siderosis: detection and clinical significance in cerebral amyloid angiopathy and related conditions. Brain. 2015;138(Pt 8):2126-2139. doi: 10.1093/brain/awv162.
18. Charidimou A, Ni J, Martinez-Ramirez S, et al. Cortical superficial siderosis in memory clinic patients: further evidence for underlying cerebral amyloid angiopathy. Cerebrovasc Dis. 2016;41(3-4):156-162. doi: 10.1159/000442299.
19. Fazekas F, Chawluk JB, Alavi A, et al. MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal ageing. Am J Roentgenol. 1987;149(2):351-356. doi: 10.2214/ajr.149.2.351.
20. Pasi M, Pongpitakmetha T, Charidimou A, et al. Cerebellar microbleed distribution patterns and cerebral amyloid angiopathy. Stroke. 2019;50(7):1727-1733. doi: 10.1161/STROKEAHA.119.024843.
21. Good CD, Ng VW, Clifton A, et al. Amyloid angiopathy causing widespread miliary haemorrhages within the brain evident on MRI. Neuroradiology. 1998;40(5):308-311. doi: 10.1007/s002340050590.
22. Hemphill JC 3rd, Greenberg SM, Anderson CS, et al. Guidelines for the management of spontaneous intracerebral hemorrhage: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. Stroke. 2015;46(7):2032-2060. doi: 10.1161/STR.0000000000000069.
23. Meier IB, Gu Y, Guzaman VA, et al. Lobar microbleeds are associated with a decline in executive functioning in older adults. Cerebrovasc Dis. 2014;38(5):377-383. doi: 10.1159/000368998.
24. van Veluw SJ, Charidimou A, van der Kouwe AJ, et al. Microbleed and microinfarct detection in amyloid angiopathy: a high-resolution MRI-histopathology study. Brain. 2016;139(Pt 12):3151-3162. doi: 10.1093/brain/aww229.
25. Boulouis G, Charidimou A, Van Veluw S, et al. Imaging the acute formation of a cortical microbleed in cerebral amyloid angiopathy. JAMA Neurol. 2017;74(1)120-121. doi: 10.1001/jamaneurol.2016.3445.

Authors' ORCID iDs and academic degrees
Pavel Gavriliuc, MD, PhD Applicant, Assistant Professor of Neurology – https://orcid.org/0000-0002-7484-1481.
Mihail Gavriliuc, MD, PhD, Professor of Neurology – https://orcid.org/0000-0002-5789-2842.
Stanislav Groppa, MD, PhD, Academician, Professor of Neurology – https://orcid.org/0000-0002-2120-2408.
Ronen Leker, MD, PhD, FAHA, Professor of Neurology – https://orcid.org/0000-0003-4794-0334.

Authors' contribution
PG and RL researched literature and conceived the study. RL wrote the first draft of the manuscript. MG and SG revised and approved the manuscript. All the authors were involved in protocol development, patients' recruitment, and data analysis, reviewed, edited and approved the final version of the manuscript.

Funding
This study was supported by the Peritz and Chantal Scheinberg Cerebrovascular Research Fund.

Ethical approval and Informed consent
The ethics committee of Hadassah-Hebrew Univerity Medica center approved this study and waived the need for obtaining informed consent. Guarantor: RL.

Conflict of Interests
All the authors declare that there is no conflict of interests.

DOI: 10.5281/zenodo.3958531
UDC: 611.147.33

Vena saphena magna – peculiarities of origin, trajectory and drainage

***Anastasia Bendelic, Ilia Catereniuc**

Department of Anatomy and Clinical Anatomy
Nicolae Testemitanu State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova

Authors' ORCID iDs, academic degrees and contributions are available at the end of the article

*Corresponding author: anastasia.bendelic@usmf.md

Manuscript received July 25, 2020; revised manuscript August 14, 2020; published online August 26, 2020

Abstract

Background: *Vena saphena magna* (VSM) – one of the two superficial venous collectors of the lower limb, the longest vein of the human body, is often accompanied by parallel veins, of which clinical significance may be different. The objective of the study was to investigate the individual anatomical variability of the VSM, on macroscopic aspect, in cadavers, of which variability is important for the vascular surgeon and / or for the cardiac surgeon.
Material and methods: This study was conducted on 22 formolized lower limbs using classical dissection methods. The observed anatomical variants were recorded and photographed.
Results: The dorsal venous arch of the foot, the origin of the VSM, was double in 2 cases (9.1%), and it was absent in one case (4.55%), thus two dorsal metatarsal veins continued proximally with two medial marginal veins. In the leg, VSM was double in one case (4.55%), and in other 14 cases (63.63%) it was accompanied by accessory saphenous veins. In the thigh, it was double in 3 cases (13.6%), and in 10 cases (45.5%) it was accompanied by accessory saphenous veins. The saphenofemoral junction was located at 4.23±0.64 cm distance from the pubic tubercle; at 12.25±1.1 cm away from the anterior superior iliac spine and at 4.3±0.65 cm below the middle of the inguinal ligament.
Conclusions. The anatomical variability of the VSM includes its duplicity and/or presence of the accessory saphenous veins. The dorsal venous arch may be double or absent. The saphenofemoral junction is relatively fixed in relation to the neighboring bone landmarks.
Key words: great saphenous vein, accessory saphenous veins.

Cite this article
Bendelic A, Catereniuc I. *Vena saphena magna* – peculiarities of origin, trajectory and drainage. *Mold Med J.* 2020;63(3):26-31. doi: 10.5281/zenodo.3958531.

Introduction

The venous anatomy of the lower extremity is substantially more variable and complicated than the corresponding arterial anatomy. The lower limb venous system includes superficial, deep, and perforating veins. The two major superficial veins of the lower limb, the great and small saphenous veins, are located above the deep or muscular fascia, within the subcutaneous tissue. The deep veins lie beneath the muscular fascia and accompany all major arteries. The perforating veins penetrate the muscular fascia and connect the superficial and deep veins [1-4]. A series of bicuspid valves together with venous muscle pumps ensure the return of blood against gravity to the heart. [5].

The *vena saphena magna* or great saphenous vein (GSV), the longest vein in the body, arises from the medial aspects of the dorsal pedal venous arch and empties into the femoral vein just below the inguinal ligament. During its course, it ascends anterior to the medial malleolus and along the medial border of the tibia, then it passes posterior to the medial condyle of the femur, and further it travels along the medial aspect of the thigh. In the proximal segment of the femoral trigon, the GSV pierces the *fascia cribrosa,* transverses the saphenous opening of the *fascia lata* and drains into the femoral vein forming the saphenofemoral junction [1-3]. The GSV lies in the saphenous compartment that is bordered superficially by saphenous fascia and deeply by the muscular fascia, but its tributaries are external to the compartment [6, 7].

The GSV remains an essential component in strategies for coronary artery bypass grafting in humans. The vessel is used alone or in combination with arterial grafts and has the advantage of being available autologous vascular tissue in most patients in need of such surgeries [5]. The clinical usage of the GSV has made its anatomical variations noteworthy.

The GSV is often accompanied by its tributaries and at times tributaries can be confused with the GSV or be mistaken for GSV duplication. Accessory saphenous veins, tributaries of the great saphenous vein, also may be important in the pathophysiology of the chronic venous disease. There are two main saphenous tributaries in the calf, the anterior (or anterior arch vein) and the posterior (or posterior arch (Leonardo`s) vein) accessory great saphenous veins, that join the GSV just distal to the knee [8, 9]. The clinically important posterior tibial perforating veins (Cockett perforators) join the posterior arch vein rather than the main trunk of the GSV [3]. In the thigh, the anterior and posterior accessory great saphenous veins ascend parallel to the GSV, external to the saphenous fascia [8, 9]. The venous drainage from the perineum and lower abdominal wall (superficial

external pudendal, superficial epigastric and superficial circumflex iliac veins) commonly joins the GSV near the saphenofemoral junction. The newly introduced term *confluens venosus subinguinalis* (confluence of superficial inguinal veins) is a part of the GSV bordered by two valves: terminal valve, located close to the estuary of the vein into common femoral vein, and preterminal valve situated 3-5 cm against flow [9].

Numerous and variable perforating veins of the lower limb can be grouped into three groups of clinical significance – those of the foot, the calf and thigh perforators. The foot perforators are unique in that they normally direct flow toward the superficial veins [10, 11], while all others normally direct flow to the deep system. The medial calf perforators, including the paratibial (Sherman and Boyd perforators) and the posterior tibial perforators (Cockett perforators), are clinically most important. The perforators of the adductor canal (hunterian perforators) connect the GSV proximal to the knee with femoral or popliteal vein.

A thorough understanding of the high variable venous anatomy is essential for understanding the underlying pathophysiology as well as in directing treatment. Knowledge of anatomical variability of great saphenous vein is of great importance when assessing the surgical anatomy of the GSV.

As a consequence, the objective of this study was to evaluate anatomical variation of the GSV, its origin, course, tributaries and perforators that would be of great help in planning varicose vein treatment and coronary bypass procedures where it is used as autograft.

Material and methods

In the present study, twenty-two formolized lower limbs were dissected at the Department of Human Anatomy, in order to study the origin, course, tributaries and perforators of the GSV. The dissection of the GSV was performed by traditional techniques. After exposing the vein, we measured its external diameters, as well as distances from the saphenofemoral junction to the adjacent landmarks. The anatomical variants were recorded, analyzed and compared with those of the previous studies.

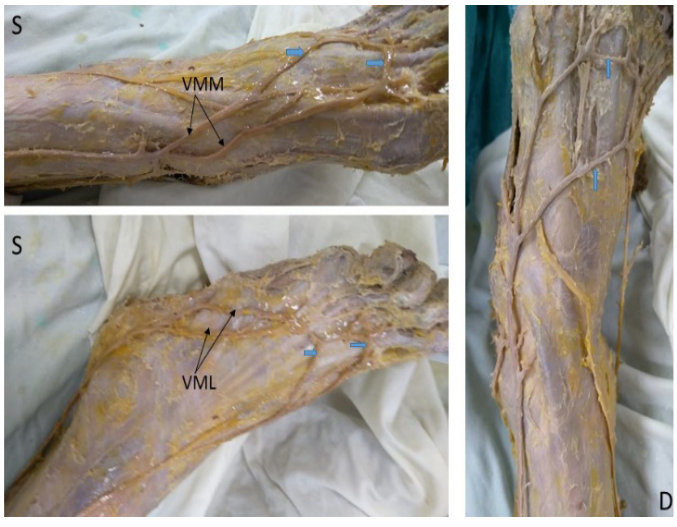

Fig. 1. Double dorsal pedal venous arch (arrows) that continues proximally with double medial and lateral marginal veins

VMM – venae marginales mediales, VML – venae marginales laterals.

Results

At the confluence of the medial extremity of the dorsal pedal venous arch with the *vena digitalis dorsalis pedis prima*, the medial marginal vein was formed, that ascends in front of the medial malleolus to continue proximally with the GSV. Such a situation was found in 86.35% of cases (19 limbs). In 9.1% of cases (2 limbs) at the dorsal faces of both feet of the same male corpse the double dorsal venous arch was found (fig. 1). It continued proximally with the double medial and lateral maginal veins, but at the distal extremity of the medial malleolus two medial marginal veins joined to form a single venous trunk that continued cranially with the GSV. In 4.55% of cases (1 limb) the dorsal venous arch was absent, and two dorsal metatarsal veins continued cranially with two medial marginal veins, which in turn continued in the calf with double GSV (fig. 2).

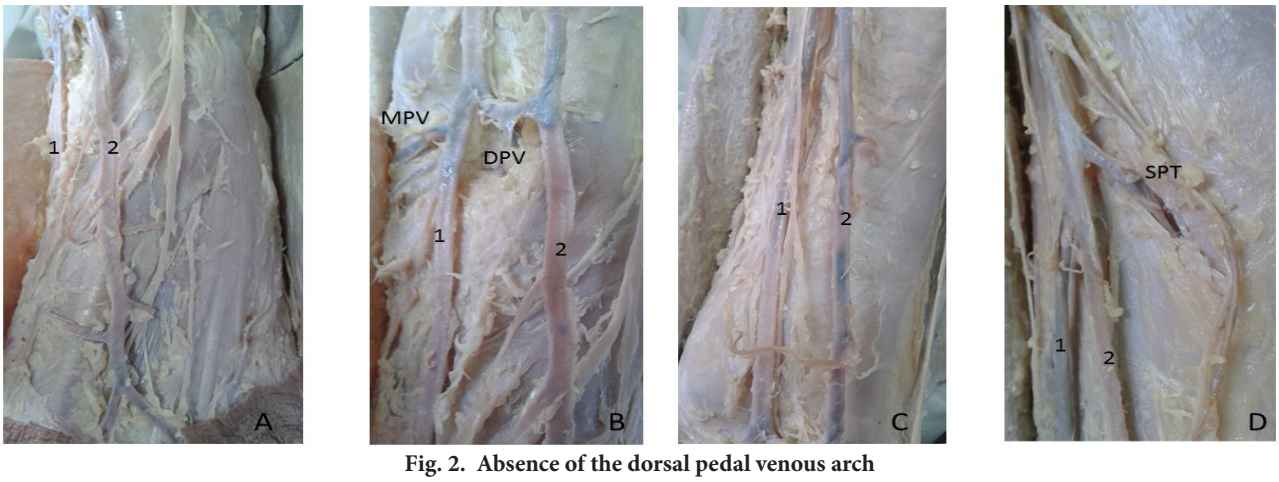

Fig. 2. Absence of the dorsal pedal venous arch

A) Two medial marginal veins on the dorsal surface of the foot, B) Two medial marginal veins at the ankle, with a communicating vein between them, receiving a dorsal perforating vein of the ankle, C) Two GSV in the distal third of the calf, D) Two GSV in the proximal third of the calf.

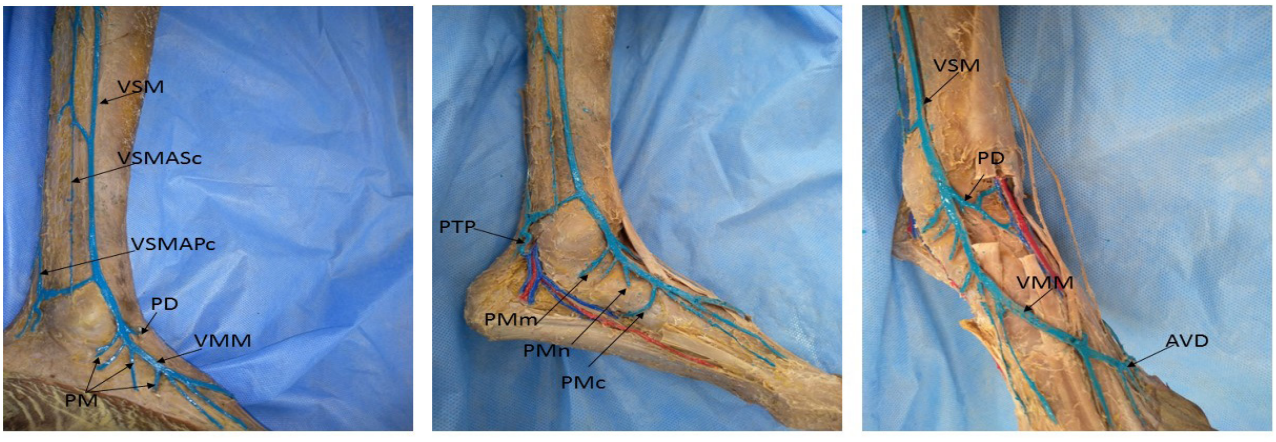

Fig. 3. The medial marginal vein and medial foot perforating veins

ADV – arcus venosus dorsalis pedis, VMM – vena marginalis medialis, PM (PMm, PMn, PMc) – venae perforantes pedis mediales, PD – vena perforans tarsalis dorsalis, PTP – vena perforans cruris tibialis posterior, VSM – vena saphena magna, VSMAPc – vena saphena magna accessoria posterior cruris, VSMASc – vena saphena magna accessoria superficialis cruris.

The medial marginal vein commonly received 3-4 medial foot perforating veins that connected the medial marginal vein with the medial plantar veins. According to the topography there were malleolar, navicular and cuneiform perforating veins. At the ankle, the medial marginal vein also received a dorsal perforating vein that connected the above-mentioned vein with the anterior tibial or / and dorsal pedal veins (fig. 3).

In the calf the diameter of the GSV was ranged from 1 mm to 4 mm, and it was accompanied by the saphenous nerve. In 31.82% of cases (7 limbs) the GSV was solitary, in 4.55% of cases (1 limb) it was double, and in 63.63% (14 limbs) it was accompanied by accessory saphenous veins (fig. 4).

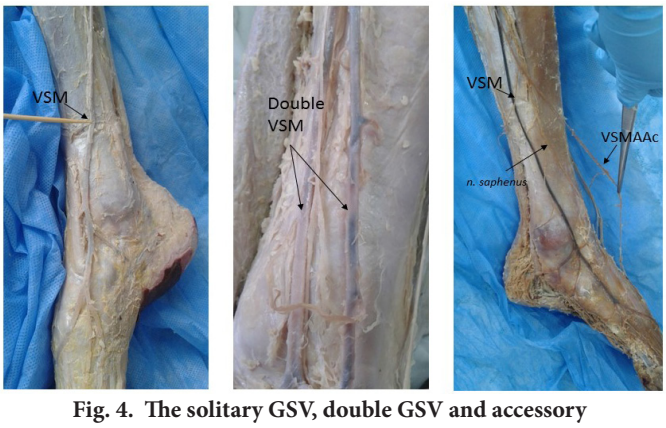

Fig. 4. The solitary GSV, double GSV and accessory saphenous veins of the calf

VSM – vena saphena magna, VSMAAc – vena saphena magna accessoria anterior cruris.

Among the tributaries of the GSV were the anterior accessory great saphenous vein of the leg (11 limbs), the posterior accessory great saphenous vein of the leg (13 limbs), and the superficial accessory great saphenous vein of the leg (2 limbs). The anterior accessory saphenous vein was the only accessory vein in 9.1% (2 limbs), the posterior accessory saphenous vein was the only accessory vein in 13.6% (3 limbs), both the anterior and posterior accessory saphenous veins were present in 40.9% (9 limbs). The posterior accessory saphenous vein was accompanied by the superficial accessory saphenous vein in 4.55% (1 limb), and three accessory saphenous veins were present in 4.55% (1 limb), (tab. 1).

In the distal third of the calf the posterior tibial perforating veins (Cockett perforators) connected to the GSV or to the posterior accessory great saphenous vein were found, in the proximal third of the calf the paratibial perforating veins were observed.

A short segment of the GSV ascending cranially posterior to the medial condyle of the femur, was connected to the small saphenous vein by 1-2 intersaphenous veins (fig. 5).

In the thigh the diameter of the GSV was ranged from 2.5 mm to 6 mm. A single trunk of the GSV was observed in 40.9% of cases (9 limbs), a duplicated vein was found in 13.6% of cases (3 limbs), and in 45.5% (10 limbs) the GSV was accompanied by the anterior or superficial accessory great saphenous veins. The anterior accessory great saphenous vein of the thigh was identified in 40.9% (9 limbs), the superficial accessory saphenous vein was observed in 9.1% (2 limbs), but the posterior accessory saphenous vein of the

Table 1

The incidence of the duplicated and accessory great saphenous veins

	Double GSV*	AAGSV*	PAGSV*	SAGSV*
In the calf	4.55% (1 limb)	50% (11 limbs)	59.1% (13 limbs)	9.1% (2 limbs)
In the thigh	13.6% (3 limbs)	40.9 (9 limbs)	68.2% (15 limbs)	9.1% (2 limbs)

*GSV – great saphenous vein; *AAGSV – anterior accessory great saphenous vein; *PAGSV – posterior accessory great saphenous vein; *SAGSV – superficial accessory great saphenous vein.

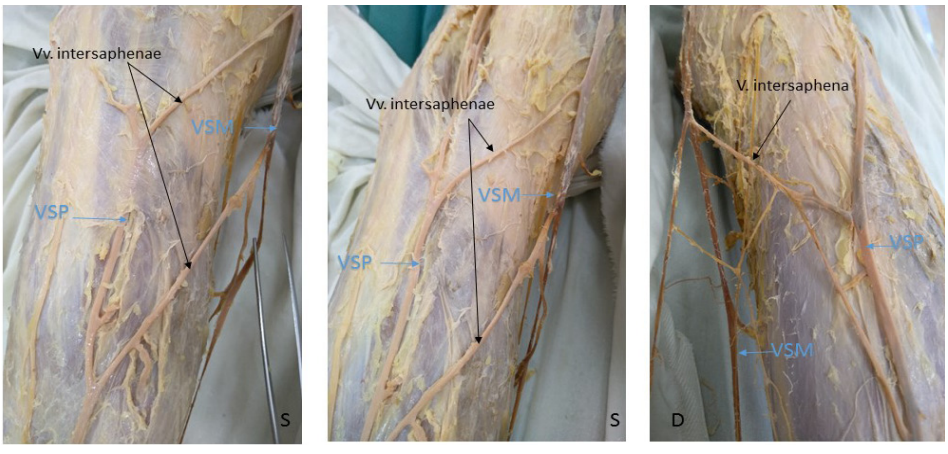

Fig. 5. The intersaphenous veins. *VSM – vena saphena magna, VSP – vena saphena parva*

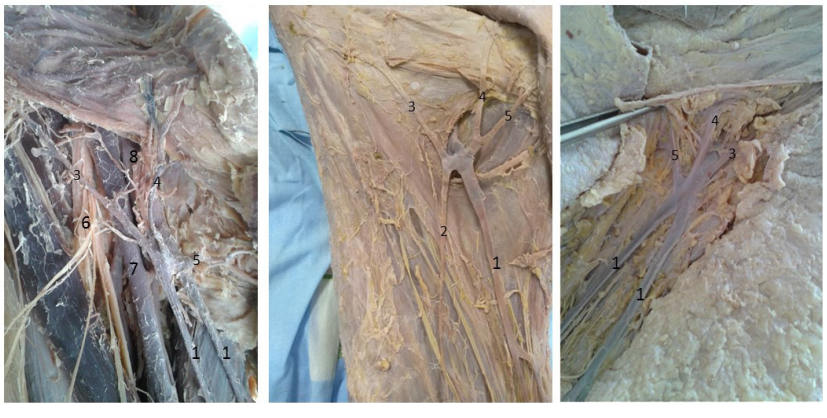

Fig. 6. "Venous star" described by Paturet
1 – vena saphena magna; 2 *– vena saphena magna accessoria anterior femoris,* 3 *– vena circumflexa ilium superficialis,*
4 *– vena epigastrica superficialis,* 5 *– vena pudenda externa superficialis,*
6 *– nervus femoralis,* 7 *– arteria femoralis,* 8 *– vena femoralis*

thigh was found in 68.2% (15 limbs) (tab. 1). In case of the double GSV, as well as in case of presence of the anterior or superficial accessory saphenous veins, the *confluens venosus subinguinalis* had the appearance of the "venous star" described by Paturet (fig. 6). The confluence received the following tributaries: the superficial circumflex iliac vein in 72.7% (16 limbs), the superficial epigastric vein in 68.2% (15 limbs), the superficial external pudendal vein in 68.2% (15 limbs), the deep external pudendal vein in 9.1% (2 limbs), the anterior accessory great saphenous vein of the thigh in 40.9% (9 limbs), the superficial accessory great saphenous vein of the thigh in 4.55% (1 limb). The posterior accessory great saphenous vein drained into the GSV distinctly below the saphenofemoral junction (from 4 cm to 10 cm).

One of the characteristics of the saphenofemoral junction is its anatomical fixity in relation to the adjacent anatomical landmarks: the anterior superior iliac spine, pubic tubercle and inguinal ligament (fig.7). The distance from the saphenofemoral junction to the anterior superior iliac spine showed an average value of 12.25±1.1 cm, the distance from the junction to the pubic tubercle was 4.23±0.64 cm, and the distance from the junction to the middle of the inguinal ligament had an average value of 4.3±0.65 cm.

Fig. 7. Distances from the saphenofemoral junction to the adjacent anatomical landmarks
SIAS – spina iliaca anterior superior, TB – tuberculum pubicum,
mij lig. in. – middle of the *ligamentum inguinale*

Discussion

Although the venous patterns described in this study generally resembled previous descriptions, there were some differences between our own observations and those in the literature.

We observed some variation in the origin of the GSV. The presence of double dorsal venous pedal arch or its absence have not been previously mentioned in the literature. This doubling may have a physiological significance in the venous return mechanism. In turn, the absence of the dorsal venous pedal arch may be accompanied by double GSV.

A phlebographic study showed that a single trunk of the GSV was present in the calf in 45% of patients and in the thigh in 65% [12]. In our study the GSV was solitary in the calf in 31.9%.and in the thigh in 40.9%.

A rare anatomical variation which consists in duplication of the GSV in our study was found in the calf in 4.55% (1 limb) and in the thigh in 13.6% (3 limbs). Two criteria make the diferences between duplicated GSV and accessory saphenous vein. The two GSV lie in the same plane, parallel to the skin and run along the deep or muscular fascia. These two GSV will also have the same caliber draining a common cutaneous territory. An accessory saphenous vein is usually smaller in size and does not drain the same cutaneous territory [13]. Though the literature suggests that duplication of GSV is present in 1-20% of cases [14], true duplication defined by Union International de Phlebologie (UIP) in 2006 is present in 1.6 – 2% of cases [14]. The duplication of GSV can be described in three types based on its relationship with the femoral vein: duplication with a common junction, duplications with separate junctions, insular duplication with a common junction [13]. In our study the duplicated GSV drains in femoral vein separately at the saphenofemoral junction.

According to Yuce et al. [15] the frequency of posterior accessory great saphenous vein of the leg is more common in the left limbs (54%) than the right limbs (45%). The type of joining of the accessory veins to the GSV is above the knee level in 4%, at the knee level in 14%, and below the knee level in 70% of patients [15]. In our study the posterior accessory vein of the leg was identified in 59.1% (13 limbs), but four of them drained into the small saphenous vein.

Mühlberger D. et al. [16] found an average of 3.7 tributaries veins entering the GSV close to the saphenofemoral junction. The superficial epigastric vein was found in 78.3% of limbs, the superficial external pudendal vein was most frequent and was identified in 90.3% of limbs, the superficial circumflex iliac vein was found in 82.9% of limbs, the anterior and posterior accessory great saphenous veins were less frequently observed. The anterior accessory vein discharged into the GSV in 50.7%, and the posterior accessory vein in 67.7% [16]. According to Souroullas P. et al. [17], the median number of the saphenofemoral junction tributaries is 4, in 43% of cases at least one tributary drains directly into the femoral vein, commonly the deep external pudendal vein (91.9%). The anterior accessory great saphenous vein of the thigh was identified in 35.8%, and the posterior accessory great saphenous vein of the thigh in 53.8% [17]. In our study we found the anterior accessory great saphenous vein in 40.9%, the posterior accessory great saphenous vein in 68.2%, and the superficial accessory great saphenous vein in 9.1%. A topic of discussion deals with posterior accessory great saphenous vein, that was situated distinctly below the saphenofemoral junction (from 4 cm to 10 cm).

According to Ndiaye A. et al. [18], on average, the saphenofemoral junction is projected out 10.88 cm from the anterior superior iliac spine, 3.83 cm from the pubic tubercle and 4.19 cm from the inguinal ligament. In our study the distances from the saphenofemoral junction to the adjacent landmarks were respectively 12.25 cm, 4.23 cm and 4.3 cm.

Mühlberger D. et al. [16] attests a complete "venous star" with five tributaries discharging into the GSV in 18.4% [16], but Ndiaye A. et al. states that a conventional type in a "vein star" shape is not the most frequent [18].

Conclusions

Dissection of 22 cadaveric feet revealed the presence of a previously unreported double dorsal venous pedal arch and its absence. This doubling may have a physiological significance in the venous return mechanism. In turn, the absence of the dorsal venous pedal arch may be accompanied by double GSV.

Duplication and persistence of accessory GSV were two major clinically significant anatomical variations of the GSV. Locating such variations of GSV is a challenging task for both diagnostic and therapeutic tactics.

References

1. Standring S, Borley NR, Gray H, editors. Gray's anatomy: the anatomical basis of clinical practice. 40th ed., anniversary ed. [Edinburgh]: Churchill Livingstone/Elsevier; 2008. 1576 p. ISBN: 978-0443066849.
2. Moore KL, Dalley AF, Agur AMR. Clinically oriented anatomy. 6th ed. Baltimore: Lippincott Williams & Wilkins; 2010. 1134 p. ISBN: 978-0-7817-7525-0.
3. Meissner MH. Lower extremity venous anatomy. Semin Intervent Radiol. 2005;22(3):147-156. doi: 10.1055/s-2005-921948.
4. Black CM. Anatomy and physiology of the lower-extremity deep and superficial veins. Tech Vasc Interv Radiol. 2014;17(2):68-73. doi: 10.1053/j.tvir.2014.02.002.
5. Portugal IB, Ribeiro Ide L, Sousa-Rodrigues CF, Monte-Bispo RF, Rocha AC. Distribution of saphenous vein valves and its practical importance. Rev Bras Cir Cardiovasc. 2014;29(4):564-568. doi:10.5935/1678-9741.20140038.
6. Caggiati A. Fascial relations and structure of the tributaries of the saphenous veins. Surg Radiol Anat. 2000;22(3-4):191-196. doi:10.1007/s00276-000-0191-3.
7. Caggiati A. The „interfascial" veins of the lower limbs. Ital J Anat Embryol. 2005;110 (2 Suppl 1):97-102.
8. Caggiati A, Bergan JJ, Gloviczki P, et al. Nomenclature of the veins of the lower limb: extensions, refinements, and clinical application. J Vasc Surg. 2005;41(4):719-724. doi: 10.1016/j.jvs.2005.01.018.
9. Kachlik D, Pechacek V, Baca V, Musil V. The superficial venous system of the lower extremity: new nomenclature. Phlebology. 2010;25(3):113-123. doi: 10.1258/phleb.2009.009046.
10. Uhl J-F, Gillot C. Anatomy of the foot venous pump: physiology and influence on chronic venous disease. Phlebology. 2012;27(5):219-30. doi:10.1258/phleb.2012.012b01.

11. Uhl J-F, Lo Vuolo M, Gillot C. Anatomy of foot and ankle perforator veins. Phlebolymphology [Internet]. 2017;24(2):105-112 [cited 2019 Oct 19]. Available from: https://www.phlebolymphology.org/anatomy-of-foot-and-ankle-perforator-veins/
12. Shah DM, Chang BB, Leopold PW, Corson JD, Leather RP, Karmody AM. The anatomy of the greater saphenous venous system. J Vasc Surg. 1986;3(2):273-283.
13. Chen SS, Prasad SK. Long saphenous vein and its anatomical variations. Australas J Ultrasound Med. 2009;12(1):28-31. doi:10.1002/j.2205-0140.2009.tb00004.x
14. Kockaert M, de Roos KP, van Dijk L, Nijsten T, Neumann M. Duplication of the great saphenous vein: a definition problem and implications for therapy. Dermatol Surg. 2012;38(1):77-82. doi:10.1111/j.1524-4725.2011.02154.x.
15. Yuce I, Oguzkurt L, Eren S, et al. Assessment of posterior accessory great saphenous vein of the leg using ultrasonography: a preliminary study. Surg Radiol Anat. 2016;38(1):123-126. doi:10.1007/s00276-015-1523-7.
16. Mühlberger D, Morandini L, Brenner E. Venous valves and major superficial tributary veins near the saphenofemoral junction. J Vasc Surg. 2009;49(6):1562-1569. doi: 10.1016/j.jvs.2009.02.241.
17. Souroullas P, Barnes R, Smith G, Nandhra S, Carradice D, Chetter I. The classic saphenofemoral junction and its anatomical variations. Phlebology. 2017;32(3):172-178. doi:10.1177/0268355516635960.
18. Ndiaye A, Ndiaye A, Ndoye JM, Diarra O, Diop M, et al. The arch of the great saphenous vein: anatomical bases for failures and recurrences after surgical treatment of varices in the pelvic limb. About 54 dissections. Surg Radiol Anat. 2006;28(1):18-24. doi:10.1007/s00276-005-0046-z.

Authors' ORCID iDs and academic degrees
Anastasia Bendelic, MD, Assistant Professor of Anatomy – https://orcid.org/0000-0002-2838-3168.
Ilia Catereniuc, MD, PhD, Professor of Anatomy – https://orcid.org/0000-0002-5479-4198.

Authors' contribution
AB designed the trial, performed it, interpreted the data, drafted the first manuscript. IC interpreted the data, revised the manuscript. Both authors revised and approved the final version of the manuscript.

Funding
The study was supported by *Nicolae Testemitanu* State University of Medicine and Pharmacy. The trial was the authors' initiative. The authors are independent and take the responsability for the integrity of the data and accuracy of the data analysis.

Ethics approval and consent to participate
The protocol of this study was approved by Ethics Research Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova (protocol No 30/32 of 29.05.2014).

Conflict of Interests
The authors declare the absence of conflict of interests.

DOI: 10.5281/zenodo.3958549
UDC: 543.544.5:547.794.3

Development and validation of the high-pressure liquid chromatographic method for the quantitative determination of propylthiohinothiadiazole

Andrei Uncu

Scientific Center for Drug Research, *Nicolae Testemitanu* State University of Medicine and Pharmacy Chisinau, the Republic of Moldova

Author's ORCID iD, academic degrees and contributions are available at the end of the article

*Corresponding author: andreiuncu1990@gmail.com

Manuscript received July 28, 2020; revised manuscript August 14, 2020; published online August 26, 2020

Abstract

Background: Propylthiohinothiadiazole is a thiadiazole derivative with pronounced antimycobacterial action. Substance is researched in order to develop methods of analysis and standardization, analytical documents regulating quality for use as an active principle in the development of pharmaceutical forms.
Material and methods: 3 series of propylthiohinothiadiazole, internal reference standard of 2-(propylthio)-5H-[1,3,4]-thiadiazole[2,3-b]-quinazolin-5-one substance with concentration 99.98%, chromatographic system Shimadzu LC-20AD high-pressure liquid chromatography (HPLC) with UV-VIS detector, acetonitrile and dimethylsulfoxide of purity grade "pro HPLC analysis" (Sigma Aldrih and Merck), bidistilled purified water.
Results: Linearity is proved for concentrations of 5-30 μg/ml, the linear regression equation is y=36472x-44580, R^2=0.9999. The limit of detection is 0.729 μg/ml and the limit of quantification is 2.210 μg/ml. It was established that the method is accurate (mean recovery values at 80%, 100% and 120% concentration levels were close to 100%). The accuracy of the method was expressed by repeatability and intermediate accuracy. The variation of the chromatographic conditions established that the method is robust. For all validation parameters, relative standard deviation was less than 1.
Conclusions: The validation results show that the developed HPLC method is simple, fast, accurate and reproducible.
Key words: high-pressure liquid chromatography, propylthiohinothiadiazole, dosing, validation.

Cite this article
Uncu A. Development and validation of the high-pressure liquid chromatographic method for the quantitative determination of propylthiohinothiadiazole. *Mold Med. J.* 2020;63(3):32-37. doi: 10.5281/zenodo.3958549.

Introduction

Tuberculosis remains one of the most common infectious diseases worldwide. According to the World Health Organization (WHO), there is an incidence of 10 million cases, which would be equivalent to 133 cases per 100.000 people [1]. The Republic of Moldova is among the 18 countries in the European Region where tuberculosis control is a priority and among the 30 countries in the world with a high burden of multidrug-resistant tuberculosis (MDR TB) [2]. Currently, the WHO recommends 11 common international drug names for the treatment of tuberculosis, of which 4 are first-line (isoniazid, rifampicin, pyrazinamide, ethambutol) and 7 are second-line (aminoglycosides, quinolones, thioamides, etc.) [3, 4].The evolution of pharmaceutical technologies and clinical trials have reduced the duration of treatment for tuberculosis from 24 months to 6-8 months of intensive treatment, the basic method being the administration of therapeutic combinations, with preparations from the first and second line simultaneously [5]. However, the latest studies show a rate of resistance to treatment in about 73% of patients, 35% of whom have multi-drug resistance since the first weeks of treatment [3]. There are several causes: non-compliance with treatment; stopping or abandoning treatment at the first reduction of symptoms, then resuming it after a while; late diagnosis; mycobacterial mutations over time and lack of alternative medication [3, 4, 6].

Thus, finding and obtaining new compounds with antimycobacterial potential is an important and very current research topic. A promising direction is the development of new substances with a high activity against MDR mycobacteria by valuing thiadiazole (quinazolinone) derivatives with structures similar to Triptantrine alkaloid [7, 8].

Propylthiohinothiadiazole (2-(propylthio)5H-[1,3,4] thiadiazolo-[2,3-b]quinasolin-5-one) is a thiadiazole derivative, synthesized in the Laboratory of Organic Synthesis and Biopharmaceuticals of the Institute of Chemistry, Chisinau,being one of the most promising substances in the series of about 80 derivatives obtained. This compound has a pronounced antimycobacterial activity ($\wp$99%) and low toxicity [9, 10, 11]. The substance is being researched in order to develop methods of analysis and standardization, of the Analytical Documents of Quality Standardization (DAN) for use as an active principle in the development of antimycobacterial pharmaceutical forms.

Evolutionary development of analytical methods provides researchers a wide range of modern, advantageous and cost-effective techniques. High-pressure liquid chromatography (HPLC) is one of them, being based on the separation of components between two phases under pressure,

allows the identification and assay of individual substances in pharmaceutical forms, but also the possible impurities from synthesis or degradation. Quantitative determination of medicinal substances is one of the most important stages of the analysis, being also the main quality parameter. The requirements assigned to a quantitative method in the process of pharmaceutical analysis are stipulated in various guidelines, which regulate the analytical procedures. The validation of analytical methods, in accordance with the guidelines of the International Conference on Harmonization (ICH), aims to "demonstrate that the method is appropriate to the proposed purpose" [12].

Propylthiohinothiadiazole, being a newly-synthesized compound, has no formalized methods of analysis. Thus, this study proposes the development for the first time of the HPLC method for propylthiohinothiadiazole assay and its validation in accordance with the provisions of the ICH guidelines.

Material and methods

The experimental researches were performed within the Laboratory of analysis, standardization and control of drugs (LASCD) of *Nicolae Testemitanu* State University of Medicine and Pharmacy.

The elaboration of the propylthiohinothiadiazole assay method was performed based on the requirements of the ICH guideline "Q2R1: For analytical and validation procedures" [12].

The study used the drug substance propylthiohinothiadiazole, synthesized in the Laboratory of Organic Synthesis and Biopharmaceuticals of the Institute of Chemistry, synthesis series: MF1-001, MF1-002, MF1-003; internal reference standard for 2-(propylthio)5H-[1,3,4]thiadiasolo-[2,3-b] quinasolin-5-one substance, purified by recrystallization from LASCM (concentration 99.98%).

Apparatus: Shimadzu LC-20AD HPLC chromatographic system, *Zorbax Eclipse Plus* C18 analytical column, 5 mm, 4.6 x 250 mm was used; UV-VIS detector, wavelength – 300 nm.

Chemicals: Acetonitrile (ACN) purity grade "pro HPLC analysis" (Sigma Aldrich), dimethylsulfoxide (DMSO) purity grade "pro analysis" (Merck), bidistilled purified water.

Chromatography conditions: The mobile phase was prepared by mixing ACN with purified water in proportions of 80:20 by volume, filtering the solution under vacuum through a *Millipore XF 5423050* capron filter (0.2-0.45μm), and degassing in the DONAU-LAB SONIC DLS 660 T/H. The temperature of the chromatographic column was 30°C; injection volume 20 μl; mobile phase flow – 1 ml/min. The retention time was 4.5 min.

Preparation of the standard solution: Approximately 0.001 g (exact mass) of the internal reference standard is placed in a 25.0 ml volumetric flask, dissolved in 5 ml of DMSO, stirred until dissolved, 10 ml of mobile phase is added and mixed, then made up with the same solvent to the quota and mixed (stock standard solution). Place 5 ml of standard stock solution in a 10 ml volumetric flask and make up to the mark with the same solvent. The solution is used freshly prepared.

Chromatographic system utility control: Before performing the analysis, the chromatographic column is stabilized with mobile phase for 10 min, then chromatographed 20 μl standard solution until an established retention time (4.5 min) is obtained after two consecutive injections; at least 3 chromatograms are obtained. The chromatographic system is considered useful when: the performance of the column, calculated after the propylthiohinothiadiazole peak, is at least 2000 theoretical plates; the asymmetry coefficient of the peak, calculated at the level ½ of the peak height does not exceed 1.5; the relative standard deviation of the peak area does not exceed 2.0%.

Preparation of the sample solution: Approximately 0.001 g (exact mass) of propylthiohinothiadiazole was accurately weighed and transferred into a 25.0 ml volumetric flask, 5 ml of DMSO was added and stirred until the substance dissolved. 10.0 ml of mobile phase was added, then it was mixed and made up to the level with the same solvent. 5.0 ml of obtained solution was placed into a 10 ml volumetric flask and made up to the mark with the same solvent.

Quantitative determination: Each 20 μl of sample solution and standard solution were chromatographed, obtaining at least 5 chromatograms of each solution under the same conditions indicated above. Then it was determined the mean value of the propylthiohinothiadiazole peak area from the chromatograms of sample solution (San) and standard solution (Sst). The chromatograms of the standard and sample solutions with a concentration of 20 μg/ml are shown in fig.1.

The propylthiohinothiadiazole content (X%) in the substance is determined according to the formula:

$$X\% = \frac{S_{an} * m_{st} * W_{an} * P_0 * [100-U] * 100}{S_{st} * m_{an} * a_x * W_{st} * 100}$$ in which:

S_{an} – the mean value of the peak area, calculated from the chromatograms of the sample solution;

S_{st} – the average value of the peak area, calculated from the chromatograms of the standard solution;

m_p – mass of the substance, g;

U_s – humidity of the standard substance, %;

m_{st} – mass of standard substance, g;

P_0 - active substance content, %;

W_{st} and W_{an} – the volumes of solutions for the standard and the analyte.

Validation of the method. The method was validated according to the ICH guide, being determined by the parameters: linearity, accuracy, precision, sensitivity (LOQ and LOD) and robustness, solution stability [12].

Linearity. The linearity of the results of an analysis procedure represents its ability to obtain results directly proportional to the analyte concentration in the sample. The linearity of the HPLC method of propylthiohinothiadiazole assay on the concentration ranges 5-30 μg/ml was investigated. Thus, the standard stock solutions of propylthiohi-

nothiadiazole 40 μg/ml were initially prepared. From the stock standard solution, 5 samples with concentrations 5, 10, 15, 20 and 30 μg/ml were prepared by dilution, using mobile phase as solvent and then were injected into the chromatograph, obtaining chromatograms and peak areas. The determinations were performed in triplicate, being constructed by the calibration curve (fig. 2). Linear regression analysis was used to evaluate the linearity of the calibration curve using the least squares method.

Accuracy. Accuracy is defined as a characteristic of the approximation of analytical results to true value and is a measure of the deviation of the mean value found by analysis from true value. It is evaluated by applying the method to samples with known concentrations [13]. To determine the accuracy of the HPLC propylthiohinothiadiazole assay method, the standard addition (sample enrichment) method was used by analyzing in triplicate solutions with concentrations of 80%, 100% and 120% (8.0 μg/ml, 10 μg/ml and 12.0 μg/ml) and the percentage recovery of the amount of substance was calculated, the relative standard deviation (RSD) value being evaluated for each concentration level (tab. 3).

Precision. Determination of the precision of the method was performed by evaluating repeatability and intermediate accuracy [14]. Repeatability was determined for 6 samples, at the concentration level of the drug substance of 100%, on the same day, respecting the same conditions (tab. 4). The intermediate accuracy was determined using the same procedure for identical samples, in the same laboratory, by different operators, using different equipment and in a specific time interval. The intermediate accuracy was investigated in 2 different days, under the same conditions, performing 6 determinations (tab. 5).

Sensibility (LOQ and LOD). The limit of detection (LOD) and limit of quantification (LOQ) of propylthiohinothiadiazole were determined by analyzing the substance solutions and measuring the signal-to-noise ratio. The limit of detection (LOD) is the concentration, which is due to the signal/noise ratio of about 3:1, while the limit of quantification (LOQ) is the concentration that gives a signal/noise ratio of about 10:1 with RSD values (n = 3) less than 10%.

Robustness. The robustness of an analytical method is demonstrated by assessing the ability of the method to remain unaffected by small, deliberate variations in parameters. It was determined by varying the following chromatographic conditions: flow rate of the mobile phase by ± 0.1 ml/min, amount of acetonitrile in the mobile phase by ± 2% and column temperature by ± 5°C [12] (tab. 6).

Stability of the solution. The stability of standard and sample analytical solutions was determined by analyzing them immediately after preparation and after 24 hours of refrigeration and room temperature (25°C). Three determinations were performed, the peak areas were evaluated, the analyte concentration in the sample (relative to a freshly prepared reference solution) and the RSD were calculated (tab. 7).

Statistical analysis. Statistical analysis was carried out by using the Statistical Package for the Social Sciences (IBM SPSS Statistics) 10.5 software.

Results and discussion

Method Development and Optimization. Preliminary study of the physicochemical properties of propylthiohinothiadiazole allowed the development of the HPLC analytical method by preliminary selection of chromatographic conditions, including detection wavelength, mobile phase, stationary phase, and sample preparation procedure. For this purpose, a series of tests were performed varying the ratio between acetonitrile and water, optimizing the chromatographic conditions on the *Zorbax Eclipse Plus* C18 column, 5 μm, 4.6 x 250 mm. The results of the method optimization are summarized in tab. 1. The mobile phase consists of acetonitrile and water in a ratio of 80:20 v/v with a flow rate of 1 ml/minute, injection volume 20 μl, running time 6 minutes and column temperature 30^{0}C at wavelength (λ) 300 nm. It was determined that these are the optimal conditions under which propylthiohinothiadiazole was eluted forming symmetrical peaks, resolution, and adequate analysis time, with retention time around 4.5 minutes (fig. 1.A and fig. 1.B).

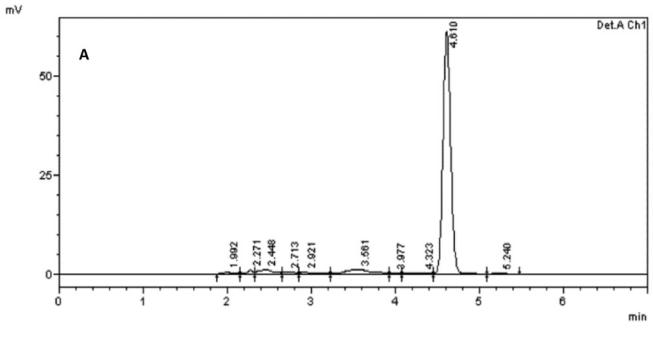

Fig. 1.A. Chromatograms of propylthiohinothiadiazole solutions with concentration 20 μg/ml: A – standard solution.

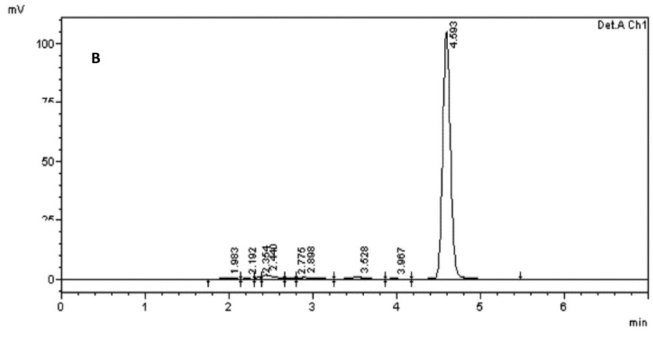

Fig. 1.B. Chromatograms of propylthiohinothiadiazole solutions with concentration 20 μg/ml: B – sample solution.

Linearity. By evaluating the averages of the peak areas obtained in the HPLC determinations, represented in relation to the corresponding concentrations, the calibration graph was obtained. The results of the linearity study (tab. 2, fig. 2) showed a linear relationship over the concentration range of 5-30 μg/ml for propylthiohinothiadiazole.

From the regression analysis, a linear equation was obtained: y=36472x-44580, and the correlation coefficient (r^2) was 0.9999, which indicates a linear relationship between the analyte concentration and the area of the chromatographic peak.

Table 1

Results of the optimization of the HPLC method of propylthiohinothiadiazole dosing

Used Column	Mobile phase	Flow rate	Wavelength	Observation	Result
Nucleosil 100, C-18, 15 µm., 4 x 150 mm	Acetonitrile: water 80:20	1.0 ml/min	235 nm	Poor resolution 1.3	Method rejected
Zorbax Eclipse Plus C18, 5 mm, 4.6 x 250 mm	Acetonitrile: water 80:20	1.0 ml/min	300 nm	Good resolution 2.4	Method accepted
Zorbax Eclipse Plus C18, 5 mm, 4.6 x 250 mm	Acetonitrile: water 65:35	1.0 ml/min	300 nm	Poor resolution 1.8	Method rejected

Table 2

Calibration data for propylthiohinothiadiazole

Solution concentration, µg/ml	Retention time, min	Peak area
5	4.483	135014.0
10	4.487	270657.0
15	4.471	405158.0
20	4.477	541687.0
30	4.479	823649.0

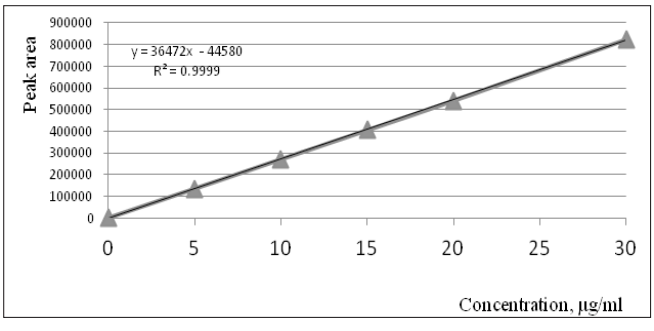

Fig. 2. Calibration curve of standard propylthiohinothiadiazole solution.

Table 3

Results of accuracy of developed method of assay of propylthiohinothiadiazole

Concentration levels, %	Number of determinations	Theoretical concentration of spiked sample, µg/ml	Peak area	Concentration in spiked sample, µg/ml	% Recovery	Average, % RSD
80	1	8	6746764.55	8.045	100.56	100.530
	2	8	6747213.28	8.046	100.58	
	3	8	6739989.94	8.037	100.46	0.061
100	1	10	8433455.69	10.045	100.45	100.710
	2	10	8460275.02	10.077	100.77	
	3	10	8472697.44	10.091	100.91	0.240
120	1	12	10110546.81	12.033	100.28	100.260
	2	12	10108824.57	12.031	100.26	
	3	12	10105489.14	12.027	100.23	0.031
Note: RSD – relative standard deviation						

Accuracy. Determining the accuracy of the analytical quantitative procedure, the selectivity of the results obtained in this method was found to achieve the true value. As indicated in table 3, RSD values are between 0.03-0.24. The triplicate analysis of solutions with concentrations of 80%, 100% and 120% (8.0 µg/ml, 10 µg/ml and 12.0 µg/ml) demonstrated a percentage recovery of the substance of at least 100.23% and maximum 100.91%, values that were within the accepted limits from 98.0% to 102.0%, which indicates the applicability of the method for quantitative analysis.

Precision. The precision expresses the narrowness of the agreement (degree of dispersion, coefficient of variation) between a series of measurements that come from several series of the same homogeneous sample (independent results) under identical working conditions. Precision provides data on random errors and has no relation to the true value. Because all measurements contain random errors, the result of a single measurement cannot be accepted as true. An estimate of this error is required to predict the range in which the true value is found, which is done by repeating the measurement several times. From this process two important parameters are obtained, namely the average value and the variability of the measurements [12]. The results of the determinations showed that the method is accurate within acceptable limits. RSD was calculated for retention time, peak area and concentration, all values being less than 1% (tab. 4 and tab. 5).

Sensibility: detection limit and quantification limit (LOD and LOQ). The limit of detection (LOD) is the smallest amount of analyte in a sample that can be detected, but not necessarily quantified, while the limit of quantity (LOQ) is the smallest amount of analyte in a sample that can be determined quantitatively with adequate accuracy [15]. The results of the determinations showed LOD of 0.729 µg/ml and LOQ of 2.210 µg/ml respectively.

Robustness. The robustness of the analytical method was tested to assess the lack of influence of minor changes

Table 4

Results of determinations of repeatability of the HPLC method for propylthiohinothiadiazole assay

No	Retention time, min	Peak area	Assay, %
1	4.483	8433455.69	100.45
2	4.487	8460275.02	100.77
3	4.471	8472697.44	100.91
4	4.477	8439984.05	100.53
5	4.479	8433455.69	100.45
6	4.484	8439984.05	100.53
Average	4.480	8446641.98	100.60
RSD, %	0.128	0.191	0.188

Table 5

Results of intermediate precision determinations in the validation of the propylthiohinothiadiazole HPLC method

Day 1				Day 2		
No.	**Retention time, min**	**Peak area**	**Assay, %**	**Retention time, min**	**Peak area**	**Assay, %**
1	4.483	8433455.69	100.45	4.495	9275724.35	101.73
2	4.487	8460275.02	100.77	4.937	9303352.96	102.03
3	4.471	8472697.44	100.91	4.922	9189046.32	100.78
4	4.477	8439984.05	100.53	4.956	9388533.41	102.96
5	4.479	8433455.69	100.45	4.988	9307879.46	102.08
6	4.484	8439984.05	100.53	4.943	9268835.80	101.65
Average	4.480	8446641.98	100.60	4.949	9288895.38	101.87
RSD, %	0.128	0.191	0.188	0.450	0.698	0.698

Table 6

Results of the determination of the robustness of the HPLC method for dosing propylthiohinothiadiazole

Variable parameters		Retention time, min	Peak area	Assay, %
Mobile phase flow 0.9 ml/min		4.482	8434755.24	99.72
0,5		4.472	8461005.87	100.03
Acetonitrile: purified water 80:20		4.486	8473097.57	100.17
Acetonitrile: purified water 81.6:18.4		4.479	8439784.07	99.78
Column temperature 30^0 C		4.481	8432915.21	99.70
Column temperature 35^0 C		4.482	8438584.13	99.77
The average value		4.480	8.45E+06	99.86
Standard deviation	S^2	2.19E-05	2.70E+08	3.77E-02
	S	0.005	16418.795	0.194
Relative standard deviation	RSD	0.104	0.194	0.194

Table 7

Results of solution stability testing for HPLC propylthiohinothiadiazole assay method

Determined parameters	Storage period	Retention time, min	Peak area	Assay, %	RSD for the peak area
Standard solution	0 h	4.483	8457894.12	-	0.03
	24 h at 25^0C	4.479	8457387.08	100.09	0.17
	24 h at 4^0C	4.482	8457614.21	100.10	0.07
Sample solution	0 h	4.484	8434755.24		0.05
	24 h at 25^0C	4.484	8432915.21	99.70	0.21
	24 h at 4^0C	4.479	8433089.57	99.71	0.11

in the conditions of chromatography using for obtaining the results of the analysis. The test results showed that at a minor change in the method conditions, such as the composition and flow rate of the mobile phase, the temperature of the column, the method is robust. A good separation has been achieved, and the RSD values are within the limits and do not exceed 2.0% (tab. 6).

Stability of the solution. After testing the stability of the solution, the concentration of propylthiohinothiadiazole in the solutions varied from 99.7% to 100.1% and the RSD was not higher than 1.0%, which indicates a high stability of the sample and standard solutions during 24 hours both by refrigeration and under normal conditions. The results of the solution stability test are presented in Table 7.

Conclusions

The HPLC method of assay of propylthiohinothiadiazole was developed and validated, which proved to be simple, fast, accurate and precise, sensitive and robust. The selected chromatographic conditions and mobile phase provide a good resolution for the test substance. The retention time does not exceed 5 min. During the experiments, the rigors of the ICH guidelines regarding the validation of the analysis methods were taken into account. The developed and validated HPLC method can be included in the Quality Standardization Analytical Documents for the propylthiohinothiadiazole quantitative determination.

References

1. Torfs E, Piller T, Cos P, Cappoen D. Opportunities for overcoming Mycobacterium tuberculosis drug resistance: emerging mycobacterial targets and host-directed therapy. Int J Mol Sci. 2019;20(12):2868. doi: 10.3390/ijms20122868.
2. Alexandru S, Vilc V, Tudor E, et al.; [Ministry of Health of the Republic of Moldova]. Tuberculoza la adult: Protocol clinic național [Tuberculosis in adult: National clinical protocol] [Internet]. Chisinau: The Ministry; 2020. 152 p. (PCN-123) [cited 2020 June 11]. Available from: http://ftiziopneumologie.asm.md/files/u1/PCN-123_Tuberculoza_la_adult.pdf. Romanian.
3. Jnawali HN, Ryoo S. First- and second-line drugs and drug resistance. In: Mahboub BH, Vats MG, editors. Tuberculosis – current issues in diagnosis and management [Internet]. London: IntechOpen; 2013 [cited 2020 June 11]. Available from: https://www.intechopen.com/books/tuberculosis-current-issues-in-diagnosis-and-management/first-and-second-line-drugs-and-drug-resistance
4. Kumar K, Kon OM. Diagnosis and treatment of tuberculosis: latest developments and future priorities. Ann Res Hosp. 2017;1:37. doi: 10.21037/arh.2017.08.08.
5. Borovitsky VS. [Current treatment of chronic cavitary tuberculosis using the example of fibrous cavitary tuberculosis]. [Pulmonology]. 2014;(1):102-108. doi:10.18093/0869-0189-2014-0-1-102-108. Russian.
6. Iseman MD. Tuberculosis therapy: past, present and future. Eur Respir J Suppl. 2002;36:86s-94s. doi: 10.1183/09031936.02.00309102.
7. Macaev F, Ribkovskaia Z, Pogrebnoi S, Boldescu V, Rusu G, Shvets N, Dimoglo A, Geronikaki A, Reynolds R. The structure-antituberculosis activity relationships study in a series of 5-aryl-2-thio-1,3,4-oxadiazole derivatives. Bioorg Med Chem. 2011;19(22):6792-6807. doi: 10.1016/j.bmc.2011.09.038.
8. Duca G, Pogrebnoi S, Boldescu V, Aksakal F, Uncu A, Valica V, Uncu L, Negres S, Nicolescu F, Macaev F. Tryptanthrin analogues as inhibitors of enoyl-acyl carrier protein reductase: activity against Mycobacterium tuberculosis, toxicity, modeling of enzyme binding. Curr Top Med Chem. 2019;19(8):609-619. doi: 10.2174/1568026619666190304125740.
9. Macaev F, Pogrebnoi S, Zveaghinţeva M, Boldescu V, Duca G, inventors. 2-(Propylthio)-5h-[1,3,4]-thiadiazole[2,3- b]-quinazoline-5-one compound, exhibiting antituberculous activity and method for synthesis thereof. The Republic of Moldova patent (MD) no. 4404. 2016 March 18. BOPI [Off Bull Intellect Prop] (Chisinau). 2016;(2):34-35.
10. Pogrebnoi S, Chiriță C, Valica V, Macaev F, Chifiriuc MC, Kamerzan C, Uncu L, Uncu A, Negreș S, Ionică FE, Nicolescu F, Marandiuc I, Ştefănescu E. Studies on the antimycobacterial action of a novel compound of the thiadiazole class, 2-(propyl-thio)-5h- [1,3,4]-thiadiazole[2,3-b]-quinazoline-5-one. Farmacia (Bucharest). 2017;65(1):69-74.
11. Macaev F, Rusu G, Pogrebnoi S, Gudima A, Stingaci E, Vlad L, Shvets N, Kandemirli F, Dimoglo A, Reynolds R. Synthesis of novel 5-aryl-2-thio-1,3,4-oxadiazoles and the study of their structure-anti-mycobacterial activities. Bioorg Med Chem. 2005;13(16):4842-4850. doi: https://doi.org/10.1016/j.bmc.2005.05.011.
12. ICH Expert Working Group. ICH Q2(R1) Validation of analytical procedure: Text and methodology: ICH Harmonized tripartite guideline. In: International Conference on Harmonization of technical requirements for registration of phar- maceuticals for human use. Geneva; 2005 [cited 2020 Feb 12]. Available from: https://database.ich.org/sites/default/files/Q2_R1__Guideline.pdf
13. Uncu L, Donici E, Valica V, Vîslouh O, Gonciar V, Parii S. Development and validation of an assay method for ciprofloxacin hydrochloride determination in combination ear drops. Chem J Mold. 2019;14(2):56-61. doi: http://dx.doi.org/10.19261/cjm.2019.607.
14. Bibire N, Vieriu M, Panainte A, Agoraei L, Uncu L, Vlase V, Vlase A. A new high performance liquid chromatographic analysis method for ciprofloxacin. Rev Chim (Bucharest). 2015;66(9):1463-1466.
15. Forootan A, Sjoback R, Bjorkman J, Sjogreen B, Linz,and L, Kubista M. Methods to determine limit of detection and limit of quantification in quantitative real-time PCR (qPCR). Biomol Detect Quantif. 2017;12:1-6. doi: 10.1016/j.bdq.2017.04.001.

Author's ORCID iD and academic degrees
Andrei Uncu, PharmD, PhD Applicant – https://orcid.org/0000-0002-6419-2596.

Author's contribution
AU designed the study, conducted the laboratory work and performed the analytical part of the laboratory work, interpreted the data, drafted, revised and approved the manuscript.

Funding
This study was supported by *Nicolae Testemitanu* State University of Medicine and Pharmacy. The trial was the author's initiative. The author is independent and takes responsibility for the integrity of the data and accuracy of the data analysis.

Ethics approval and consent to participate
No approval was required for this study.

Conflict of Interests
No competing interests were disclosed.

DOI: 10.5281/zenodo.3958553
UDC: 616-001-037:577.152.34

Survival predictive model for severe trauma patients using proteases/antiproteases system components

[*1,2]Oleg Arnaut, [1]Ion Grabovschi

[1]Department of Human Physiology and Biophysics, [2]*Valeriu Ghereg* Department of Anesthesiology and Intensive Care
Nicolae Testemitanu State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova

Authors' ORCID iDs, academic degrees and contributions are available at the end of the article

*Corresponding author: oleg.arnaut@usmf.md
Manuscript received August 01, 2020; revised manuscript August 14, 2020; published online August 26, 2020

Abstract

Background: Assessing the traumatic injuries severity, as well as estimating the severe trauma patient's prognosis are the key moments in their management. Predictive models for severe trauma outcome need improvement.
Material and methods: In the clinical study (65 severe trauma patients), proteases, antiproteases and treatment outcome (survival/non-survival) were considered. There were used two statistical instruments – dimension reduction analysis (principal component analysis) to prepare the data for modeling and modeling itself through multivariate logistic regression.
Results: Principal component analysis evidenced 12 "latent" factors grouped in four models. The survival predictive model had the following characteristics: calibration χ^2=1.547, df=7, p=.981; determination – 0.759; discrimination, sensitivity – 90.7%, specificity – 81.8 %, area under ROC curve – 0.95 (95%CI 0.912, 1.000). The model enrolled four "latent" factors (three destructive and one protective), male gender and ARDS development.
Conclusions: In our research, the survival predictive model for severe trauma patients on base of proteases/antiproteases system components after dimension reduction procedure was elaborated. The model showed good characteristics and needs validation to be implemented in daily clinical practice.
Key words: trauma, survival predictive model, proteases, antiproteases.

Cite this article
Arnaut O, Grabovschi I. Survival predictive model for severe trauma patients using proteases/antiproteases system components. *Mold Med J.* 2020;63(3):38-42. doi: 10.5281/zenodo.3958553.

Introduction

Trauma remains an important medical problem. A series of scores and algorithms were elaborated to estimate the severity and to predict the treatment results for injured patients, but, actually, there are no any universal scores [1]. Moreover, existing scores applied in different medical systems or different populations with their specific demographic structure showed different ability to predict the outcomes [2, 3]. This, in turn, makes researchers be active in efficient variables/biomarkers identification that will increase the potential models' predictive power.

The potential source for biomarkers in trauma patients is the components of proteases/antiproteases system. These elements are some active immune response participants in trauma and especially in severe trauma, activated by Systemic Inflammatory Response Syndrome (SIRS) development with proteases release by neutrophils in both, injured and non-injured tissues [4, 5]. Thus, the proteases and their antagonists, antiproteases, could be used as predictors in survival predictive models. At the same time, the relationships between proteases/antiproteases system elements are, evidently, complex and this is why it is more rational to use some integrative indicators for analyses. This allows characterizing pathophysiological process/processes instead of individual proteases or antiproteases effect/effectsPrincipal component analysis represents a statistical instrument for dimension reduction. Analyzing dataset, this method extracts the "latent" factors, which are the numerical expression (quantification) of pathophysiological/fundamental factors/processes involved in traumas evolution and outcome, including the appearance of indirect lesions. Each extracted factor is estimated by linear regression technique, having at least two predictors and is used for modeling instead of initial variables [6].

The aim of this research was to elaborate a survival predictive model for severe trauma patients on base of proteases/antiproteases system components after dimension reduction procedure.

Material and methods

Ethical committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (Chisinau, the Republic of Moldova) approved the study design (Protocol 33/46 of 16.12.2016).

In a clinical prospective analytical study, 65 severe trauma patients admitted within first 24 hours after trauma in Intensive Care Unit of Emergency Medicine Institute (ICU EMI), Chisinau, The Republic of Moldova, were enrolled. Plasma samples (venous blood) were collected at 3, 6, 12 and 24 hours after traumatic impact, the potential model being

applicable at 24 hours after trauma. For every blood sample, there were estimated 10 proteases/antiproteases system parameters – two antiproteases (α_2-macroglobulin (α_2M), α_1-antitrypsin (α_1AT)) and eight proteases (Cathepsin D activity (CDA), Cathepsin H activity (CHA), Cathepsin L activity (CLA), Cathepsin G activity (CGA), Elastase activity (EA), Trypsin activity (TA), Adenosinedesaminase activity (ASDS) and Adenilatdesaminase activity (ALDS). In addition, gender (binary variable), aging (continuous variable), Acute Respiratory Distress Syndrome (ARDS) development (binary variable) were considered for modeling. The outcome (dependent variable) was trauma patient survival (survived or non-survived). Criteria for severe trauma was NISS more than 15 points [7], ARDS diagnosis being determined considering Berlin definition [8].

To achieve the aim of study, two statistical instruments – dimension reduction (principal component analysis (PCA, varimax rotation) and multivariate logistic regression were used. Through dimension reduction analysis were extracted as named "latent" factors, maximal three for every PCA model. Also, taking into account the relatively small patients sample Kaiser-Meyer-Olkin (KMO) (measures of how suited your data is for factor analysis) and Bartlett (if the number of extracted factors was appropriate) tests were applied. If KMO was more than .5 and Bartlet test was significant (Bonferroni correction for multiple comparisons) the model was considered for further analysis. In addition, the PCA models were optimized by variable elimination if correlation coefficient was less than .6 or if a parameter was associated with two extracted factors and difference between the absolute correlation values was less than .3.

Extracted "latent" factors, being estimated quantitatively (continuous variable), together with other potential predictors mentioned above were considered for modeling through multivariate logistic regression (backward conditional method). To estimate the model's predictive potential, were considered the following characteristics: determination (Nagelkerke R Square), calibration (Hosmer–Lemeshow test and classification plots) and discrimination (specificity, sensibility, area under ROC curve and sensibility/specificity optimization by cut-off changing). In addition, were appreciated model's stability (resampling by bootstrapping) and multicollinearity analysis.

Results

According to PCA results (tab. 1), there were evidenced 4 models, corresponding to 4 blood samples, collected at 3, 6, 12 and 24 hours after trauma. Each model had 3 "latent" (extracted) factors. Every factor, in turn, enrolled between 2 and 5 proteases/antiproteases system components. In addition, taking into account the parameters that estimated the factors, it was possible to identify, at least presumably, the potential factors roles, divided into potentially destructive or potentially protective.

Within multiple regression analysis for outcome prediction, the data collected at 24 hours after the trauma were considered (ARDS diagnosis, Age, Gender and "latent" factors estimated before). The *null hypothesis* – the model's covariates are not able to predict the survival probability better than a model with constant only. The a*lternative hypothesis* – at least one covariate is able to predict the survival probability better than a model with constant only. The *null hypothesis* was rejected by *Omnibus Test of Model Coefficients* ($\chi^2 = 51.569$, df=6, p<.001, significance level (α) being .05.

Determination coefficient (Nagelkerke R Square), showed the value .759 (75.9%) – more than 75% from survival probability dispersion was explained by the elaborated model's covariates.

The calibration (Hosmer–Lemeshow test) had closed to ideal value of $\chi^2 = 1.547$, df=7, p=.981, being nonsignificant, and confirmed the results fidelity.

The discrimination properties (cut-off was increased up to .55 for accuracy increasing) had the optimal values for specificity 81.8% (18 out of 22), sensibility 90.7% (39 out of

Table 1

The principal component analysis output (varimax rotation)

Sample timing, hours after trauma	Extracted factors	Components	Potential effect
3 hours	1st Factor	α_2M_3, CHA_3, TA_3	Protective
	2nd factor	$ASDS_3$, $ALDS_3$	Destructive
	3rd factor	EA_3, CDA_3	Destructive
6 hours	1st Factor	$ASDS_6$, $ALDS_6$, α_2M_6 (negative correlation)	Destructive
	2nd factor	CHA_6, TA_6, α_2M_6	Protective
	3rd factor	EA_6, CGA_6, CDA_6	Destructive
12 hours	1st Factor	α_1AT_{12} (negative correlation), TA_{12}, $ASDS_{12}$, EA_{12} (negative correlation), α_2M_{12}	Protective
	2nd factor	$ALDS_{12}$, CDA_{12}, CLA_{12}	Destructive
	3rd factor	CHA_{12}, CGA_{12}	Destructive
24 hours	1st Factor	EA_{24}, α_1AT_{24}	Destructive
	2nd factor	TA_{24}, α_2M_{24}, CGA_{24}	Protective
	3rd factor	$ASDS_{24}$, $ALDS_{24}$	Destructive

α_2-macroglobulin (α_2M), α_1-antitrypsin (α_1AT), Cathepsin D activity (CDA), Cathepsin H activity (CHA), Cathepsin L activity (CLA), Cathepsin G activity (CGA), Elastase activity (EA), Trypsin activity (TA), Adenosinedesaminase activity (ASDS) and Adenilatdesaminase activity (ALDS).

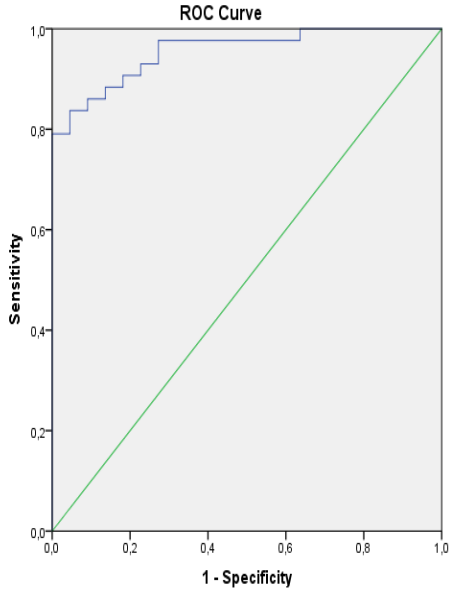

Fig. 1. Area under ROC curve for survival predictive model based on data collected within first 24 hours after trauma

43) and overall percentage (87.7%), all these parameters being higher than target value of 80%. Area under ROC curve for proposed model was estimated at level of .956 (95%CI 0.912, 1) and represented significance in relation to the value of .5 ($p<0.001$) (fig. 1).

The final model included the constant (B=7.816), ARDS diagnosis (B=-4.731), male gender (-.333), the values of factor $2_{\text{model 2}}$ (B=4.038), factor $3_{\text{model 1}}$ (-2.752), factor $2_{\text{model 3}}$ (-2.623) and factor $2_{\text{model 4}}$ (-2.623) (tab. 2). The variable Age and other extracted factors did not show significance, the proposed model being represented as follows:

$$p = \frac{1}{1+e^{-b}} \text{ (formula 1), where}$$

p – 3ability to survive

b = 7.816 + 4.038 x factor 2_{model2} *- 2.752 x factor* 3_{model1} *- 2.623 x factor* 2_{model3} *- 1.504 x factor* 2_{model4} *- 3.333 x male - 4.731 x ARDSe (exponent) – constant equal to 2.71828*

The resampling procedure by bootstrapping (999 samples) showed the significance of potential predictors evidenced in elaborated models, without signs inversion, 95% CI for coefficients being relatively wide (tab. 2b).

Discussion

The alternative approach, used in this research, especially "preparing" the data for modeling by PCA allowed taking into account proteases/antiproteases components in complex, as physiopathological elements instead of individual role of separated elements. Finally, four significant extracted factors took part from elaborated predictive model (tab. 1 and 2).

Table 2

Coefficients for elaborated model equation (a) and bootstrapping for variables in the equation (b)

a. Final model coefficients								
	B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I. for EXP(B)	
							Lower	Upper
ARDS diagnosis	-4.731	1.739	7.397	1	.007	.009	.000	.267
factor $3_{\text{model 1}}$	-2.752	.883	9.723	1	.002	.064	.011	.360
factor $2_{\text{model 2}}$	4.038	1.292	9.767	1	.002	56.693	4.506	713.222
factor $2_{\text{model 3}}$	-2.623	.950	7.617	1	.006	.073	.011	.468
Male gender	-3.333	1.513	4.851	1	.028	.036	.002	.693
factor $2_{\text{model 4}}$	-1.504	.751	4.011	1	.045	.222	.051	.968
Constant	7.816	2.555	9.362	1	.002	2480.270		

b. Bootstrap for Variables in the Equation						
	B	Bias	S.E.	Sig.	95% Confidence Interval for B	
					Lower	Upper
ARDS diagnosis	-4.731	-89.957	715.811	.002	-615.579	-2.512
factor $3_{\text{model 1}}$	-2.752	-45.593	305.169	.003	-389.184	-1.728
factor $2_{\text{model 2}}$	4.038	86.037	528.509	.002	2.488	671.286
factor $2_{\text{model 3}}$	-3.333	-62.414	372.735	.006	-562.397	-.793
Male gender	-2.623	-53.494	413.554	.001	-381.894	-1.756
factor $2_{\text{model 4}}$	-1.504	-22.276	133.442	.004	-143.446	-.403
Constant	7.816	140.631	1045.749	.001	5.189	1136.440

Constant – equation constant's value, B – B coefficients, S.E. – standard errors, Wald – Wald statistics, df – degrees of freedom, Sig. – significance threshold, Exp (B) – odds ratio values, 95% C.I. for EXP(B) – 95% confidence interval for odds ratio.

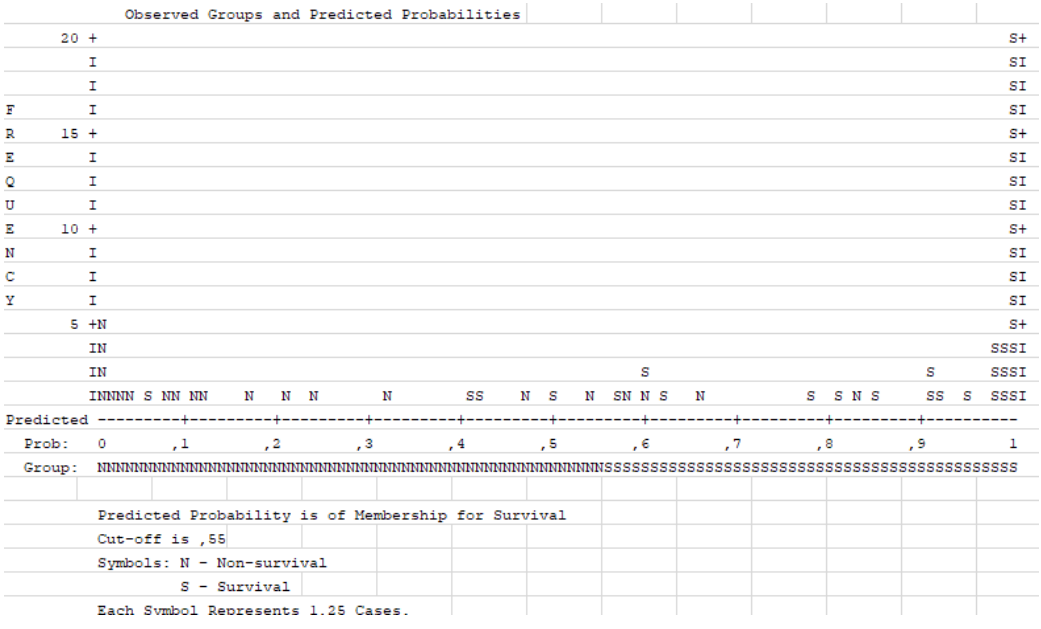

Fig. 2. Classification plot for survival predictive model in severe trauma based on data collected within first 24 hours after trauma

The third extracted factor (PCA model at 3 hours) was in positive association with two proteases – AE_3 and ACD_3, the factor was estimated as destructive one (tab 1). In accordance with literature the elastase represents the key substance in both, direct and indirect lung lesions [9], having multiple destructive effects as degradation the elastin fibers, ciliar apparatus lesions, apoptosis amplification etc. [10]. Moreover, the elastase represents multiple proinflammatory effects [11]. Cathepsin D, in turn, was demonstrated to have ability for enzyme, hormones, antigen and neuropeptide processing, having a function to activate Caspase 8, an important element in apoptosis cascade. In addition, Cathepsin D showed complement activation and increasing for trauma patients [12].

The second extracted factor (PCA model at 6 hours) was, probably, protective because of positive association with trypsin and α_2-macroglobulin. The last one represents the antiprotease substance, that is able to inactivate almost all proteases [13]. Trypsin, unexpectedly, in different research showed a series of positive effects as anti-inflammatory, antiedematic, antioxidant, first two effects demonstrated for trauma patients [14, 15]. The Cathepsin H positive association could be explained by incomplete determination coefficient abilities in linear regression equation for extracted factors in PCA.

The PCA model at 12 hours evidenced 3 factors, second one being significant for final predictive model. This factor was estimated as destructive because of positive association with three proteases – AAMP, ACD and ACL. Cathepsin L, in accordance with literature data, represented a proteolytic activity for almost all proteins, including enzymes and receptors [16]. It is associated with Cathepsin D, controlled apoptosis and neovascularization, enrolled in immune response [17].

The second extracted factor from the fourth PCA model was explained by trypsin, α_2-macroglobulin and cathepsin G values, all components having positive association. Taking into account this, especially first two, the "latent" factor was interpreted as protective one. However, the equation for "latent" factor quantitative estimation included cathepsin G, which represents a series of potential negative effects. It is able to activate coagulation cascade, immune response, to destroy the vascular matrices and to generate edema [18-21]. This, in turn, probably, determined the destructive nature of this parameter, trypsin, α_2-macroglobulin being just associated with severity of potential lesions without direct implication.

Regarding the final predictive model, the main result of this study, it is important to mention good characteristics despite of relatively small sample size (one of the limitations). At the same time, resampling showed the reproductibility of the experiment. The model had relatively high determinant coefficient, the value being close to 80%. Calibration and discrimination showed good model fit and good sensibility/specificity abilities. The most important covariate was factor $3_{\text{model 1}}$ (AE and ACD as predictors). It determined 23% of dependent variable dispersion. Speculating, AE and AED inhibition within first 3 hours after trauma could be a perspective direction for further researches. On the second place was factor $2_{\text{model 2}}$ (17.1%), followed by ARDS diagnosis (12.7%), male gender (10.3%), factor $2_{\text{model 3}}$ (7.3%) and factor $2_{\text{model 4}}$ (5.5%). These data suggest idea about the antiprotease treatment optimization in dependence on the admission timing, that, was not taken into consideration before – possible reason for antiproteases treatment failure [22, 23].

In accordance with elaborated model's results, ARDS, factor $3_{\text{model 1}}$, factor $2_{\text{model 3}}$, factor $2_{\text{model 4}}$ and male gender were the parameters that decrease survival probability (OR=.009, 95%CI .000, .267; OR=.064 95%CI .011, .360; OR=.073, 95%CI .011, .468; OR=.222, 95%CI .051, .968 and OR=.036, CI95% .002, .693, respectively). Factor $2_{\text{model 2}}$, had a protec-

tive effect, OR being 56.693 (CI 95% 4.506, 713.222) (Table 2a). Resampling (bootstrapping, 999 samples) showed wide confidence intervals for model's covariates with significance and no changing signs in front of parameters. In addition,it is important to mention no multicollinearity between predictors included in the final model.

Conclusions

In our research, the survival predictive model for severe trauma patients on base of proteases/antiproteases system components after dimension reduction procedure was elaborated. The model showed good characteristics and needs validation to be implemented in daily clinical practice.

Dimension reduction analysis with "latent" factor extraction has a perspective in research with numerous potential biochemical parameters, taking into account their complex interactions, estimated factors, probably, being quantitatively estimated for different pathophysiological mechanisms.

References

1. Arnaut O, Croitoru D, Grabovschi I, Sandru S. Trauma scoring systems. Mold Med J. 2020;63(1):64-74. doi: 10.5281/zenodo.3685673.
2. Nassar AP, Mocelin AO, Nunes ALB, Giannini FP, Brauer L, Andrade FM, Dias CA. Caution when using prognostic models: a prospective comparison of 3 recent prognostic models. J Crit Care. 2012;27(4):423.e1-423.e7. doi: 10.1016/j.jcrc.2011.08.016.
3. Deng Q, Tang B, Xue C, Liu Y, Liu X, Lv Y, Zhang L. Comparison of the ability to predict mortality between the injury severity score and the new injury severity score: a meta-analysis. Int J Environ Res Public Health. 2016;13(8):825. doi: 10.3390/ijerph13080825.
4. Al-Mahdi W, Ibrahim MM, Spiegel DA, Arkader A, Nance M, Baldwin K. Is systemic inflammatory response syndrome relevant to pulmonary complications and mortality in multiply injured children? J Pediatr Orthop. 2020 Jan;40(1):1-7. doi: 10.1097/BPO.0000000000001085.
5. Nussler AK, Wittel UA, Nussler NC, Beger HG. Leukocytes, the Janus cells in inflammatory disease. Langenbeck's Arch Surg. 1999;384(2):222-32. doi: 10.1007/s004230050196.
6. Sell SL, Widen SG, Prough DS, Hellmich HL. Principal component analysis of blood microRNA datasets facilitates diagnosis of diverse diseases. PLoS One. 2020;15(6):e0234185. doi: 10.1371/journal.pone.0234185.
7. Ali Ali B, Lefering R, Fortun Moral M, Belzunegui Otano T. Epidemiological comparison between the Navarra Major Trauma Registry and the German Trauma Registry (TR-DGU®). Scand J Trauma Resusc Emerg Med. 2017;25(1):1-12. doi: 10.1186/s13049-017-0453-2.
8. Rawal G, Yadav S, Kumar R. Acute respiratory distress syndrome. J Transl Int Med. 2018;6(2):74-7. doi: 10.1515/jtim-2016-0012.
9. Wang T, Zhu Z, Liu Z, et al. Plasma neutrophil elastase and elafin as prognostic biomarker for acute respiratory distress syndrome: a multicenter survival and longitudinal prospective observation study. Shock. 2017;48(2):168-174. doi: 10.1097/SHK.0000000000000845.
10. Brusselle GG, Van Braeckel E. Sputum neutrophil elastase as a biomarker for disease activity in bronchiectasis. Am J Respir Crit Care Med. 2017;195(10):1289-91. doi: 10.1164/rccm.201612-2476ED.
11. Lee WL, Downey GP. Leukocyte elastase: physiological functions and role in acute lung injury. Am J Respir Crit Care Med. 2001;164(5):896-904. doi: 10.1164/ajrccm.164.5.2103040.
12. Huber-lang M, Denk S, Fulda S, Erler E, Kalbitz M, et al. Cathepsin D is released after severe tissue trauma *in vivo* and is capable of generating C5a *in vitro*. Mol Immunol. 2012;50(1-2):60-5. doi: 10.1016/j.molimm.2011.12.005.
13. Rehman AA, Ahsan H, Khan FH. Alpha-2-macroglobulin: a physiological guardian. J Cell Physiol. 2013;228(8):1665-1675. doi: 10.1002/jcp.24266.
14. Shah D, Mital K. The role of trypsin:chymotrypsin in tissue repair. Adv Ther. 2018;35(1):31-42. doi: 10.1007/s12325-017-0648-y.
15. Miller RR, De Young DV, Paxinos J. Enzymes for trauma. Postgrad Med J. 1970;46:154-6. doi: 10.1136/pgmj.46.533.154.
16. Frade R, Rousselet N, Jean D. Intratumoral gene delivery of anti-cathepsin L single-chain variable fragment by lentiviral vector inhibits tumor progression induced by human melanoma cells. Cancer Gene Ther. 2008;15(9):591-604. doi: 10.1038/cgt.2008.51.
17. Kanbak G, Kartkaya K, Ozcelik E, Guvenal AB, Kabay SC, et al. The neuroprotective effect of acute moderate alcohol consumption on caspase-3 mediated neuroapoptosis in traumatic brain injury: the role of lysosomal cathepsin L and nitric oxide. Gene. 2013;512(2):492-5. doi: 10.1016/j.gene.2012.10.012.
18. Gale AJ, Rozenshteyn D. Cathepsin G, a leukocyte protease, activates coagulation factor VIII. Thromb Haemost. 2008;99(1):44-51. doi: 10.1160/TH07-08-0495.
19. Faraday N, Schunke K, Saleem S, Fu J, Wang B, et al. Cathepsin G-dependent modulation of platelet thrombus formation *in vivo* by blood neutrophils. PLoS One. 2013;8(8):e71447. doi: 10.1371/journal.pone.0071447.
20. Gao S, Zhu H, Zuo X, Luo H. Cathepsin G and its role in inflammation and autoimmune diseases. Arch Rheumatol. 2018;33(4):498-504. doi: 10.5606/ArchRheumatol.2018.6595.
21. Campanelli D, Detmers PA, Nathan CF, Cabay JE. Azurocidin and a homologous serine protease from neutrophils. Differential antimicrobial and proteolytic properties. J Clin Invest. 1990;85(3):904-15. doi: 10.1172/JCI114518.
22. Zeiher BG, Artigas A, Vincent J-L, Dmitrienko A, Jackson K, et al. Neutrophil elastase inhibition in acute lung injury: results of the STRIVE study. Crit Care Med. 2004;32(8):1695-702. doi: 10.1097/01.ccm.0000133332.48386.85.
23. Pu S, Wang D, Liu D, Zhao Y, Qi D, et al. Effect of sivelestat sodium in patients with acute lung injury or acute respiratory distress syndrome: a meta-analysis of randomized controlled trials. BMC Pulm Med. 2017;17(1):1-9. doi: 10.1186/s12890-017-0498-z.

Authors' ORCID iDs and academic degrees
Oleg Arnaut, MD, PhD, Associate Professor – https://orcid.org/0000-0002-5483-8672.
Ion Grabovschi, MD, Assistant Professor – https://orcid.org/0000-0002-7716-9926.

Authors' contribution
OA conceptualized the idea, conducted literature review, wrote the manuscript, revised and approved the final text; GI and OA interpreted the data.

Funding
This study was supported by *Nicolae Testemitanu* State University of Medicine and Pharmacy. The trial was the authors' initiative. The authors are independent and take responsibility for the integrity of the data and accuracy of the data analysis.

Ethics approval and consent to participate
The research project was approved by Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (Protocol No 46, 16.12.2016).

Conflict of Interests
No competing interests were disclosed.

DOI: 10.5281/zenodo.3958557
UDC: 615.451.3.014.4:616.28-002

Stability studies of combined ear drops for the treatment of otitis

[*]Eugeniu Nicolai, Oxana Vislouh, Vladimir Valica, Sergiu Parii, Livia Uncu

Scientific Center for Drug Research, *Nicolae Testemitanu* State University of Medicine and Pharmacy
Chisinau, the Republic of Moldova

Authors' ORCID iDs, academic degrees and contributions are available at the end of the article

[*]Corresponding author: eugeniu.nicolai@usmf.md
Manuscript received 30 July, 2020; revised manuscript August 14, 2020; published online August 26, 2020

Abstract

Background: The stability of a drug is an important factor in ensuring its quality.The studied combined ear drops have an increased tendency of degradation, which requires an extensive stability study and obtaining data to determine the shelf life and establish the storage conditions. The purpose of the work: Stability studies of ear drops containing ciprofloxacin, dexamethasone, loratadine and volatile basil oil.
Material and methods: International Harmonized Guideline ICH Q1A (R2) stability testing methodology; 3 series of ear drops; reference standards for the active substances (Sigma Aldrich, USA); Shimadzu LC-20AD liquid chromatograph with UV-VIS detector; Fungilab Smart R viscometer; pH meter inoLab 7110; solvents, reagents in accordance with the European Pharmacopoeia.
Results: Ciprofloxacin is stable in acid medium, degrades in alkaline medium after 3 hours (approximately 10.0%), under oxidation (19.7%) and light action (17.1%). Dexamethasone degrades in acid medium (by 7.7%) and under oxidation (by 19.9%), it is stable in alkaline medium and under the action of light. Loratadine degrades in acid medium (by 3.0%), is stable in alkaline medium, under oxidation and action of light. In real-time storage conditions (25°C±2°C and RH 60%±5%), it was found that the pharmaceutical form did not change its quality parameters for 24 months.
Conclusions: The stability studies under stress and in real time conditions allowed us to select the packaging, the optimal storage conditions and to establish the provisional shelf life for the combined auricular pharmaceutical form during 2 years.
Key words: stability, combined ear drops, otitis, shelf life.

Cite this article
Nicolai E, Vislouh O, Valica V, Parii S, Uncu L. Stability studies of combined ear drops for the treatment of otitis. *Mold Med J.* 2020;63(3):43-50. doi: 10.5281/zenodo.3958557.

Introduction

Stability study is one of the most important areas, in relation to the registration of pharmaceutical products, as it predicts shelf life and storage instructions for batches. It also determines degradation of products, mechanism of breakdown and conditions under which the breakdown occurs. With the help of stability studies, any parameter subject to change within the ear drop during storage can be measured, such as appearance, pH, viscosity and density (where relevant), solubility time (reconstitution and appearance thereof) sterility, preserving ability and preservative content (where relevant). Tests are also performed to ensure compatibility between the container-closure system and the product. Stability testing is the cornerstone of drug development or formulation [1]. In addition to the degradation of the unstable product into toxic decomposition products, loss of activity up to 85% level of the concentration indicated on the label may lead to the failure of therapy. For this reason, data on stability studies are absolutely necessary [2].

Stability testing and study is a mandatory step in drug development [3]. The need for stability studies has a major impact in order to obtain the following information: providing evidence on how product quality changes and degrades; data on optimal storage conditions; establishing a validity period with determination of shelf life; choosing of a justified packaging for the pharmaceutical product storage; obtaining an inoffensive drug for the patient's life; setting high quality and safety conditions [4]. Depending on the mode, stages and conditions of the stability research, two study methods are used: real-time stability and stress stabi-lity studies [5].

For real-time stability studies, the dosage form, at the preformulation stage, is stored for an indefinite period (long time), under normal conditions, which are typical for the dosage form and each component in particular. This method is performed in order to obtain the data that will provide information about the degradation and change of characteristics (properties) of the product under the normal conditions recommended for storage. The test period does not have a normal time, depending on the properties and the actual stability of the product, which should be as long as possible. During testing, the data are collected with a regular frequency of time in order to observe a change scale characteristic for the dosage form [5]. This type of research can value the performance of the dosage form based on its physico-chemical characteristics, the product must maintain its good quality for a pre-designed period [6].

Accelerated stability studies provide extremely valuable information for finding alternative dosage forms, which is the best way to obtain data depending on optimal storage

conditions, packaging materials, manufacturing process etc. As a rule, accelerated stability testing is performed at the same time as the beginning of the real-time test [5].

Forced degradation studies allow the evaluation of the main degradation pathways of the active principles: the delimitation of the degradation products of the active substances from those formed by the auxiliary compounds; obtaining data about the structure of degradation products, their toxic impact on the drug; determining the intrinsic stability of all substances used in the formulation; obtaining more data about the physicochemical properties of the substances; prevention of additional costs during the formulation and production process, by introducing some changes at early stages [7].

Data on the behavior of each substance exposed to different stress conditions are extremely valuable, especially for the choice of excipients and packaging methods [8]. The stress condition that has the greatest influence on the stability of the preparation is the temperature increase, it is important to have a higher temperature than the one possibly considered normal for the given form [9]. In addition to temperature, it is also necessary to test the drug under stress conditions, such as humidity, light, pH change, the action of oxidants and reducing agents [10].

Stability is one of the key problems in development of the combined pharmaceuticals, as it can be influenced by chemical interaction between the combined active principles in a formulation [11].

Within the Scientific Centre for Drug Research, there are in the process of ongoing the research of elaboration and development of combined ear drops for the treatment of otitis with the following composition: ciprofloxacin hydrochloride (antibiotic), dexamethasone (corticosteroid, anti-inflammatory), loratadine (antihistamine, desensitizing) and volatile basil oil (calming, regenerating and natural antiseptic) [12, 13]. This pharmaceutical form has undergone the stability testing process in order to obtain data that would highlight any physico-chemical changes in the components and to establish the shelf life and storage conditions.

Material and methods

For stability studies in stress and real-time conditions were used 3 series of ear drops prepared in laboratory conditions (Laboratory Analysis, Standardization and Drug Control of the Scientific Center for Drug Research within *Nicolae Testemitanu* State University of Medicine and Pharmacy) where all experimental research was performed.

Materials: Medicinal substances (ciprofloxacin hydrochloride, loratadine, dexamethasone and volatile basil oil), excipients (Methyl paraben (Methyl-4-hydroxybenzoate, nipagen), Polysorbate 20, KOLLISILV ™ PEG E 400) and former procured standards at SIGMA-ALDRICH, USA, meet all the requirements of the European Pharmacopoeia.

Apparatus: The HPLC method, previously developed, was used for the assay of the active principles [14]. Shimadzu LC-20AD HPLC chromatographic system was used, analytical column NUCLEOSIL® 100-5 C18, 5 µm, 4.0 x 150 mm; UV-VIS detector, wavelength 280 nm. The pH on the pH meter inoLab 7110, and the viscosity on the Fungilab Smart R viscometer were determined.

Chemicals: Acetonitrile (ACN) and methanol (MeOH) were used in purity grade "pro HPLC analysis" (Sigma Aldrich), orthophosphoric acid and triethylamine purity grade "pro analysis" (Merck), bidistilled purified water.

Chromatography conditions: 0.1% orthophosphoric acid was mixed with ACN in 65:45 proportions by volume (sol. A). Solution A was mixed with MeOH in 80:20 proportions, homogenized and adjusted with triethylamine to pH 3.0; filtered under vacuum through a Millipore XF 5423050 capron filter (0.2-0.45 µm), degassed in the DONAU-LAB SONIC DLS 660-T / H. The temperature of the chromatographic column was 400°C; injection volume 20 µl; mobile phase flow – 1 ml / min.

Preparation of *standard solutions:* 0.01 g (exact mass) of each standard substances ciprofloxacin hydrochloride, loratadine and dexamethasone were accurately weighed and transferred into a 25 ml volumetric flask. Then it was dissolved in the mobile phase and made up to the level with the same solvent.

Preparation of *the sample solution:* 5.0 ml of pharmaceutical form was placed in a 25 ml volumetric flask and diluted with the mobile phase to the mark.

Stress degradation methods.

Preparation of the solution *for acid degradation:* 2.5 ml of pharmaceutical form was mixed with 2.5 ml of 0.1 mol / l hydrochloric acid, then it was homogenized and left for 24 hours at room temperature (25^0C). 4.0 ml of solution was diluted with mobile phase to 10 ml in a volumetric flask.

Preparation of the solution *for basic degradation:* 5.0 ml of pharmaceutical form was mixed with 0.5 ml of 0.1 mol / l sodium hydroxide, then it was homogenized and left for 24 hours at room temperature (25^0C). 5.0 ml of solution was diluted with mobile phase to 25 ml in a volumetric flask.

Preparation of the solution *for oxidative degradation:* 2.5 ml of pharmaceutical form was mixed with 2.5 ml of 3% hydrogen peroxide, then it was homogenized and left for 24 hours at room temperature (25^0C). 4.0 ml of solution was diluted with mobile phase to 10 ml in a volumetric flask.

Preparation of the solution *for photolytic degradation:* 5.0 ml of pharmaceutical form was irradiated with UV light (300 nm) for 48 hours. 5.0 ml of UV treated solution was diluted with mobile phase to 25 ml in a volumetric flask.

Preparation of the solution *for thermal degradation:* 5.0 ml of pharmaceutical form was stored in a thermostat at 600 °C for 48 hours. 5.0 ml of solution under thermal stress was diluted with mobile phase to 25 ml in a volumetric flask.

Chromatography technique: In the chromatographic column, 20 µl of standard and sample solutions (for all stress conditions) were injected alternatively, preventively filtered through a 0.45 µm membrane filter, obtaining at least 3-5 chromatograms for each solution.

Real-time stability study.

The pharmaceutical form, packed in dark glass vials with plastic stoppers, was stored for a long time, under normal conditions, at a temperature of 25 °C ± 2 °C; relative humidity 60% ± 5%. The periodicity of the real-time test was performed at equal time intervals: 0, 3, 6, 9, 12, 18, 24 months.

During this period, the main quality parameters were monitored: appearance, identification of active ingredients (ciprofloxacin hydrochloride, dexamethasone and loratadine), pH, viscosity and assay.

Statistical analysis.

Statistical analysis was done by using the Statistical Package for the Social Sciences (IBM SPSS Statistics) 10.5 software.

Results and discussion

To evaluate the degradation processes under stress, the assay variations of the active principles in pharmaceutical form were followed. For the assay of the active ingredients, the chromatograms of the standard solutions of drug substances and of the sample solution were recorded before the application of the stress conditions (fig. 1).

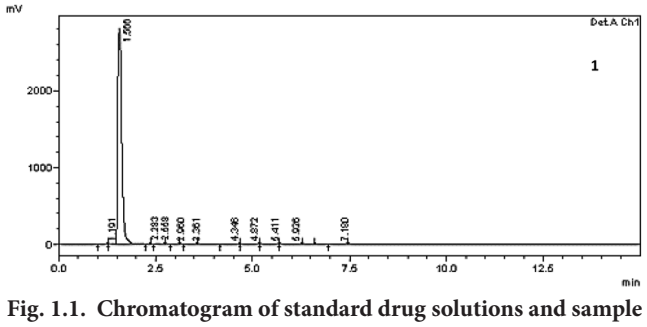

Fig. 1.1. Chromatogram of standard drug solutions and sample solution before application of stress conditions: 1 – standard solution of ciprofloxacine hydrochloride

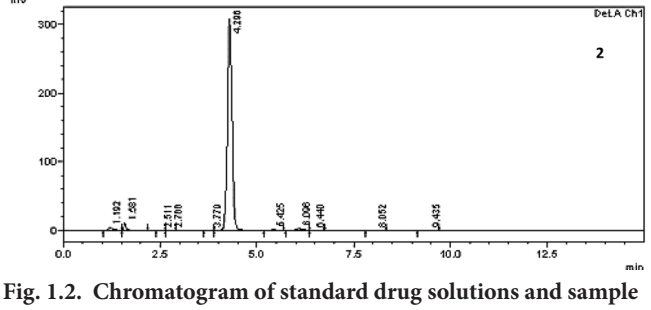

Fig. 1.2. Chromatogram of standard drug solutions and sample solution before application of stress conditions: 2 – standard solution of dexamethasone

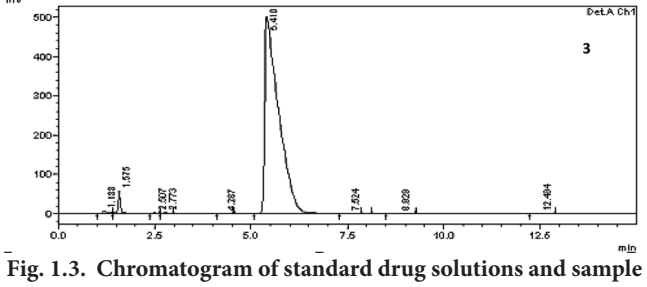

Fig. 1.3. Chromatogram of standard drug solutions and sample solution before application of stress conditions: 3 – standard solution of loratadine

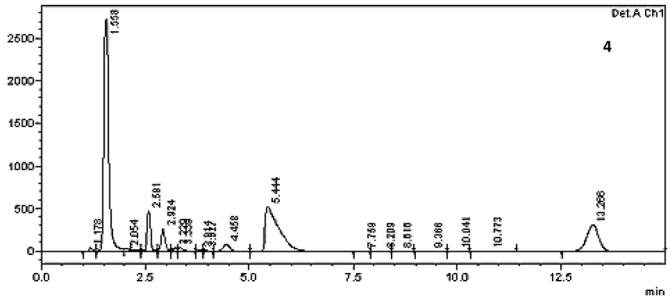

Fig. 1.4. Chromatogram of standard drug solutions and sample solution before application of stress conditions: 4 – sample solution

Degradation under stress conditions

Hydrolytic stress

Hydrolysis is one of the most common chemical degradation pathway over a wide range of pH variations. The hydrolytic study in acid and basic conditions involves the catalysis of the ionizable functional groups present in the molecule. Testing by acid or basic stress involves the forced degradation of a drug substance [15, 16].

Chromatograms of stressed solutions in acid (fig. 2.1) and basic medium (fig. 2.2) were recorded. The chromatograms show the appearance of additional peaks, which denotes the onset of degradation processes.

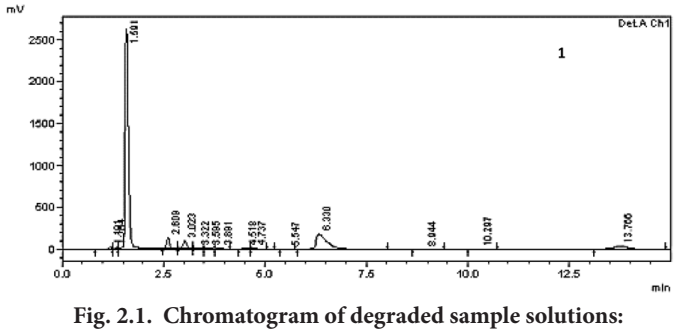

Fig. 2.1. Chromatogram of degraded sample solutions: 1 – in acid medium

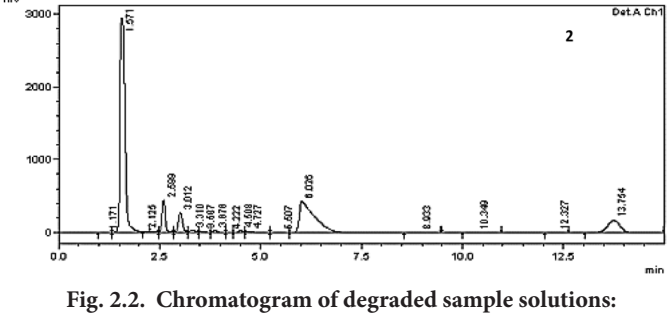

Fig. 2.2. Chromatogram of degraded sample solutions: 2 – in alkaline medium

The results of the quantitative determinations of the active principles were statistically evaluated (table 1). The standard relative deviation of assay results after acid and basic hydrolysis does not exceed 1% for ciprofloxacin hydrochloride and loratadine, and constitutes 3.646 and 2.279 for dexamethasone, respectively.

As can be seen from table 1 and figure 3.1., in acid medium, ciprofloxacin degraded practically insignificantly, while

Table 1

The results of stability studies of ear drops following acid and alkaline hydrolysis

	Test period hours	Ciprofloxacin hydrochloride				Dexamethasone				Loratadine			
		#$t_{R, min}$	#S	#C, g	RSD	#$t_{R, min}$	#S	#C, g	RSD	#$t_{R, min}$	#S	#C, g	RSD
Acid degradation	**0**	1.566	30561193.83	0.29914	0.333	4.294	259125.85	0.01926	3.646	5.402	1118775.74	0.01876	0.741
	3	1.556	30459876.57	0.29814		4.294	249173.46	0.01852		5.403	1102373.41	0.01848	
	24	1.564	30358432.48	0.29715		4.294	239098.89	0.01777		5.410	1091954.81	0.01831	
Basic degradation	**0**	1.566	30161093.87	0.29522	0.208	4.294	256125.85	0.01903	2.279	5.410	1148875.57	0.01926	0.302
	3	1.556	30059876.57	0.29423		4.294	249173.46	0.01852		5.403	1146373.47	0.01922	
	24	1.566	30161193.83	0.29522		4.294	241098.89	0.01792		5.410	1141954.79	0.01915	

Note: The average results for three series of ear drops are shown

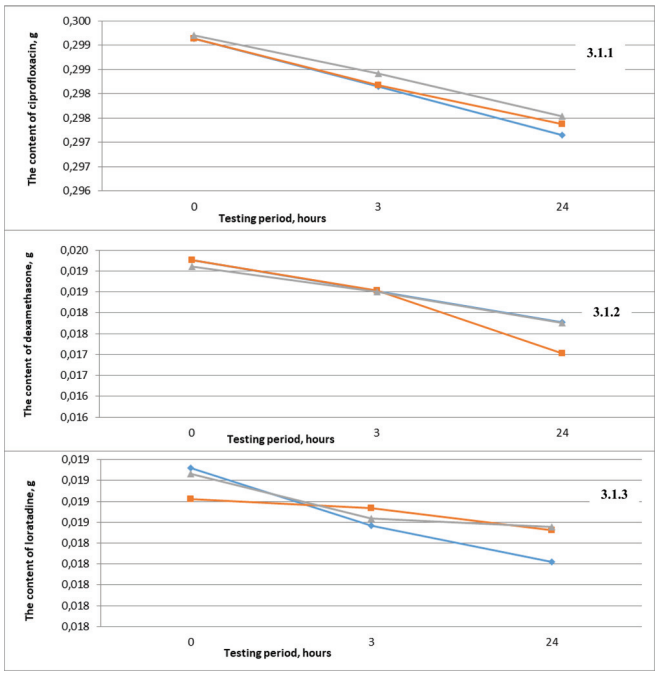

Fig. 3.1. Modification of the concentration of active principles following acid hydrolysis (for 3 series of pharmaceutical form): 3.1.1. – ciprofloxacine hydrochloride; 3.1.2. – dexamethasone; 3.1.3. – loratadine

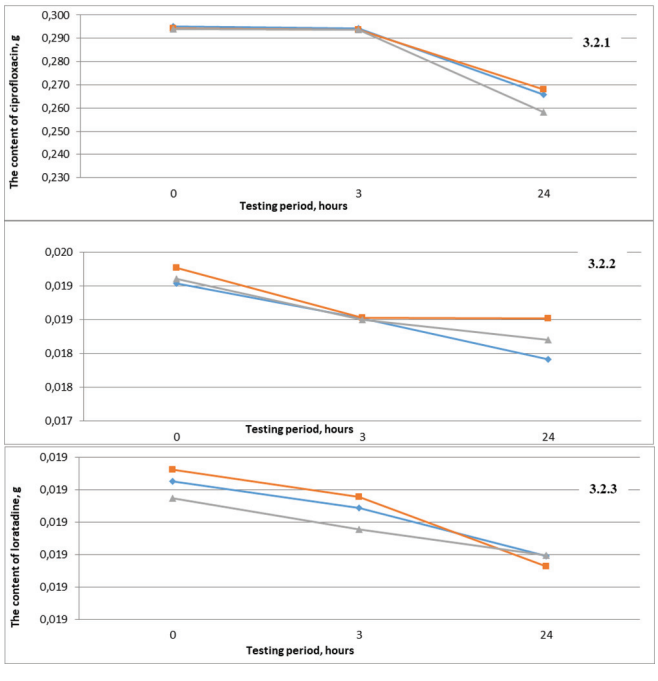

Fig. 3.2. Modification of the concentration of active principles following alkaline hydrolysis (for 3 series of pharmaceutical form): 3.2.1. – ciprofloxacine hydrochloride; 3.2.2. – dexamethasone; 3.2.3. – loratadine

dexamethasone began to degrade after the first 3 hours, with the decrease of the substance concentration after about 24 h. Loratadine, showed its degradation as well as dexamethasone, after 24 h from exposure to acid medium.

In the alkaline medium (fig. 3.2.), ciprofloxacin shows considerable degradation, which begins in about 3 hours, with the formation of the precipitate. Dexamethasone and loratadine in alkaline medium, have a greater tendency of stability, the relative degradation begins only after 24 h.

Oxidative stress

Oxidative degradation of the drug substance involves an electron transfer mechanism to form reactive anions and cations [17]. Molecular oxygen is a key element that affects the physico-chemical stability of the drug system. Strong oxidizers, such as 3% hydrogen peroxide, can trigger irreversible processes in the investigated medicinal substances, which, when studied, allow to establish the nature of the toxic degradation products and the methods of prevention of these processes [15, 18, 19].

The chromatogram of the pharmaceutical form subjected to oxidative stress was recorded (fig. 4).

The results of the quantitative determination of the active principles were statistically evaluated (table 2). The standard deviation does not exceed 1% for loratadine, and is high for ciprofloxacin hydrochloride (12.173) and dexamethasone (13.254).

Following the evaluation of the oxidation results (fig. 5), it is found that a more significant degradation suffer cipro-

Table 2

The result of stability studies of ear drops following oxidation

	Test period, hours	Ciprofloxacin hydrochloride				Dexamethasone				Loratadine			
		#$t_{R, min}$	#S	#C, g	RSD	#$t_{R, min}$	#S	#C, g	RSD	#$t_{R, min}$	#S	#C, g	RSD
Oxidative degradation	**0**	1.574	29961193.83	0.29326	12.173	4.730	261125.85	0.01941	13.254	6.014	1199214.51	0.02010	0.034
	3	1.576	29959876.57	0.29325		4.704	209173.46	0.01555		5.903	1199199.41	0.02009	
	24	1.574	24058432.48	0.23549		4.704	209098.89	0.01554		5.910	1199204.84	0.02009	

Note: The average results for three series of ear drops are shown

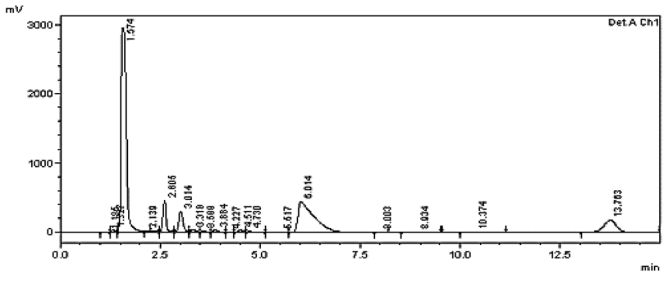

Fig. 4. Chromatogram of the sample solution subjected to oxidative degradation

floxacin and dexamethasone, starting with the first hours, and after about 24 hours it reaches its maximum point. Loratadine has been shown to be more stable to oxidants, being slightly degraded after about 3 hours.

Photolytic and thermal stress

Photostability studies are performed to generate primary degradants of drug substances by exposure to UV or fluorescent conditions. The most acceptable wavelengths are

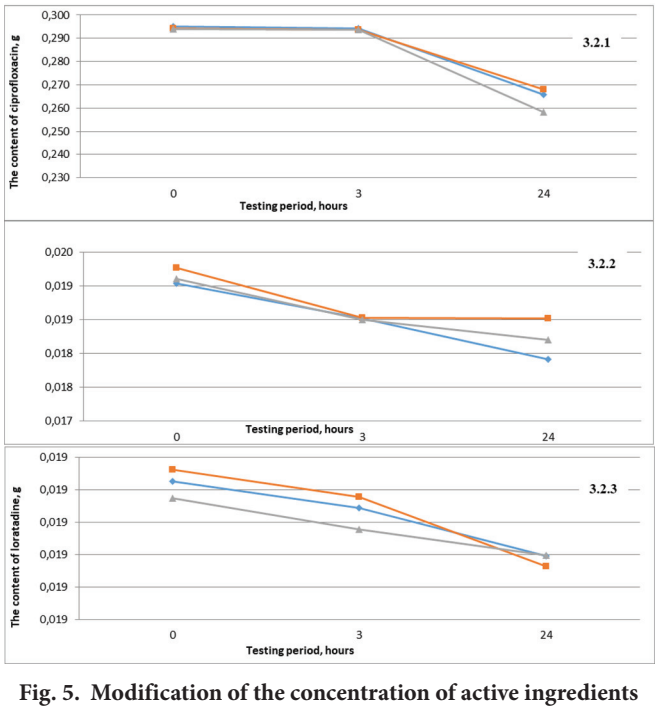

Fig. 5. Modification of the concentration of active ingredients following oxidation (for 3 series of pharmaceutical form): 5.1. – ciprofloxacine hydrochloride; 5.2. – dexamethasone; 5.3. – loratadine

usually between 300 and 800 nm to cause photolytic degradation. Mild stress conditions can induce photochemical oxidation through the mechanism of free radicals [20, 21].

Temperature changes sometimes have drastic effects on the stability of the drug. Increasing the temperature usually increases the rate of drugs hydrolysis. The effect of temperature on stability is described by Arrhenius' equation [22-25].

Chromatograms of the pharmaceutical form subjected to oxidative and thermal stress were recorded (fig. 6).

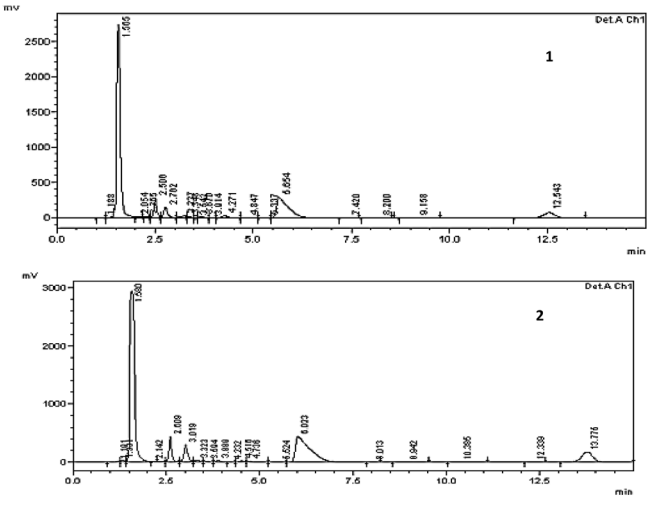

Fig. 6. Chromatograms of the sample solutions subjected to photolytic and thermal degradation: 1 – photolytic stress, 2 – thermal stress

The results of the quantitative determination of the active principles after photolytic degradation were statistically evaluated (tab.3). The standard deviation does not exceed 1% for loratadine, it is about 1.0 for dexamethasone and 10.414 for ciprofloxacin hydrochloride on photochemical degradation. In the case of heat stress, the standard deviation does not exceed 1% for ciprofloxacin hydrochloride, it is about 1.0 for dexamethasone and 3.140 for loratadine.

As can be seen from Figure 7.1, light is the decisive factor for the degradation of ciprofloxacin, the first 3 h curve being linear, and already from 3 h there is a significant decrease in concentration. Dexamethasone has a relatively slight degradation, so it is not particularly affected by the action of light. Loratadine was shown to be more stable, showing degradation after approximately 24 h.

Table 3

The result of stability studies of ear drops following exposure to light and temperature

	Test period, hours	Ciprofloxacin hydrochloride				Dexamethasone				Loratadine			
		#$t_{R, min}$	#S	#C, g	RSD	#$t_{R, min}$	#S	#C, g	RSD	#$t_{R, min}$	#S	#C, g	RSD
Photolytic degradation	0	1.674	29161193.83	0.28543	10.414	4.289	276135.67	0.02052	1.122	6.014	1196775.34	0.02010	0.289
	24	1.656	29059876.57	0.28444		4.291	272273.64	0.02023		5.903	1196473.37	0.02006	
	48	1.664	24158432.48	0.23647		4.291	270188.89	0.02007		5.910	1192954.67	0.01999	
Termal degradation	0	1.580	30299973.67	0.29658	0.036	4.124	276589.27	0.02056	1.023	5.92	1198775.74	0.0201	3.140
	24	1.588	30289876.24	0.29648		4.125	274192.38	0.02038		5.911	1192393.27	0.01999	
	48	1.588	30278432.61	0.29637		4.127	271001.37	0.02014		6.033	1131959.28	0.01898	

Note: The average results for three series of ear drops are shown

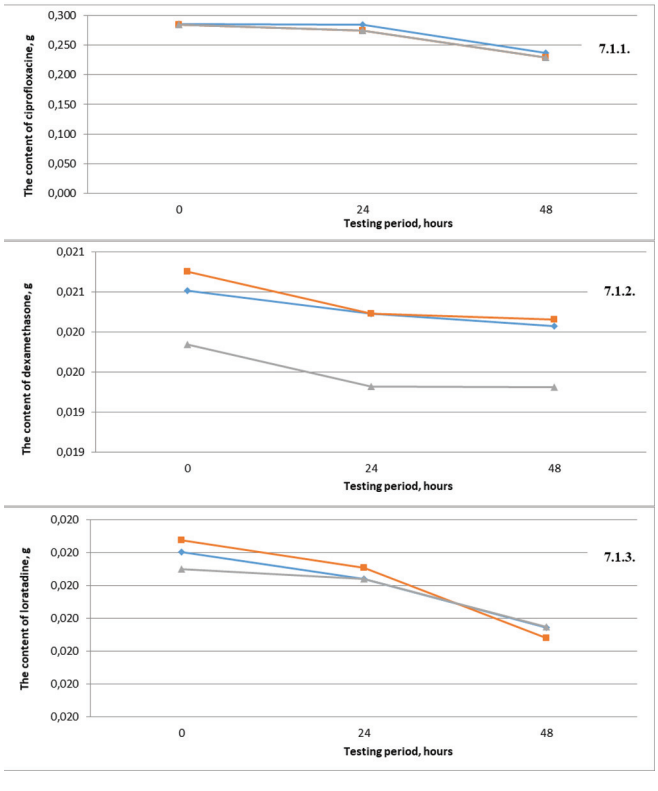

Fig. 7.1. Modification of the concentration of active principles following photolytic degradation (for 3 series of pharmaceutical form): 7.1.1. – ciprofloxacine hydrochloride; 7.1.2. – dexamethasone; 7.1.3. – loratadine

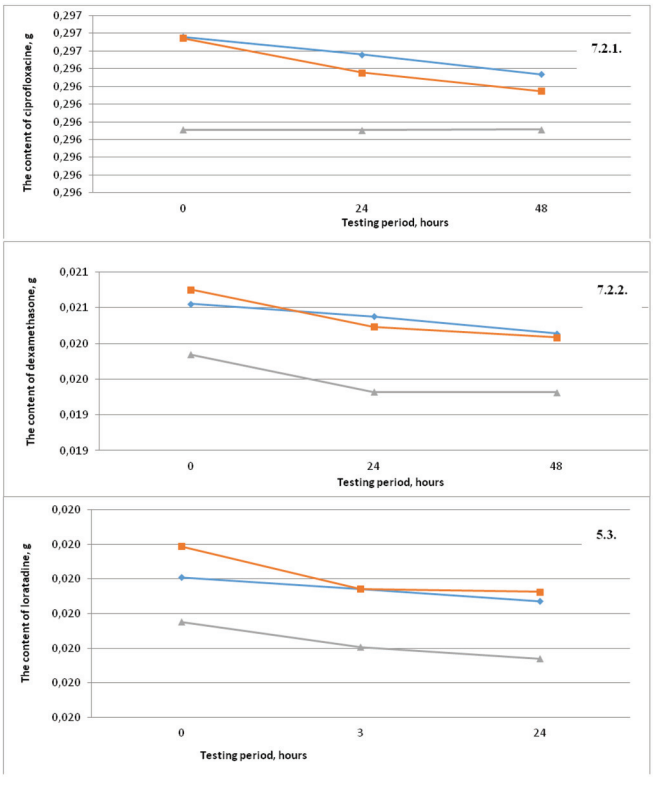

Fig. 7.2. Modification of the concentration of active principles following thermal degradation (for 3 series of pharmaceutical form): 7.2.1. – ciprofloxacine hydrochloride; 7.2.2. – dexamethasone; 7.2.3. – loratadine

Thermal stress practically does not influence ciprofloxacin and dexamethasone, which are stable during the test period (48 hours). Loratadine is temperature labile, degrading by about 5% for 48 hours of exposure to 600°C (fig. 7.2).

Real-time stability study

In the stability study process, it is very important to establish the shelf life of the product, during which the drug can be used according to medical prescriptions. The International Harmonized Guideline ICH Q1E recommends that a shelf life test period be established. For this purpose, the regression analysis of the stability data is used, the degradation being the primary factor in the analysis of duration (long term) [5].

The main quality parameter, which denotes the presence or absence of degradation processes of the multicomponent pharmaceutical form, is the medicinal product assay. Thus, for the entire storage period (24 months), the dosing of the active principles by HPLC method was performed, along with the other parameters. At the same time, the parameters were monitored: appearance, identity of active principles, viscosity, pH of the pharmaceutical form (tab. 4).

As can be seen from table 4, after storage in real-time

Table 4

The result of real-time ear drops stability studies

Periodicity of testing, months	Analyzed parameters and admissibility conditions						
	Appearance milky suspension, with a yellowish shade, with a specific smell, bitter taste	**Identity HPLC (Ret. time)** Ciprofloxacine hydrochloride – 1.53-1.57 Dexamethasone - 4.10-4.45 Loratadine - 5.40-5.43	**pH** 4.5 – 6.0	**Viscosity** 10 - 30 P 10^2	**Assay, HPLC**		
					Cipro-floxacine hydrochloride 0.29-0.31g	Dexa-methasone 0.019-0.021g	Loratadine 0.019-0.021g
Batch 1							
0	Corresponds	Corresponds	5.5	25.7	0.30012	0.02000	0.02010
3	Corresponds	Corresponds	5.5	25.8	0.30010	0.02001	0.02009
6	Corresponds	Corresponds	5.4	25.8	0.30009	0.02000	0.02000
9	Corresponds	Corresponds	5.5	25.8	0.29999	0.02000	0.01995
12	Corresponds	Corresponds	5.6	25.7	0.29996	0.01999	0.01979
18	Corresponds	Corresponds	5.7	25.9	0.29950	0.01998	0.01977
24	Corresponds	Corresponds	5.7	25.9	0.29949	0.01997	0.01976
Batch 2							
0	Corresponds	Corresponds	5.6	27.4	0.30012	0.02000	0.01958
3	Corresponds	Corresponds	5.6	27.4	0.30013	0.02001	0.01957
6	Corresponds	Corresponds	5.4	27.5	0.30010	0.02000	0.01958
9	Corresponds	Corresponds	5.5	27.5	0.29952	0.02000	0.01958
12	Corresponds	Corresponds	5.7	27.5	0.29951	0.01999	0.01957
18	Corresponds	Corresponds	5.6	27.6	0.29951	0.01987	0.01955
24	Corresponds	Corresponds	5.7	27.7	0.29951	0.01987	0.01954
Batch 3							
0	Corresponds	Corresponds	5.4	26.1	0.29949	0.01999	0.01998
3	Corresponds	Corresponds	5.4	26.2	0.29939	0.01999	0.01997
6	Corresponds	Corresponds	5.5	26.2	0.29950	0.01999	0.01999
9	Corresponds	Corresponds	5.5	26.1	0.29939	0.01999	0.01995
12	Corresponds	Corresponds	5.6	26.2	0.29921	0.01999	0.01993
18	Corresponds	Corresponds	5.6	26.2	0.29926	0.01989	0.01993
24	Corresponds	Corresponds	5.6	26.1	0.29926	0.01989	0.01992

conditions for 24 months, the drug substances underwent no significant changes regarding the concentration, pH and viscosity, the values being within the admisible limits. Storage at 25°C ± 2°C and relative humidity 60% ± 5% continues, to establish the maximum shelf life. Currently, it can be said with certainty that the pharmaceutical form *Combination ear drops containing ciprofloxacin, dexamethasone, loratadine and volatile basil oil* is valid for 2 years.

Conclusions

The stress factors degree of influence on the pharmaceutical form was established by evaluating the degradation process under temperature, acid and alkaline hydrolysis, light, oxidative stress. Ciprofloxacin is stable under acid medium and temperature action, degrades in alkaline medium after 3 hours (approximately by 10.0%), under oxidation (by 19.7%) and light action (by 17.1%).

Dexamethasone degrades in acid medium (by 7.7%) and under oxidation (by 19.9%), but it is stable in alkaline medium and under light and temperature action. Loratadine degrades in acid medium (by 3.0%) and under temperature action (by 5%), but is stable in alkaline medium, under oxidation and light action. Stability studies under stress storage conditions have allowed the selection of optimal packaging and storage conditions for the investigated pharmaceutical form: dark colored containers (protected from light), tightly closed (inaccessible to oxygen from the air and humidity), at temperatures not more than 25 °C ± 2 °C and 60% RH ± 5%. These results will be included in the analytical quality standardization documentation for the researched product.

In real-time storage conditions (it was found that the pharmaceutical form did not change its quality parameters for 24 months (by April 2020). Real-time stability studies for this product continue.

References

1. Waterman KC, Adami RC. Accelerated aging: prediction of chemical stability of pharmaceuticals. Int J Pharm. 2005;293:101-125. doi: 10.1016/j.ijpharm.2004.12.013.
2. Jamrógiewicz, M. Consequences of new approach of chemical stability tests of active pharmaceutical ingredients (APIs). Front Pharmacol. 2016;7:17. doi: 10.3389/fphar.2016.00017.
3. World Health Organization. Stability testing of active pharmaceutical ingredients and finished pharmaceutical products. WHO Technical Report Series, No. 953 [Internet]. Geneva: WHO; 2009 [cited 2020 June 12]. Available from: https://extranet.who.int/prequal/sites/default/files/documents/TRS1010_Annex10.pdf.
4. Colgan ST, Watson TJ, Whipple RD, Nosal R, Beaman JV, De Antonis DM. The application of science- and risk-based concepts to drug substance stability strategies. J Pharm Innov. 2012;7(3-4):205-13. doi:10.1007/s12247-012-9135-9.
5. European Medicines Agency. ICH Topic Q1A (R2): Stability testing of new drug substances and products [Internet]. Amsterdam: EMA; 2003 [cited 2020 Apr 12]. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-1-r2-stability-testing-new-drug-substances-products-step-5_en.pdf.
6. Singh R, Rehman Z. Current trends in forced degradation study for pharmaceutical product development. J Pharm Educ Res. 2012;3(1):54-63.
7. Singh A, Singh P, Shukla D. Technical considerations of forced degradation studies of new drug substances and product: regulatory perspectives. J Drug Deliv Ther. 2018;8(2):163-168. doi: 10.22270/jddt.v8i2.1681.
8. Naveed S, Basheer S, Qamar F. Stability of a dosage form and forced degradation studies. J Bioequivalence Bioavailab. 2016;8:191-3. doi: 10.4172/jbb.1000292.
9. Singh S, Junwal M, Modhe G, Tiwari H, Kurmi M, et al. Forced degradation studies to assess the stability of drugs and products. TrAC - Trends Analyt Chem. 2013; 49:71-88. doi: 10.1016/j.trac.2013.05.006.
10. Maggio RM, Vignaduzzo SE, Kaufman TS. Practical and regulatory considerations for stability-indicating methods for the assay of bulk drugs and drug formulations. TrAC - Trends Anal Chem. 2013;49:57-70. doi: 10.1016/j.trac.2013.05.008.
11. Savkare AD, Wakhare RK. Review on the fixed dose combination drugs and their incompatibilities. Int J Pharm Sci Res. 2017;8(7):2798-2807. doi: 10.13040/IJPSR.0975-8232.8(7).2798-07.
12. Maniuc M, Valica V, Parii S, Uncu L, Ababii Polina, Nicolai E, Stefarta R, inventors. Medicinal preparation for the treatment of otitis. The Republic of Moldova patent (MD) no. 4291. 2013 Dec 27. BOPI [Off Bull Intellect Prop] (Chisinau). 2014;(7):22-23.
13. Uncu L, Donici E, Valica V, Vîslouh O, Gonciar V, Parii S. Development and validation of an assay method for ciprofloxacin hydrochloride determination in combination ear drops. Chem J Mold. 2019;14(2):56-61. doi: http://dx.doi.org/10.19261/cjm.2019.607.
14. Nicolai E, Parii S, Valica V, Uncu L. Evaluarea conținutului de impurități de etilendiaminoderivat de ciprofloxacină în studiul stabilității picăturilor auriculare combinate [Evaluation of the impurity content of ethylenediamine derivative of ciprofloxacin in the study of the stability of combined ear drops]. In: [Challenges of contemporary pharmacotherapy: National Conference of Clinical Pharmacy, 2nd edition; 2017 June 15-17; Bucharest, Romania]. p. 195-198. ISBN 978-973-0-24609-4. Romanian.
15. Blessy M, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability indicating studies of drugs: a review. J Pharm Anal. 2014;4(3):159-165. doi: 10.1016/j.jpha.2013.09.003.
16. Wall GM, Stroman DW, Roland PS, Dohar J. Ciprofloxacin 0.3 %/dexamethasone 0.1 % sterile otic suspension for the topical treatment of ear infections: a review of the literature. Pediatr Infect Dis J. 2009;28:141-144. doi: 10.1097/inf.0b013e31818b0c9c.
17. Alsante KM, Ando A, Brown R, et al. The role of degradant profiling in active pharmaceutical ingredients and drug products. Adv Drug Deliv Rev. 2007;59(1):29-37. doi: 10.1016/j.addr.2006.10.006.
18. Sharma MK, Murugesan M. Forced degradation study an essential approach to develop stability indicating method. J Chromatogr Sep Tech. 2017;8(1):349. doi: 10.4172/2157-7064.1000349.
19. Pyka A, Babuska-Roczniak M, Bochenska P. Determination of hydrocortisone in pharmaceutical drug by TLC with densitometric detection in UV. J Liq Chromatogr Relat Technol. 2011;34(9):753-769. doi: 10.1080/10826076.2011.563891.
20. Baertschi SW, Thatcher SR. Sample presentation for photo stability studies: problems and solutions. In: Piechocki J, Thoma K, editors. Pharmaceutical photostability and stabilization technology. New York: Taylor & Francis; 2006. p. 445-450.
21. Glass BD, Novák C, Brown ME. The thermal and photostability of solid pharmaceuticals. J Therm Anal Calorim. 2004;77(3):1013. doi: 10.1023/B:JTAN.0000041677.48299.25.
22. Tollefson AE, Hermiston TW, Lichtenstein DL, Colle CF, Tripp RA, Dimitrov T, Toth K, Wells CE, Doherty PC, Wold WS. Forced degradation of Fas inhibits apoptosis in adenovirus-infected cells. Nature. 1998;392(6677):726-30. doi: 10.1038/33712.
23. Naveed S, Naseem Y, Samie S, Khan S, Ramzan S. Degradation study of five different brands of ciprofloxacin using UV visible spectrophotometer and their comparative study. Int Res J Pharm. 2014;5(3):189-190. doi: 10.7897/2230-8407.050339.
24. Aksoy B, Kucukguzel I, Rollas S. Development and validation of a stability-indicating HPLC method for determination of ciprofloxacin hydrochloride and its related compounds in film-coated tablets. Chromatographia. 2007;66:57-63. doi: 10.1365/s10337-007-0287-6.
25. Sharma EA, Shah NJ. Stability indicating RP-HPLC method for combination of pseudoephedrine sulphate and loratadine hydrochloride in pharmaceutical formulation. Int J Pharm Sci Res. 2018;9(2):599-607. doi: 10.13040/IJPSR.0975-8232.9(2).599-07.

Authors' ORCID iDs and academic degrees

Eugeniu Nicolai, PharmD, PhD Applcant – https://orcid.org/0000-0003-4666-9335.
Oxana Vislouh, PharmD, – https://orcid.org/0000-0001-7537-3758.
Vladimir Valica, PharmD, Pharm PhD, Professor – https://orcid.org/0000-0002-1068-5504.
Sergiu Parii, MD, PhD, Associate Professor – https://orcid.org/0000-0003-2229-4444.
Livia Uncu, PharmD, Pharm PhD, Associate Professor – https://orcid.org/0000-0003-3453-2243.

Authors' contribution

EN performed the technological part, interpreted the data, drafted the first manuscript; OV performed the analytical part of the laboratory work; VV interpreted the data, revised the manuscript; SP interpreted the data, revised the manuscript; LU designed the study, conducted the laboratory work, interpreted the data and revised the manuscript. All the authors revised and approved the final version of the manuscript.

Funding

This study was supported by *Nicolae Testemitanu* State University of Medicine and Pharmacy. The trial was the authors' initiative. The authors are independent and take responsibility for the integrity of the data and accuracy of the data analysis.

Ethics approval and consent to participate

No approval was required for this study.

Conflict of Interests

No competing interests were disclosed.

DOI: 10.5281/zenodo.3958563
UDC: 616.211-006.5-092:546.172.6-31

Correlation between nitric oxide levels and clinical features in patients with nasal polyposis

Ala Istratenco

Department of Laboratory Medicine, Department of Otorhinolaryngology
Nicolae Testemitanu State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova

Author's ORCID iD, academic degrees and contribution are available at the end of the article

Corresponding author: ala.istratenco@usmf.md
Manuscript received July 30, 2020; revised manuscript August 14, 2020; published online August 26, 2020

Abstract

Background: Nasal polyposis (NP) is a multifactorial pathology with negative impact on quality of life. The pathogenesis of NP has not been fully elucidated. This limits the pathogenic treatment. Recent studies suggest that nitric oxide (NO) and its metabolites are involved in pathophysiological events of NP. Purpose of the study: to evaluate the tissue NO and nitrite/nitrate (NO_2^-/NO_3^-) levels in patients with and without NP and to establish the relationship between NO levels and some clinical features.
Material and methods: 86 recruited patients were divided into a case group (N_1=43), patients with NP and a control group (N_2=43), patients with septal deviations and turbinate hypertrophies. Visual Analog Scale (VAS) was used to evaluate the severity of nasal obstruction and olfactory disturbances. NO and NO_2^-+NO_3^- concentrations in tissue specimens were measured by spectrometric method.
Results: Case group had significantly lower NO (U=173.5, p<0.001) and NO_2^-+NO_3^- levels (U=123.5, p<0.001). A negative correlation was found between VAS for nasal obstruction and NO levels (r_s = -0.379, p<0.05), between VAS for olfactory disturbances and NO levels (r_s = -0.531, p<0.001), and between endoscopic score and NO levels (r_s = -0.758, p<0.05).
Conclusions: Our results corroborate the previous findings. This underlines that NO levels depend on the patency of sinus ostium and the state of osteomeatal complex. Further studies, which take into account the role of NO in different rhinosinusitis endotypes, are needed to be performed in order to improve the NP management.
Key words: nasal polyposis, nitric oxide, nasal obstruction, olfactory dysfunction, endoscopy score.

Cite this article
Istratenco A. Correlation between nitric oxide levels and clinical features in patients with nasal polyposis. *Mold Med J.* 2020;63(3):51-57. doi: 10.5281/zenodo.3958563.

Introduction

Nasal polyposis (NP), one of the clinical phenotypes of chronic rhinosinusitis, is recognized as being a major health problem, frequently encountered in otorhinolaryngological practice. NP is a chronic inflammatory condition of the upper respiratory tract with incompletely understood etiology [1-5]. NP is characterized by formation of nasal polyps, benign formations, arising from the mucosa of the paranasal sinuses or nasal cavity, and often manifested by a high tendency of recurrent growth even after surgical excision [1, 5-7]. NP is responsible for affecting the patient's quality of life (QoL) more than other chronic diseases, resulting in enormous socioeconomic consequences [1, 5, 8]. Olfactory dysfunction developed in NP can be debilitating with substantial impact on QoL [9]. Patients with NP express varying sleep disturbances in high proportion leading to an impairment of cognitive function and depression [10]. NP is still generating significant healthcare costs: the direct and indirect cost of lost working days [8, 11-13].

NP is attracting considerable interest due to its misunderstood pathogenesis. NP is widely considered a multifactorial pathology, inflammation playing one of the most important roles. Nevertheless, the causes that determine the persistence of chronic inflammation with nasal polyps formation, have not yet been fully established [1-3, 13-15]. There is a considerable amount of literature on the role of multiple factors (infectious and noninfectious inflammation, anatomic and genetic abnormalities, oxidative stress, aspirin intolerance/sensitivity, environmental factors) involved in the pathogenesis of NP [1, 16-18]. Therefore, pathogenic treatment of NP remains a challenging issue. Approximately 20% of patients are facing an uncontrolled pathology despite adequate medical therapy and modern sinus surgery [7, 19].

In recent years there has been considerable growing interest regarding the involvement of nitric oxide (NO) in the pathogenesis of NP [20-24]. Current understanding supports that NO and its counterpart reactive nitrogen species may participate in pathophysiological events in a variety of inflammatory diseases, including NP [20, 25]. The primary source of NO in the respiratory tract seems to be the paranasal sinuses. NO is involved in upper airway homeostasis

and immunity by modulating blood flow, regulating mucociliary clearance and acting as an antiviral and antimicrobial agent. Notwithstanding the fact that NO protects, it may expresses toxic effect under certain condition [20]. The exact role of NO in respiratory homeostasis and pathophysiology is still unclear. The purpose of this study was consequently to further current knowledge of involvement of NO in NP pathogenesis and to determine relationship between NO level and some clinical features.

Material and methods

This study involved eighty-six patients from the Otolaryngology Department of *Timofei Mosneaga* Republican Clinical Hospital (Chisinau, the Republic of Moldova). Prior to subject recruitment, the study protocol was reviewed and approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (SUMPh), the Republic of Moldova (Report No 30 of 29.03.2016). Written informed consent was obtained from all patients at the beginning of the study. From 2016 to 2019, eighty-six patients with NP, septal deviations and middle concha hypertrophies were recruited for the study. Patients were divided into two groups: group 1 – Case (N_1= 43) consisted of patients with NP, and group 2 – Control (N_2= 43) consisted of patients with septal deviations and middle concha hypertrophies. Patients with septal deviations and middle concha hypertrophies have nasal obstruction, comparable according to VAS to that of NP. The diagnosis was based on history, anterior rhinoscopy, endoscopic examination and paranasal sinus computed tomography (CT), CT was done by all the patients with NPs and some cases of middle concha hypertrophies. Endoscopy findings were scored according to the Lund and Kennedy scoring system [26]. The parameters, such as presence or absence and extent of nasal polyps, edema, and discharge were graded. For nasal polyps, 0 was given for the absence of polyps, 1 for polyps present within the middle meatus, and 2 for polyps beyond the middle meatus.

Routine blood sampling, blood biochemistry and urinalysis were performed to verify the presence of any illness that would be the exclusion criteria. The exclusion criteria included the following: age <18 years; pregnancy; severe chronic diseases (liver, kidney, cardiovascular, respiratory, malignancy, diabetes mellitus); chronic alcoholism; recent use (last four weeks) of topical or systemic glucocorticoids; refusal to participate in the study.

The Visual Analog Scale (VAS) was used to evaluate the severity of the nasal obstruction and severity of olfactory disturbances by scoring them on a continuous 10-cm horizontal line on which 0 cm represented no complaints and 10 cm – serious complaints, associated with nasal obstruction and olfactory disturbances, respectively. The SinoNasal Outcome Test-22 (SNOT-22) test was used in order to evaluate the severity of nasal symptoms and their influence on the QoL.

Tissue specimens were collected from all patients involved in the study. Polyp specimens were taken from all patients who underwent endoscopic surgery for NP. Control specimens (nasal mucosae) were obtained from patients who underwent an operation for septoplasty or concha hypertrophy. The freshly obtained tissue samples were immediately transferred to laboratory and stored at -70° C until needed (laboratory evaluation of NO levels). It is well known that NO rapidly degrades to nitrite and nitrate (NO_2^- and NO_3^-) in aqueous solution. Therefore, the nitrate and nitrite levels were estimated, to provide an index of NO production. Concentration of NO and its metabolites (NO_2^- and NO_3) was measured by spectrometric method in homogenized polyp and control specimens. Laboratory analyses were performed in the Scientific Biochemistry Laboratory of *Nicolae Testemitanu* SUMPh.

All statistical analyses were performed using Statistical Package for Social Sciences (IBM SPSS Statistics for Windows, version 22.0). The Kolmogorov-Smirnov test was used to test the normality of data distribution. The data were expressed as mean and standard deviations. The chi-square test was used to compare categorical variables between groups. The independent sample T-test and Mann Whitney-U test were used to compare continuous variables between the two groups. Spearman's rank correlation analyses were used to examine the association between variables. Correlations were considered negligible if $r_s < 0.3$, low if $0.3 < r_s < 0.5$, moderate if $0.5 < r_s < 0.7$, high if $0.7 < r_s < 0.9$, and very high if $0.9 < r_s < 1.0$. Differences were considered statistically significant at p value of less than 0.05.

Results

The group 1 (case) was composed of 43 patients with nasal polyposis: 26 men and 17 women, with a mean age of 48.09±13.56 years (range, 22 to 76 years). The group 2 (control) was composed of 43 patients with septal deviations and middle concha hypertrophies: 30 men and 13 women, with a mean age of 33.67±11.44 years (range, 19 to 65 years). Characteristics of the study populations are given in tab. 1. There were no statistically significant differences between the two groups regarding gender, residence, use of nasal decongestants and VAS score for nasal obstruction ($p > 0.05$), except in terms of distribution of the age. In addition, patients with NP had a greater VAS score for olfactory disorders and SNOT-22 score ($p < 0.05$), (tab. 1).

Comparison of NO and NO_2^-+NO_3^- concentrations

There were found significant differences between mean NO_2^-+NO_3^- and NO concentrations in polyp tissues and control specimens. Compared to group 2 (control), group 1 (case) had significantly lower NO_2^-+NO_3^- concentration in tissue specimens ($U=123.5$, $p < 0.001$) (tab. 2, Fig. 1B). The mean levels of NO were significantly lower in NP tissues than in control specimens ($U=173.5$, $p < 0.001$) (tab. 2, fig. 1A).

Correlation analysis between NO levels and some clinical features in study groups

The statistical analysis, performed using Spearman's coefficient, showed significant correlations between NO level and VAS for nasal obstruction, VAS for olfactory disorder

Table 1

Comparison of the demographic and clinical characteristics of all patients

Characteristics	Value		p
	Group 1 *(case)* (N_1=43)	***Group 2*** *(control)* (N_2=43)	
Age (years), Mean±SD	48.09±13.56	33.67±11.44	< 0.001[a]
Gender (male/female), N	26/17	30/13	0.249[b]
Residence (urban/rural), N	17/26	18/25	1.0[b]
Nasal decongestants (naphazoline/ xylometazoline/ oxymetazoline/ combined/ several types), N	9/18/2/0/14	5/15/2/1/20	0.482[b]
VAS score for nasal obstruction, Mean±SD	8.28±1.054	7.81±0.982	0.051[a]
VAS score for olfactory dysfunctions, Mean±SD	8.35±1.744	1.47±2.25	< 0.001[a]
Lund-Kennedy endoscopic score, Mean±SD	9.28±2.529	-	
SNOT-22 score, Mean±SD	61.79±15.875	50.30±15.26	0.001[c]

Note: SD, Standard deviation; N, number; VAS, Visual Analog Scale; SNOT-22, SinoNasal Outcome Test-22; – Mann-Whitney-U test; [b] – Chi-square test; [c] – Independent sample T-test.

Table 2

Comparison of NO and NO_2^-+NO_3^- concentrations in tissue specimens taken from patients

Characteristics	Value		p
	Group 1 *(case)* (N_1=43)	***Group 2*** *(control)* (N_2=43)	
NO_2^-+NO_3^- (µM/g.prot.) Mean ±SD	1.15±0.54	2.89±1.02	< 0.001[a]
NO (µM/g.prot.), Mean±SD	0.79±0.49	1.99±0.85	< 0.001[a]

Note: SD, Standard deviation; [a] – Mann-Whitney-U test.

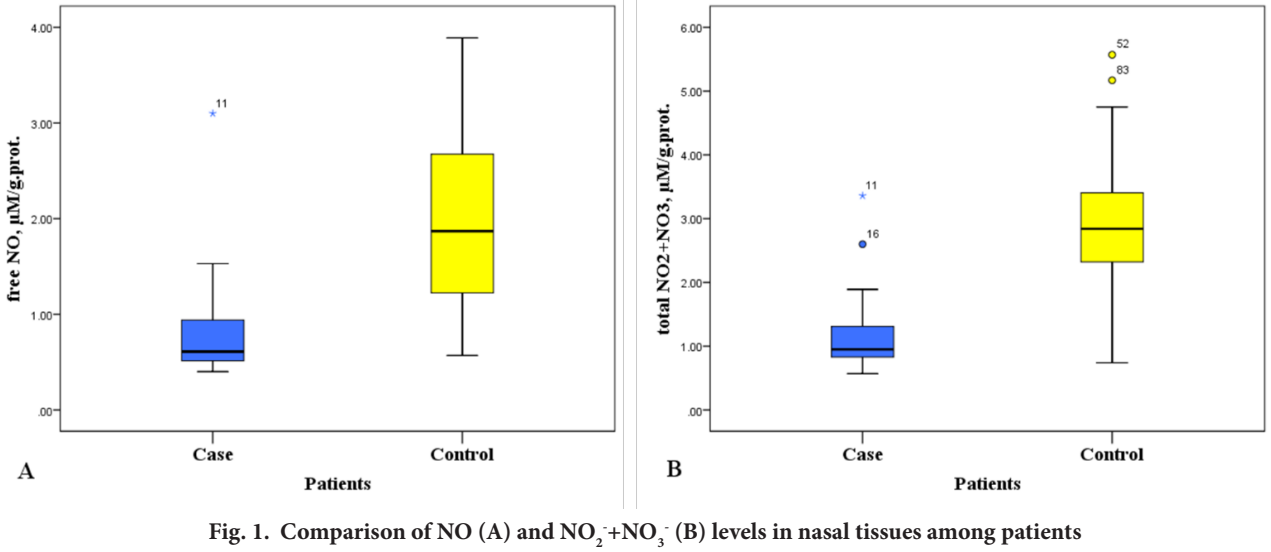

Fig. 1. Comparison of NO (A) and NO_2^-+NO_3^- (B) levels in nasal tissues among patients

and Lund-Kennedy endoscopic score in case group (tab. 3). However, no significant correlations were observed between NO level and nasal obstruction, and olfactory disorder in control group ($p > 0.05$ for all) (tab. 3). Furthermore, correlations were found negligible.

While analysing the correlation between the VAS for nasal obstruction and NO levels in case group, it was observed a low, negative correlation (r_s = -0.379, $p < 0.05$) (tab. 3). From the scatterplot (fig. 2A), which relates the VAS for nasal obstruction to the NO levels, it may be concluded that the increase of nasal obstruction corresponds to the decrease of NO values. A more severe nasal obstructive symptoms corresponded to the lower levels of NO. A moderate, negative correlation was found between VAS for olfactory dysfunctions and NO levels (r_s = -0.531, $p < 0.001$) (tab. 3). From the scatterplot (fig. 2 B) it may be concluded that patients reporting the worst olfactory status were associated with the lowest mean of NO. A Spearman's correlation run to determine the relationship between Lund-Kennedy endoscopic score and NO levels showed a high, negative correlation between endoscopic score and NO levels (r_s = -0.758, $p < 0.05$), (tab. 3 and fig. 2C).

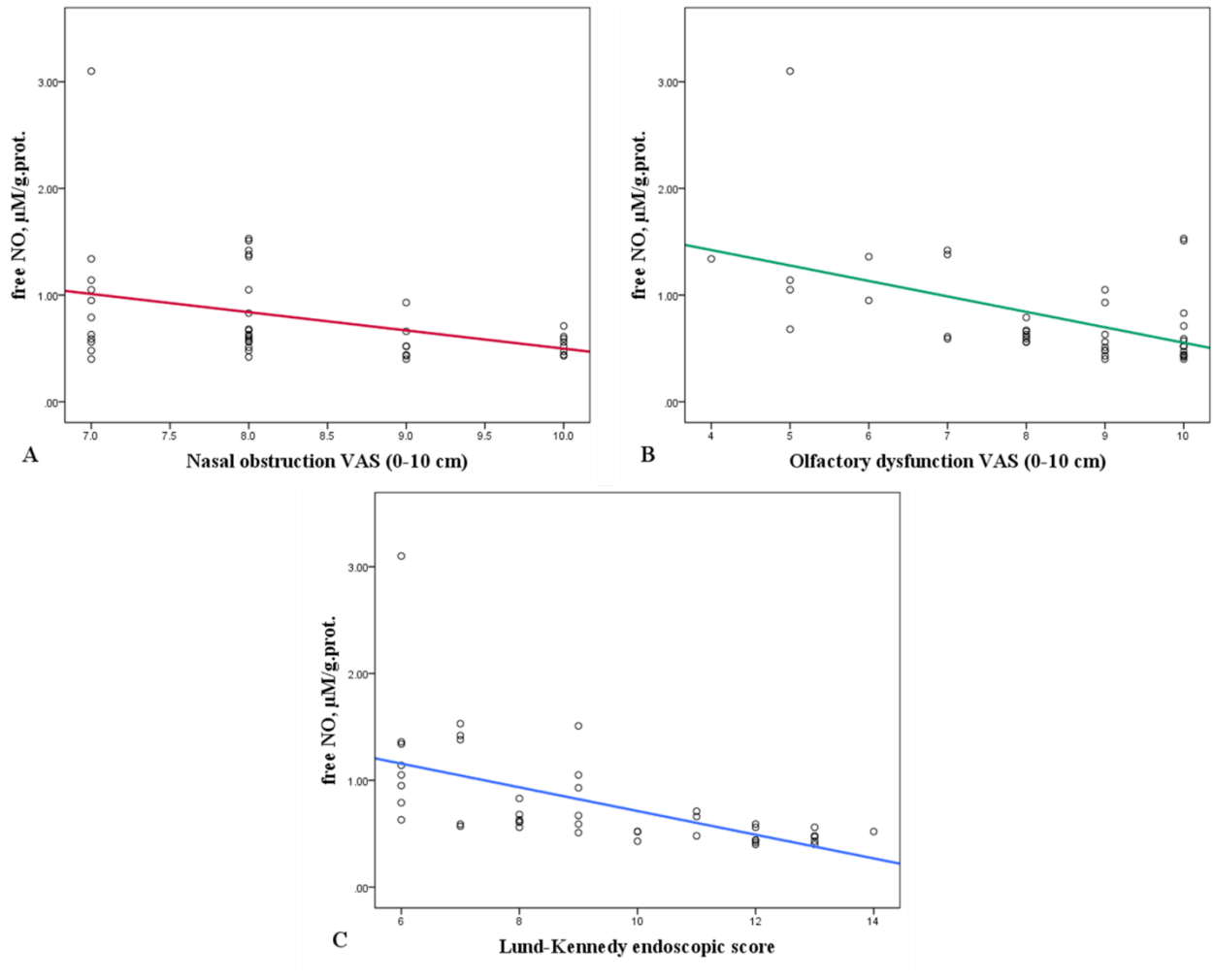

Fig. 2. Correlation between NO levels and some clinical findings: VAS for nasal obstruction (A), VAS for olfactory dysfunctions (B), and Lund-Kennedy endoscopic score (C)

Discussion

The main findings of this study are summarized as follows: (1) NO and NO_2^-+NO_3^- levels in tissues of patients with NPs were lower than in patients without NP; (2) Patients who had lower NO levels reported a more increased VAS score for nasal obstruction and olfactory dysfunction, and a more increased endoscopic score.

NO is a reactive molecule with physiological and pathological effects, mediated by its metabolites rather than the NO molecule itself, being involved in a variety of vital processes, such as antimicrobial/antiviral activity, blood flow regulation, cell metabolism, neurotransmission, immunity, inflammation control, platelet function, etc. [16]. NO production is catalysed by nitric oxide synthase (NOS). Once synthesized, NO acts as an intracellular or extracellular messenger. There are known three isoforms of NOS: NOS-1 (nNOS) and NOS-3 (eNOS) are constitutive isoforms, found in neurons and endothelial cells, and NOS-2 (iNOS) is in-

Table 3

Correlations between variables using Spearman's coefficient

		Nasal obstruction VAS (cm)	Olfactory dysfunctions VAS (cm)	Lund-Kennedy endoscopic score
Case group (patients with NP)				
NO, µM/g.prot.	Spearman's correlation	-0.379*	-0.531**	-0.758*
	P value	0.012	< 0.001	< 0.001
Control group (patients without NP)				
NO, µM/g.prot.	Spearman's correlation	-0.084	-0.038	
	P value	0.59	0.80	

Note: * - Correlation is significant at the 0.05 level; ** - Correlation is significant at the 0.01 level.

ducible isoform, found in activated cells during infection or inflammation [20, 21]. A number of studies have found that exhaled NO is mainly produced in the upper airways [20, 25], where NO is generated by the paranasal sinus epithelium and then diffuses into the nasal cavities. Therefore, the paranasal sinuses appear to be the primary source of NO in the respiratory system [21, 27-29].

In this study, there were estimated the NO and NO_2^- +NO_3^- levels in tissue specimens, finding the lowest NO and NO_2^-+NO_3^- levels in patients with NP. These results are in line with some previous results. Frendo et al. [30] reported that nasal NO was significantly lower in patients with NP compared with controls. Torretta et al. [31] also showed that median nasal NO levels were lower in NP than in controls. Arnal et al. [29] demonstrated that NO concentration in nasal nonallergic polyposis was significantly decreased compared with both controls and polyposis with allergy. These findings contrast with previous results reported by Karlida et al. [23], showing an increased tissue NO levels in patients with NP. They suggested that inflammatory cells cause more excessive free oxygen radicals (FORs), including NO, and that natural antioxidants are inadequate for detoxification of these FORs. Therefore, undetoxified FORs may cause tissue damage and NP [23].

The NO_2^-+NO_3^- concentrations are hardly distinguishable from those of Bugdayci et al. [32], who demonstrated the change in NO_2^-/NO_3^- levels of nasal polyp patients, reporting a higher level of NO_2^- and NO_3^- than in normal tissue ($p<0.05$). They mentioned that further studies are required concerning the significance of changes in lipid peroxidation and nitrite levels in pathogenesis of NP [32].

Sadek et al. [25] studying histological and genetical expression of iNOS to evaluate the role of NO in the pathogenesis of allergic and non-allergic NP detected that the expression of iNOS in both epithelial and stromal layers was greater in NP than in control tissues. The allergic NP group showed more iNOS expression than those of non-allergic NP group. Similarly, Kang et al. [33], studying the expression of iNOS, and the production of peroxynitrite represented by the formation of 3-nitrotyrosine (3-NT) by immunohistochemistry in nasal polyps, demonstrated that the stromal cells of the nasal polyp had higher labeling intensity for both iNOS and 3-NT.

Although our results regarding NO levels differ from those of Karlidag et al. [23], and NO_2^-+NO_3^- levels differ considerably from those of Bugdayci et al. [32], the difference could nevertheless be explained. Furthermore, several studies, for instance [20, 25, 33], performed on iNOS and the levels of NO, showed more iNOS expression and upregulation in NP despite lower nasal NO levels than in controls. These are possibly related to mechanical blockage of the ostiomeatal complex by nasal polyp tissue and failure of NO generated constitutively and inducible in the sinuses to reach the nasal airway and to diffuse into nasal tissue. Colantonio et al. [34] supported that the rise of NO after medical and surgical treatment is due to decrease of obstruction of sinus ostium. Colantonio et al. propose the following scenario. NO levels, being the result of inducible plus constitutive production, in uncomplicated allergic rhinitis with patent sinus ostia tend to be elevated, but when inflammation is sufficient to obstruct sinus ostium (as observed in NP), NO levels fall.

Patient-reported subjective measures of symptoms, such as VAS for nasal obstruction and for olfactory dysfunctions, and objective measure such as Lund-Kennedy score were assessed in order to correlate the findings with the levels of NO. There were found negative correlations that are consistent with previous results [28, 31, 35, 36].

Delclaux et al. [36] conducted a study in which statistical relationships were demonstrated between nasal NO and severity scores (clinical: p=-0.31, p=0.015; endoscopic: p=-0.57, p<0.0001; CT: p=-0.46, p=0.0005). Torretta et al. [32] found that Lund-Mackay scores (CT scores) inversely correlated with median NO levels (r=-0.31; p=0.04). Jeong et al. [28] observed a significant inverse relationship between NO levels and sinus CT scores, severity of nasal obstruction, and purulent rhinorrhea in chronic rhinosinusitis with polyps. No publications were identified that assess the correlation between NO concentrations and endoscopy Lund-Kennedy score. However, Nass et al. have found that the correlation between CT and nasal endoscopic findings in chronic rhinosinusitis diagnosis constitutes 90% [37].

Landis et al. [38] summarized in their review that both nasal NO and olfactory function are worth testing routinely in any rhinology workup. Regrettably, no evidence for NO levels in NP and loss of smell was found. Elsherif et al. [39] found that olfactory function and NO concentration correlate in chronic rhinosinusitis patients but not in healthy subjects, suggesting that both parameters do not directly influence each other. The inflammatory process present in chronic rhinosinusitis presumably affects olfaction and NO levels. Nasal NO produced by the paranasal sinuses seems not to directly influence olfactory function [39].

Our results related to NO levels in NP tissue and relationship between NO levels and clinical outcomes are in agreement with those obtained in earlier studies despite some limitations regarding the methodology of assessing the NO concentrations and endotypes of recruited patients.

Conclusions

In conclusion, our results corroborate the previous findings in the literature regarding significantly lower NO levels in NP compared to controls and negative correlation between NO levels and nasal obstruction, olfactory disturbances, and endoscopic appearance in patients with NP.

This study provides additional insight into complicated biological pathways of NO involvement in the pathogenesis of NP. Taken together, these results would seem to suggest the idea that the levels of NO, generated mainly in paranasal sinuses in normal state and pathological one, depend on the patency of sinus ostium and the state of osteomeatal complex. NP environment is characterized by abnormalities in NO metabolism. Further studies, which take into account

the role of NO in different chronic rhinosinusitis endotypes, are needed to be undertaken in order to allow an earlier and more accurate diagnosis, noninvasive follow-up monitoring, and development of new therapeutic approaches that will prevent the harmful direct and indirect effects mediated by NO.

References

1. Fokkens WJ, Lund VJ, Hopkins C, Hellings PW, Kern R, Reitsma S, et al. European position paper on rhinosinusitis and nasal polyps 2020. Rhinology. 2020;58(Suppl 29):1-464. doi: 10.4193/Rhin20.600.
2. Lam K, Schleimer R, Kern RC. The etiology and pathogenesis of chronic rhinosinusitis: a review of current hypotheses. Curr Allergy Asthma Rep. 2015;15(7):41. doi: 10.1007/s11882-015-0540-2.
3. Ickrath P, Kleinsasser N, Ding X, Ginzkey C, Beyersdorf N, Hagen R, et al. Characterization of T-cell subpopulations in patients with chronic rhinosinusitis with nasal polyposis. Allergy Rhinol. 2017;8(3):139-147. doi: 10.2500/ar.2017.8.0214.
4. Cain RB, Lal D. Update on the management of chronic rhinosinusitis. Infect Drug Resist. 2013;6:1-14. doi: 10.2147/IDR.S26134.
5. Esmatinia F, Bakhshaei M. Recurrent sinonasal polyposis after the endoscopic sinus surgery. Rev Clin Med. 2014;1(2):86-92. doi: 10.17463/RCM.2014.02.010.
6. Newton JR, Ah-See KW. A review of nasal polyposis. Ther Clin Risk Manag. 2008;4(2):507-512. doi: 10.2147/tcrm.s2379.
7. Istratenco A. Oxidative stress-related pathophysiology in chronic rhinosinusitis with nasal polyps: research challenges. Rom J Rhinol. 2019;9(34):71-77. doi: 10.2478/rjr-2019-0008.
8. Bhattacharyya N, Villeneuve S, Joish VN, Amand C, Mannent L, Amin N, et al. Cost burden and resource utilization in patients with chronic rhinosinusitis and nasal polyps. Laryngoscope. 2019;129(9):1969-1975. doi: 10.1002/lary.27852.
9. Victores AJ, Chen M, Smith A, Lane AP. Olfactory loss in chronic rhinosinusitis is associated with neuronal activation of c-Jun N-terminal kinase. Int Forum Allergy Rhinol. 2018;8(3):415-420. doi: 10.1002/alr.22053.
10. Mahdavinia M, Schleimer RP, Keshavarzian A. Sleep disruption in chronic rhinosinusitis. Expert Rev Anti Infect Ther. 2017;15(5):457-465. doi: 10.1080/14787210.2017.1294063.
11. Wang DY, Ghoshal AG, Bin Abdul Muttalif AR, Lin H-C, Thanavirata-nanich S, Bagga S, et al. Quality of life and economic burden of respiratory disease in Asia-Pacific Burden of Respiratory Diseases Study. Value Heal Reg issues. 2016;9:72-77. doi: 10.1016/j.vhri.2015.11.004.
12. Lourijsen ES, Fokkens WJ, Reitsma S. Direct and indirect costs of adult patients with chronic rhinosinusitis with nasal polyps. Rhinology. 2020;58(3):213-217. doi: 10.4193/Rhin19.468.
13. Hulse KE, Stevens WW, Tan BK, Schleimer RP. Pathogenesis of nasal polyposis. Clin Exp Allergy. 2015;45(2):328-346. doi: 10.1111/cea.12472.
14. Dinarte VRP, Santos ARD dos, Araújo LF de, Reis MGA dos, Tamashiro E, Valera FCP, et al. Polymorphisms in chronic rhinosinusitis with nasal polyps – a systematic review. Braz J Otorhinolaryngol. 2017;83(6):705-711. doi: 10.1016/j.bjorl.2017.03.002.
15. Ball SL, Mann DA, Wilson JA, Fisher AJ. The Role of the Fibroblast in Inflammatory Upper Airway Conditions. Am J Pathol. 2016;186(2):225-233. doi: 10.1016/j.ajpath.2015.09.020.
16. Holecek V, Rokyta R, Slipka J. Free radicals in nasal and paranasal diseases. In: Miller J, Le Prell CG, Rybak L, editors. Free radicals in ENT pathology. Cham: Humana Press; 2015. p. 479-492. doi: 10.1007/978-3-319-13473-4_24.
17. Duda R. The role of inflammatory mediators in the pathogenesis of nasal polyposis: literature review. Rom J Rhinol. 2015;5(18):81-85. doi: 10.1515/rjr-2015-0009.
18. Akyigit A, Keles E, Etem EO, Ozercan I, Akyol H, Sakallioglu O, et al. Genetic polymorphism of antioxidant enzymes in eosinophilic and non-eosinophilic nasal polyposis. Eur Arch Otorhinolaryngol. 2017;274(1):267-273. doi: 10.1007/s00405-016-4259-z.
19. Wagenmann M, Scheckenbach K, Chaker AM. Endotypes in chronic rhinosinusitis: biomarkers based on a mechanistic insight for targeted treatment? ORL J Otorhinolaryngol Relat Spec. 2017;79(1-2):78-84. doi: 10.1159/000455721.
20. Tamashiro E, Banks CA, Cohen NA. New areas for investigation: Nitric Oxide. In: Önerci TM, Ferguson BJ, editors. Nasal polyposis: pathogenesis, medical and surgical treatment. Heidelberg: Springer; 2010. p. 175-183. doi: 10.1007/978-3-642-11412-0_20.
21. Serrano C, Valero A, Picado C. Nasal nitric oxide. Arch Bronconeumol. 2004;40(5):222-230. doi: 10.1016/s1579-2129(06)70088-x.
22. Liu C, Zheng M, He F, Wang X, Zhang L. Role of exhaled nasal nitric oxide in distinguishing between chronic rhinosinusitis with and without nasal polyps. Am J Rhinol Allergy. 2017;31(6):389-394. doi: 10.2500/ajra.2017.31.4480.
23. Karlidag T, Keles E, Ilhan N, Yalçin S, Kaygusuz I, Yildiz M. Roles of free radicals, nitric oxide, and scavenging enzymes in nasal polyp development. Ann Otol Rhinol Laryngol. 2005;114(2):122-126. doi: 10.1177/000348940511400207
24. Lee JM, McKnight CL, Aves T, Yip J, Grewal AS, Gupta S. Nasal nitric oxide as a marker of sinus mucosal health in patients with nasal polyposis. Int Forum Allergy Rhinol. 2015;5(10):894-899. doi: 10.1002/alr.21598.
25. Sadek AA, Abdelwahab S, Eid SY, Almaimani RA, Althubiti MA, El-Readi MZ. Overexpression of inducible nitric oxide synthesis in allergic and nonallergic nasal polyp. Oxid Med Cell Longev. 2019;2019:7506103. doi: 10.1155/2019/7506103.
26. Lund VJ, Kennedy DW. Quantification for staging sinusitis. The staging and therapy group. Ann Otol Rhinol Laryngol Suppl. 1995;167:17-21.
27. Carey RM, Chen B, Adappa ND, Palmer JN, Kennedy DW, Lee RJ, et al. Human upper airway epithelium produces nitric oxide in response to *Staphylococcus epidermidis*. Int Forum Allergy Rhinol. 2016;6(12):1238-1244. doi: 10.1002/alr.21837.
28. Jeong JH, Yoo HS, Lee SH, Kim KR, Yoon HJ, Kim SH. Nasal and exhaled nitric oxide in chronic rhinosinusitis with polyps. Am J Rhinol Allergy. 2014;28(1):e11-6. doi: 10.2500/ajra.2014.28.3984.
29. Arnal JF, Flores P, Rami J, Murris-Espin M, Bremont F, Pasto L Aguilla M, et al. Nasal nitric oxide concentration in paranasal sinus inflammatory diseases. Eur Respir J. 1999;13(2):307-312. doi: 10.1034/j.1399-3003.1999.13b15.x.
30. Frendo M, Hakansson K, Schwer S, Ravn AT, Meteran H, Porsbjerg C, et al. Exhaled and nasal nitric oxide in chronic rhinosinusitis patients with nasal polyps in primary care. Rhinology. 2018;56(1):59-64. doi: 10.4193/rhin17.111.
31. Torretta S, Cappadona M, Cairoli D, Pignataro L. Airborne nitric oxide and nasal cytology in patients with chronic rhinosinusitis and nasal polyps. J Biol Regul Homeost Agents. 2015;29(4):969-976.
32. Bugdayci G, Kaymakci M. Nitrite/nitrate and malondialdehyde levels in nasal polyp. Cell Mol Biol (Noisy-le-grand). 2008;54 Suppl:OL1043-5.
33. Kang BH, Huang NC, Wang HW. Possible involvement of nitric oxide and peroxynitrite in nasal polyposis. Am J Rhinol. 2004;18(4):191-196. doi: 10.1177/194589240401800401.
34. Colantonio D, Brouillette L, Parikh A, Scadding GK. Paradoxical low nasal nitric oxide in nasal polyposis. Clin Exp Allergy. 2002;32(5):698-701. doi: 10.1046/j.1365-2222.2002.01379.x.
35. Yoshida K, Takabayashi T, Imoto Y, Sakashita M, Narita N, Fujieda S. Reduced nasal nitric oxide levels in patients with eosinophilic chronic rhinosinusitis. Allergol Int. 2019;68(2):225-232. doi: 10.1016/j.alit.2018.09.005
36. Delclaux C, Malinvaud D, Chevalier-Bidaud B, Callens E, Mahut B, Bonfils P. Nitric oxide evaluation in upper and lower respiratory

tracts in nasal polyposis. Clin Exp Allergy. 2008;38(7):1140-1147. doi: 10.1111/j.1365-2222.2008.03006.x.
37. Gupta D, Gulati A, Singh I, Tekur U. Endoscopic, radiological, and symptom correlation of olfactory dysfunction in pre-and postsurgical patients of chronic rhinosinusitis. Chem Senses. 2014;39(8):705-710. doi: 10.1093/chemse/bju042.
38. Landis BN, Lacroix JS. Olfactory function and nasal nitric oxide. Curr Opin Otolaryngol Head Neck Surg. 2009;17(1):18-22. doi: 10.1097/MOO.0b013e32831fb580.
39. Elsherif HS, Landis BN, Hamad MH, Hugentobler M, Bahig SM, Gamaa AM, et al. Olfactory function and nasal nitric oxide. Clin Otolaryngol. 2007;32(5):356-360. doi: 10.1111/j.1749-4486.2007.01534.x.

Author's ORCID iD and academic degrees
Ala Istratenco, MD, PhD Applicant – https://orcid.org/0000-0001-6776-4820.

Author's contribution
AI conceptualized the idea, conducted literature review, collected the data, interpreted the data, wrote the manuscript, revised and approved the final text.

Funding
This study was supported by *Nicolae Testemitanu* State University of Medicine and Pharmacy. The trial was the author's initiative. The author is independent and takes responsibility for the integrity of the data and accuracy of the data analysis.

Ethics approval and consent to participate
The study protocol was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (Report No 30 of 29.03.2016). It was obtained an informed consent from all participants in the study.

Conflict of Interests
There is no known conflict of interests to declare.

DOI: 10.5281/zenodo.3958567
UDC: 616.126.52-007.271-089.843(478)

Transcatheter aortic valve implantation. First experience of minimally invasive treatment in the Republic of Moldova

[*1,3]Marcel Abras, [1,3]Artiom Surev, [2]Mariuca Vasa-Nicotera, [3]Vitalie Moscalu, [1,3]Andrei Grib, [3]Ion Popovici, [1]Catalina Beiu

[1]Department of Internal Medicine, Cardiology, *Nicolae Testemitanu* State University of Medicine and Pharmacy Chisinau, the Republic of Moldova
[2]Department of Cardiology, Cardiac Catheterization and Structural Heart Program University Hospital of Frankfurt, Germany
[3]Department of Cardiac Catheterization, Institute of Cardiology, Chisinau, the Republic of Moldova

Authors' ORCID iDs, academic degrees and contributions are available at the end of the article

*Corresponding author: marcel.abras@usmf.md
Manuscript received on July 02, 2020; revised manuscript August 17, 2020; published online August 26, 2020

Abstract

Background: Aortic stenosis (AS) is the most prevalent primary heart valve disease and the third most prevalent cardiovascular disease in the world, having a severe and slowly progressive evolution. The implementation of Transcatheter Aortic Valve Implantation (TAVI) is necessary because the population is constantly ageing, and most elderly people have various comorbidities, which places them among patients with high surgical risk. The aim was to report immediate and 9 months follow-up results of first TAVI implantations in the Republic of Moldova.
Material and methods: Three patients with severe symptomatic AS and high surgical risk underwent TAVI implantation using Portico valves.
Results: The mean age was 76.7 ± 1.2 years, 2 women and 1 man. The mean Logistic EuroSCORE II was 5.68 ± 0.67%. Procedural success was achieved in all (100%) patients. After the intervention, it was shown that the GP mean decreased by an average of 20.27±1.61 mmHg, the Vmax of the jet through AoV decreased on average by 2.05±0.19 m/s, and they were maintained all over the nine months follow-up period. Quality of life, using the TASQ (Toronto Aortic Stenosis Quality of Life) questionnaire, was improved by 62.3 ± 2.2 points.
Conclusions: TAVI, as a new minimally invasive method of treatment for aortic valve stenosis appears to be safe, with a low rate of early and late complications in elderly patients at high surgical risk, with good clinical outcomes at 9 months. Its implementation in the Republic of Moldova is welcome in the conditions of continuously ageing population and the prevalence of associated comorbidities.
Key words: aortic stenosis, high surgical risk, Transcatheter Aortic Valve Implantation, elderly patients.

Cite this article
Abras M, Surev A, Vasa-Nicotera M, Moscalu V, Grib A, Popovici I, Beiu C. Transcatheter aortic valve implantation. First experience of minimally invasive treatment in the Republic of Moldova**.** *Mold Med J.* 2020;63(3):58-65. doi: 10.5281/zenodo.3958567.

Introduction

Aortic stenosis (AS) is the most prevalent primary heart valve disease [1] and the third most prevalent cardiovascular disease in the world, having a severe and slowly progressive evolution [2]. This pathology is defined by firbro-calcific remodeling of the normal aortic valve (AoV) (tricuspid) or congenital abnormal AoV (biscupid), which results in the narrowing of the valve opening through a degenerative mechanism. Its evolution is specific for elderly patients, who have other comorbidities and is associated with progressive heart dysfunction. Patients with severe symptomatic AS, without any intervention, reach a fatal rate of about 50% at 2 years and the only way to stop the progression to a terminal heart disease is valve replacement [2, 3].

A meta-analysis of studies conducted in Europe and USA concluded that the prevalence of AS is 12.4% among the population, and for those over the age of 75 years is 3.4% [4], so that more than 1 out of 8 people over the age of 75 suffer from this pathology [5]. As the population ages, AS becomes an important issue both in terms of declining patients' quality of life and in terms of the impact on the health care system [5].

Until recently, the golden standard and the only solution in treating AS was surgical aortic valve replacement (SAVR). However, the risks associated with SAVR are increased in elderly patients and those with severe systolic heart failure or concomitant coronary heart disease, as well as in patients with comorbidities such as cerebrovascular and peripheral arterial disease, chronic renal disease and chronic respiratory failure. Approximately one third of the patients with AS are inoperable or have a high surgical risk with the SAVR method [5, 6].

Thus, in 2002, Alain Cribier came up with an alternative for the replacement of stenotic AoV, namely the Transcatheter Aortic Valve Implantation (TAVI) [7]. The introduction of the new method of treatment has revolutionized the management of aortic stenosis [8]. Several studies, where first-generation devices have been used, have established the superiority of TAVI compared to medical therapy

that includes valvuloplasty in patients at high surgical risk [8]. Subsequently, TAVI has been proposed as a valuable alternative to SAVR in an ever-widening spectrum of patients with severe symptomatic AS. The safety and efficacy of TAVI were initially established in high surgical risk patients in the PARTNER Study 1A, US CoreValve High-risk Trial who presented comparable clinical results [9] non-inferior to SAVR [8, 10].

This intervention is minimally invasive and is based on the percutaneous implantation of a self-expanding AoV prosthesis, replacing the dysfunctional native valve, through a catheter, using transfemoral approach (also the transapical or transaortic approach might be considered). Due to the indications of TAVI, this represents an excellent opportunity for patients with an increased or intermediate surgical risk, or those who are inoperable [11]. The basic indications for TAVI, respecting the clinical aspects, would be STS / EuroSCORE II > 4%, presence of severe comorbidities, age > 75 years, previously undergone cardiac surgery, patient fragility which is associated with increased morbidity and mortality after surgery and TAVI, reduced motility and conditions that would affect the post-procedural rehabilitation process. Also, the anatomical aspects of the patients are taken into account, first of all a favorable transfemoral access for TAVI, porcelain aorta, the presence of coronary bypass with previous sternotomy, possible patient-prosthesis mismatch, severe thoracic deformities, post-radiation thoracic sequelae (tab. 1) [1].

At this stage, companies producing new-generation TAVI devices are developing important technological refinements, including reduced size delivery systems, trying to avoid or minimize paravalvular leakage. Broadly speaking, for clinical use, there are two main categories of AoV transcatheter prostheses: balloon-expandable valves and self-expandable valves [8].

The Portico Valve System (St. Jude Medical; Minneapolis, Minnesota, USA) is a second-generation transcatheter AoV, a self-expandable prosthesis that consists of a trifoliate bovine pericardial valve and a porcine pericardial sealing cuff attached to a nitinol frame, all meant to reduce paravalvular leakage. The delivery system allows full resheathing and repositioning prior to complete valve deployment until 80-90% of deployment, which is potentially one of the most important advantages of this transcatheter heart valve. At this stage, the annular section of the prosthesis has full contact, allowing the assessment of placement and hemodynamic function before its final release. In addition, the leaflets are designed to function at an intra-annular aortic position, which helps maintain hemodynamic stability during implantation [12].

As a result of a fruitful collaboration with the specialists of the University Hospital of Frankfurt, in October 2019, as a premiere for the Republic of Moldova, transcatheter AoV prostheses were implanted. Being the first team of doctors who performed TAVI in our country, we have the opportunity to report the data of a series of cases, with clinical follow-up at 9 months after the procedure.

Material and methods

I. The team

The team consisted of two cardiologists, a cardiovascular surgeon, an anesthesiologist and an echocardiographist. Interventional cardiologists underwent a training program in the University Hospital of Frankfurt. The first three cases were performed under the supervision of Doctor Mariuca Vasa-Nicotera, Frankfurt, Germany.

II. The patients

The first patients to benefit from the procedure were 2 women and a man, the mean age was 76.7 ± 1.2 years, with a severe degree of AoV calcification, who could not undergo SAVR due to the high surgical risk. Patients underwent coronary angiography, echocardiography and computed tomography to determine the coronary blood flow, structural cardiac parameters, AoV size and the surgical approach.

Patient No 1, woman, 79 years old.

She was admitted with severe AS documented following M-mode and two-dimensional echocardiography, with a mean pressure gradient of 58.33 mmHg and a maximal jet velocity through the AoV of 5.06 m/s, moderate AoV regurgitation, marked left ventricular (LV) myocardial hypertrophy with accelerated flow in the LV outflow tract, ejection fraction being 59% (fig. 1). On computed tomography was determined aortic valve with an annulus perimeter of 68.8 mm, its area of 363.8 mm^2, and a diameter of 21.5 mm (fig. 2). Also, the patient had a porcelain aorta (fig. 3). Coronary angiography revealed no stenotic lesions

Table 1

Indications for aortic stenosis interventions and the recommendations for choosing the type of intervention [1].

Symptomatic patients with aortic stenosis	Class	Level
The intervention is indicated for symptomatic patients with severe aortic stenosis, severe high gradient (AV Vmax >4 m/s or mean gradient >40 mm Hg)	I	B
Indicated in patients with low-flow low-gradient severe AS (<40 mmHg) with reduced ejection fraction and evidence of contractile reserve excluding pseudo-severe AS.	I	C
Intervention should be considered in patients with symptomatic low-flow low-gradient severe AS (<40 mmHg), with preserved ejection fraction, after careful confirmation of severe AS.	IIa	C
Intervention should be considered in patients with low-flow low-gradient severe AS with reduced ejection fraction without evidence of contractile reserve especially where CT calcium scoring confirms severe AS.	IIa	C

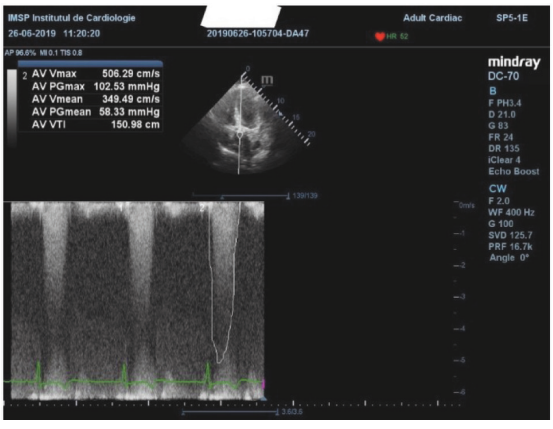

Fig. 1. Echocardiography, patient No 1

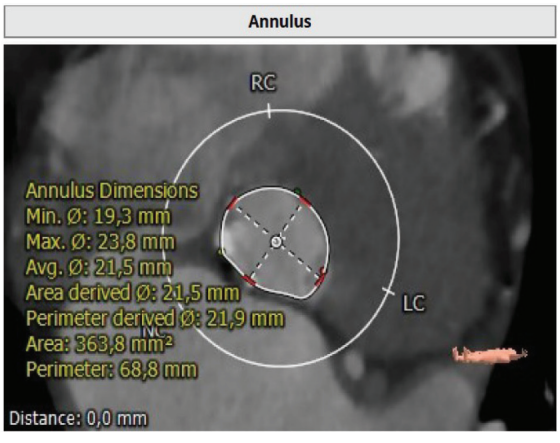

Fig. 2. CT of aortic valve, patient No 1

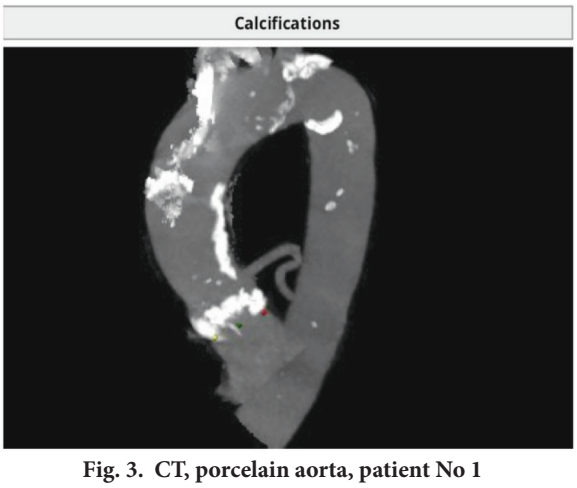

Fig. 3. CT, porcelain aorta, patient No 1

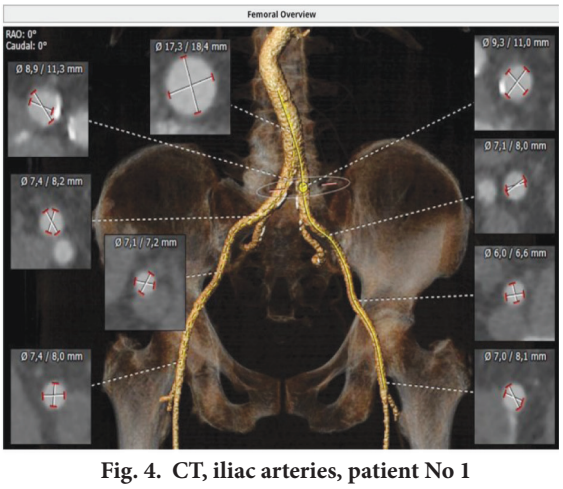

Fig. 4. CT, iliac arteries, patient No 1

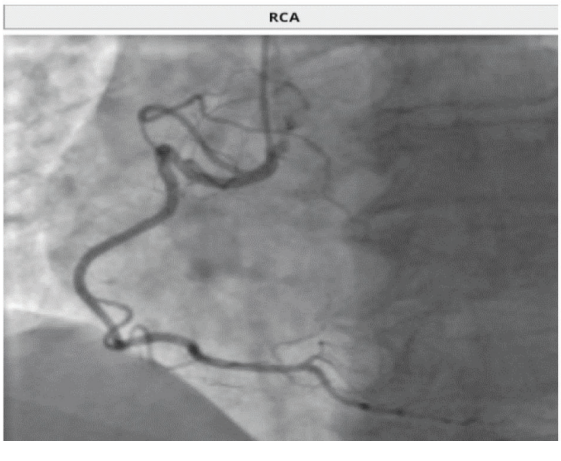

Fig. 5. Coronarography, patient No 1

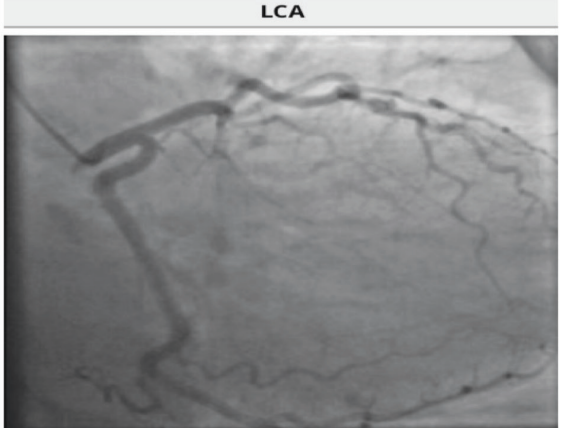

Fig. 6. Coronarography, patient No 1.

of the coronary arteries (fig 5, 6). Calculated in advance, the patient had an Euroscore II of 6.14%.

The size of the selected valve, according to the above parameters, was 25 mm. After analysing the anatomical aspects of the iliac and femoral arteries (fig. 4), the approach for intervention was chosen, namely the right femoral one.

Patient No 2, man, 76 years old.

At admission, he presented with severe AS and several comorbidities such as dyslipidemia, type 2 diabetes, chronic bronchitis in partial remission, left renal cysts, right eye blindness, obesity class II, discirculatory encephalopathy. Euroscore II calculated was 6.53%.

Following M-mode and two-dimensional real-time cardiac ultrasonography, AoV critical stenosis was determined with a mean pressure gradient at the aortic valve of 36.57 mmHg, the maximal aortic jet velocity being 3.84 m/s and the ejection fraction of the left ventricle 36% (fig. 7). The computed tomography showed an aortic valve annulus with a diameter of 24.7 mm, an area of 450.5 mm^2 and a perimeter of 77.9 mm (fig. 8, 9). Coronary angiography did not de-

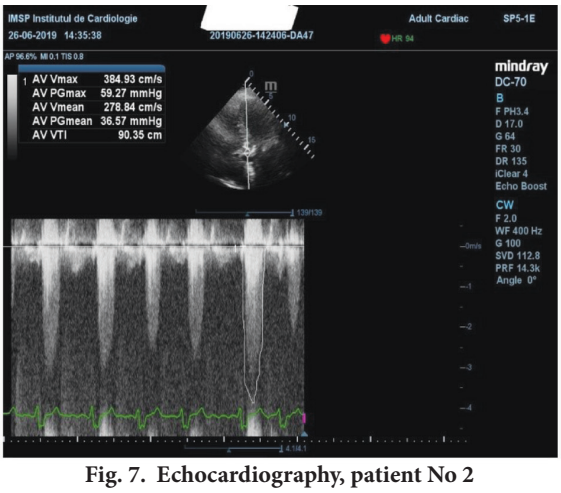

Fig. 7. Echocardiography, patient No 2

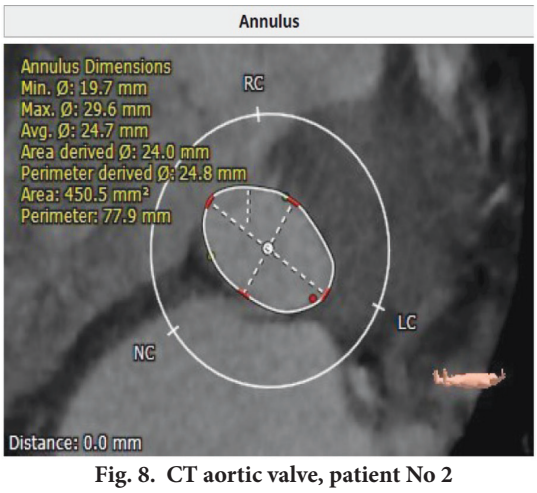

Fig. 8. CT aortic valve, patient No 2

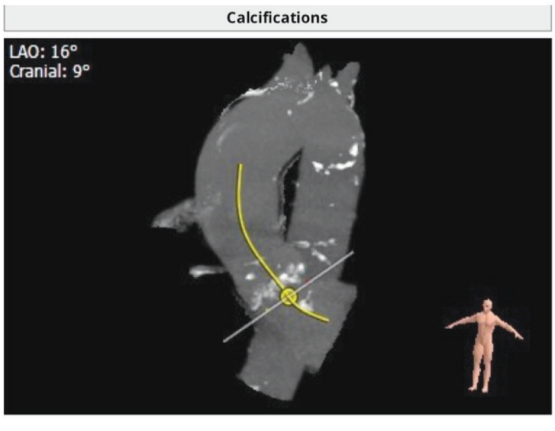

Fig. 9. CT, aorta, patient No 2

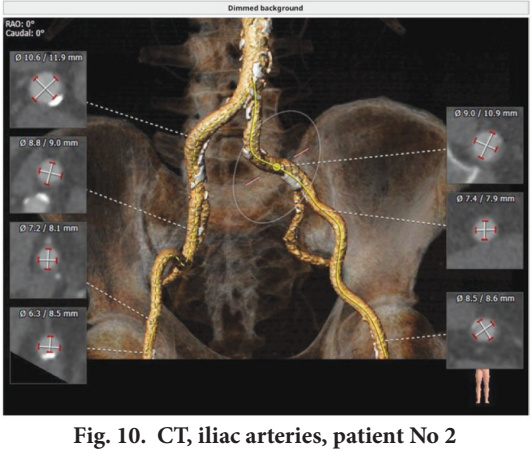

Fig. 10. CT, iliac arteries, patient No 2

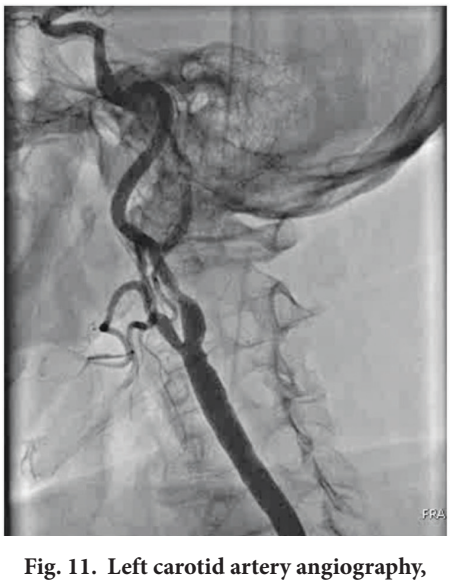

Fig. 11. Left carotid artery angiography, patient No 2

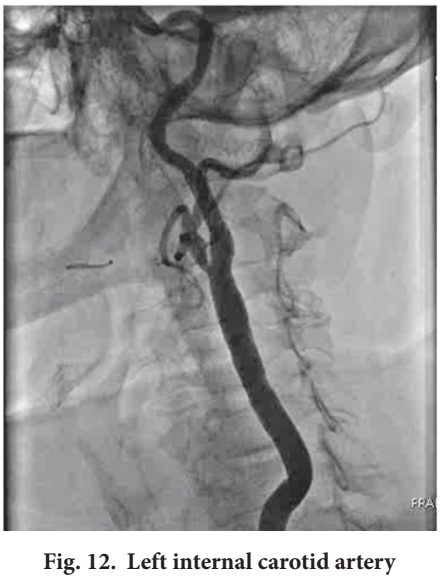

Fig. 12. Left internal carotid artery angiography, after angioplasty

termine stenoses on the coronary arteries but revealed critical stenosis with the appearance of unstable plaque on the left internal carotid artery (fig. 11). Two weeks before the procedure, the patient successfully underwent carotid angioplasty with self-expanding stent implantation (PROTEGE Rx) (fig. 12).

According to these data, the size of the valve should be 27 mm, but due to ellipticity of the valve, a 29 mm one was chosen. The geometric construction of the valve allows it to shape itself to various anatomical variations, but also to easily attach to the calcifications of the native valve, without deforming the nitinol frame. Analysing the anatomical as-

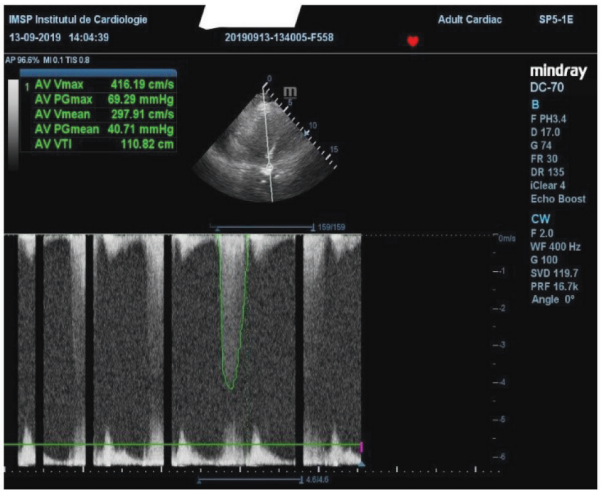

Fig. 13. Echocardiography, patient No 3

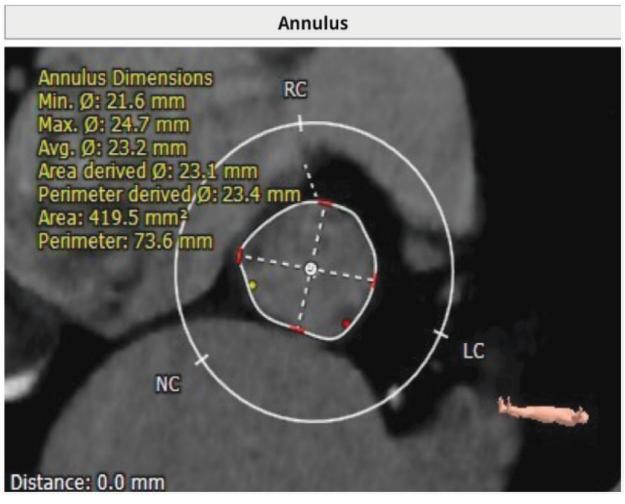

Fig. 14. CT of the aortic valve, patient No 3

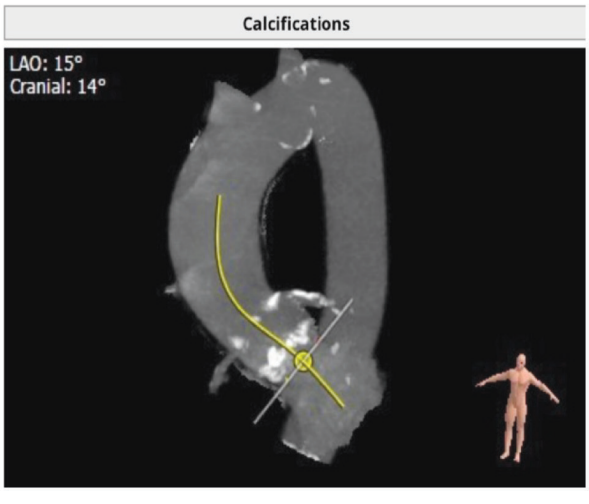

Fig. 15. CT of aorta, patient No 3

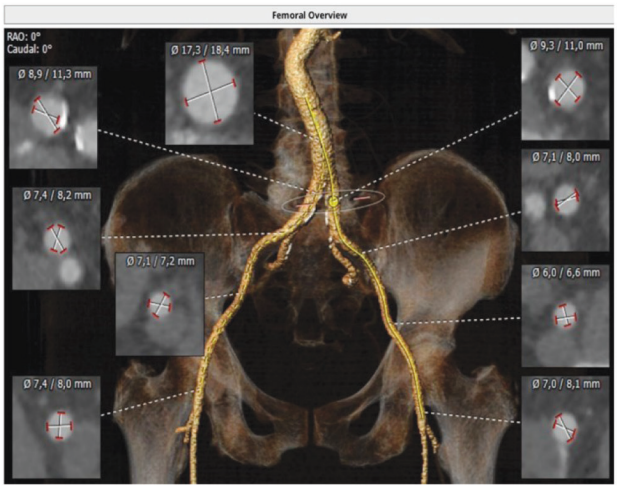

Fig. 16. Iliac arteries CT, patient No 3

pect of the iliac and femoral arteries (fig. 10), it was decided to select the approach for intervention – the left femoral.

Patient No 3, woman, 75 years old.

The patient was admitted with diagnosis of severe AS, left bundle branch block, permanent atrial fibrillation, high thromboembolic risk (CHADS VASc = 6), NYHA class III heart failure, stage III hypertension, very high additional risk, dyslipidemia, ischemic stroke endured in 2015. Euroscore II, calculated, was 4.36%. Following M-mode and two-dimensional real-time cardiac ultrasonography, severe AS with a mean pressure gradient of 40.71mmHg was determined, the maximal jet velocity at AoV level being 4, 16 m / s, mild AoV insufficiency, moderate mitral valve and tricuspid valve insufficiency, ejection fraction of 55% (fig. 13).

On the computed tomography, the aortic valve annulus has a perimeter of 73.6 mm, an area of 419.5 mm^2, and a diameter of 23.2 mm (fig. 14, 15). Coronary angiography does not determine stenotic lesions of the coronary arteries (fig. 17, 18). Analysing the above data, it was decided to implant a 27 mm valve. Examining the anatomical aspect of the iliac and femoral arteries (fig. 16), it was decided to select the approach for the intervention, right femoral artery.

I. Devices

Portico prosthetic valves, which were used for all 3 patients, consist of a self-expandable nitinol frame, with rhomboid spaces in which pericardial tissue of bovine or porcine origin is arranged, with an available diameter of 23 to 29 mm (tab. 2). This valve undergoes a Linx AC (anti-calcification) treatment, based on ethanol, which has been shown to be effective in reducing the calcification in animal tissues [12]. The mandatory component of the valve is a flexible and easy-to-navigate Portico delivery system, which has an 18Fr / 19Fr (6.0 / 6.5 mm) delivery sheath.

Table 2

Anatomical parameters of prosthetic valve selection

Portico Valve size	Aortic annulus diameter (mm)	Aortic annulus area (mm^2)	Aortic annulus perimeter (mm)
23 mm	19-21	277-346	60-66
25 mm	21-23	338-415	66-73
27 mm	23-25	405-491	72-79
29 mm	25-27	479-573	79-85

II. The procedure

Patients were given clopidogrel and aspirin prior to intervention, immediately before the procedure – antibiotics for the prophylaxis of potential infections, and unfractionated heparin (100 U/kg) throughout the procedure. All three patients underwent general anaesthesia.

The minimally invasive intervention was performed in a cardiac catheterization room, in sterile conditions. In all 3 cases, the transfemoral approach was used, the femoral artery being cannulated using the Seldinger technique with placement of the femoral sheath on the contralateral side, a femoral access was obtained for aortic angiography. Also, a venous access was made for a temporary pacemaker, later used for rapid ventricular pacing, its proper placement being verified by a rapid ventricular pacing test at the rate of 200 beats per minute. At the same time, the angiography of the aorta was made, choosing the optimal projection in which all the aortic cusps are aligned. Through the femoral artery, the valve delivery sheath was inserted (18F for the 25 mm valve, and 19F for the 27 mm and 29 mm valves). A left Amplatz catheter (AL) on a J-shaped tip guidewire was used to pass the arch of aorta, then this guidewire was changed to a straight-tipped one, the native aortic valve being crossed through the non-coronary cusp, then advancing the AL catheter into the left ventricle. To avoid perforation of the ventricle, the AL was changed on a pigtail catheter, and the straight guidewire, again, on a J-shaped type.

During a rapid ventricular pacing, 200 rpm, under fluoroscopic guidance, balloon valvuloplasty was performed (fig. 17), its role being in cracking the calcifications of the affected valve. After removing the balloon, the prosthetic valve and its delivery system were inserted through the delivery sheath, advanced through the descending aorta, carefully crossing the aortic arch, then the ascending aorta. Again, under fluoroscopic guidance, the valve was positioned accordingly (fig. 18). Rapid ventricular pacing was initiated, and when systolic pressure reached its lowest level, the aortic valve was expanded (fig. 19).

At the next stage, the pacing was stopped and a repeated angiography and echocardiography were performed, so that the good functionality of the valve and the lack of paravalvular regurgitation might be appreciated. Subsequently, it proceeded to the stage of releasing the valve from the delivery system. One by one, the delivery system, the catheter and the delivery sheath were removed. At the time of removal, blood pressure was carefully monitored, with simultaneous injection of contrast, to rule out a possible vascular rupture.

Hemostasis after femoral arteriotomy was performed using the Perclose ProGlide occlusion system. After TAVI, patients had to take clopidogrel for 6 months, and aspirin for indefinite amount of time.

Echocardiography follow-ups were performed immediately after the procedure, at 24h, 1 week, 1 month, 3 months, 6 months and 9 months after implantation.

Quality of life (QoL) is an important consideration for aortic stenosis patients. The TASQ is questionnaire (Toronto Aortic Stenosis Quality of Life), is a QoL tool that was developed to evaluate the psychometric properties of patients who underwent TAVI. TASQ is a questionnaire of 16 items, with 4 subdivisions, namely: symptoms – 2 items, physical limitations – 4 items, emotional impact – 7 items, social limitations – 2 items and one item that reflects health expectations. For each item, patients should expose the impact of aortic stenosis on their quality of life scale anchored by "not very much" – 1 point, to "very much" – 7 points. Total scores can range from 16 to 112, with the highest reflecting a better quality of life [13]. The patients respond at this questionnaire before the intervention and at 9 months after it.

Results

Procedure results and complications

Procedural success was achieved among all (100%) patients included in this case series analysis. All patients underwent the procedure through the femoral approach, which was afterwards sutured using Perclose ProGlide device, without any further complications at the puncture site. Patient No 2 developed a complete atrioventricular block after the placement of the valvular prosthesis and needed a permanent cardiac stimulator to be implanted. Other peculiarities during the performance of TAVI did not interfere and no serious complications occurred in the recovery process of the patients (tab. 3). The average length of the hospital stay was 6 days.

Following TAVI, in all 3 patients who benefited from the intervention, the functional parameters of the heart improved considerably. Post-procedurally, the ventriculog-

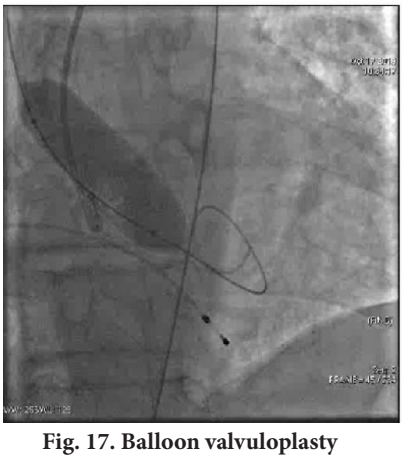

Fig. 17. Balloon valvuloplasty

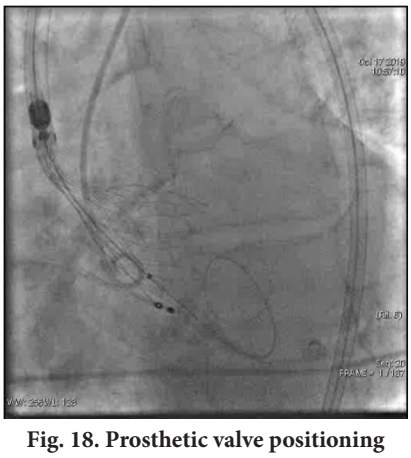

Fig. 18. Prosthetic valve positioning

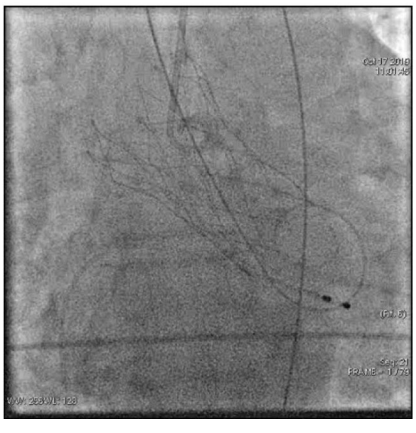

Fig. 19. Expanded prosthetic valve

raphy showed normal transvalvular flow at the AoV level, without mitral regurgitation, with a clearly improved ejection fraction.

Table 3

Possible post-procedural complications

Aortic regurgitation > grade I	1/3
Paravalvular leak > mild	0/3
Valve embolization	0/3
Valve-in-valve implantation	0/3
Coronary obstruction	0/3
Myocardial infarction	0/3
Left ventricular perforation	0/3
Permanent pacemaker	1/3
Vascular complications in trans-femoral approach	0/3
Transient ischemic attack	0/3
Procedure-related death	0/3

One week after the intervention, following a repeated echocardiography, it was shown that the GP mean decreased by an average of 20.27±1.61 mmHg, the Vmax of the jet through AoV decreased on average by 2.05±0.19 m/s, with an aortic orifice, obviously of a normal size. At nine month the GP mean decreased by an average of 28.4±3.21 mmHg, the Vmax of the jet through AoV decreased on average by 2.32±0.03 m/s in patient 1, a mild paravalvular leak was found, but without any symptoms (tab. 4).

Quality of life was assessed using the TASQ (Toronto Aortic Stenosis Quality of Life) questionnaire. We can mention a growth in the perception of quality of life in patient 1 from 30 to 95 points, in patient 2 from 34 to 98 points and in patient 3 from 38 to 96.

Discussion

This first experience in the Republic of Moldova, regarding TAVI, gives results similar to the ones from other studies and centres with experience in performing these minimally invasive interventions [8, 12]. The success rate depends on a good training of the team, adequate selection of the patients and the proper choice of the valve prosthesis, as well as the careful supervision of an experienced proctor.

Until the implementation of TAVI in the Republic of Moldova, experience in minimally invasive approach on aortic pathology was only in the case of aortic coarctation [14]. The large number of patients with severe AS over the age of 75, with multiple comorbidities and high surgical risk, to whom the replacement of the valve by surgery is often refused, imposed the need to implement these interventions in the Republic of Moldova.

The Portico TAVI system study demonstrates that the treatment of elderly patients, with severe AS, using the full range of sizes of self-expandable, resheathable, repositionable and retrievable Portico valves leads to significant improvements in aortic valve function at 1 year. Importantly, the improvement was observed in the presence of a low rate of paravalvular regurgitation and new pacemakers implantations [12] similar to the results obtained in our patients. Conduction disorders that require implantation of a permanent pacemaker after TAVI are generally more common in patients who have implanted self-expanding transcatheter valves compared to those who have balloon-expandable devices. A large US registry reported a 30-day permanent pacemaker implantation rate of 25.1% among patients who received a Portico self-expanding valve, that is in line with pacemaker implantation rates reported for other TAVI self-expanding devices (22%-26%) [15].

Adequate hemodynamic performance was observed by echocardiographic evaluation, with stable hemodynamic characteristics between 30 days and 1 year of follow-up. Paravalvular regurgitation is a rare phenomenon, due to the better patient selection, using multidetector computed tomography system, continuous increasing of experience among the operators and the possibility of repositioning the device. No patients reported severe paravalvular regurgitation at one-year follow-up [8].

The symptoms associated with AS can lead to a much diminished quality of life, interfering in the patient's ability to integrate into normal daily activities but also in relationships with other people. Understanding the effect of the pathology on the patient's life is an essential step in planning the proper treatment that will optimize the management

Table 4

Survival, symptomatic (NYHA) and echocardiographic data, compared over time

	Patient 1			Patient 2			Patient 3		
	IF	PP	9M	IF	PP	9M	IF	PP	9M
Survival	+	+	+	+	+	+	+	+	+
NYHA class	III	II	II	III	II	II	III	II	II
AVA (mm^2)	363.8	NA	NA	450.5	NA**	NA	419.5	NA	NA
MPG (mmHg)	58.33	36.0	23.6	36.57	16.4	12.3	40.71	22.4	14.5
Peak velocity (m/s)	5.06	3.0	2.7	3.84	1.6	1.5	4.16	2.3	1.9
Annulus (mm)	21.5	NA	NA	24.7	NA	NA	23.2		NA
Ejection fraction	59%	62%	62%	36%	44%	48%	55%	63%	63%
AR grade	II	II	II	II	II	II	I	I	I

*IF – initial features, PP – post-procedural, 9M – 9 months after the procedure, **NA – Non-appreciable,
AVA – aortic valve area, MPG – mean aortic valve pressure gradient, AR grade – grade of aortic valve insufficiency.

of the pathology and the quality of the results. Comparing the patients' responses before the intervention with those at 9 months after it, we can say with certainty that TAVI has changed their perception of life, giving them both satisfaction and hope to live as long as possible.

The implementation of TAVI in the Republic of Moldova is necessary because we can say that the population is constantly aging, and most elderly people have various comorbidities, which place them among patients with high surgical risk. This method is a minimally invasive alternative that gives us predictable and reproducible results, progressively becoming safe and effective. A multidisciplinary team approach is recommended in the management of the patients with AS, because the decisions that should be taken in this pathology are very complex [1].

However, the high costs of this intervention and the limited financial resources available in a developing country are the main impediments to the widespread application of this new method of treating AS in the Republic of Moldova.

Conclusions

Transcatheter aortic valve implantation, as a new minimally invasive method of treatment for aortic valve stenosis appears to be safe, with a low rate of early and late complications in elderly patients at high surgical risk, with good clinical outcomes at 9 months. Its implementation in the Republic of Moldova is welcome in the conditions of continuously aging population and the prevalence of associated comorbidities.

References

1. Baumgartner H, Falk V, Bax JJ, et al. 2017 ESC/EACTS Guidelines for the management of valvular heart disease. Eur Heart J. 2017;38(36):2739-2791. doi: 10.1093/eurheartj/ehx391.
2. Nathaniel S, Saligram S, Innasimuthu AL, et al. Aortic stenosis: an update. World J Cardiol. 2010 June 26;2(6):135-139. doi: 10.4330/wjc.v2.i6.135.
3. Lindman BR, Clavel MA, Mathieu P, et al. Calcific aortic stenosis. Nat Rev Dis Primers. 2016 Mar 3;2:16006. doi: 10.1038/nrdp.2016.6.
4. Osnabrugge RL, Mylotte D, Head SJ, et al. Aortic stenosis in the elderly: disease prevalence and the number of candidates for transcatheter aortic valve replacement: a meta-analysis and modeling study. J Am Coll Cardiol. 2013;62(11):1002-12. doi: 10.1016/j.jacc.2013.05.015.
5. Bouma BJ, van Den Brink RB, van Der Meulen JH, et al. To operate or not on elderly patients with aortic stenosis: the decision and its consequences. Heart. 1999 Aug;82(2):143-8. doi: 10.1136/hrt.82.2.143.
6. Iung B, Cachier A, Baron G, et al. Decision-making in elderly patients with severe aortic stenosis: why are so many denied surgeries? Eur Heart J. 2005 Dec;26(24):2714-20. doi: 10.1093/eurheartj/ehi471.
7. Cribier A, Eltchaninoff H, Bash A, et al. Percutaneous transcatheter implantation of an aortic valve prosthesis for calcific aortic stenosis: first human case description. Circulation. 2002;106(24):3006-3008. doi: 10.1161/01.cir.0000047200.36165.b8.
8. Giordano A, Corcione N, Ferraro P, et al. Comparative one-month safety and effectiveness of five leading new-generation devices for transcatheter aortic valve implantation. Sci Rep. 2019;9(1):17098. doi: 10.1038/s41598-019-53081-w.
9. Siontis GCM, Overtchouk P, Cahill TJ, et al. Transcatheter aortic valve implantation vs surgical aortic valve replacement for treatment of symptomatic severe aortic stenosis: an updated meta-analysis. Eur Heart J. 2019;40(38):3143-3153. doi: 10.1093/eurheartj/ehz275.
10. Tzamalis P, Alataki S, Bramlage P, et al. Comparison of valve durability and outcomes of transcatheter aortic valve implantation versus surgical aortic valve replacement in patients with severe symptomatic aortic stenosis and less-than-high-risk for surgery. Am J Cardiol. 2020;125(8):1202-1208. doi: 10.1016/j.amjcard.2020.01.015.
11. Adams DH, Popma JJ, Reardon MJ, et al. Transcatheter aortic-valve replacement with a self-expanding prosthesis. N Engl J Med. 2014;370:1790-1798. doi: 10.1056/NEJMoa1400590.
12. Linke A, Holzhey D, Mollmann H, et al. Treatment of aortic stenosis with a self-expanding, resheathable transcatheter valve: one-year results of the international multicenter Portico transcatheter aortic valve implantation system study. Circ Cardiovasc Interv. 2018;11:e005206. doi: 10.1161/CIRCINTERVENTIONS.117.005206.
13. Styra R, Dimas M, Svitak K, et al. Toronto aortic stenosis quality of life questionnaire (TASQ): validation in TAVI patients. BMC Cardiovasc Disord. 2020;20(1):209. doi: 10.1186/s12872-020-01477-2.
14. Popovici I, Liuba P, Surev A, Grib A, Abras M, Calenici E, Damascan S, Esanu A. Short-term clinical follow-up of the treatment of aortic coarctation with a new generation of device. Rom J Cardiol. 2018;28(2):160-167.
15. Fadahunsi OO, Olowoyeye A, Ukaigwe A, et al. Incidence, predictors, and outcomes of permanent pacemaker implantation following transcatheter aortic valve re-placement: analysis from the U.S. Society of Thoracic Surgeons/ American College of Cardiology TVT Registry. JACC Cardiovasc Interv. 2016;9(21):2189-2199. doi: 10.1016/j.jcin.2016.07.026.

Authors' ORCID iDs and academic degrees

Marcel Abras, MD, PhD, Associate Professor – https://orcid.org/0000-0003-2640-978X.
Artiom Surev, MD, Assistant Professor – https://orcid.org/0000-0002-8303-2256.
Mariuca Vasa-Nicotera, MD, PhD, Professor – https://orcid.org/0000-0002-6794-4941.
Vitalie Moscalu, MD, PhD, Associate Professor – https://orcid.org/0000-0001-7129-9298.
Andrei Grib, MD, Assistant Professor – https://orcid.org/0000-0002-4627-3819.
Ion Popovici, MD, PhD, Associate Professor – https://orcid.org/0000-0002-0371-0791.
Catalina Beiu, MD, Undergraduate – https://orcid.org/0000-0002-8610-6441.

Authors' contribution

AG, CB and IP acquired, interpreted the data, drafted the first manuscript, MA, AS, MN and VM performed most of the analyzed interventions, MA designed the trial and revised the manuscript critically. All the authors revised and approved the final version of the manuscript.

Funding

The study was supported by *Nicolae Testemitanu* State University of Medicine and Pharmacy. The authors are independent and take responsibility for the integrity of the data and accuracy of the data analysis.

Ethics approval and consent to participate

The research was approved by the Research Ethic Board research (protocol No 12 of September 20, 2019) of *Nicolae Testemitanu* State University of Medicine and Pharmacy. Written informed consent was obtained from all participants in the study.

Conflict of Interests: The authors have no conflict of interest to declare.

DOI: 10.5281/zenodo.3958615
UDC: 616.718.19-001.5-089.227.84:618.5

Pelvic ring stabilization in the peripartum injuries

[*1,3]Vladimir Kusturov, [1]Gheorghe Ghidirim, [2,3]Anna Kusturova, [1]Irina Paladii

[1]*Nicolae Anestiadi* Department of Surgery No1, [2]Department of Orthopedics and Traumatology
Nicolae Testemitanu State University of Medicine and Pharmacy
[3]Laboratory "Polytrauma", Institute of Emergency Medicine
Chisinau, the Republic of Moldova

Authors' ORCID iDs, academic degrees and contributions are available at the end of the article

[*]Corresponding author: kusturov@mail.ru
Manuscript received August 03, 2020; revised manuscript August 17, 2020; published online August 26, 2020

Abstract

Background: Parturition-induced pelvic injuries after spontaneous vaginal delivery sometimes happen causing deformation of the pelvic ring. The frequency of these lesions is from 1:300 to 1:30000 births, creating many problems for young women in postpartum life.
Material and methods: Under our follow-up there were 14 patients with disruption to the pelvic ring II-III degree during labor. Special bed position was recommended for the patients (n=4) with rupture of the pubic symphysis II degree. Sacroiliac joint subluxation was reduced by manual traction in multiparous patients (n=3) with disruption of III degree. Closed pelvis osteosynthesis by external device was performed.
Results: Osteosynthesis was performed in trauma clinic, taking into account that patients had breast-fed children. The maximum anesthesia time was up to 30 minutes. Closed reposition of the pelvic bones and stabilization by an external device without additional blood loss were performed. Reposition of damaged bone surfaces was achieved in all cases. In 3-4 hours after surgery, the patients were returned to their children. From the second day they were able to get up, move around the room, take care of the children. Long-term results of treatment were evaluated from 1.5 to 14 years and rated as good.
Conclusions: Rupture of the pelvic ring in women during childbirth requires qualified diagnostics and appropriate treatment. Conservative treatment does not give stable clinical result in multiparous women with disruption of II degree.
Key words: rupture, pubic symphysis, delivery, external fixation.

Cite this article
Kusturov V, Ghidirim G, Kusturova A, Paladii I. Pelvic stabilization in the peripartum injuries. *Mold Med J.* 2020;63(3):66-69. doi: 10.5281/zenodo.3958615.

Introduction

The widening of the pelvic ring joints in the third trimester of pregnancy is physiological and helps to prepare the woman's birth canal for a successful delivery [1, 2]. However, activity of the hormone relaxin and its metabolites in the blood or a high sensitivity of a pregnant woman to it can cause excessive relaxation of the pelvic ligaments and cartilage [3-6]. Numerous factors including multiparity, macrosomia, cephalo-pelvic disproportion, previous trauma, abnormality due to congenital dysplasia, osteomalacia, chondromalacia, rickets, tuberculosis, rapid progression of second stage of labor and rapid descent of presenting part, and epidural anesthesia have been suggested as possible risk factors for the diastasis of the pubic symphysis and sacroiliac joints or damage to the coccyx [7, 8]. The frequency of these lesions is from 1:300 deliveries to 1:30000 deliveries [9, 10]. Diastasis between the pelvic bones, formed during childbirth and preserved in the postpartum period for more than 2-3 weeks, is a pathological condition that causes deformation of the pelvic ring due to the onset of instability [11-13]. These rare complications can cause significant disability in young women in the postpartum life [14, 15]. The aim is to determine the criteria for early stabilization of the pelvic ring when it is damaged due to childbirth, taking into account the individual particularities of the woman in labor.

Material and methods

The study group included 14 patients with diastasis of the pubic symphysis of the II-III degree, that was formed during childbirth. The age of the women in labor was from 23 to 37 (28.5±1.4, $p<0.05$). Four patients were primiparous, the rest were multiparous. The damage to the pubic symphysis was determined in the maternity in 8 patients, by the attending physician and they were transferred to a specialized trauma clinic. Three of them were examined by an orthopedist in the first 24 hours after delivery. They had a rupture of the pubic symphysis III degree with subluxation in the sacroiliac joint, that was accompanied by functional limb shortening and severe pain. A hematoma gradually spread 3-5 days after delivery, along the medial surface of the thigh, along the adductor muscles (*mm. adductor longus et brevis*), from top to bottom. It indicated the presence of the intrapelvic bleeding after rupture of the symphysis pubis and damage to the hypertrophied vascularization of the small pelvis, paravesical venous plexus.

Six patients came by themselves and were consulted

within a month after childbirth. Their main complaints were: characteristic pain in the symphysis area, which intensified turning in bed, trying to actively move the legs. Women could not get out of bed without help, could not walk. Patients took an unusual position in bed – "the pose of a frog", lying on their back with their abducted hips and slightly bent knees. Constant pain in the lumbar region, "duck gait" or positive Trendelenburg sign were also present.

All patients were examined by comprehensive clinical and laboratory tests upon admission. Therefore, special attention was paid to the orthopedic status of the women: the condition of the pelvic ring and lower limbs. Pathological rupture of the pelvic bones was determined from 14-20 mm to 50-70 mm and the patients felt severe painful sensations immediately after childbirth, especially primiparous. Multiparous patients suffered from mild or moderate pain in the pelvic ring and in the region of medial surface of the middle third of the thigh. Bladder dysfunction, gait disturbance, loss of active movement and difficulty in caring were present in some patients. Patients with coccyx lesion (n=2) suffered from the clinic of coccydynia – pain in the sacrococcygeal zone that progressed during a long sitting, or standing up and bending frontwards.

X-ray examination of the pelvis included the anterior-posterior and inlet views visualizing the pubic symphysis and sacroiliac joints. The diastasis between the pubic bones was 14-20 mm (II degree) in 6 patients. Pubic symphysis rupture between 22-34 mm (III degree) was radiographically determined in 5 patients, with various vertical displacement. The diastasis between the pubic bones was up to 70 mm in other 3 patients with articular space enlargement of the sacroiliac joint on the left side. They had a third degree rupture of the pubic symphysis and subluxation of the sacroiliac joint, which were accompanied by functional shortening of the limb and severe pain.

After childbirth, when diagnosing damage to the symphysis pubis, the parturient patients were placed on the left side, with a weight (2-3 kg) on the right, that avoids compression on the inferior vena cava by the uterus, decreased venous return and associated hypotension. In the supine position, compression of the inferior vena cava by the uterus is observed, with the possibility of a 30% reduction in cardiac output, which significantly worsens the general condition of the patient and changes the resuscitation process [16].

In the group of multiparous (n=3), after radiography and determination of a third degree pubic symphysis rupture with subluxation in the sacroiliac joint, the subluxation was removed by manual traction and the pelvis and lower limb were immobilized.

Case A. A 34-year-old, 79 kg woman (gravid: 3, parity: 3) was examined in maternity for anterior groin, lower-back (on the left side), and hip pain associated with leg movements. She had delivered an infant weighing about 3800 g 18 hours previously. The delivery occurred at 38 gestational weeks after an uncomplicated prenatal course and followed 7-8 hours of continuous second-stage labor attended by a physician. No medication was administered to induce or augment labor. During and immediately after delivery, the patient felt a shearing pain in the area of the pubic symphysis and reported that she was unable to move from the table.

Clinical examination: the patient lay on her back, the left leg was abducted, in the supination position, functional shortening of the limb. X-ray showed rupture to the pubic symphysis and left ilio-sacral syndesmosis with displacement of the left pelvic bone in the cranial and lateral directions. After anesthesia, according to emergency indications, the subluxation was reduced by manual traction, the limb was immobilized. Immobilizing bandages were applied at the level of the pelvis, trochanteric region of the thigh and the upper third of the leg. The patient was transferred to a specialized trauma department and urgently operated – closed osteosynthesis of the pelvic ring was performed using an external fixation device.

When the patients were admitted to our trauma department, to perform a stabilizing operation on the pelvis, a repeated complex clinical, radiological and laboratory examination was carried out, with a mandatory examination by a gynecologist. The osteosynthesis was performed in this group of patients, taking into account that their children were breastfed. The maximum anesthesia time was up to 30 minutes. Bladder catheterization was obligatory, to exclude infringement of its wall. Closed reposition of the pelvic bones was performed on an orthopedic table and a stabilizing version of the external pelvic fixation device was applied. In the presence of pubic diastasis up to 20 mm and the absence of free bone fragments (n=5), the diastasis was immediately reduced, and interfragmental compression was created. In the remaining cases (n=9), after the application of the external pelvic fixation device, prolonged reduction was performed until the fragments were completely adapted, followed by their compression. Patients were discharged home to the children in 3-4 hours after the operation, after X-ray control and with a stable satisfactory condition of the puerperas. From the second day they could get up without help, move around the room, take the children in their arms and feed them. Three patients with damage to the symphysis pubis and ilio-sacral syndesmosis were recommended to use additional support (crutches) for 6 weeks. A protective cover was used to exclude traumatization by the device parts in the postoperative period. X-ray control was performed once a month.

Results and discussions

The symphysis pubis is a semi-joint with extremely limited possibilities for movement. Normally, the width of the symphysis (the distance between the pubic bones) can be up to 10 mm. During the day, this distance can change, depending on the water and electrolyte balance of the body, on the amount of joint fluid [1, 17]. The true distance between the pubic bones is determined by analyzing X-rays in dynamics, which, despite their objectivity, introduce certain errors into the pelviometry system. According to A. M. Novikov (cited by [9]), the width of the pubic symphysis changes with age:

it is ~ 6 mm at the age of 18-20 years, and it is ~ 2 mm in older women. Of course, these are, averaged values. But these values must be added to the projection magnification – up to 32% of the true one, and then we obtain an X-ray image of diastasis between the pubic bones on radiograms.

According to clinical and radiological data, there is a gradation of the pubic symphysis ruptures (more than physiological widening – 5-6 mm) [9]:

I degree – up to 5-9 mm;

II degree – up to 10-20 mm;

III degree – more than 20 mm.

This classification is relative, since the position of the pubic bones at the moment of maximum discrepancy in childbirth and during radiography is different. Physiological widening between the pubic bones up to 10 mm, without damaging the joints and soft tissue structures of the birth canal is the norm, because the body of a pregnant woman is preparing for childbirth. If a postpartum woman has a clinic of ligaments lesion in the region of the pubic symphysis with displacement of bones and the formation of significant diastasis, in such cases the result of treatment is determined by correct orthopedic diagnosis and early stabilization of the pelvis.

Until now, most surgical interventions for reduction of the pubic symphysis diastasis were performed using internal fixation – plates and screws installed on the pubic bones [12, 18]. Although internal fixation provides good structural support, this technique cannot be used if the patient with postpartum diastasis of the pubic symphysis has significant reproductive organs damage and there is a high risk of soft tissue infection or osteomyelitis, that has not received sufficient attention in the medical literature nowadays [19, 20].

The analysis of treatment outcomes of the pelvic ring injuries during childbirth by the external fixation device has indicated that the optimal method of treatment for this category of patients is early external fixation of the pelvis. The method is minimally invasive, does not cause additional blood loss, is not prolonged during implementation [13]. The reduction of diastasis between the pubic bones facilitated the alignment of the articular surfaces in the sacroiliac joint and no additional interventions were required.

In this prospective study, all 14 patients completed treatment without complications. The duration of fixation in primiparous patients was 46 ± 1.1 days; in the rest of the patients, the period of fixation of the pelvis with external device was on average 58.66 ± 2.6 days ($p<0.05$). The surgical treatment outcomes of patients with peripartum pelvis injuries during the first year were assessed using S. A. Majeed scale (1989) as excellent (n=9) and good (n=5).

Long-term results from 1.5 to 12 years were studied in 9 patients. No complaints are present, the women walk without limp. Diastasis of the pubic symphysis after closed pelvic osteosynthesis remained at the level of 4-6 mm.

Conclusions

The widening of the pubic articulation is a result of the ongoing physiological changes in the body of a pregnant woman, as an adaptive process to facilitate childbirth, i.e. these are normal changes. But the rupture of the pubic symphysis in excess of the physiological discrepancy of 5-6 mm becomes the concern of orthopedists, who must promptly establish the correct diagnosis and conduct surgical treatment at early stage. External pelvic fixation is an alternative treatment for symphysis pubis diastasis. Conservative treatment of the II-III degrees of the symphysis rupture does not give a stable clinical result and transfers young women to chronic patients.

References

1. Jain N, Sternberg LB. Symphyseal separation. Obstet Gynecol. 2005;105(5 Pt 2):1229-1232. doi: 10.1097/01.AOG.0000149744.82912.ea.
2. Khorashadi L, Petscavage JM, Richardson ML. Postpartum symphysis pubis diastasis. Radiol Case Rep. 2015;6(3):542. doi: 10.2484/rcr.v6i3.542.
3. Sanz-Salvador L, García-Pérez MÁ, Tarín JJ, Cano A. Bone metabolic changes during pregnancy: a period of vulnerability to osteoporosis and fracture. Eur J Endocrinol. 2015;172(2):R53-R65. doi: 10.1530/EJE-14-0424.
4. Muench MV, Canterino JC. Trauma in pregnancy. Obstet Gynecol Clin North Am. 2007;34(3):555-xiii. doi: 10.1016/j.ogc.2007.06.001.
5. Culligan P, Hill S, Heit M. Rupture of the symphysis pubis during vaginal delivery followed by two subsequent uneventful pregnancies. Obstet Gynecol. 2002;100(5 Pt 2):1114-1117. doi: 10.1016/s0029-7844(02)02155-5.
6. Chawla JJ, Arora D, Sandhu N, Jain M, Kumari A. Pubic symphysis diastasis: a case series and literature review. Oman Med J. 2017;32(6):510-514. doi: 10.5001/omj.2017.97.
7. Sujana B, Keepanasseril A, Maurya DK. Diastasis of the pubic symphysis following vaginal delivery. Int J Gynaecol Obstet. 2017;139(1):102-103. doi: 10.1002/ijgo.12242.
8. Yoo JJ, Ha YC, Lee YK, Hong JS, Kang BJ, Koo KH. Incidence and risk factors of symptomatic peripartum diastasis of pubic symphysis. J Korean Med Sci. 2014;29(2):281-286. doi: 10.3346/jkms.2014.29.2.281.
9. Ailamazian EK, Kulakov VI, Radzinskii VE, Savel'eva GM. Akusherstvo: Natsional'noe rukovodstvo. [Obstetrics: National guidelines] Moscow: GEOTAR-Media; 2013. 1200 p. Russian.
10. Hou Z, Riehl JT, Smith WR, Strohecker KA, Maloney PJ. Severe postpartum disruption of the pelvic ring: report of two cases and review of the literature. Patient Saf Surg. 2011;5(1):2. doi: 10.1186/1754-9493-5-2.
11. Çıçek H, Keskın HL, Tuhanıoğlu U, Kiliçarslan K, Oğur HU. Simultaneous disruption of the pubic symphysis and sacroiliac joint during vaginal birth. Case Rep Orthop. 2015;2015:812132. doi: 10.1155/2015/812132.
12. Gostev VS. Operativnoe lechenie svezhikh razryvov simfiza [Operative treatment of recent symphysis ruptures]. Ortopediia, Travmatologiia i Protezirovanie [Orthop Traumatol Prosthet]. 1978;(1):49-53. Russian.
13. Kusturova A, Kusturov V. Treatment of disruptions of pelvic ring in the time of childbirth. 3rd Medical Scientific Conference for students and young doctors; 2004 May 15-17; Pleven, Bulgaria. Abstract book. Pleven; 2004. p. 56-57.
14. Coelho KS, Shintre H, Shyamkul A, Rani B. Rent in the vent: a rare event: Parturition-induced rupture of pubic symphysis and dislocation of sacroiliac joint after spontaneous vaginal delivery. J Obstet Gynaecol India. 2016;66(Suppl 2):590-593. doi: 10.1007/s13224-015-0798-2.
15. Kharrazi FD, Rodgers WB, Kennedy JG, Lhowe DW. Parturition-induced pelvic dislocation: a report of four cases [published correction appears in J Orthop Trauma 1997 Oct;11(7):543]. J Orthop Trauma. 1997;11(4):277-282. doi: 10.1097/00005131-199705000-00009.
16. Buitendyk M, Brennan B, Vora P, Smith P, Winsor S. Acute intrapartum rupture of the pubic symphysis requiring resuscitation and surgical intervention: a case report. J Obstet Gynaecol Can. 2018;40(1):68-71. doi: 10.1016/j.jogc.2017.05.028.
17. Schoellner C, Szöke N, Siegburg K. Der schwangerschaftsassoziierte Symphysenschaden aus orthopädischer Sicht--Untersuchungen zu Veränderungen an der Symphysis pubica in der Schwangerschaft, unter der Geburt und post partum [Pregnancy-associated symphysis damage from the orthopedic viewpoint – studies of changes of the pubic sym-

physis in pregnancy, labor and post partum]. Z Orthop Ihre Grenzgeb. 2001;139(5):458-462. doi: 10.1055/s-2001-17991. German.
18. Herren C, Sobottke R, Dadgar A, et al. Peripartum pubic symphysis separation – current strategies in diagnosis and therapy and presentation of two cases. Injury. 2015;46(6):1074-1080. doi: 10.1016/j.injury.2015.02.030.
19. Gräf C, Sellei RM, Schrading S, Bauerschlag DO. Treatment of parturition-induced rupture of pubic symphysis after spontaneous vaginal delivery. Case Rep Obstet Gynecol. 2014;2014:485916. doi: 10.1155/2014/485916.
20. Saeed F, Trathen K, Want A, Kucheria R, Kalla S. Pubic symphysis diastasis after an uncomplicated vaginal delivery: a case report. J Obstet Gynaecol. 2015;35(7):746-747. doi: 10.3109/01443615.2014.992873.

Authors' ORCID iDs and academic degrees
Vladimir Kusturov, MD, PhD, Associate Professor – https://orcid.org//0000-0001-7934-092.
Gheorghe Ghidirim, MD, PhD, Academician, Professor – https://orcid.org/ 0000-0002-9047-0596.
Anna Kusturova, MD, PhD, Assistant Professor – https://orcid.org/0000-0002-5330-3203.
Irina Paladii, MD, PhD, Researcher – https://orcid.org/0000-0002-9005-2693.

Authors' contribution
VK and AK acquired, interpreted the data, VK and GG drafted the first manuscript, VK and AK performed most of the analyzed interventions, VK designed the trial, GG and IP revised the manuscript critically. All the authors revised and approved the final version of the manuscript.

Funding
The study was supported by *Nicolae Testemitanu* State University of Medicine and Pharmacy. The authors are independent and take responsibility for the integrity of the data and accuracy of the data analysis.

Ethics approval and consent to participate
The research was approved by the Research Ethic Board of *Nicolae Testemitanu* State University of Medicine and Pharmacy (protocol No 6 of March 3, 2020). Written informed consent was obtained from all participants in the study.

Conflict of Interests
The authors have no conflict of interests to declare.

DOI: 10.5281/zenodo.3958628
UDC: 612:001.89:378.661(091)(478-25)

The historical review of the research studies in Physiology conducted within *Nicolae Testemitanu* State University of Medicine and Pharmacy (1945-2020)

Victor Vovc

Department of Physiology and Biophysics, *Nicolae Testemitanu* State University of Medicine and Pharmacy Chisinau, the Republic of Moldova

Author's ORCID iD, academic degrees and contributions are available at the end of the article

Corresponding author: victor.vovc@usmf.md
Manuscript received July 10, 2020; revised manuscript July 23, 2020; published online August 26, 2020

Abstract

Background: Undoubtedly, the research studies have played both a decisive and edifying role in the emergence and development of Physiology as a separate science. The research and particularly, the experimental studies, were the milestones for the development of physiology as a distinct science and discipline, used in both general and specific biological and medical education. Physiology is an important and permanent source of research and modeling methods of vital processes among the fundamental and preclinical medical and biological sciences, such as pathophysiology and pharmacology. This short essay does not claim to comprehensively describe the development of physiological research studies within *Nicolae Testemitanu* State University of Medicine and Pharmacy over 75 years since the university foundation. However, this present chronological presentation with elements of analysis provides historical and current data on the researchers and their research papers, while working as collaborators within the Department of Physiology at our university.
Conclusions: This historical review brings conclusive evidence upon the research work conducted by the academic staff and their study results, since the foundation of the Department of Physiology, which have greatly reflected the international research trends in the field of physiological sciences. Current physiological research studies, conducted at the Department of Physiology, are aimed at studying the physiological markers and predictors of different diseases, involving cardiovascular and respiratory systems by means of psychophysiology.
Key words: physiology, history, milestones, vital processes, conclusive evidence.

Cite this article
Vovc V. The historical review of the research studies in Physiology conducted within *Nicolae Testemitanu* State University of Medicine and Pharmacy, 1945-2020. *Mold Med J.* 2020;63(3):70-73. doi: 10.5281/zenodo.3958628.

Introduction

Undoubtedly, the research studies have played a decisive or even an edifying role in the emergence and development of Physiology as a separate science. The research and experimental studies, particularly, provided a background for the development of physiology as a distinct science and discipline, used in both general and specific biological and medical education. Among the fundamental and preclinical medical-biological sciences, such as pathophysiology, pharmacology, physiology has an important role, being a permanent source of research methods and modeling of vital processes. Physiology is also closely related to other premedical sciences, such as functional anatomy, histology, cytology, molecular biology, genetics, biochemistry, and morphopathology, since these domains use physiological research methods, as well as due to the widely applied morphological, histological, genetic, biochemical, molecular, biological and other approaches within the physiological research studies during the recent decades. Furthermore, over the last 50-70 years, the physiological research methods have been introduced into a variety of clinical and medical fields, ranging from psychiatry to traumatology, from neurology to surgery, from functional diagnosis to physiotherapy and medical rehabilitation, etc.

The impact of international medical breakthroughs on the local research studies in physiology

Since the middle of the 20th century until today, the local science of physiology has passed through periods of onset and fruitful progresses. The physiology research studies during the Moldovan Soviet Socialist Republic (MSSR) period and later on, in the Republic of Moldova, were definitively influenced by the development of international physiological sciences. The 1950s and 1960s of the 20th century were characterized by a considerable research advance of electrophysiological methods, regarding the central and peripheral nervous system, which resulted in famous discoveries in physiology, as for example, of the reticular formation of the brainstem and the description of the upper parts of the central nervous system. Nevertheless, further development of local physiological research studies was also quite representative, due to the extensive and in-depth researches concerning stress and global adaptation, leading to important discoveries in neuroendocrinology and physiological cellular, subcellular and molecular processes. There have been significant study results of local researchers, which are more precisely described in this present review. Over 1970s and 1990s, physiology drew a special attention to scientific researches related to the cardiovascular system, worldwide. The

systemic and organ-specific physiological processes were described, as well as their disorders, which led to the occurrence and development of cardiovascular diseases, being the main cause of global mortality over the last 50 years. It should be mentioned, that important national and international research publications, as well as significant study results were also reported by the collaborators of the department of Physiology from the State University of Medicine and Pharmacy. Recently, the research methods used in physiology have been greatly expanded by physiologists from our university in the following clinical fields: neurology, resuscitation, medical rehabilitation, etc. In this respect, it should be noted that our academic staff has followed the current international trends in terms of various discoveries in the field of physiology, prophylaxis and non-drug treatment of functional and psycho-neurological disorders, as well as in the development of medical rehabilitation means of cardiovascular and neurological disorders.

Contributions of local researchers

The Department of Physiology was founded in 1945, at that time Moldova was one of the republics of the USSR, by Professor A. Zubkov, Emeritus in Science, Habilitated Doctor in Medicine. Professor A. Zubkov started his scientific research activity under the guidance of Professor M. Saternicov, who was a disciple of the outstanding physiologist – Ivan Sechenov. His scientific contributions included multilateral research studies like those related to pancreatic secretion and the importance of reticular formation for nervous activity.

The university Professor Anatolii Zubkov (fig. 1) had a major role in founding and developing scientific research studies in the field of physiology at the Chisinau State Institute of Medicine. Due to his extensive knowledge and scientific interest, the university Professor A. Zubkov organized and conducted physiological research studies at the Department of Physiology of the State Institute of Medicine in Chisinau in 1945, 1951-1967. Another major contribution of this outstanding physiologist was the foundation of the first physiology laboratory (in 1957) within the MSSR branch of the USSR Academy of Sciences. The physiological researchers of those times contributed substantially to the development of universal physiological science, by providing new experimental and theoretical data in neurophysiology, neuroendocrinology, experimental cardiology, physiology of farm animals in the agricultural sector, etc. [1].

It is worth mentioning the research results of the disciples and collaborators of the university Professor A. Zubkov during the 60s - 70s of the 20th century. O. Dobromaslova (habilitated doctor in medicine and university professor) studied the changes of the afferent impulses in the nerve trunks due to different actions on the energetic metabolism of the peripheral receptors. Other researches included studies on the occurrence of spontaneous and evoked afferent impulses due to excitation of the cutaneous receptors and of the small intestine. The changes in the function of receptors, resulting from excitation of the reticular formation within the brainstem were also described. O. Sherstneva (habilitated doctor in medicine and university professor) studied the afferent impulses generated in peripheral receptors under inflammatory conditions. She also proved the dependence between the spontaneous afferent impulses and the impulses evoked by the inflammatory stages. The author studied the influence of desimpathization of the animal limbs on the evolution of the inflammatory processes, as well as the occurrence of the afferent impulses in the muscles. The study highlighted that in these conditions, the inflammatory process is reduced or even inhibited; moreover, the afferent impulses in the nerves of desimpathizes limbs also decreased.

Professor A. Zubkov also guided a number of physiological research studies closely related to the practical medicine requirements. In this context, the university lecturers A. Babin and T. Sorocean, who conducted relevant researches, should also be mentioned. These scientific works analyzed the electrophysiological changes in the central nervous system functio/ning, while developing a traumatic and blood transfusion shock [1, 2].

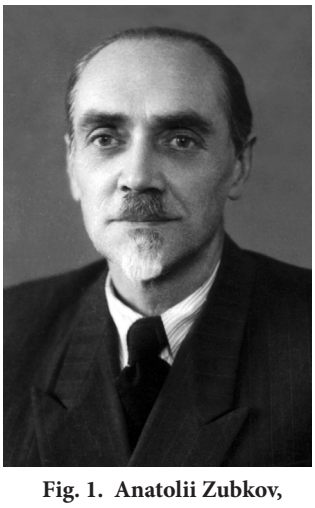

Fig. 1. Anatolii Zubkov, MD, PhD, Emeritus Professor

Professor A. Zubkov also conducted and personally participated within a series of important scientific researches as to the role of reticular formation of the brainstem in regulating various body functions, within the Moldovan SSR branch of the USSR Academy of Sciences. Thus, the fundamental work of T. Furdui (habilitated doctor in Biology, university professor, academician of ASM) on cortico-reticular-thyroid interaction, namely, on "Regulation of thyroid gland functions and the mechanism of neurogenic thyrotoxicosis", published in 1967 was of great importance, as well. This research was the first successful attempt to design an experimental model of neurogenic thyrotoxicosis due to chronic stress, thus arousing great interest to the studies on physiology and pathology of the thyroid gland.

Being more concerned with the theoretical issues of physiology, Professor A. Zubkov paid more attention to the applied aspects of physiology, such as the work physiology. In this regard, the research paper of G. Rudi (habilitated doctor in medicine and university professor) referred to the work physiology of the agricultural workers of the viticulture sector. The study findings were implemented in practical recommendations on reducing fatigue and providing proper work management[3].

Furthermore, O. Sherstneva (fig. 2), one of Professor A. Zubkov's disciples, habilitated doctor in medicine, university professor, and head of the Physiology Department of the Chisinau State Institute of Medicine, took over and developed further studies in the field of physiology during the years 1967–1988. This period was characterized by the development of experimental neurophysiological research studies on "Central-peripheral relationships under the action of various stress factors upon the body". The contemporary electrophysiological methods used in those times (spontaneous and evoked biopotential recordings of the central and peripheral structures of the nervous system), provided important scientific results on

Fig. 2. Olga Sherstneva, MD, PhD, Professor

the regulatory importance of the central and peripheral interaction of the nervous system in different experimental models: the action of myorelaxants and their combinations was studied by university assistant V. Cuzor; experiments on hypothermia were made by university lecturer I. Chiaburu; the role of radiotoxins in the afferent activity was investigated by Associate Professor A. Saulea; the afferent impulse in allotransplantation – by university lecturer N. Demishcan [4].

During the second half of aforementioned period, a group of young researchers was formed within the scientific and academic staff of the Department of Physiology, aimed to diversify and adjust the scientific research studies to the global requirements and tendencies for the development of physiological sciences. A technical-material and experimental basis was developed, used for advanced scientific research, in terms of experimental cardiology on models of adaptation to hypobaric hypoxia and containment stress, as well as on hyperbaric oxygen therapy. The study findings were successfully implemented within two doctoral theses by V. Vovc and N. Bolocan and one habilitation thesis in medicine by A. Saulea. These research studies exhibited actuality and scientific significance of the new field of research, a fact proved by the development of fruitful cooperation of the aforementioned collaborators with the leading laboratories in the field of physiology within the former USSR.

In 1988, A. Saulea (fig. 3) habilitated doctor in medical sciences, university professor, corresponding member of the Romanian Academy of Medical Sciences, and Emeritus, was appointed the head of the Department of Physiology. The scientific investigations of the department were oriented towards the study of the impact of stressful mechanisms on the cardiovascular system. The original concept, described by the university professor A. Saulea (1989), regarding the role of the trypsin-antitrypsin system in the pathogenesis of stress lesion of cardiomyocytes was a conclusive evidence, found in the research papers of the following collaborators: V. Rotari (MD, 1993) and T. Tazlavan (MD, 1993). The Associate Professor O. Arnaut presented this concept in terms of clinical and physiological application within his PhD thesis. A scientific research laboratory in the field of psychophysiology was founded within the Department of Physio-logy, which allowed studies to be conducted on the impact of stressors on human psychophysiological parameters, as well as the development of a psychophysiological assessment on vegetative deficiency and its related disorders. The 1980s – 1990s were characterized by a multilateral development of physiological research, showing a more applicative value. The collaborators of the department conducted investigations in the field of sports medicine, medical rehabilitation and gerontology.

Therefore, the department started joint projects with the relevant departments from the entire republic, from Romania, Ukraine, and the Russian Federation. The scientific findings were presented at various republican and international events. There was set up much successful scientific activities with the University of Physical Education and Sports (Chisinau), the Technical University of Moldova, the Universities of Medicine and Pharmacy from Iasi, Cluj-Napoca and Bucharest (Romania), as well as with Rene Decartes University (Paris).

In 1990, Professor A. Saulea jointly with Professor F. Meerson (Moscow) published a monograph on "Post-stress disorders of myocardial functioning", reporting new conclusive data on the harmful effect of excessive stress factors on the myocardium functioning. Two other monographs of Professor A. Saulea, "Balneophysiotherapy" and "Gerontology" were published in 1993 and 2009, respectively.

In 1994, the Department organized a symposium on sports medicine under the auspices of the International Olympic Committee of Medical Commission. In 1990-1994, the department staff participated in the organization of the Republican Center for Medical and Social Rehabilitation and conducted scientific researches in the field of gerontology and geriatrics. The department also organized an international scientific conference and four symposia on current physiology-related issues and on medical and gerontological recovery. Furthermore, the academic staff attended international congresses and conferences with original scientific reports, thus following the European and world standards of research in the field of physiology and its related fields [4, 5].

Fig. 3. Aurel Saulea, MD, PhD, Emeritus Professor

During 1990-2000, the department staff carried out a series of scientific research, predominantly focused on the physiology of adaptive medicine. In 1993, the Associate Professor V. Vovc (later on, habilitated doctor in medicine and university professor) defended the Habilitation thesis on the cardio-protective effect of organism adaptation to hypobaric hypoxia and stress. At the same time, adaptive physiological reserves of the elderly were studied in order to develop and implement geriatric rehabilitation methods. Studies in the field of physiological gerontology resulted in a successful presentation of two PhD theses in medical sciences: G. Vasilache (2001) and L. Munteanu (2001). There was conducted a series of experimental studies on the physiological mechanisms of vascular smooth muscle contractility (V. Ojog); on the reactions of blood components to the action of physical factors, like helium plasma, and millimeter waves (S. Lozovanu, MD, 2006); the diagnostic value of physiological tremor recording and analysis (B. Dragan, MD, 2006) [6].

Research studies in the field of physiology were also carried out in the years 1999–2010, by the Department of Biophy-

Fig. 4. Victor Vovc, MD, PhD, Emeritus Professor

sics, Informatics and Human Physiology, which have been continued since 2010 till present, within the current Department of Physiology and Biophysics, run by the head of the Department, university professor and Habilitated Doctor in Medicine – Victor Vovc (fig. 4). There was made a number of researches on changes in the respiratory patterns of different experimental models and certain neurological disorders; ventilatory and cardiac effects of voluntary hyperventilation in healthy subjects and in patients suffering from panic disorders (T. Besleaga, PhD, 2011); cardiovascular interactions in respiratory pattern changes (A. Ganenco). A scientific collaboration with the Laboratory of Respiratory Physiology of Joseph Fourier University (Grenoble, France) is currently being developed. The scientific activities between the French scientists and those from the Department of Neurology of our university, guided by the university professor Ion Moldovanu, resulted in joint scientific papers, aimed at analyzing the parameters of the respiration patterns as a new type of assessment for functional diagnosis, which is applied for psychoneurovegetative disorders, Parkinson's disease, etc. Currently, applied physiological research studies are being conducted on "The importance of oscillatory physiological processes in regulating the cardiovascular and respiratory system functioning" (V. Tonu, PhD student); "Objective criteria of mechanisms inducing functional heart rate disorders" (L. Sidorenko, PhD student); "The morning headaches in patients with breathing-related sleep disorders" (a clinical and psycho-physiological study) (A. Lupushor, PhD student). The department is also working on two Habilitation theses, conducted by the Associate Professor S. Lozovanu, regarding an extensive and in-depth research on the psychophysiology of respiration; whereas the university lecturer O. Arnăut studies the possibility of mathematical modeling in vital organ disorders due to polytraumas, by developing the assessment criteria based on the study of physiological indices. Since 2013, when the Somnology Center was founded, the scientific department showed great interests in the field of physiology and disorders of sleep and wakefulness. The relevant studies are being carried out by the university professor V. Vovc and A. Lupushor, the university assistant and PhD student [7].

Conclusions

This short essay does not claim to comprehensively describe the development of physiological research studies within *Nicolae Testemitanu* State University of Medicine and Pharmacy over 75 years since the university foundation. This present chronological presentation with elements of analysis provides historical and current data on the researchers and their research papers, while working as collaborators within the Department of Physiology at our university.

This present paper has provided conclusive evidence that since its foundation, the Department of Physiology has conducted a variety of research studies, whereas the study results have largely reflected the international trends in the field of physiological sciences. The past and contemporary researchers achieved valuable research results in the field of electrophysiology of the central and peripheral nervous system, cardiovascular system physiology, stress and adaptation physiology, work physiology, psychophysiology, modeling of physiological processes and predicting the transition of physiological processes into pathological ones.

Current physiological research studies, conducted at the Department of Physiology, are aimed at studying the physiological markers and predictors for different diseases, involving cardiovascular and respiratory systems by means of psychophysiology. These research features correspond to the up-to-date trends of research development that refer to physiological sciences.

References

1. Babskii EB, Zubkov AA. Ocherk zhizni i nauchnoi deiatel'nosti [Life and scientific activity review]. In: Problemy sovremennoi fiziologii: Sbornik statei posviashchennykh pamiati professora A. A. Zubkova. [Problems of modern physiology: Collection of articles dedicated to the memory of prof. A. A. Zubkov]. Chisinau: Cartea moldoveneasca; 1969. p. 5-24. Russian.
2. Grossu I. Universitatea de Stat de Medicina si Farmacie "Nicolae Testemitanu" [*Nicolae Testemitanu* State University of Medicine and Pharmacy]. Chisinau: Inconcom; 1995. 512 p. Romanian.
3. Anatolii Zubkov o personalitate notorie in fiziologie – 100 de ani [Anatolii Zubkov a famous personality in physiology – 100th anniversary]. Chisinau: Medicina; 2000. 32 p. (Savanti-medici ilustri [Famous Scientists and Doctors]). Romanian.
4. Universitatea de Stat de Medicina si Farmacie "Nicolae Testemitanu" la 60 de ani [*Nicolae Testemitanu* State University of Medicine and Pharmacy at the 60th anniversary]. Chisinau: Medicina; 2005. 448 p. Romanian.
5. Baciu Gheorghe. Profesorii universitari, absolventi ai USMF "Nicolae Testemitanu" [University professors, graduates of *Nicolae Testemitanu* SUMPh]. Chisinau: [publisher unknown]; 2012. 296 p. Romanian.
6. Aurel Saulea: alocuțiune aniversara – 70 de ani [Aurel Saulea – 70th anniversary]. Chisinau: Medicina; 2012. 40 p. (Savanti-medici ilustri [Famous Scientists and Doctors]). Romanian.
7. Victor Vovc: alocutiune aniversara – 70 de ani [Victor Vovc – 70th anniversary]. Chisinau: Medicina; 2018. 72 p. (Savanti-medici ilustri [Famous Scientists and Doctors]). Romanian.

Author's ORCID iD and academic degrees: Victor Vovc, MD, PhD, Emeritus Professor of Physiology – https://orcid.org/0000-0001-7624-9644.

Author's contribution: VV initiated the review, gathered and interpreted the data and revised the manuscript critically. The author revised the final version of the manuscript.

Funding: This study was supported by *Nicolae Testemitanu* State University of Medicine and Pharmacy. The review was the author's initiative. The author is independent and takes responsibility for the integrity of the data and accuracy of the data analysis.

Ethics approval and consent to participate: No approval was required for this review study.

Conflict of Interests: No competing interests were disclosed.

GUIDE FOR AUTHORS

The manuscript has to be sent electronically to editor@moldmedjournal.md by the author, responsible for the correspondence, using the **Authorship Statement Form** and **License Agreement**.

The authors are kindly requested to visit our web site www.moldmedjournal.md and strictly follow the directions of the **Publication Ethics and Malpractice Statement.**

Details about submission and article processing charge can be found on the journal's website: www.moldmedjournal.md.

All papers are to be executed in the following manner:

1. The manuscript should be typed in format A4, 1.5-spaced, with 2.0 cm margins, printing type 12 Times New Roman, in Microsoft Word.

2. The original article (presents new and original scientific findings, explains research methodology and provides data) has to be less than 16 pages long and should consist of an Introduction, Material and methods, Results, Discussion, Conclusions and be followed by not more than 40 references.

3. The review article (provides an overview of a field or subject, synthesizes previous research) must not exceed 25 pages and contain not more than 100 references.

4. The title page should include the first and family name of all the authors, their academic degrees, the name of the department and institution from which the paper has arrived, the phone number and e-mail address of the corresponding author.

5. The abstract should be written on the title page and limited from 220 to 240 words. The abstract of original article should have 4 parts: Background, Material and methods, Results, Conclusions.

The abstract of review article should have 2 parts: Background and Conclusions. The abstract should end with 3 to 6 key words.

6. The tables and figures must be typed, consecutively numbered and followed by an explanatory text. The figures that have to emphasize a comparison or details are published in color. If colored figures are to be placed, the author must pay an additional fee of €100 per page (1-8 figures on a page).

7. The references are to be listed in order of their appearance in the text, and the appropriate numbers are to be inserted in the text in square brackets in proper places.

The list of references should contain more than 50% in Scopus or WoS, more than 80% with DOI and not more than 30% of monographs or conference abstracts.

The references must comply with the general format outlined in the Uniform Requirements for the Manuscripts Submitted to Biomedical Journals developed by the International Committee of Medical Journal Editors (www.icmje.org), chapter IV.A.9.

The references in the Cyrillic script should be transliterated into Latin script using the American Library Association and Library of Congress Romanization Tables as follows: А=A, Б=B, В=V, Г=G, Д=D, Е=E, Ё=E, Ж=ZH, З=Z, И=I, Й=I, К=K, Л=L, М=M, Н=N, О=O, П=P, Р=R, С=S, Т=T, У=U, Ф=F, Х=KH, Ц=TS, Ч=CH, Ш=SH, Щ=SHCH, Ъ=", Ы=Y, Ь=', Э=E, Ю=IU, Я=IA.

Immediately after the transliteration the translation of the title in English in the square brackets should follow. For example: Ivanov IV, Shchukin NF, Men'shikov VM, Ad"yunktov AM. Transplantatsiia organov i tkanei [Transplantation of organs and tissues]. Vestnik Khirurgii. 2010; 26(6):45-49. Russian.

Address of the Editorial Office

192, Stefan cel Mare Avenue, Chisinau, MD-2004, the Republic of Moldova
Telephone: +373 22 205 209, +373 79 429 274 mobile
www.moldmedjournal.md editor@moldmedjournal.md