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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.

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ORIGINAL RESEARCHES

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Isolated renal hydatid cyst in the primary generation of the larval cyst

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Abstract

Background: In the Republic of Moldova echinococcosis has a uniform geographical distribution, with an incidence of 10/100000 inhabitants. Renal hydatidosis occupies an important place in surgical pathology. Today's modern diagnostics and treatments of renal hydatidosis include, in addition to surgery, the drug therapy as well: Helmex, Albendazole, Mebandazole, etc. The surgical techniques in renal hydatidosis are adapted according to the clinical polymorphism of hydatidosis, the cyst topography, the multitude of hydatid vesicles, the volume of the cyst, as well as its complications.
Material and methods: The study materials included the anamnestic data, the preoperative diagnostic imaging methods, such as ultrasound, computed tomography and laboratory data, the surgical resolution, followed by the morphopathological assessment of the postoperative renal hydatid cyst specimen.
Results: Clinical and paraclinical assessment, characterized by a predominantly pain-related morbidity status, included the diagnostic imaging findings, which revealed a parapelvic cyst with no infiltrative or obstructive processes present. The histopathological examination of the cyst specimen showed the presence of proligerous membrane and daughter vesicles, which confirmed the diagnosis of a renal hydatid cyst.
Conclusions: Currently, the hydatid diseases, including the renal one, remain a major health problem, requiring specific surgical approaches and techniques. The surgical treatment of hydatid cysts is an individualized approach depending on their size and location, which might range from cystectomy or cyst enucleation to a complete removal of the impaired kidney.
Key words: renal diagnostic techniques, surgical treatment, children.

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Introduction

Renal echinococcosis is a rare nosological entity, commonly revealed, due to the early patient's referral and diagnosis of thoracic and hepatic hydatidosis.

Secondary echinococcosis results from the evolution of the "small cycle". The parasitic echinococcus develops from scolex, which might graft on any living tissue. The "small cycle" may evolve on the same patient showing the following characteristics and mandatory stages: the rupture of the primitive vesicle, their release, grafting and the evolution of the host tissue scolices. The fertile scolices might evolve either into an adult form when they reach the intestine of the dog, or into a parasitic form, when they get into contact with the host's tissue due to a hydatid rupture.

The ruptured hydatid cyst may result from medical maneuvers, but commonly due to traumas, particularly in large cysts [1].

Thus, according to the aforementioned, the hydatid cysts denote the larval stage of a parasitic infection or echinococcosis, caused by *Echinococcus granulosus*, an endemic parasite, showing a medical and biological impact and being commonly encountered in many countries worldwide [2, 3], including the Republic of Moldova.

The hydatid cyst disease was first described by Hippocrates, however it was more accurately defined by Goeze, J. A. E. [4] and Leuckart R. [5] , who discovered its parasitic origin. Vogel H. [6] describes the evolutionary cycle of the parasite: (*Taenia echinococcus*), the larval stage in humans – intermediate host – hydatid disease.

The major steps in highlighting the etiopathogenesis, diagnosis and treatment of this parasitic disease were made at the beginning of the 20th century by F. Deve, who was also considered as founder of Modern Hydatidology [7].

The biological, epidemiological, and clinical aspects of the disease were further revealed by Eckert J. and Deplazes P. [8]. In the Republic of Moldova, many outstanding surgeons like professors Constantin Țîbîrnă, Natalia Gheorghiu, Eva Gudumac, Gh.Ghidirim, Gh. Grosu, Stanislav Babuci and

Alexandru Jalbă performed surgical interventions on hydatid cysts in children, thus describing a number of diagnostic techniques, surgical resolutions and prophylaxis for preventing recurrences [9-15].

Over the last decades, the morbidity rate in the southern regions of the Republic of Moldova accounted for 9.13-15.5% per 100000 inhabitants [16, 17]. The most commonly reported human cases involved liver, followed by impaired lungs, and other organs, including kidneys that make up 8-10% [18, 19]. Isolated primary renal echinococcosis in primary human infection accounts for 1.5%-5.0% [20], however, its occurrence being briefly reported in single clinical cases among children [21, 22].

The purpose of this study is to highlight the diagnostic and surgical management applied for isolated hydatid cyst with primary generation of larval cysts in kidneys.

Material and methods

Over the last 20 years, 4 cases with renal cysts were examined and operated at *Natalia Gheorghiu* Clinic of Pediatric Surgery. It should be mentioned that 2 patients underwent surgical interventions on renal hydatidosis with concomitant pulmonary hydatid cysts on both lungs (1), both lungs + liver (1), two isolated cases on the kidneys, of which one patient exhibited a "crushed eggshell" type due to trauma. The assessment of the anamnestic data, ultrasound or CT preoperative diagnosis, the intraoperative characteristics and the morphopathological studies were carried out within the postoperative period of the patients enrolled in the study. The biological specimens were collected according to the current research principles, approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (positive opinion dated on May 13, 2015, report No 55). The histological examination was carried out by assessing and processing the pericystic surgical material and larval cysts, according to the standard histological procedures [23].

Results

The diagnosis included the ultrasound and CT scan exams. The diagnostic strategy, based on the technical findings and interpreted according to specific principles, was followed by a detailed study of the adjacent organ relationships, being a main stage of a CT diagnosis in cases of renal hydatid cysts. The CT assessment of the surrounding organs allows to establish the compression degree of an organ, due to an expansive process, invasion of the hollow organ wall, displacement of a hollow organ, or abnormal rotation of the impaired kidneys. The immunological tests are of great importance, though, they are not always available and do not reveal the anatomical location of a hydatid cyst [21]. The diagnostic strategy also involves both anamnestic and imaging data (abdominal and urinary ultrasound, computerized tomography (CT), magnetic resonance imaging (MRI), and renal scintigraphy), which allow to establish not only the cyst location, but also its relation with other surrounding organs, density measurement, the cyst size, as well as images from sagittal planes, etc. [1].

CT scanning is a basic imaging method for diagnosing a renal hydatid cyst. The contrast medium administration (in CT angiography) will be differentiated and adjusted to each individual case depending on the particularities, in order to exclude possible involvement of the vascular structures.

It is worth mentioning that, there has not been developed any complete efficient surgical approach for the treatment of this pathology, so far. However, the current method of choice for the resolution of the hydatid cyst, including the renal one is still the surgical treatment, being maximally conservative and oriented towards the maintenance of renal function [24].

The specialized literature has described a number of techniques for diagnosis and surgical management of hydatidosis, including those encountered in children, which mainly attempt to solve two major problems: surgical removal of the hydatid cyst and drainage of the pericystic cavity. However, the abundance of the surgical techniques actually proves that no method is an ideal criterion for solving these two major problems. A major concern related to hydatidosis, is that like any parasitic disease, it might graft on any healthy organs or those impaired by various acquired congenital disorders such as hydronephrosis, renal hypoplasia, renal multicystosis and ureteral calculi, which might also affect both the proper diagnosis and positive disease prognosis [25]. There is a difficulty in establishing the diagnosis of this disease, since only 4 cases of renal hydatid cyst, caused by activity outside the disease- endemic area, have been detected in the Republic of Moldova. The impact of diagnostic errors might trigger an inadequate individualized surgical approach, as well as improper prophylactic treatment of these patients. Therefore, we analyzed and presented the anamnestic and diagnostic data , as well as the surgical resolution, followed by the morphopathological study of the postoperative specimen of a recently operated clinical case, dated on 10.02.2020.

We believe that this clinical case will be useful for young specialists, who are interested in this pathology.

Clinical case study

The morbidity data. According to the hospital registry data, a 6-year-old female patient, A.D., was admitted to the Department of Urology, at the Mother and Child Institute and at *Natalia Gheorghiu* Clinic of Pediatric Surgery, with abdominal and low back pain lasting for 6 months since the disease onset. Over 6 years the patient did not present any renal-related complaints. At the age of 5 years 6 months, the patient began to feel pain in the abdomen and in left lumbar region. The patient underwent a treatment within the outpatient department, by administering antibiotics and antispasmodics. Even though the pain subsided, it persisted periodically, accompanied by febrile syndrome. The patient was assessed for the causative factor of the morbid status within our clinic, based on the diagnostic, imaging and paraclinical research protocols.

Imaging diagnosis. The patient underwent the primary ultrasound assessment of the urinary system on 06.02.20, which revealed an anatomical dissociation of the kidney size with the following data: the right kidney – 80x31mm, the parenchyma – 10 mm, the right renal pelvis – 2mm, slightly deviated left kidney compared to the right one – 88x31 mm, the parenchyma – 10 mm and the left renal pelvis – 2 mm. A 55x29 mm formation was determined in the left lower middle part of kidney, featured by a solid + fluid content and regular renal contour (fig. 1A). The dynamic contrast-enhanced spiral CT (fig. 1B) of the kidneys (arterial and nephrogenic phase), dated on 05.02.20, revealed a more detailed assessment of the cystic lesion. CT images showed 7.6 cm for longitudinal right diameter and 7.5 for left diameter. Homogeneous right parenchymal and left intraparenchymatous patterns were revealed on the lateral-medial surface. A cyst formation with a size of 4.8x3.8x2.6 cm (vertical x transverse x anteroposterior) was found within the middle 1/3 of the kidney that extended into the parapelvic

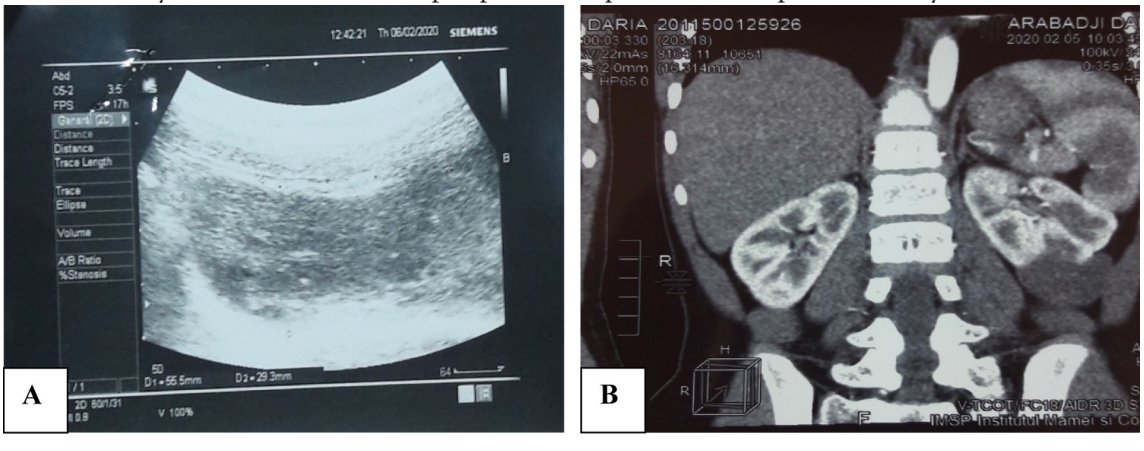

Fig. 1. Ultrasound and CT imaging patterns: A – The left kidney ultrasonography, 55x29mm cyst formation, solid + fluid content, regular contour in the lower and middle pole; B – The left papelvical renal cyst of Bosniak type I.

and retroperitoneal space of a polygonal shape, clearly contoured with homogeneous content (native density + 12 UH, post-contrast – at minimum amplification, no intrastromal septa, wall thickness, no calciform inclusions, moderate exert of mass effect on the left kidney). Anterior rotation of the lower pole. Equal bilateral corticomedullary density. Normal right pelvis system was determined over 3 minutes after the intravenous introduction of the iodine substance. A compressed left pelvis system was also found on the aforementioned formation. Conclusion: Imaging data suggested a left parapelvic solitary renal cyst of Bosniak type I.

The paraclinical diagnosis included the hemolithogram, blood biochemistry, and urinalysis. On 05.02.2020, the hemolithogram revealed: Hb – 112 (reference range 115 – 137 g / l), RBC – 3.60 (reference range 4.1-4.5 x 1012 / L), color index – 0.92 (reference range 0.71-1.01), WBC – 8.8 (reference range 5.7-8.7 x 109 / L), non-segmented neutrophils – 9.0 (reference range 1-3%), segmented neutrophils – 41 (reference range 43-59%), eosinophils – 5.0 (baseline blood range 3.0-4.6%), monocytes – 3.0 (baseline blood range 4-8%).

Blood biochemistry, dated on 5.02.2020, revealed the following levels of albumin – 46.3 (reference range 38-54 g / l), aspartate aminotransferase – 14.5 (reference range 0-32 U/L), direct bilirubin – 0.50 (reference range 0-5 μmol / l), total bilirubin – 12.6 (reference range 0-21 μmol / l), serum creatinine – 46.0 (reference range 64-83 μmol / l), total protein – 65.0 (normal range 60-80 g / l), fibrinogen – 2.1 (normal range 2-4 g / l), INR values – 1.08 (normal range 0.85-1.25), Quick prothrombin values – 83.3 (normal range 70-130%).

On 6.02.20, the urinalysis showed the following: the urine amount – 40 ml, yellow urine color, density – 1020, acidic urine reaction, transparent, absence of proteins, flat epithelial cells 4-6/hpf, WBC 3-6/hpf. The clinical and paraclinical assessment showed a predominant pain-related morbidity status, whereas the diagnostic imaging data revealed the presence of a parapelvic cyst with no infiltrative or obstructive processes. No serological investigations were available at that moment. The location and size of the cyst, the risk of developing complications such as obvious compression of the pelvicaliceal system, as well as an infection overlap with a parapelvic cyst were considered. Simple cysts are commonly asymptomatic but might be complicated by intrarenal or paranephral rupture. A surgical intervention was performed based the parent's written consent.

Surgical treatment

On February 10 2020, the child underwent a classic surgical intervention via lumbotomy incision on the left, which revealed a cystic white-yellowish formation within the paranephral / retroperitoneal space that was 5.0 x 2.5 cm in size. A jelly-like fluid with a translucent capsule was found at the opening of the thickened tissue capsule, suggesting a hydatid cyst (fig. 2). A conservative surgical approach was considered in order to preserve normal kidney functioning. Simple internal endocyst excision, followed by internal excision of the capsule and pericyst were performed after the removal of the cystic content. The residual wall cavity was carefully processed with 10% polyamine iodide solution. Subsequently, the hemostatic tamponade of the cyst cavity was performed through the paranephron tissue, followed by application of drainage and a layered wound suturing.

In this case, the surgical technique was adapted according to the location and size of the renal hydatid cyst, in or-

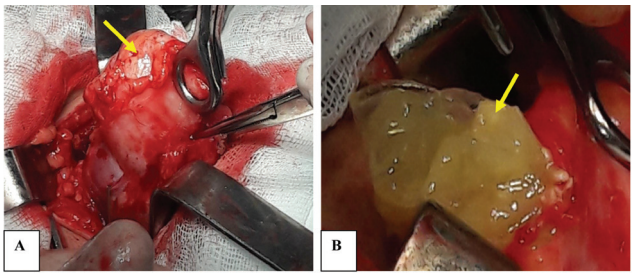

Fig. 2. Intraoperative features of the hydatid cyst: A – The lower pole of the left kidney distorted by the cyst; B – Opening of the pericyst, translucent yellowish-colored hydatic cyst, with a small yellowish-pale plateau.

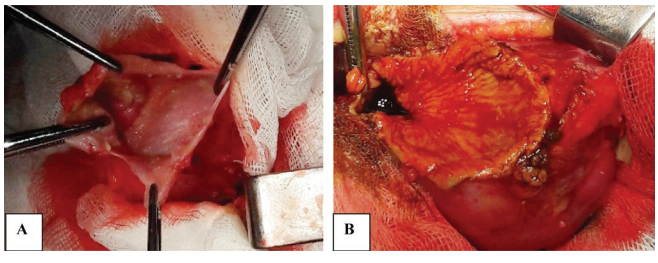

Fig. 3. Intraoperative pericyst features. A – The pericystic cavity after the extraction of the larval cyst; B – The remaining superior renal pole after the resection of the pericyst and treatment with 10% Betadine solution.

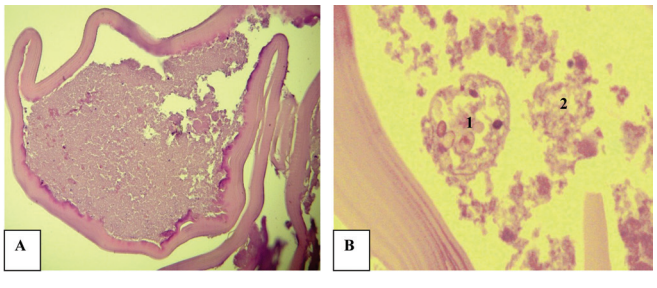

Fig. 4. The general pattern of the hydatid cyst: A – Lamellar cuticle with a microgranular mass of hydatid fluid × 25. H-E coloring; B – The germinating layer – degenerting dead protoscoleces (1), disintegrated proliger membrane (2). ×200. H-E coloring.

der to perform the intact ablation of the hydatid vesicle with preservation of the pericyst.

The intraoperative morphological patterns are reflected within the following figures (fig. 3, 4, 5, 6 and 7). A small prominent yellow-pale plateau in the lumen was observed through the translucent larval cystic wall.

After the removal of the larval cyst contents, the pericyst exhibited elasticity, with a minimum thickness of 0.1-0.15 cm, sometimes up to 0.3 cm. The internal surface revealed a pinkish colored appearance with small, non-significant yellowish foci, and no blood vascular fistulas present.

The histological examination of the excised surgical specimen of the translucent cyst and pericyst confirmed the parasitic origin, as well as the morphological particularities of the renal cyst formation (fig. 4). A primary larval stage of the Echinococcosis granulosus cestode was detected from the proliferating membrane and lamellar cuticle with dystrophic and necrotic changes that characterized a dead lar-

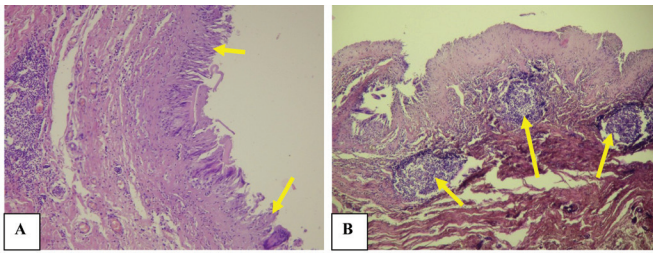

Fig. 5. General pericyst pattern: A – The inner surface of the pericyst in polynuclear giant simplast layers in palisade × 50 H-E coloration; B – Sclerosis of the pericyst surface, hyalinized by well-circumscribed lymphoid follicles in chain. ×25. H-E coloring.

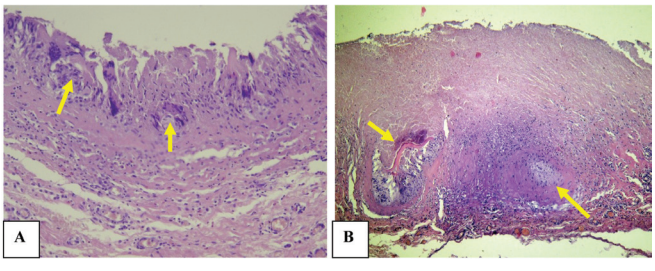

Fig. 6. Extra germ migration patterns: A – Necrotic simplast layers in palisade with formation of epithelial nodules surrounding the parasitic microvesicles × 50. H-E coloring; B – Small dead cysts in the pericyst region within necrotic areas. ×25. H-E coloring.

val cyst, though a former outbreak fertilization activity was observed intraoperatively in the plateau aspect. Cyst fertility was proved by presence of degenerating protoscoleces in the larval mass (fig. 4).

The pericyst was mostly characterized by tissues of the host organ with fibrillar connective tissue and a reduced focal fibrosis, whereas the internal surface consisted of simplast polynuclear palisade cells. The fibrillar and connective features of the pericyst showed a chaotic, slightly dispersed lymphocyte cellular component, whereas some lymphoid follicular structures were found between the pericyst and parenchyma border (fig. 5A). The subcapsular area of the upper pole revealed areas, which were lacking simplast palisade cells with a marked sclerosis, including hyalinization with discrete cellular aspect, while the perifocal area showed multiple well-circumscribed lymphoid follicles in chain (fig. 5B).

Some areas concomitantly exhibited superficial necrotic foci on the simplast layer of palisade cells, as well as the presence of actively proliferating cell nodules with pseudo-microvesicular inclusions within their surrounding areas and on the borders with the pericyst. A distinct eosinophilic cell component was found in the fibrillar structure of the connective region (fig. 6A). Therefore, we might conclude that these particularities of common pericyst with degenerative changes of the larval cyst justify the pathway for germ cell migration. It was also confirmed by the presence of marked necrotic changes of the pericyst within some regions, or being transformed into a mass of cellular debris with smaller mortified cysts, followed by a pronounced inflammatory

response (fig. 6B). The pericystic area revealed no renal parenchyma. The pericystic vascular network had no endo- or perivasculitis, thrombi or parasitic elements. A non-significant sclerosis with solitary glomeruli was observed adjacent to its borders.

The final morphopathological diagnosis: A primary renal hydatid cyst (*echinococcus granulosus*) with a maternal mortified larval cyst and peri-larval mortified daughter vesicles were identified, as well as reactive focal and inflammatory necrolytic features within the pericyst, suggesting a risk for spread. The intensive postoperative therapy was aimed at managing the volume loss and maintaining the fluid-clotting balance, as well as the active assessment of the liver and kidney functions.

The postoperative period did not show any complications. Over 10 days, the child had a satisfactory condition and underwent an USI scanning. The child was discharged, being referred to and supervised by the family doctor. The patient was administered the following drug therapy: Escazole 400 mg, 1 tablet/day, per oral during mealtimes for 5 days. After being discharged from the Pediatric Urology Clinic, the patient data were included in a dispensary protocol carried out by the family doctor. The child was examined on the 20th day after the hospital discharge. The child proved to be healthy with no signs of renal disorder or other distant complications.

Discussion

Human echinococcosis, due to its medical, social and economic impact, remains a constant issue for surgeons, parasitologists and epidemiologists, who are interested in its invasive-destructive patterns affecting the liver and lungs, rarely involving kidneys and spleen [11, 14, 15, 18, 26, 27]. This disease is considered hyperendemic in Moldova. However, recent studies of the molecular biology of cystic echinococcosis in humans and pigs have reported both *Echinococcus granulosus* sensu stricto and *Echinococcus canadensis* G6 / G7 within several outbreaks from our country [28].

In the Republic of Moldova, renal echinococcosis, especially in children, rarely occurs, thus making it difficult to establish the diagnosis, particularly in cases of malignancy [17, 20].

A number of studies have highlighted the particular epidemiological patterns of this disorder, namely, its evolution towards the cystic extension and total destruction of renal parenchyma, the uncommon and severe features of this cyst type, specific precautions that should be considered by the surgeons during the surgical intervention, as well as the preventive measures to avoid the infection [17].

Most studies report that the hydatid cysts are spherical fluid-filled, unilocular vesicles, defined as "rock water". Three membranes limit the hydatid larva: 1. Internal, fragile, or proligerous membrane. 2. External, with a milky yellowish appearance. 3. Peripheral, the surrounding tissues are removed, showing a sclerotic reaction of the interstitial connective tissue, which forms a third membrane, thus justifying the absence of the cleavage plane during the surgical procedure. The anatomical and pathological evolution is oriented towards the increase of the cyst volume. The third membrane becomes sclerotic and calcified, being visible on CT [20, 23].

Renal hydatid cyst may be primary or secondary, single or multiple, with or without concomitant parasitic damage of the adjacent organs. Sometimes, complicated evolutions of the disease have been reported. The most serious complications include the cyst rupture within the excretory pathways, characterized by hematuria, puria, hydraturia, etc. Ruptures in the surrounding organs have also been described i.e. pleura, lungs, peritoneum [3, 17, 29, 30]. Therefore, cysts can affect different organs. Typically, 70% of tapeworm eggs are filtered within the liver; however, if these pass through the liver border, they might get into the lungs. Commonly, the cysts might be accidentally discovered on an occasional ultrasound imaging, thus a differentiated diagnosis is required to exclude other cystic formations that can be located in the liver and less frequently in the kidneys. This disease causes moral and cost prejudices within population, thus the medical and veterinary authorities are facing the problem for improving the prevention and control measures of echinococcosis [20, 27].

Conclusions

1. Echinococcosis, including the renal one, remains one of the most significant problems, requiring specific surgical approaches and techniques.

2. The severity of the parasitic cyst infections arises from their multiple localization and the superimposed surface, which leads to associated complications.

3. The disease is encountered in most countries worldwide and currently affects about one million people. Albendazole is used in patients with hydatic cyst before and after the surgery, if a cyst rupture occurred or if live cysts have been discovered during the surgical intervention, due to a percutaneous drainage of the cyst for diagnostic or therapeutic purposes. Pre-operative administration of mebendazole has been applied in all reported cases. The direct echinococcosis-related mortality rate is low (0.29-0.60%), though being accompanied by recurrent incidence in nearly 10% of cases.

4. The individualized surgical treatment is selected based on the size and location of the hydatid cysts, ranging from cystectomy treated by enucleation to a complete removal of the impaired kidney.

5. A mandatory medical treatment with Mebendazole or Albendazole (dose of 5 mg / kg / day, post-operatively for a period of 28 days, with a 14-day break) is given both before and after surgery, to prevent hydatid recurrences. Pre- and post-operative intensive treatment with antibiotics was also administered to control the suppurative, inflammatory phenomena, followed by uroseptic drugs (Furamag, Furagin, No-Cyst).

6. Hydatid diseases, including the renal ones require an adequate selection and a collaboration between various specialists like pediatric surgeons, pediatric anesthesiologists and reanimatologists, imaging specialists and pathomorphologists.

7. Despite the popularity of the medical treatment, the surgical approach is still a method of choice in treatment of a hydatid disease, especially in cases of giant and ruptured cysts, as well as in those suppurative and calcified ones.

8. The selection of a surgical approach depends on the clinical situation, associated with administration of antiparasitic therapy (Albendazole, Escazole, Doxycycline, and Helmex), which allows to prevent the post- hydatid disease, characterized by cavity remnants, hemorrhage and secondary recurrences.

9. The follow-up of all the surgical cases, carried out at 1-6 month intervals did not reveal any recurrent cases.

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JB drafted the first manuscript, VP acquired and interpreted the morphological data, VR interpreted the data, AC collected the data, EG designed the trial and revised the manuscript critically. All the authors revised and approved the final version of the manuscript.

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The authors have no conflicts of interests to declare.

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Thiol-disulfide metabolism in kidney tissue at the administration of some copper coordination compounds

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Abstract

Background: Thiol-disulfide metabolism is essential for normal function of the organism. Thus the interest of the scientists in this area of research continues to grow.
Material and methods: Copper coordination compounds (CCC), derivatives of thiosemicarbaside (CMD-4, CMJ-33, CMT-67), action on thiol-disulfide metabolism in the healthy *Ratta albicans* kidneys were studied. The animals were divided in 6 groups of 7 rats each. The control group included healthy rats which were injected i/m physiological solution 3 times a week, for 30 days. The rats from groups 2-6 have got 3 times a week, for 30 days, i/m injections of CCC. The activity of following thiol-disulfide metabolism enzymes in the renal supernatant has been measured: glutathion-reductase (GR), glutathion-peroxidase (GPO), glutathion-S-transferase (G-S-T), γ-glutamyl transpeptidase (γ-GTP), glutaredoxin (Grx), as well the amount of the protein SH-groups and of the total glutathione, reduced glutathione (GSH) and oxidized glutathione (GSSG) in renal tissue.
Results: The compounds exhibit different actions: CMT-67 in the dose of 0.1 μM/kg influenced the activity of the glutathione metabolism enzymes – activated γ-glutamyl transpeptidase (γ-GTP) and glutaredoxine (Grx) and inhibited glutathione reductase (GR), while CMD-4 in doses of 0.1 μM/kg and 1.0 μM/kg and CMJ-33 in the dose of 1.0 μM/kg significantly diminished the reduced glutathione (GSH) level and increased the amount of the oxidised one (GSSG).
Conclusions: Selective action of the copper coordination copounds established by this study opens new possibilities of their usage in the therapy of kidney diseases.
Key words: thiol-disulfide metabolism, copper coordination compounds, kidney tissue.

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Introduction

Thiol-disulfide metabolism is a factor, which largely determines the functional activity of human body. Its intensity conditions the speed of the most diverse reactions of biosynthesis, growth and development of cells and organs, transport and repair processes and many other aspects of vital activity. This explains the constant interest of scientists in the exploring of various aspects of thiol-disulfide metabolism [1-3].

A very topical issue is the need to study the new coordination compounds of transition metals, especially the thiosemicarbazon derivatives, which can serve as a basis in the development of the medicinal preparations for the prophylaxis and the treatment of various diseases, including the renal ones and the stimulation of the regenerative processes in the tissues. Many medicinal remedies that contain metals exhibit modulatory effects on the thiol-disulfide system, which provides an opportunity to understand the molecular mechanisms of their action on the body and allow the development of new strategies for the treating of different diseases [4].

Due to their high biological activity, especially, to influence the thiol redox system, the thiosemicarbazon derivatives are increasingly used for the treatment of various diseases, including cancer [5]. The performed preliminary researches have revealed their therapeutic efficacy and the prospects for using of these compounds [6].

In the scientific studies with reference to the problem in question, the data that reveal the changes of thiol-disulfide metabolism in the renal tissue in the use of new thiosemicarbazon derivatives, complexed with metal ions are lacking.

The aim of the study was to elucidate the peculiarities of thiol-disulfide metabolism and its pathogenic importance in the renal tissue of laboratory healthy animals while administering of some new copper coordination compounds (CCC), derivatives of thiosemicarbazide.

Material and methods

The new local CMD-4, CMJ-33 and CMT-67 copper coordinative compounds, derivatives of the thiosemicarbazide, synthesized in the Laboratory of Advanced Materials in Biopharmacy and Technique of the State University of Moldova, have been studied [6-8].

Experiments have been performed on white laboratory rats (No 42) weighting 180-240 g. The research protocol was approved by the Research Ethic Board of *Nicolae Testemitanu* State University of Medicine and Pharmacy (No 43 of June 18, 2015) and the tests have been done according to the contemporary principles in biological standardization of experiences and Declaration of Helsinki with further amendments (Somerset West Amendment, 1996).

The animals have been randomly divided in 6 groups of 7 rats each, with chow and water available *ad libitum*. The control group included healthy rats which were injected i/m physiological solution 3 times a week, for 30 days. The rats from groups 2-6 have got 3 times a week, for 30 days, i/m injections of copper coordination compounds as following: group 2 – CMD-4 (0.1 μM/kg), group 3 – CMD-4 (1.0 μM/kg), group 4 – CMJ-33 (0.1 μM/kg), group 5 – CMJ-33 (1.0 μM/kg) and group 6 – CMT-67 (0.1 μM/kg). The experimental animals have been euthanized 24 hours after the last administration of CCC and the kideys have been removed.

The kidneys have been homogenized in 0.1 M (pH 7.4) phosphate buffer solution that contained 1 mM EDTA, final dilution 1:10. Membranes have been destroyed by Triton X-100 processing (final concentration 0.1%). All procedures have been performed in glacial media. The obtained homogenate has been subjected to centrifugation for 15 min at 5000 rotations per minute and the supernatant has been stored at (-) 40°C.

The activity of following thiol-disulfide metabolism enzymes in the renal supernatant has been measured: glutathion-reductase (GR), glutathion-peroxidase (GPO), glutathion-S-transferase (G-S-T), γ-glutamyl transpeptidase (γ-GTP), glutaredoxin (Grx), as well the amount of the protein SH-groups and of the total glutathione, reduced glutathione (GSH) and oxidized glutathione (GSSG) in renal tissue. Spectrophotometric micromethods adapted for Synergy H1 Hybrid Rider (BioTek Instruments, USA) have been applied [9, 10].

Data were subjected to statistical analysis in StatsDirect Statistical Software (version 1.9.5., 2001) using *U Mann-Whitney* non-parametric test to determine the differences between means at the significant level of $p<0.05$.

Results

Table 1 shows the activities of the thiol-disulfide metabolism enzymes (GR, GPO, G-S-T, γ-GTP and Grx) in the kidney homogenate after the administration of the CCC. The data reveals that the CCC exhibit different action upon the studied glutathione-dependent enzymes activities in the kidneys. The GR and G-S-T activities decreased by 14-35%, while GPO – non-significantly, under the influence of all studied compounds. γ-GTP was inhibited by CMJ-33 administrated in dose of 1.0 μM/kg, while the rest of the CCC have not changed the activity of the enzyme in comparison with the control group. Compound CMD-4 in both doses has not induced significant changes of Grx activity, which was considerably increased (up to 39-95%) by both doses of CMJ-33 and CMT-67 in the dose of 0.1 μM/kg.

The research results of the influence of studied compounds on the level of total, reduced and oxidized glutathione and protein SH groups in the kidney tissue are presented in table 2. Studied compounds have not induced statistically significant changes of the total glutathione level in the kidneys. Administration of both doses of CMD-4 and CMJ-33 in the dose of 1.0 μM/kg has produced a significant decrease (by 75-86%) of GSH along with a considerable increase of GSSG level – more than 2 times in comparison with the control group. Subsequently the GSH/GSSG ratio was notably reduced. The amount of protein SH groups manifested a decreasing tendency by the studied CCC, but the attested decrease has proved to be of no statistical relevance.

Discussion

The influence of CCC on the thiol-disulfide metabolism in the kidneys of healthy rats has been studied. GPO, G-S-T, GR, γ-GTP, Grx and reduced glutathion (GSH) are the elements of a complex antioxidant system that neutralizes the hydrogen peroxide and organic peroxides both in the cellular environments and biological membranes. Organic peroxides having a high damaging capacity can induce a series of chain reactions that will deteriorate the cellular structures [11].

We have identified a statistic significant decrease of the GR and G-S-T activities after the administration of the minimal dose of CCC (0.1 μM/kg), while the high dose (1 μM/kg) has not produced a significant effect on the enzymes activity ($p>0.05$).

The biological role of GR is to maintain a high level of GSH and low one of GSSG, which is particularly important, because glutathion performs its biological functions only in the reduced form. GR, having high activity in the kidneys, represents the enzyme that reduces GSSG and thus plays a cardinal role in the GSH recycling. We have attested the reduction of the GR activity in the kidneys after the administration of low doses of CCC, thus the enzyme can not restore the GSH level by reducing GSSG. The inability of GR to reduce GSSG and maintain the optimal level of GSH will disturb the proper functions of GPO and GST, that require GSH for peroxides reduction, and/or of Grx, that reduces the disulfides [11-13]. Thus, the CCC, diminishing the activity of GR, can influence the activities of GPO, G-S-T and Grx through the reduction of GSH amount in the kidneys.

Our study revealed a significant decrease of the G-S-T activity in the kidneys after CCC administration. In the kideys G-S-Ts are expressed in the tubules and acount for 2% of the total soluble cytoplasmic proteins. Recently was established that G-S-Ts are involved in the metabolism of xenobiotics and can neutralize the final products of lipid

peroxidation – a,b-highly reactive unsaturated aldehydes, such as 4-hydroxynonenal (4-HNE), and the latter compound, via a feedback mechanism, is involved in the regulation of the multiple cell signaling pathways.

The pronounced inhibition of GST activity in the renal tissue, detected in our research, may induce the increase of 4-HNE concentration, which in turn causes inhibition of pro-proliferative mechanisms, as well as DNA and protein synthesis, which may result in apoptotic or necrotic eventual death of cells [14]. Thus, inhibition of GSTs at the cellular level, along with other mechanisms, could underlie the antiproliferative, antitumor properties of these compounds and, it is not excluded, could produce effects, manifested also by a certain degree of nephrotoxicity. Participation of G-S-Ts as mediators of the signaling pathways involved in cell proliferation and death have been highlighted by numerous previous studies [15-17]. In other words, GSTs regulate cellular homeostasis by modulating intracellular levels of 4-HNE, and the latter functions as a sensor that at low concentrations directs cells to proliferation, survival, differentiation, and at increased concentrations causes inhibition of pro-proliferative processes, which induces apoptosis and necrosis [18, 19].

Therefore, G-S-T activity can be an important tissue marker of the effects on cell signaling, but also of the mechanisms underlying toxic/side effects of the chemical compounds tested as new pharmaceutical remedies.

GPO is a family of antioxidant enzymes, present in the tissues under several isoenzymes. The kidney isoform – GPO-3, is a tetrameric selenoprotein that is attached to the basal membrane of the proximal renal tubes. The GPO-3 is responsible for the reduction of hydrogen peroxide and of some organic peroxides using GSH as a donor of reducing equivalents. The GPO activity was not significantly decreased by the administration of CCC and the ability to reduce H_2O_2 to water and the potential for annihilation of organic peroxides was preservered, thereby the kindey cells were protected from oxidative damage.

The kidney is the main organ that regulates the plasma level of GSH, due to the high activity of γ-GTP in the renal convoluted tubules [20]. The enzyme is essental in the γ-glutamyl cycle, a pathway responsible for amino acids and peptides transfer through membranes into the cell, as well as in protein synthesis, synthesis and cleavage of glutathione, detoxification of medicines and xenobiotics [11, 13, 21, 22]. γ-GTP may also exhibit prooxidative properties in some circumstance and induce metal-dependent DNA damage [23-25].

Studied CCC maintained the γ-GTP activity at the level specific for control group animals that proves that the CCC do not influence the γ-GTP-dependent processes mentioned above. As an exception a significant decrease was revealed after the administration of CMJ-33 in maximal dose of 1.0 μM/kg.

This fact, probably, not only affects the translocation of amino acids and proteosynthetic processes in the renal cell, but can also be regarded as a process oriented to the reducing of the oxidative damage of cellular DNA dependent on metal ions, such as Fe, Cu, etc. In the literature it has been reported that γ-GTP may also exhibit prooxidative properties in some circumstance and in the presence of metal ions, such as Fe^{2+}, Cu^{+}, increases the level of DNA damage and nitrogenous bases [24, 25]. Subsequently CMJ-33 is not only having an impact on the amino acids transfer into the cell and protein synthesis in the kidneys, but also can prevent the oxidative damage of the DNA from metal ions like Fe^{2+}, Cu^{+}, etc.

Thus, the tissue level of γ-GTP activity can be an important marker of protein synthesis and DNA metal-ion dependent damage in preclinical and clinical studies of potential medicines, as long in the research of pathogenic mechanism of kidney diseases.

Grx was activated by CMJ-33 and CMT-67 revealing the capacity of these compounds to induce the reactions of reduction/deglutathionilation of proteins. Such reactions are catalysed by glutaredoxines/thioltransferases (Grx/TT) – members of the GSH-dependent thioldisulfide oxidoreductases, involved in reduction of disulfides or GSH mixed disulfides [26-28]. Grx and thioredoxin are catalyzing the reduction of protein disulfides, sulfoxides and sulfenic acid, as well as the reduction of ribose in the pathway of deoxyribonucleotide synthesis [29]. It is known that GSH modulates the DNA synthesis by maintaining glutaredoxin and/or thioredoxin in a reduced state, which is required for the activity of ribonucleotide reductase, enzyme that limits DNA synthesis in the cells [30].

Grx regulates through redox mechanisms the nuclear kB factor (NF-kB) expression and subsequently the chemokine production and inflammation in the kidneys [31]. Also these mechanisms involve Grx in the regulation of the eneregy state of the kidneys [32]. Therefore, Grx can be a valuable tissue marker, not only for assessing the detailed mechanisms of action of various chemical compounds tested as new therapeutic agents *in vivo*, but also for an in-depth study of the pathogenesis of kidney diseases.

The changes of GSH and GSSG amounts, as long of the GSH/GSSG ratio have been studied in the kidneys after administration of the CCC in different doses. The statistic significant decrease of GSH, increase of GSSG and decline of the GSH/GSSG ratio have been revealed in our study. Those changes can be a consequence of the reduction of the GSH recycling rate at enzyme level or/and enhancement of GSSG formation due to oxidative stress exacerbation.

The optimal GSH/GSSG ratio is important for cell vitality, and alteration of the intracellular GSH balance has been reported in many diseases, including tumors [33, 34]. Abnormal GSH/GSSG ratio can trigger significant changes of the redox-dependent cellular signaling mechanisms that are involving G-S-T and Grx.

Likewise, it is important to note that shifting the GSH/GSSG redox toward the oxidizing state activates several signaling pathways, like nuclear factor κB, c-Jun N-terminal kinase, apoptosis signal-regulated kinase 1, protein kinase

B, protein phosphatases, etc., thereby reducing cell proliferation and increasing apoptosis [35].

It is known that reduced glutathion plays an important role in antioxidant protection and is involved in regulation of major cellular processes like gene expression, protein synthesis, apoptosis, signal transduction, cytokine production and immune response and glutathionylation of proteins [2, 27, 33, 34, 36]. In reduced form, this tripeptide is involved in many reactions and processes, due to the high reactivity of the SH group, which by giving an electron pair can achieve a wide range of reversible or irreversible chemical combinations. GSH can directly or through some enzymatic reactions, acting as a coenzyme of GPO or GST, efficiently capture free radicals and other reactive oxygen species and neutralize endogenous or/and exogenous toxic compounds [20, 37, 38]. Thus, its decrease can have a significant impact on the mentioned processes.

The overall changes of the thiol-disulfide metabolism in the kidneys after the administration of the CCC offer new incites on this metabolic branch role in tissue homeostasis and possibilities of CCC usage in the treatment of the kidney diseases, as well as new directions of research of the therapeutical and preventive potential of the novel copper coordination compounds derivatives of thiosemicarbazide in kidney diseases.

Conclusions

1. The main indices of thiol-disulfide metabolism that can reveal the efficiency of the novel copper coordination compounds derivatives of thiosemicarbazide, in the kidneys of healthy animals have been estimated.

2. Evaluation of the activities of the thiol-disulfide metabolism enzymes in the kidney homogenate after the ad-

Table 1

The influence of copper coordination compounds derivatives of the thiosemicarbazide – CMD-4, CMJ-33 and CMT-67 on the indices of thiol-disulfide metabolism in the kidney tissue of rats

Groups of study	GR (nM/s.g.prot.)	G-S-T (nM/s.g.prot.)	GPO (nM/s.g.prot.)	γ-GTP (μM/s.g.prot.)	Grx (nM/s.g.prot.)
Control group	17.2±1.21 (100%)	16.4±0.66 (100%)	42.3±2.90 (100%)	8.2±0.35 (100%)	9.7±1.07 (100%)
CMD-4 0.1 μM/kg	**13.2±1.16* (77%)**	**14.1±0.38** (86%)**	34.8±2.53 (82%)	7.7±0.71 (93%)	8.7±0.63 (89%)
CMD-4 1.0 μM/kg	17.9±2.37 (104%)	14.58±0.85 (89%)	40.8±2.63 (96%)	7.54±0.55 (91%)	11.6±1.91 (118%)
CMJ-33 0.1 μM/kg	**13.0±0.81** (75%)**	**13.9±0.42** (85%)**	40.1±3.74 (95%)	8.6±0.19 (104%)	**13.6±1.52* (139%)**
CMJ-33 1.0 μM/kg	15.5±1.70 (88%)	15.1±0.71 (92%)	38.9±2.89 (92%)	**4.6±1.02*** (56%)**	**19.0±2.13*** (195%)**
CMT-67 0.1 μM/kg	**11.1±1.00*** (65%)**	**12.1±0.41*** (74%)**	40.8±5.81 (96%)	8.8±0.83 (106%)	**15.3±2.19* (157%)**

Note: * – *statistically significant difference with the control group, p <0.05; ** – p <0.01; *** – p <0.001.*

Table 2

The influence of copper coordination compounds derivatives of the thiosemicarbazide – CMD-4, CMJ-33 and CMT-67 on the indices of thiol-disulfide metabolism in the kidney tissue of rats

Groups of study	Total GSH (μM/g tissue)	GSH, (μM/g tissue)	GSSG, (μM/g tissue)	GSH/GSSG ratio	SH-group of prot. (mol/g.prot.)
Control group	3.72±0.07 (100%)	3.29±0.09 (100%)	0.44±0.09 (100%)	7.48±1.03 (100%)	17.18±1.48 (100%)
CMD-4 0.1 μM/kg	3.56±0.17 (96%)	**2.48±0.13** (75%)**	**1.07±0.10** (243%)**	**2.32±0.93*** (31%)**	15.08±0.76 (88%)
CMD-4 1.0 μM/kg	3.56±0.18 (96%)	**2.56±0.19* (78%)**	**1.00±0.10** (227%)**	**2.56±1.76* (34%)**	15.68±1.61 (91%)
CMJ-33 0.1 μM/kg	4.27±0.24 (114%)	3.21±0.06 (97%)	**1.06±0.22* (240%)**	**3.03±0.29** (41%)**	15.81±0.71 (92%)
CMJ-33 1.0 μM/kg	3.41±0.19 (86%)	**2.83±0.09* (86%)**	0.58±0.11 (132%)	**4.88±0.68* (65%)**	16.84±0.4 (98%)
CMT-67 0.1 μM/kg	3.89±0.85 (104%)	3.36±0.21 (102%)	0.53±0.15 (120%)	6.34±0.81 (85%)	15.21±0.58 (88%)

*Note: * – statistically significant difference with the control group, p <0.05; ** – p <0.01; *** – p <0.001.*

ministration of the CCC revealed that GR and GST activities were inhibited by CMD-4, CMJ-33 and CMT-67 (0.1 μM/kg), γ-GTP – by CMJ-33 (1.0 μM/kg), Grx activity was considerably increased by CMJ-33 (both doses) and CMT-67 (0.1 μM/kg), while GPO function did not change.

3. CMD-4 (both doses) and CMJ-33 (1.0 μM/kg) induced a significant increase of GSSG along with a considerable decrease of GSH and of the GSH/GSSG ratio.

4. The study of the copper coordination compounds derivatives of the thiosemicarbazide – CMD-4, CMJ-33 and CMT-67 influence on the thiol-disulfide metabolism, opens new possibilities of research with focus on the elucidation and analysis of the mechanism of CCC preventive and therapeutical action in health and disease.

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Authors' contribution
VS – designed the research, did statistics and interpreted the data; VP – conducted/performed the laboratory work; AG – interpreted the data and drafted the manuscript; OT – conceptualized the project and designed the research; LA – conducted/performed the laboratory work; IS – conducted/performed the laboratory work; VT – drafted the manuscript.
VG – conducted the laboratory work, revised the manuscript critically. All authors revised and approved the final version of the manuscript.

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Ethics approval and consent to participate
The research protocol was approved by the Research Ethic Board of Nicolae Testemitanu State University of Medicine and Pharmacy (No 43 of June 18, 2015) and the tests have been done according to the contemporary principles in biological standardization of experiences and Declaration of Helsinki with further amendments (Somerset West Amendment, 1996).

Conflict of Interests
No competing interests were disclosed.

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The validation of New Injury Severity Score for severe and critical trauma patients

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Abstract

Background: Considerable part of traumatized patients has criteria for severe/critical trauma. There is no international consensus concerning the most accurate traumatic scores. Their utilization in Moldova requires optimization. This article's goal is to validate the predictive ability of New Injury Severity Score (NISS) in sever/critical trauma patients admitted in Moldovan trauma center.
Material and methods: The retrospective cohort study, trauma patients (n=476) were admitted to Intensive Care Unit (ICU). The cohort was divided into severe (NISS > 15) and critical (NISS > 24) traumas. To achieve the aim, the multivariate logistic regression was used. The results were adjusted to gender, age and the mechanical ventilation use.
Results: Severe trauma model had an acceptable determination coefficient (Nagelkerke R Square=0.541). The calibration was poor (Hosmer-Lemeshow test, χ^2=17.430, df=8, p=0.026). The discrimination parameters, sensibility and specificity, were 85.9% and 85.1%. The determination coefficient for critical trauma model was 0.568, the calibration ability being normal (χ^2=7.249, df=8, p=0.510). The sensibility and specificity were 70.9% and 94.7%, respectively.
Conclusions: In this study, were proposed two mathematical models that validated NISS as an instrument to predict the outcomes in sever/critical trauma patients admitted in Moldovan trauma center. In general, the model's characteristics (determination, calibration and discrimination) could be appreciated as good ones with some limitations. Taking into account the advantages and disadvantages, both models could be recommended for daily practice usage in condition of ICU from Emergency Medicine Institute.
Key words: new injury severity score, severe trauma, critical trauma, score validation.

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Introduction

Trauma remains medical, social and economic problem being the main worldwide cause of death for the patients younger than 40 years [1]. In addition, while the death rate from oncological and cardiac diseases has favorable trend, the death rate from trauma is rising faster than the parallel population increase. This is a sign that, in the long perspectives, probably, the trauma will prevail in general lethality structure [2].

The trauma structure analysis has shown that considerable part of traumatized patients, almost 40%, has criteria for severe or critical trauma. These two categories of trauma require treatment in the intensive care unit (ICU) department considering the increased risk of complications and death. The trauma completes condition for severe if NISS (New Injury Severity Score) > 15, and when NISS > 24, the trauma is classified as critical [3, 4]. Taking into account the risks, it is crucial to estimate the trauma's lesions severity. Unfortunately, today, there is no international consensus on what kind of scores or algorithms of trauma assessment are optimal. As a consequence, different medical systems use different scores [5, 6], considering particularities of these systems, of demographic structure or sometimes geographical factors [7].

The rational (optimal) utilization of the traumatic scores in the Republic of Moldova remains a serious problem. Because of that, at the patient's evaluation there are some disagreements on the prediction, different scores often estimating the outcomes completely different. According to the open resources at least, RTS (Revised Trauma Score), AIS (Abbreviated Injury Score) and ISS (Injury Severity Score) algorithms have been used, all these models having no any statistical validation [8]. The same statement is avai-lable for Acute Physiology, Age, Chronic Health Evaluation II score (APACHE II), used by the anesthesiologists to predict mortality rate for critical patients in ICU condition [9] and A Severity Characterization of Trauma (ASCOT) used for patients with associated trauma and Traumatic Brain Injury (TBI) lesions [10]. There are only two validated trauma scores. Both, the MPMoIII (Mortality Probability Admission Model) and NISS were tested for survival prediction in severe trauma patients (criteria being NISS > 15) transferred from regional hospitals to Emergency Medicine Institute (EMI). These models were used in a pilot research

with relatively reduced number of respondents and, of course, the accuracy of coefficient needs improvement, results being extrapolated on the specific category of trauma patients and as a result cannot be used for patients admitted directly in EMI [11].

The described problems need solution. This can be done using the strategy, described above [12]. It is based on three main elements – revision of potential models, their adaptation or alternative model/models ela-boration and, finally, comparative evaluation. The first element was performed and the main existing trauma scoring systems were revised to highlight the potential scoring systems that in perspective can be validated in the Moldovan medical system. Totally, we have analyzed 33 potential mo-dels divided into three main groups: anatomical, physiological and mixed.

In this list of scores for trauma patient evaluation, the ISS and NISS, mentioned above, derivate from AIS, are the most popular anatomical scores being widely used in different medical systems. To estimate ISS, we have to use the following formula: $ISS = AIS_1^2 + AIS_2^2 + AIS_3^2$, where AIS_1, AIS_2, AIS_3 are the highest AIS values present in six topographic regions [5]. NISS in comparison with ISS, estimates trauma severity taking into account three maximal values of AIS, without taking into account the lesion's localization [6]. For example, in case of trauma when $AIS_{abdomen} = 2$, $AIS_{head\ and\ neck} = 3$, $AIS_{head\ and\ neck} = 3$ and $AIS_{limbs} = 5$, the NISS value will be higher ($NISS=5^2 + 3^2 + 3^2 = 43$) in comparison with ISS ($ISS=5^2 + 3^2 + 2^2 = 38$). Both, the ISS and NISS can vary from 0 up to 75. In condition if there is a topographical region with AIS = 6, ISS or NISS automatically is considered equal to 75. A meta-analysis shows similar sensibility and specificity of NISS and ISS mortality prediction in trauma patients [13]. We suppose that this result can be explained by insufficient determination coefficient in equations that use NISS or ISS. At the same time, there are data that the NISS is more precise, especially for patients with multiple injuries [14].

This article's goal is to validate the NISS as an instrument to predict the outcomes in sever/critical trauma patients admitted in Moldovan trauma center in order to use it for survival prediction. The validation has two elements – the confirmation of predictive power and adaptation of NISS regression coefficients for sever and critical trauma patients admitted in ICU, EMI of Chisinau, the Republic of Moldova.

Material and methods

The retrospective cohort study was performed. The injured patients (n = 467) were admitted in acute trauma period (the first 72 hours after impact) to ICU of EMI, Chisinau, the Republic of Moldova. The severity of lesions was appreciated using the NISS (AIS 2015), criteria for severe trauma being the value over the 15 points (n = 467 patients), the cut-off for critical trauma was considered the NISS value over the 24 points (n = 225 patients). Taking into account particularities related to the patients with mental disorders (psychoses) as the reason for admission to ICU, these ones were not considered in this research.

Statistical analysis

Descriptive statistics had the following components. The continuous data were represented using the central tendency indicators (mean, median) and dispersion parameters (standard deviation, interquartile range). To describe the dichotomous data have been used the frequencies and proportions. To analyze the precision of obtained data the 95% confidence intervals were estimated. To achieve the aim of this research, taking into account that the outcome (death/survival) depends on a variety of factors and it represents a dichotomous variable, the multivariate logistic regression procedure was used. The results were adjusted to gender, age and the mechanical ventilation (MV) use. In order to match the regression analysis conditions the data was tested for multicollinearity. The potential model was characterized using determination (*Nagelkerke R Square*), calibration (*Hosmer-Lemeshow test*) and discrimination (*sensibility, specificity, mean validation, ROC curve and classification graph, cut-off* modification) parameters. In addition, the proposed models stability analysis was performed (resampling using *bootstrapping*). According to literature data, we expected better discrimination and calibration in critical vs severe trauma patients [4].

Results

According to the table 1 data, becomes evident the prevalence of males in both cohorts, the proportion being similar (70.2% for severe and 72% for critical trauma, 95% CI having approximately the same range, 65.9, 74.2 and 65.8, 77.5, respectively). Similar affirmation is valid for age variable (median 43 vs 44, interquartile range being 26 and 24, respectively for severe and critical trauma).

Intrahospital lethality was estimated at level 13.7% (CI 95% 10.9, 17.1) and 24.4% (CI 95% 19.3, 30.5), evidently predominating in critical trauma patients. It is important to mention that CI 95% are not large and there is no any intersection between them. This is a sign for significant differences in outcomes for these two cohorts and the lethality in critical trauma is twice higher (OR=2.04, CI 95% 1.36, 3.05).

The NISS variable, without any doubts, is significantly higher in critical trauma patients (mean=43, 95% CI 40.8, 45.2 for critical and mean=26.6, 95% CI 25.6, 27.6 for severe trauma patients). Regarding MV, it can be concluded that, in condition of critical trauma, the proportion of alternative approach is similar with traditional one that is not characteristic for severe trauma patients (39% vs 61%), (tab. 1).

The predictive model for primary outcome (death/survival) in severe trauma

First, for the patients that meet criteria for severe trauma, were formulated the hypotheses as follows. *The null hypothesis* – the covariates included in the model (gender, age, NISS, MV) are not able to predict the death's probability in severe trauma patients better than a model that includes only the constant. *The alternative hypothesis* – at least one variable is able to predict the death's probability in severe trauma patients better than a model that includes only the constant.

Table 1

Descriptive statistics for severe (a) and critical (b) trauma patients

a. Severe trauma patients (NISS > 15)				
		Mean (Standard Deviation)/ Count (%)	**95% Confident interval Lower limit, Upper limit**	**Median (Interquartile Range)**
Gender	**Female**	139 (29.8%)	25.8, 34	
	Male	328 (70.2%)	65.9, 74.2	
Age, years		42 (17)	40.5, 43.5	42 (26)
NISS, points		26.6 (11.5)	25.6, 27.6	23 (15)
Mechanical ventilation	**Traditional**	285 (61%)	56.5, 65.3	
	Prophylactical	182 (39%)	34.7, 43.5	
Outcome	**Survival**	403 (86.3%)	82.9, 89.1	
	Death	64 (13.7%)	10.9, 17.1	
b.	**Critical trauma patients (NISS > 24)**			
Gender	**Female**	63 (28%)	22.5, 34.2	
	Male	162 (72%)	65.8, 77.5	
Age, years		43 (17)	40.8, 45.2	44 (24)
NISS, points		36 (10)	34.7, 37.3	34 (14)
Mechanical ventilation	**Traditional**	117 (52%)	45.5, 58.5	
	Prophylactical	108 (48%)	41.6, 54.5	
Outcome	**Survival**	170 (75.6%)	69.5, 80.7	
	Death	55 (24.4%)	19.3, 30.5	

The null hypothesis was rejected (*Omnibus Test of Model Coefficients* (χ^2 = 165.044, df=3, p<0.001, significance being 0.05/2 = 0.025) that means that at least one variable is able to predict the outcome, the elaborated model having the following characteristics. Determination coefficient, *Nagelkerke R Square* = 0.541 (54.1%). This result can be considered as acceptable one, but anyway it can be improved. The calibration ability was poor, *Hosmer-Lemeshow test* being significant (χ^2 = 17.430, df=8, p=0.026). The discrimination parameters, sensibility and specificity, according to the classification table, were 85.9% (55 dead patients out of 64) and 85.1% (343 survived patients out of 403), respectively, overall percentage being estimated at 85.2% (cut-off=0.13, fig. 1). Analysis of classification graph can reveal the overfitting problem.

Area under the Receiver Operating Characteristics

```
              Observed Groups and Predicted Probabilities

     160 +                                                                                                  +
         I                                                                                                  I
         I                                                                                                  I
F        I                                                                                                  I
R    120 +                                                                                                  +
E        IS                                                                                                 I
Q        IS                                                                                                 I
U        IS                                                                                                 I
E     80 +S                                                                                                 +
N        IS                                                                                                 I
C        ISS                                                                                                I
Y        ISSS                                                                                               I
      40 +SSS                                                                                               +
         ISSSS                                                                                              I
         ISSSSSS                                                                                            I
         ISSSSSSSSSSSS D      D                                                                             I
Predicted ---------+---------+---------+---------+---------+---------+---------+---------+---------+----------
  Prob:   0        ,1        ,2        ,3        ,4        ,5        ,6        ,7        ,8        ,9        1
  Group:  SSSSSSSSSSSSSDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDD

          Predicted Probability in the quantity of dead
          The Cut-off Value is ,13
          Symbols: S - Survival
                   D - Dead
          Each Symbol Represents 10 Cases.
```

Fig. 1. Classification graph for severe trauma prediction model.

Table 2

Variables in equation of severe trauma. Initial model (a), final model (b) and after resampling (c)

a. Model before the Gender elimination

	B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
							Lower	Upper
Gender	.062	.406	.023	1	.879	1.064	.480	2.357
Age, years	.050	.012	18.441	1	.000	1.052	1.028	1.076
NISS, points	.156	.018	76.982	1	.000	1.169	1.129	1.210
MV	-1.335	.432	9.533	1	.002	.263	.113	.614
Constant	-8.807	1.021	74.443	1	.000	.000		
Variable(s) entered on step 1: NISS, Age, MV, Gender								
b. Model after the Gender elimination (final model)								
Age, years	.050	.012	18.677	1	.000	1.051	1.028	1.075
NISS, points	.156	.018	77.132	1	.000	1.169	1.129	1.210
MV	-1.328	.430	9.549	1	.002	.265	.114	.615
Constant	-8.755	.960	83.249	1	.000	.000		
Variable(s) entered on step 2: NISS, Age, MV								

c. Bootstrap for Variables in the Equation

	B	Bias	S.E.	Sig.	95% Confidence Interval for B	
					Lower	Upper
Age, years	.050	.002	.012	.001	.029	.078
NISS, points	.156	.005	.021	.001	.124	.206
MV	-1.328	-.056	.488	.004	-2.440	-.489
Constant	-8.755	-.278	1.038	.001	-11.364	-7.226

Unless otherwise noted, bootstrap results are based on 1000 bootstrap samples

(ROC) curve for severe trauma model was estimated at 0.907 (95%CI 0.862, 0.952) being significant compared with 0.5 value (p<0.001), (fig. 2).

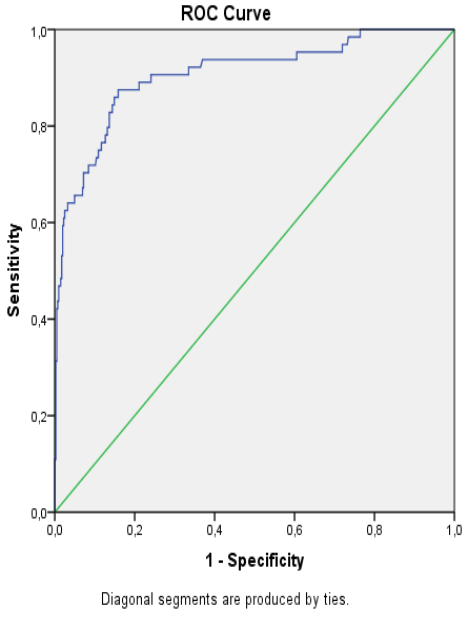

Fig. 2. ROC curve for severe trauma patients mortality predictive model.

Taking into account the data from table 2, the proposed (final) predictive model has the following formula:

$$p = 1/(1+e^{8.775 - NISS * 0.156 - Age * 0.05 + MV * 1.335}) \text{ (formula 1),}$$

where

p – probability of death in severe trauma

e (exponent) – constant equal to 2.71828

The final model includes the constant (B = -8.755), NISS (B = 0.156), Age (B =0.050) and MV (B = -.1.335) as efficient variables. Gender did not achieve the significance (p=.879). Moreover, after the elimination of this variable (tab. 2a and tab. 2b), the regression's coefficient, the Odds Ratio (OR) and the confidence intervals were without significant changes.

According to the obtained data, the most important covariate was NISS (determination coefficient .424), followed by Age (approximately 9%) and MV (3%). The quantitative expression of positive relationships of NISS and Age on the one hand and the primary outcome on the other hand (positive regression coefficients and OR more than 1, confident intervals being relatively narrow) allows to modulate different situations as follows. For example, if in ICU, we meet two similar patients according to NISS and MV criteria, having only one year age difference, the death probability is 5% higher (95% CI 2.8. 7.5) for the older patient. In the same way, the only one NISS point difference can increase

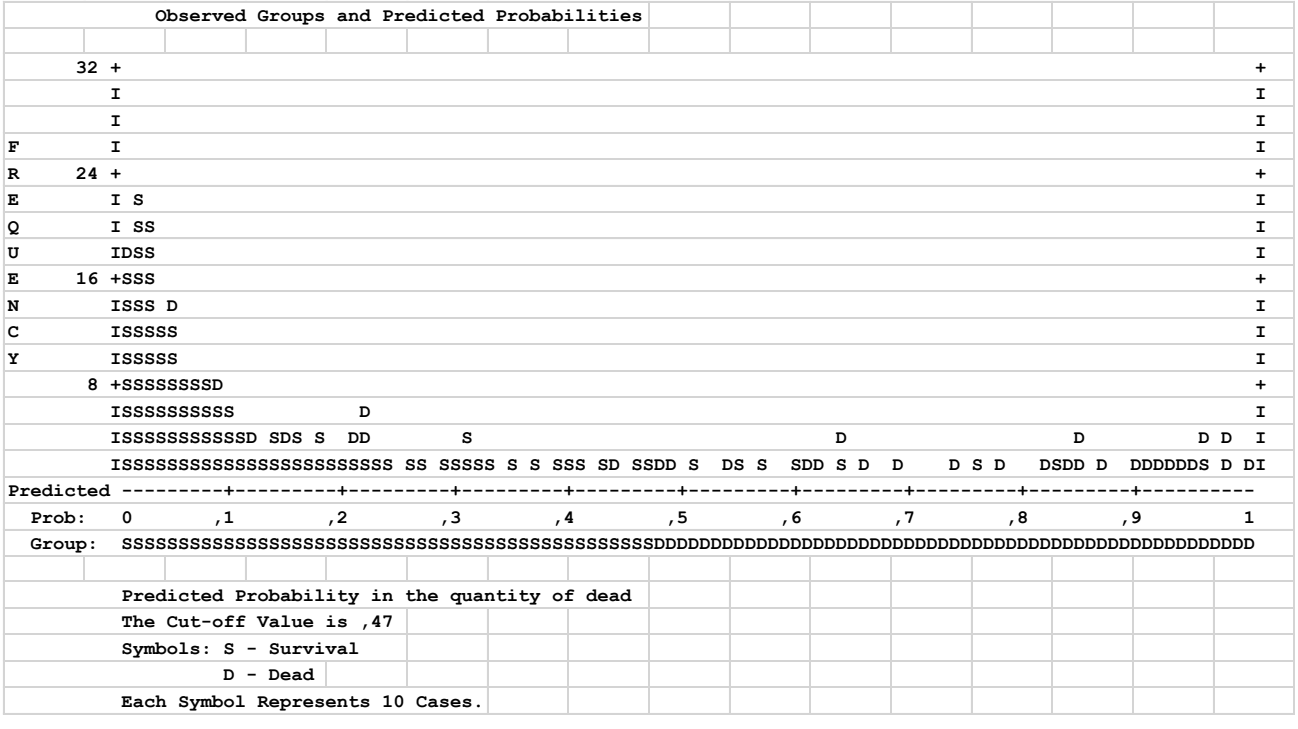

Fig. 3. Classification graph for critical trauma prediction model.

the probability of death by 16.9% (95% CI 12.9, 21).

The resampling (bootstrapping, 1000 samples) was used to prove the elaborated model's stability. All parameters were significant, regression coefficients having narrow 95% confidence intervals, these two conditions serving as arguments to consider this model as a stable one (tab. 2c).

Table 3

Variables in equation for critical trauma.
Initial model (a), final model (b) and after resampling (c)

a. Model before the Gender elimination

	B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
							Lower	Upper
Gender	-.065	.483	.018	1	.893	.937	.364	2.414
Age, years	.045	.014	10.526	1	.001	1.046	1.018	1.075
NISS, points	.180	.026	48.722	1	.000	1.197	1.138	1.259
MV	-1.448	.487	8.828	1	.003	.235	.090	.611
Constant	-9.517	1.420	44.940	1	.000	.000		
Variable(s) entered on step 1: NISS, Age, MV, Gender								
b. Model after the Gender elimination (final model)								
Age, years	.046	.014	10.700	1	.001	1.047	1.018	1.075
NISS, points	.180	.026	48.667	1	.000	1.197	1.138	1.260
MV	-1.457	.484	9.068	1	.003	.233	.090	.601
Constant	-9.572	1.363	49.321	1	.000	.000		
Variable(s) entered on step 2: NISS, Age, MV								

d. Bootstrap for Variables in the Equation

	B	Bias	S.E.	Sig.	95% Confidence Interval for B	
					Lower	Upper
Age, years	.046	.001	.015	.002	.019	.082
NISS, points	.180	.009	.032	.001	.134	.258
MV	-1.457	-.094	.565	.003	-2.710	-.553
Constant	-9.572	-.403	1.552	.001	-13.605	-7.578
Unless otherwise noted, bootstrap results are based on 1000 bootstrap samples						

The predictive model for primary outcome (death/survival) in critical trauma

As in condition of severe trauma, for critical trauma patients, were formulated the following hypotheses. *The null hypothesis* – the covariates included in the model (gender, age, NISS, MV) are not able to predict the death's probability in critical trauma patients better than a model that includes only the constant. *The alternative hypothesis* – at least one variable is able to predict the death's probability in critical trauma patients better than a model that includes only the constant.

The null hypothesis was rejected (*Omnibus Test of Model Coefficients* (χ^2 = 107.889, df = 3, p<0.001, significance being 0.05/2 = 0.025) that means that at least one variable is able to predict the probability of death in critical trauma patients. Determination coefficient, *Nagelkerke R Square*, was estimated at 0.568 (56.8%) vs 0.541 (54.1%) in severe trauma. This result can be considered as acceptable but needs improvement. The calibration ability (fidelity results evaluation) was normal, *Hosmer-Lemeshow test* being nonsignificant (χ^2 = 7.249, df = 8, p = 0.510). The discrimination parameters, sensibility and specificity, according to the classification table were 70.9% (39 dead patients out of 55) and 94.7% (161 survived patients out of 170), respectively, overall percentage being estimated at 88.9% (cut-off = 0.47, fig. 3).

Area under the ROC curve for critical trauma model was estimated at 0.905 (95% CI 0.856, 0.954) being significant vs 0.5 value (p<0.001), (fig. 4).

Taking into account the data from table 3, the proposed (final) predictive model for death prediction in critical trauma patient can be converted into the following formula:

$p = 1/(1+e^{9.572 - NISS * 0.180 - Age * 0.46 + MV * 1.457})$ (formula 2), where

p – probability of death in sever trauma

e (exponenta) – constant equal to 2.71828

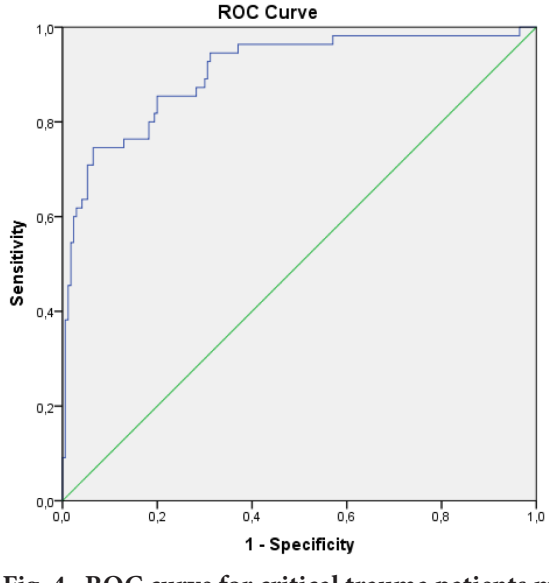

Fig. 4. ROC curve for critical trauma patients mortality predictive model.

The final model includes the constant (B = -9.572), NISS (B = 0.180), Age (B = 0.046) and MV (B = -.1.448) as significant variables, Gender being far from significance range (p = 0.893) without any effects on the regression coefficients, the OR or the confidence intervals (tab. 3a and tab. 3b) as it was mentioned in severe trauma model.

The most powerful covariate (NISS) was able to determine 43.6% of variable dispersion, a result similar with the determination coefficient values of NISS in severe trauma. The effect of Age was estimated at level of 9%, MV having 4.3% instead of 3%.

The resampling (bootstrapping) has shown stability; coefficients having significance and narrow 95% confidence intervals (tab. 3c).

Discussion

In this study, were proposed two mathematical models that validated NISS as an instrument to predict the outcomes in sever/critical trauma patients admitted in Moldovan trauma center in order to use it for survival prediction. The validation of trauma scoring scales for different medical systems prior to their practical use represents an efficient instrument with a worldwide use, net result of this process being the regression coefficients correction and optimization [3, 14, 15, 16].

The predictive potential of NISS was proven for both, critical and severe trauma patients. In addition, NISS regression coefficient was adapted to particularities of Moldovan medical system and adjusted to Age and MV strategy. The Gender variable was not significant in both trauma groups, being independent in relation to other variables. The NISS and Age covariates have shown negative effects, having relatively narrow confidence intervals. The MV has had the opposite effect with relatively large confidence intervals.

Taking into account the model's characteristics, logistic regression classified them as significantly better models than aleatory ones. In general, the determination, calibration and discrimination model's parameters could be appreciated as good ones with some following limitations.

Firstly, the research is retrospective that is why there could not be considered all risk factors and potential biomarkers that reduce the evidence level. The perspective group of potential predictors consists of components of proteases/antiproteases system. Among them are cathepsin D and a_2-macroglobulin that have shown their potential to predict the survival rate and Acute Respiratory Distress Syndrome in polytrauma patients [17, 18]. Secondly, evidently, it is not possible to extrapolate the results for all Moldovan medical system hospitals. Thirdly, both models explained less than 60% of dependent variable dispersion, optimal value being over 80%. This fact makes us think that they should be added some other efficient variables that could improve the accuracy of prediction. Fourthly, the poor calibration and potential overfitting for severe trauma model could demand precaution in practical use, other parameters being close to the optimal value [4].

In addition, it is important to mention the reduced NISS determination coefficient values for severe trauma (.424) and critical trauma (.436) vs severe trauma patients trans-

ported from regional hospitals (.641) [11]. These differences, about .2 (20%) from dispersion of dependent variable, can be considered as a benefit for patients admitted directly in trauma center because of conversion of the nonchangeable factor (trauma injuries) into potentially changeable. Increasing the ratio of the last one gives us more opportunities to influence the outcomes in order to improve them.

Conclusions

Taking into account the advantages and listed above disadvantages, both models could be recommended for daily practice usage in ICU. The arguments against these models are that they were elaborated for this particular trauma group and do not have alternative validated scores. Also, being incorporated in hospital's informational system the models could be improved in real time by adding the potential efficient variables. In addition, the model's accuracy could be raised by including new severe and critical trauma cases data.

The obtained results determine us to continue the researches in this field. Taking into consideration the experience of this study, the perspective for completely elaborated models is opening, to validate other potential models and to elaborate new scores able to predict the trauma patient's outcomes.

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OA conceptualized the idea, conducted literature review, wrote the manuscript, revised and approved the final text.

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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
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Clinico-morphological aspects and outcomes of the lean umbilical cord

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Abstract

Background: The umbilical cord forms connecting link between the fetus and placenta through which the fetal blood flows to and from the placenta. Wharton's jelly is a specialized tissue which acts as supportive and protective structure substituting for the adventitia of the umbilical vessels. The aim of the study was to reveal the clinical and morphological features of the lean umbilical cord for improving its outcomes.
Material and methods: The study included 190 patients divided into 2 groups: L_1 – 95 patients with UC abnormalities and L_0 – 95 with normal UC. Umbilical cord assessment was based on macro- and microscopic examinations. An entire umbilical cord was available from all patients and examined for diameter. Three full thickness sections of the UC were fixed in 10% formalin and stained with hematoxylin and eosin, Van Gieson's, Alcian blue for histological examination.
Results: A study of the association between the absence of Wharton's jelly and an unfavorable pregnancy outcome was undertaken. The lean umbilical cord results in reduced fetoplacental circulation, thus resulting in intrauterine growth restriction ($p<0.0001$), fetal distress and hypoxia, which require the neonatal intensive care ($p<0.0001$). Morphological examination of the umbilical cord revealed changes in Wharton's hard and fibrous jelly.
Conclusions: This study shows the importance of the knowledge of cord diameter. Clinico-morphological examination of the umbilical cord, including the study of the lean forms is of great importance in the diagnosis of the causes of adverse perinatal outcomes of fetuses and newborns in the obstetrical practice. Therefore, our study confirms that all umbilical cords should be submitted for complete examination.
Key words: lean umbilical cord, Wharton's jelly, diameter, morphology, perinatal outcome.

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Introduction

One of the most important parts of the fetoplacental unit is the umbilical cord (UC) [1]. It is the lifeline that connects and provides vascular flow between the fetus and the placenta. The fully developed umbilical cord is pearly white, 50-60 cm long with an average diameter of 1-2 cm, optimal protection which is provided by Wharton's jelly (WJ) [2]. Other studies have found that at birth, the average diameter and circumference of the umbilical cord in a normal term infant is 1.5 cm and 3.6 cm, respectively [3,4]. The ultrasound average vein diameter is 8 mm with an average artery diameter of 4 mm at term [5]. The "*lean cord*" is a rare structural abnormality, characterized by reduced or completely absent Wharton's jelly, which has seldom been described in the medical literature. Umbilical cord is lean if the middle diameter is less than 1.0 cm and the lean index is less than 0.55 g/cm in male fetus and less than 0.49 g/cm in female fetus [6].

Wharton's jelly is a specialized tissue serving many purposes for the developing fetus. The WJ is the umbilical cord stroma that originates from the extraembryonic mesoderm of allantois. It is rich in mucopolysaccharides such as hyaluronic acid and chondroitin sulfate and thus protects umbilical vessels from compression [7]. It is surrounded by myofibroblasts, which are mesenchymal cells with the characteristics of both fibroblasts and smooth-muscle cells. Myofibroblasts have both fibrogenesis and contractile functions and produce increasing amounts of type I, II, and V collagen fibrils during the pregnancy, giving Wharton's jelly elastic and contractile properties as well as microfibrils. Hyaluronic acid is the most common glycosaminoglycan – a hydrophilic component of Wharton's jelly that absorbs water and electrolytes. It has been suggested that diameter of the umbilical cord is determined by the water content of Wharton's jelly. Umbilical cord elasticity confers resistance to external pressure, and acts as a physical buffer in the regulation of fetoplacental blood circulation and umbilical vessels [8]. It has been speculated that the cells of Wharton's jelly may participate in the regulation of umbilical blood flow. In some cases, the reduction in fetal growth could be directly associated with Wharton's jelly decrement, leading to hypoplasia of the umbilical vessels. If Wharton's jelly is poorly developed, or if the vessels remain unprotected, they become more prone to compression [9]. Cord compression may be a stimulus to induce such a compromise. It is theorized that if the umbilical blood flow is slowed, then placental blood flow is also slowed. When this happens, blood

thickens in the small spaces in the placenta called the villous vessels and clots like gelatin. This, in turn, causes thromboses of the intervillous space that contains blood flow from the mother. The result is devitalized placental tissue and loss of that placental nutritional space [10, 11]. The development of multiple capillaries in the periphery and fibrosis of Wharton's jelly may be secondary consequences of the thin UC, and the capillaries can be considered collaterals for compensating for chronically compromised umbilical circulation. Thus, loss of protection by the Wharton's jelly can lead to compromised fetoplacental circulation and subsequent fetal death [12, 13].

It is believed that males have more Wharton's jelly content than do females and that good nutrition increases the amount [6]. Wharton's jelly tends to reduce with gestational age and can disappear when pregnancies go beyond forty weeks. Because these cases tend to have fetal heart rate changes, the level of Wharton's jelly is a consideration when obstetricians plan the deliveries of pregnancies low on amniotic fluid [5].

Umbilical cord is vital to fetal development and its alterations are related to perinatal complications. Though the pathogenesis of variability of umbilical cord diameter remains unclear, this study would provide information about the thin umbilical cord and its association with adverse outcomes.

There is a huge arena for research in this field as what we are seeing is just the tip of an iceberg. The challenge should be taken up, and newer equipment and strategies should be developed to analyze and avoid cord complications. This would decrease the incidences of the perinatal morbidity and mortality due to cord complications in the future and help in realizing the expections for the delivery of a healthy baby.

Material and methods

It was performed a prospective cohort study in the Department of Obstetrics and Gynecology at the clinical base of Municipal Clinical Hospital No 1, *Nicolae Testemitanu* State University of Medicine and Pharmacy. The control group (L_0) included 95 patients with normal umbilical cord and the study group (L_1) included 95 patients with umbilical cord abnormalities of which 24 had a lean UC.

Clinical details from all patients and their newborns consisted of the evaluation of patient's complaints, the anamnesis, general physical and obstetrical examination which were obtained along with the cord specimens. Umbilical cord assessment was based on macro- and microscopic examinations. The umbilical cord was available from all patients and examined for diameter (in centimeters) immediately after delivery. Abnormality included in the study group was thin UC. Three full thickness sections of the UC were fixed in 10% formalin and stained with hematoxylin and eosin (H&E), selectively histochemical staining – Van Gieson's (VG), Alcian blue pH 2.5 (AB) for histological examination.

The *inclusion* criteria in the research were: gestational age between 22^{+0}–41^{+6} weeks, spontaneous and singleton pregnancy, maternal age ≥ 18 years, research participation agreement. The *exclusion* criteria were: gestational age ≤ 21^{+6} weeks and ≥ 42^{+0} weeks, pregnancy, which occurred as a result of assisted reproduction technologies, multifetal gestation, decompensated somatic pathology of the patient, age of the patient ≤ 18 years, patients who refused voluntary participation in the research.

Statistical analysis was performed using *Statistical Package for Social Sciences* for Windows (SPSS Version 23.0), *Statistical Analysis System* (SAS Version 9.4) and Microsoft Excel 2016. The significance was tested by using a Chi-square test, the Cramer V coefficient, and the Fisher's exact test. For all quantitative characteristics in the compared groups were evaluated the arithmetic means and mean-square (standard) errors of the mean, coefficient of variation, median, mode, and quartiles. To analyze the differences among group means in a sample was used a collection of statistical model's ANOVA. *P* value of less than 0.05 was regarded as statistically significant.

Results

In our study no demographic differences could be detected between the study and control groups. The rate of lean UC was 12.63% (24) in all singleton pregnancies and the cord diameter varied from 0.4 to 0.7 (0.55±0.01) cm. The gestational age in the patients with lean UC ranged between 26-41 (38.25±0.78) weeks, there was no difference in this parameter in the studied groups (p> 0.05). There were 2 times more primiparous women than multiparous ones – 16 (8.42%) vs 8 (4.21%) cases. Patients of the study group had an imminent delivery at different periods: 4-16 weeks in 10 (5.26%) cases, 23-35 weeks in 5 (2.65%) cases, IUGR – in 4 (2.11%) cases by 2-6 weeks. The findings suggest that 19 (10%) women underwent vaginal delivery and 5 (2.63%) women had a cesarean delivery.

All children were born alive. Out of all neonates, 15 (7.89%) were male and 9 (4.74%) were female babies. The birth weight in the control group (L_0) was 2460–4780 g (mean 3470.21±463.80 g); in the study group (L_1) – 1030-3740 g (mean 2890±146.8 g) (p<0.01) (fig. 1). The newborn length in the L_0 = 45–57 cm (52.37±1.99 cm) vs 34–54 cm (49.37±1.04 cm) in the L_1 (p<0.01) (fig. 2). APGAR score at 1 min L_0 = 7-10 points (8.66±0.69) vs L_1 = 4-9 points (7.45±0.24) (p<0.05). APGAR score at 5 min L_0 = 8-10 points (9.13±0.67) vs L_1 = 5-10 points (8.12±0.23) (p>0.05).

In our study thin cords were found to be significantly associated with maternal outcome such us woman's obstetrical history (primiparity), somatic diseases, TORCH-infection (p<0.05) and perinatal outcome. The lean umbilical cord in the long run results in reduced fetoplacental circulation, thus resulting in intrauterine growth restriction (p<0.0001), fetal distress and hypoxia, which required the neonatal intensive care (p<0.0001) (tab. 1). The neonatal morbidity in the study group was observed in 16 (8.42%) cases and was

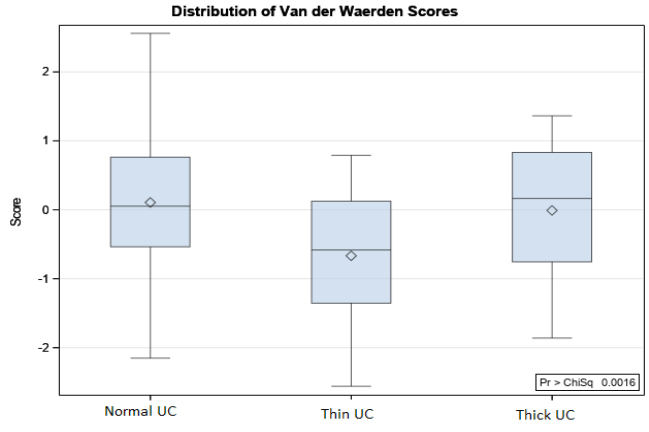

Fig. 1. Distribution of groups according to the birth weight.

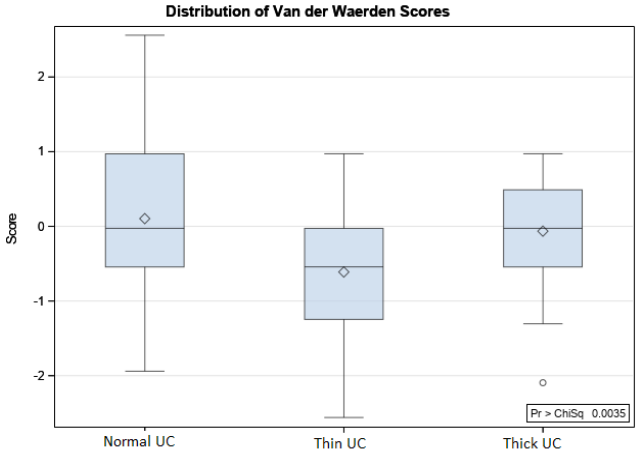

Fig. 2. Distribution of groups according to the newborn length.

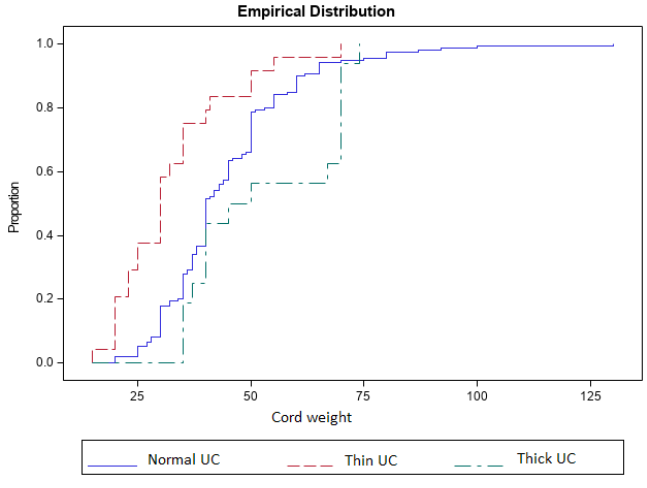

Fig. 3. Distribution of groups according to the cord weight.

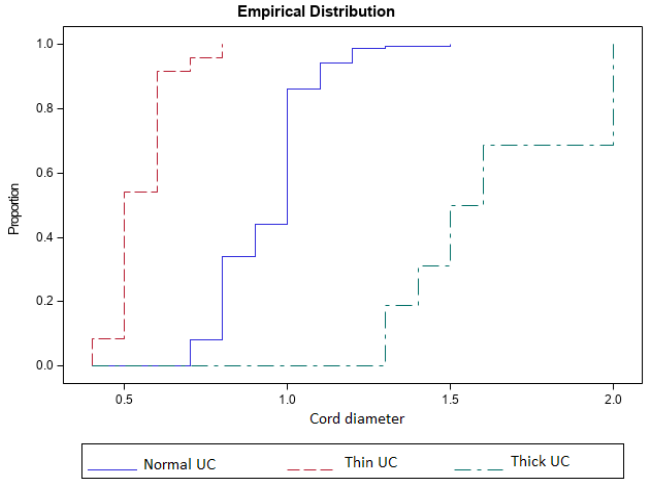

Fig. 4. Distribution of groups according to the cord diameter.

presented as infectious complications (intrauterine infection – 2 (2.53%), pneumonia – 3 (3.80%)), respiratory distress syndrome (RDS) in 5 (2.65%) cases, IUGR and anemia in 2 cases each (2.53%), metabolic disorders – 4 (5.06%), central nervous system diseases – 2 (2.53%), respiratory disorders – 10 (12.66 %). No association was found among disorders from other organs and systems.

Analysis of the morphological proprieties of the UC demonstrated that the length in the L_0 ranged between

Table 1

Maternal and perinatal factors of the lean umbilical cord

The evaluated characteristics	*χ2*	*P value*	*Cramer's V*
Primipara	9.56	0.04	0.16
Extragenital pathology:			
- ENT diseases	21.04	0.02	0.9
- diseases of the urinary system	7.10	0.02	0.2
- endocrine system diseases	37.76	0.0006	0.4
TORCH-infection (Mycoplasma)	33.55	0.006	0.3
Insufficiency of placental circulation	15.29	0.0005	0.3
IUGR	23.32	<0.0001	0.35
Fetal bradicardia	18.32	0.001	0.2
Pathological CTG	30.89	<0.0001	0.3
Fetal hypoxia	30.02	<0.0001	0.4
Pathological adaptation period	19.27	<0.0001	0.32
Neonatal morbidity	8.61	0.01	0.2
Transfer to other medical facilities	13.98	0.0009	0.3

Note: UC – umbilical cord; ENT – ear, nose, and throat; CTG – cardiotocography; IUGR – Intrauterine growth restriction.

42-69 (55.6±7.29) cm, but in the L_1 = 25-83 (53±3.37) cm ($p>0.05$) frequently with a rectilinear or slightly spiral normoform appearance and only segmentally being attested some varicose or trajectory disorders of the vascular device in cross sections. The weight of the UC varied between 20-130 (44.72±1.27) g in the control group vs 15-70 g (32.6±2.6) in the study group ($p<0.01$) (fig. 3). The diameter of the umbilical cord was ≤ 10 mm ($p<0.01$), which was lower than the 10th percentile of the normal range (fig. 4).

Histological examination in cross sections found the form disorders of the UC and structural abnormalities of the cell-matrix component of the Wharton's jelly. It showed a decrease in differentiation of the areas (perivascular, intermediate and subepithelial), being presented by a homogeneous structure, hypocellular or acellular perivascular with low or high density (fig. 5, 6).

The Van Gieson's method was used to assess the physical and chemical properties of the Wharton's jelly. It is the simplest method of differential staining of collagen and other connective tissue showed a close correlation with the umbilical vein muscles, the presence of variable density of the Wharton's connective tissue in the peripheral muscle layer without the strict differentiation of the cells and the presence of scattered myocytes (fig.7). The evaluation of the Wharton's jelly by Alcian blue (pH 2.5), which stains acid mucosubstances and acetic mucins, designated the positive features of the stroma's persistence penetration and absorption capacities. The mesenchymal mucin in the stroma of the Wharton's jelly is more pronounced compared to the vascular one (fig.8).

Thus, the macroscopic and microscopic examination in the study group attested some particularities of the thin umbilical cord such as the presence of disorders in the stromal (Wharton's jelly) and vascular components. No less significant is the presence of segmental muscle hypoplasia or vascular muscles with frequent aneurysmal deformation of the umbilical vein. The formation of capillary vessels was also detected in the peripheral region of Wharton's jelly.

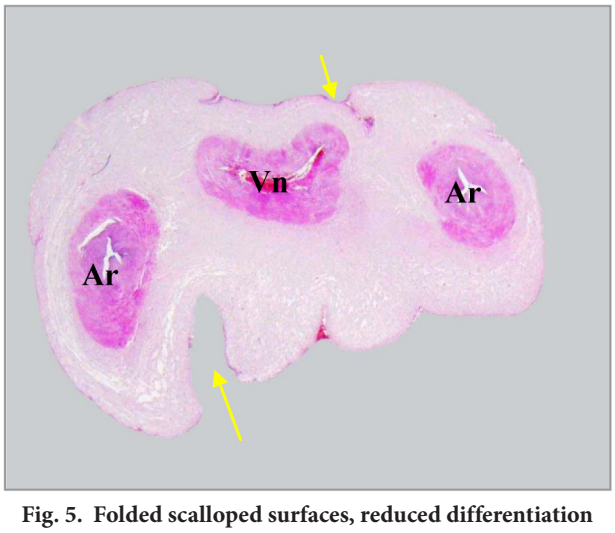

Fig. 5. Folded scalloped surfaces, reduced differentiation of the Wharton's jelly, variable density of the matrix cellular component. ×6, H&E.

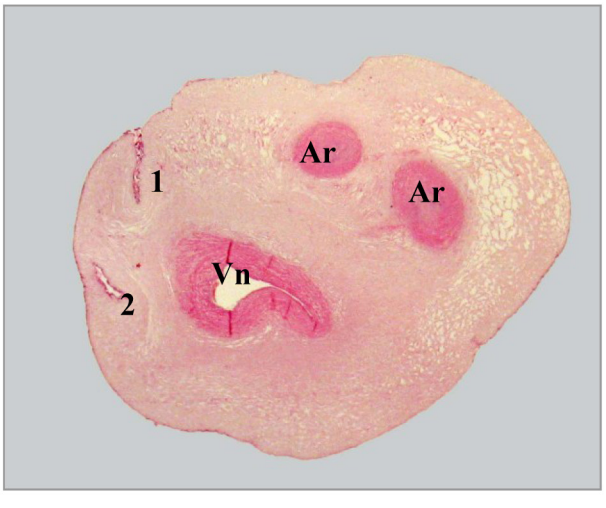

Fig. 6. Oval shape of the UC, central device with variable thickness of the Wharton's jelly, hernia of the epithelial tissue (1) in the intermediate area with formation of the cysts (2). ×6, H&E.

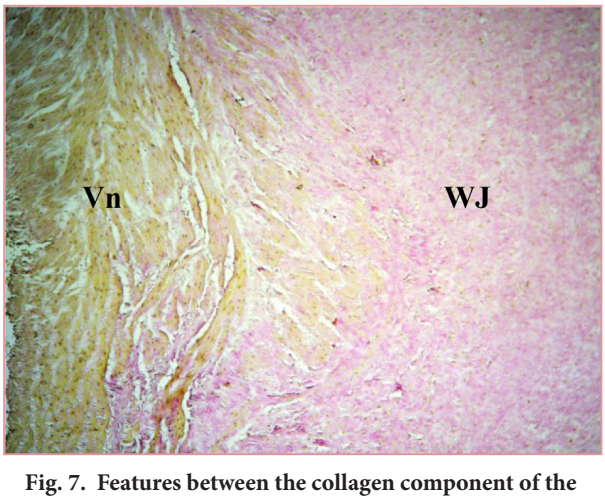

Fig. 7. Features between the collagen component of the connective tissue of the WJ and the muscle component of the umbilical vein. ×100, VG.

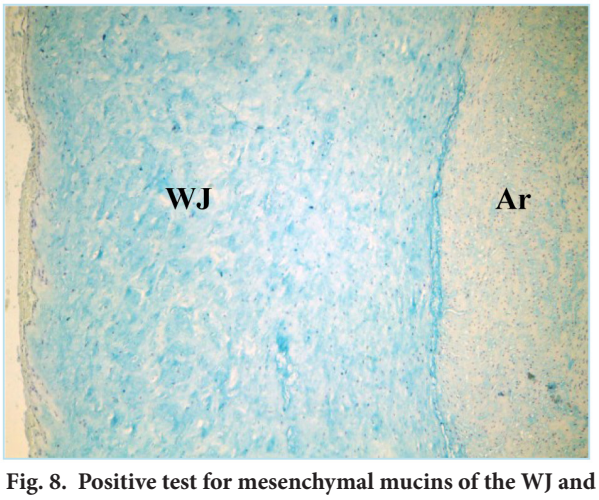

Fig. 8. Positive test for mesenchymal mucins of the WJ and vascular mucin of the umbilical artery. ×100, Alcian blue.

Discussion

Previous studies have found an association of thin cord with fetal growth restriction and other poor fetal outcomes [14,15]. Very often, a lean umbilical cord is combined with hypocoiling, which also renders umbilical cord vessels susceptible to kinking and acute obstruction. Wharton's jelly reductions have also been recognized as a possible cause of fetal death in the presence of single umbilical artery, which is the most common abnormality of the umbilical cord [16].

Proctor et al. [17] studied 497 umbilical cords of gestational ages ranging from 18 weeks to 41 weeks. They found that the umbilical cord diameter increases as the gestational age progresses until 28 weeks when it reaches a plateau at approximately 1.0 cm. These findings are in agreement with the antenatal ultrasound assessment of the umbilical cord that describes an increase in diameter with gestational age until the third trimester. Di Naro et al. [9] obtained a similar result demonstrating the sonographic umbilical cord diameter and area increase as a function of gestational age until the 32nd week of pregnancy. After that, a reduction in the diameter of the umbilical cord can be observed due to the water content of Wharton's jelly at the end of the pregnancy [9].

Using Proctor's nomogram, they identified and classified the umbilical cord diameter as thin (< 10th percentile), average (10th–90th percentile) and thick (> 90th percentile), and concluded that the umbilical cord components were responsible for the diameter variation. Their findings show that a significant increase in the vessel area (specifically an increase in the umbilical artery wall area) is responsible for a thick umbilical cord diameter, while a significant decrease in Wharton's jelly area is responsible for a thin umbilical cord diameter. Proctor et al. [17] showed that there was a relationship between the umbilical cord diameter and gross placental pathologic features. A thin umbilical cord was associated with low placental weight percentile, a single umbilical artery, and a marginal umbilical cord insertion [17]. Baergen et al. [18] demonstrated that an abnormally thin umbilical cord is associated with adverse pregnancy outcomes such as oligohydramnios, fetal growth restriction, and fetal distress.

Filiz et al. [8] investigated the relationship between the amount of Wharton's jelly and its protective role in umbilical cord vessels, and hence, in fetal growth. Their study concluded that the "quality" and characteristics of Wharton's jelly were both important in its protective role. Abnormal situations, such as a decrease in the hyaluronic acid content of Wharton's jelly and Wharton's jelly fibrosis, may affect the mechanical characteristics of the cord, which leads to impaired fetal circulation, anoxia, and fetal death.

Silver et al. [19] reported that in post-term pregnancies, the umbilical cord diameter is smaller in patients with oligohydramnios compared with normal amniotic fluid. In addition, these authors found a higher incidence of antepartum variable decelerations in patients with a small umbilical cord diameter compared with those with a normal umbilical cord. Raio et al. [20] found an association between the presence of a thin umbilical cord and the delivery of an infant who is small for its gestational age.

The possible physiopathologic mechanisms for this anomaly could be an incomplete fusion of the amniotic covering and the mesenchyme of the umbilical cord during early development, or a hypoplasia of this amniotic covering with a secondary loss of Wharton's jelly [21]. Decreased WJ area is associated with clinically-significant placental pathology and WJ area scales proportionally with placental size. These findings suggest that WJ area correlates with functional capacity of the placenta and thus merits further evaluation alongside currently-available tests of placental function in clinical practice [16].

Pathologic studies and case reports demonstrated that a thin umbilical cord is associated with oligohydramnios, fetal distress, and adverse pregnancy outcome. Careful umbilical cord examination often reveals significant lesions which may be associated with these processes. As obstetricians, we realize this when a heavy toll of fetal life is due to cord complications. Suspected fetal distress is not uncommon. Often no explanation for such intrapartum complication is apparent. Complications associated with lean umbilical cord may explain this enigma [1].

Conclusions

The present study showed that the diameter of umbilical cord is variable, but cases which had thin UC constituted abnormal cord diameter. The findings suggest that the clinico-morphological aspects of the lean umbilical cord were associated with a high risk of maternal and perinatal outcomes ($p<0.05$). The changes in the quantity or quality of Wharton's jelly affect the diameter of the umbilical cord and the hemodynamics of its vessels, leading to impaired fetal blood flow and consequently low weight gain and fetal demise. Morphometric parameters of the umbilical cord are considered as markers of fetal development disorders, and their early assessment allows to predict risk of adverse outcomes in children. In case of the thin umbilical cord, rather expressed dystrophic and degenerative changes of all the umbilical cord structures are detected. These pathomorphological changes of umbilical cord structures are supposed to play a great part in the development of fetal hypoxia. Therefore, our study attests that all umbilical cords should be submitted for complete examination.

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Authors' contributions
AA designed the trial and interpreted the data, drafted the first manuscript; VP described morphology; NC revised the manuscript. All the authors approved the final version of the manuscript.

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Ethics approval and consent to participate
The protocol of the study was approved by the Ethics Committee No 95/110 21.06.2017 of *Nicolae Testemitanu* State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova. It was obtained an informed consent from all participants in the study.

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Clinical indicators of gait freezing in Parkinson's disease

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Abstract

Background: Freezing of gait is a common (FOG) episodic gait disorder in advanced Parkinson's disease (PD). FOG is one of the main risk factors for falls; therefore FOG's consequences can be devastating. This symptom is difficult to study in a research laboratory because of its unpredictability which makes it difficult to select patients for clinical testing and make the right treatment decisions. Thus, clinical indicators may be useful to distinguish between patients with and without FOG. The aim of this study was to compare the number of steps and the time to perform a 180-degree turn in patients with Parkinson's disease with and without FOG.
Material and methods: The study was performed on 56 PD patients. Subjects were divided into 2 groups: a total of 28 patients with PD and FOG were compared with 28 patients with PD without FOG, according to item number 14, from the Unified Parkinson's Disease Rating Scale (UPDRS). The 2 subgroups were homogeneous in terms of age and disease severity.
Results: Significant differences were found between the two groups both in steps number ($P < 0.0001$) and in the time required to perform a 180-degree turn ($P < 0.0001$).
Conclusions: Performing more steps and more seconds to turn 180 degrees may be a useful indicator to distinguish the characteristics of PD patients with FOG. A PD patient which turns at 180-degree with more than 5 steps is most likely a patient with FOG.
Key words: Parkinson's disease, gait, freezing, turning, steps.

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Introduction

Freezing of gait (FOG) is a common episodic gait disorder in advanced Parkinson's disease (PD). It is defined as a "brief, episodic absence or marked reduction of forwarding progression of the feet despite having the intention to walk" [1] and the patients often describe this symptom as like the feet are glued to the floor. Approximately 50% of PD patients experience FOG at least twice a month and in the more advanced stages 80% of the patients suffer from this symptom [2]. Because 26% of falls have been shown to be related to FOG [3], it has a significant impact on the quality of life of PD patients [4].

FOG's prevalence depends on disease duration [2, 4, 5], disease stage [2, 4] and the dose of administered levodopa [4]. The pathological mechanisms are not completely comprehended [6], but several FOG hypotheses were attributed like abnormal sequencing of steps, axial akinesia, asymmetric symptom severity [7] and frontostriatal disruption [8, 9].

One challenge that adds considerable complexity to FOG research is the fact that this phenomenon is hard to study in a research laboratory because of its unpredictability. FOG was shown to occur most often during turning [10]. While turning difficulties tend to be associated with FOG, there were only limited studies to describe these difficulties. In a previous study patients experiencing FOG were found to have a higher rotational arch than patients without FOG [11], patients with FOG only get 75% of the necessary turning angle and need more time to complete the turn [12]. There are several studies showing that PD patients perform a greater number of turning steps compared to control group subjects [13, 14]. The current study hypothesizes that a greater number of steps and the longer time required to complete a 180-degree turn in patients with Parkinson's disease is an indicator of FOG, even if the patient does not have a FOG episode at the time of turn.

Material and methods

Fifty-six patients diagnosed with PD were included in the study. The patients were recruited in the Movement Disorders Clinic of the University Hospital of Kiel (Germany). The current research is a part of a larger project for research on the phenomenon of FOG in patients with PD that was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (No 44 / 53 of 12.04.2018). A special authorization for the use of patients' data for scientific and educational purposes has been signed by all patients in accordance with German law.

The inclusion criteria were: (1) PD diagnosed according to Brain Bank criteria, (2) the ability to walk independently

and perform a 180-degree turn in the OFF phase of the disease. Exclusion criteria: (1) comorbidities that could influence gait, (2) the presence of cognitive impairment, measured by mini-mental examination (MMSE <24 points). An equal number of patients was recruited with and without FOG, according to item No 14 of the Parkinson's Disease Rating Scale (UPDRS). Patients with MI were considered those who had item No 14> 0. The severity of the disease was determined according to the UPDRS scale, part III. Homogeneous patients were selected in terms of age and disease severity.

The protocol requires subjects to stand up from the chair, walk 8 m towards examiner, turn 180 degrees, and return to the chair. The number of steps and the time required for turning were calculated for each patient by 2 blind raters, who did not know the patient's status (with/without FOG). All patients were examined in the OFF phase, with levodopa being stopped for 12 hours and dopaminergic agonists for 72 hours. Patients who experienced a FOG episode during turning were excluded.

Statistical analysis was performed using SPSS, version 23.0. Data are expressed by mean and standard deviation in the case of normal distribution or median and range values for variables with non-normal distribution. The differences between the two groups of patients were analyzed with the t-test or Mann-Whitney test, as appropriate. In all analyses, p values <0.05 were considered significant.

Results

A total of 56 patients were included in the study, divided into 2 groups, 28 patients with PD and FOG were matched for age, disease severity and compared with 28 patients with PD without FOG, according to item number 14, from the Unified Parkinson's Disease Rating Scale. The demographic data and characteristics of the patients included in the study are presented in Table 1. The number of steps performed during 180 degrees turn, as well as the time required to perform the rotation are statistically significantly higher in patients with FOG than those without FOG (p = 0.0001 for both measurements).

Assessing the number of steps performed by patients with and without FOG, we determined that in the patients which have FOG median is 5 and those without FOG the median is 3 steps. We also noticed that patients without FOG have performed a maximum of 5 steps. We may therefore infer that if a PD patient performs more than 5 steps at 180 degrees turn, it is most likely a patient with FOG (fig. 1).

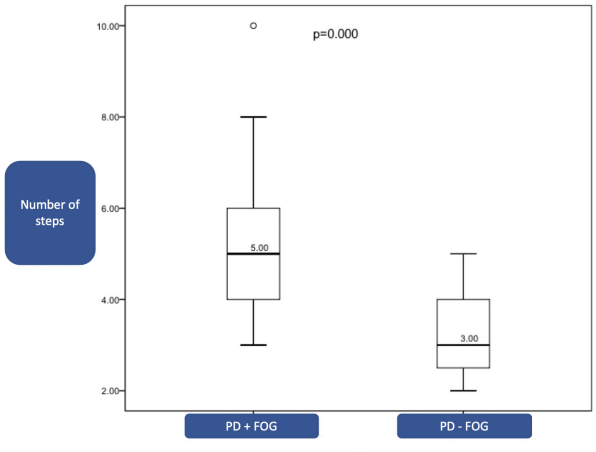

Fig. 1. The number of steps performed during the 180-degree turning in patients with PD), with and without FOG.

Also, there is a tendency to increase the number of steps with increasing FOG severity (fig. 2). As there were no patients with grade 4 severity and only 3 patients with grade 3 in the group of patients, this is not applicable for the group of most affected patients.

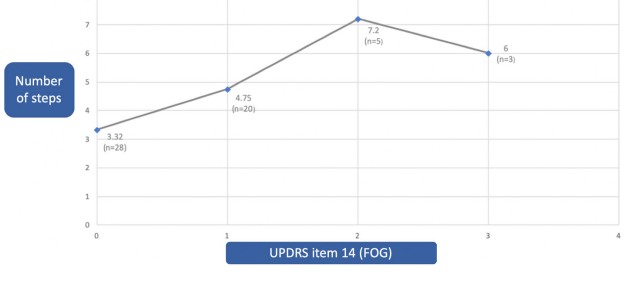

Fig. 2. Number of steps performed during the 180-degree rotation in patients with PD relative to the FOG severity according to item 14 of the UPDRS scale.

Table 1

PD patients with and without FOG

Variables	PD + FOG (n=28)	PD – FOG (n=28)	P-value
Age, years (mean ± SD)	59.2 ± 7.1	60.5 ± 6.5	0.5
UPDRS III (mean ± SD)	40.1 ± 12.1	33.7 ± 8.4	0.2
Number of steps performed during turning (median, min-max)	5 (3 - 10)	3 (2 - 5)	**0.0001**[a]
Time required to complete turning, seconds (mean ± SD)	5.2 ± 1.4	3.1 ± 1.2	**0.0001**[b]

PD – Parkinson's disease, FOG – freezing of gait, SD – standard deviation, UPDRS – Unified Parkinson's Disease Rating Scale. [a] – Mann-Whitney test. [b] – T independent test.

Discussion

This study is an attempt to identify clinical indicators of gait freezing in patients with Parkinson's disease. Finding the number of steps performed while turning 180 degrees and the time required for this maneuver proved being an excellent way to identify FOG patients.

Although there are studies that report the turning difficulty in patients with PD [12, 13, 15], to our knowledge this is the first study to confirm that this deficit is associated with FOG. Patients that experience FOG are likely to have problems with many daily activities and should therefore benefit from rehabilitation. FOG evaluation is not trivial, it is a variable symptom and difficult to provoke in clinical conditions [16]. Patients or relatives may confuse FOG with

other symptoms, such as steps shortening present in PD or falls in atypical forms of Parkinsonism like progressive supranuclear palsy. It is simple when this sign is seen in the clinic, however it does not occur during a regular examination in around half of the patients [6]. For this reason, a simple test such as the patient's 180-degree rotation, which can be easily done in the clinic that can help identify FOG, is definitely a good one. We have shown that greater number of steps and more time to turn 180 degrees is distinctive for PD patients with FOG. Therefore, we conclude that a patient with PD which performs the 180-degree rotation maneuver with more than 5 steps is probably a patient with FOG, even if he does not have an episode of FOG at the moment of examination. Though we cannot tell the opposite according to our data, a patient who performs less than 3 steps may still be a FOG patient.

Another interesting result is the severity of MS and the greater number of steps. According to item No 14 (FOG) of the UPDRS scale, the severity of the FOG is graded as: (0) FOG missing, (1) blocks rarely occur during walking, pause can occur at the beginning of walking, (2) occasional blockages while walking, (3) frequent FOG episodes, often block-related falls, (4) frequent FOG falls. The results of the current study show that the higher the number of steps, the more severe the FOG could be.

Conclusions

The present study has shown that it may be useful to distinguish the characteristic of PD patients with FOG from that of PD patients without FOG by the number of steps and the time used to perform the 180-degree turn.

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Authors' contribution

OG drafted the first manuscript; AA collected the data; MG designed the trial and revised the manuscript critically. All the authors revised and approved the final version of the manuscript.

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Ethics approval and consent to participate

The research protocol No 44 of April 12, 2018 was approved by the Research Ethic Board of *Nicolae Testemitanu* State University of Medicine and Pharmacy.

Conflict of Interests

The authors have no conflicts of interests to declare.

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Contemporary diagnosis of rhinosinusitis in children

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Abstract

Background: Inflammatory disease of the paranasal sinuses, due to its high incidence in the nosological structure of morbidity, is a constant problem of modern otorhinolaryngology. The purpose of this study was to assess the importance of modern investigative methods for establishing the diagnosis in children with inflammatory rhinosinusitis.
Material and methods: The study was conducted on a group of 55 children, aged 4 to 18 years, diagnosed with inflammatory rhinosinusitis, hospitalized during the 2017-2020 years within ENT Department of *Emilian Cotaga* Republican Clinical Hospital for Children, Chisinau. The retrospective analysis of the data, retrieved from the clinical observation sheets and their attached documents, as well as from inpatient surgical protocols, during the years 2017-2020, was carried out.
Results: The applied diagnostic methods were as following: optical endoscopy, rhinomanometry, acoustic rhinometry, mucociliary clearance, computed tomography.
Conclusions: The study revealed that computed tomography and optical endoscopy provide an accurate mapping of rhinosinusal structures. Nowadays, inflammatory rhinosinusitis is definitely diagnosed by highly accurate methods, namely the optical endoscopy and computed tomography that are "the gold standard". The functional tests such as rhinomanometry and acoustic rhinometry are used to assess permeability and endonasal geometry, as well as evaluation of respiratory function in children with rhinosinusitis. The mucociliary nasal clearance plays a major role in modern diagnostic algorithm, being an essential index in assessing the functional status of the nasal mucosa and performing the protective function.
Key words: paranasal sinus, rhinosinusitis, children, diagnosis, investigation.

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Introduction

The inflammatory disease of the paranasal sinuses, due to its high incidence of the nosological structure of morbidity, severe complications and adverse consequences on the child's health, has always been a relevant issue in modern otorhinolaryngology [1].

The inflammatory diseases of the paranasal sinuses is a major health problem in childhood, showing an incidence, ranging from 18-30% to 38-42%, which has been increasing by 1.5%-2% per year [2-4]. According to American researchers regarding the incidence of rhinosinusitis, it makes up 4.6% of all visits to general practitioner [1, 5, 6]. According to other scientists, sinusitis leads to complications in 5% cases of upper respiratory tract infections among children [7]. The data provided by ENT departments of healthcare institutions revealed that patients suffering from diseases of paranasal sinuses make up the majority in 62% of cases [5].

Pathogenesis and early diagnosis have been multicenterally studied by ENT specialists [8]. There are studies describing the importance of both anatomical and physiological features of the nose and paranasal sinuses in the pathogenesis of rhinosinusitis [3, 9-11]. The pathogenesis of recurrent and chronic sinusitis in modern relevant literature is termed as "cooperation" between the infection and the predisposing factors [12-14]. The methods of diagnosing rhinosinusitis in children have changed considerably in the last 10 years, due to the development and widespread implementation of functional diagnostic methods (acoustic rhinometry, rhinomanometry),as well as by diagnostic nasal endoscopy and CT imaging [9, 15-17].

One of the major symptoms in patients with rhinosinusitis is the nasal obstruction, which is actually a subjective parameter. An objective nose breathing assessment, to diagnose the inflammatory disease of paranasal sinuses, is currently being required by both practitioners and scientific researchers [18-20]. The emerging technologies have made it possible to investigate the physiological processes occurring within the nasal cavity, particularly the nasal breathing, as well as bring more objective evidence to these changes [15, 21, 22]. Currently, the assessment of nasal resistance via anterior active rhinomanometry has been increasingly applied in scientific research, thus allowing collecting data that are more detailed on the nasal structure [23, 24].

Both modern endoscopy techniques and complex imaging investigations, such as CT scan provide a framework for innovative research, in order to fully assess the impact of specific areas in the development and maintenance of chronic rhinosinusitis [25-27]. The endoscopic examina-

tions performed by flexible fibroscope or rigid endoscopes enable to identify specific blocked sinus areas, as well as to determine anatomical variations and bone abnormalities, which are highly important in the pathogenesis and development of the disease [1, 28].

The computed tomography of the coronary and axial sections provides a detailed examination of the abnormalities and anatomical variations of the lateral wall of the nasal fossa, as well as an objective assessment of recurrent or chronic sinusitis. CT provides a precise map of the paranasal sinuses, thus ensuring a good study of the normal anatomy of the nasal cavities and an accurate description of dangerous relationships, which might further lead to a more beneficial, exact and limited therapeutic approach [25, 28].

The anterior rhinomanometry provides a dynamic study of the nasal ventilation function, which includes the assessment of the nasal airway resistance by measuring total airflow volume and total resistance [16, 24].

Rhinomanometry also gives objective and quantitative evidences on nasal permeability that depends upon two parameters: differential pressure (Δp), flow (V). The differential pressure (Δp) is the pressure difference between the atmospheric pressure measured in nasal vestibules using a mask and the inspiratory and expiratory pressure assessed in choanae. The respiratory flow (V) refers to the airflow volume passing through the nostrils [15, 17, 18]. Anterior rhinomanometry is a method of choice. It confirms the nasal obstruction by providing objective data to the patient's subjective symptoms. Taking into account the etiological diagnosis, rhinomanometry provides detailed data upon the mechanism of nasal obstruction from an anatomical point of view, thus having an essential role in assessing the conservative or surgical treatment. Rhinomanometry justifies the importance of mucociliary clearance of the sinus cavities and is of secondary forensic relevance [15, 23].

Acoustic rhinometry is a relatively new method, based on the analysis of sound waves reflected from the nasal cavity walls, aimed to assess the lumen size of the nasal fossae, as well as the sites of a reduced airflow, thus allowing assessing the correlation between minimal cross-sectional area (MCA) of the nasal cavities and their anteroposterior dimension.

The acoustic rhinometry shows a clinical importance due to its ability to measure the dimensions of the nasal cavity, as well as to evaluate the nasal permeability in terms of a curve [16].

Normal mucociliary clearance is essential for the maintenance of a healthy sinus cavity. The mucociliary transport of the ciliated epithelium is an important defense mechanism of the nasal cavity that helps in removing foreign particles from the nasal cavity during inspiration. An impaired mucociliary clearance leads to stasis of sinonasal secretions on the pituitary surface and a decrease in sinus aeration, which leads to the development and maintainance of rhinosinusitis [15, 29].

Material and methods

The study was conducted on a group of 55 children, aged between 4-18 years old, diagnosed with inflammatory rhinosinusitis (acute, recurrent and chronic rhinosinusitis), hospitalized within the ENT department at *Emilian Cotaga* Republican Clinical Hospital for Children, Chisinau, during the years 2017-2020. The retrospective analysis of data, retrieved from the clinical observation sheets and their attached documents, as well as from inpatient surgical protocols, during the years 2017-2020, was carried out. The inclusion criteria were as following: the study of anamnesis, of general clinical and ENT investigations, CBC, nasal endoscopy, rhinomanometry, acoustic rhinometry, mucociliary clearance, paranasal sinus radiography, and CT scan.

Data analysis and processing was performed by using the Microsoft Office Excel 2016 program.

Nasal endoscopy has become an integral aspect of the diagnostic algorithm for rhinosinsitis. The study group included 44 children, who underwent endoscopic examination, which made up 80% of cases. Optical endoscopy was carried out after a proper nasal cavity toilette. While considering the children's age, their neuropsychic and behavioral patterns, as well as the increased reflexology of the pituitary, the diagnostic endoscopy was commonly performed under general anesthesia, which was applied immediately before the procedure. The anesthetic, used to numb the nasal pituitary, was performed 15-20 minutes before the patients were transported to the operating room, in order to "skeletalize" the lateral wall of the nostrils, which is a key moment in endoscopic examination.

Pediatric patients, who were more cooperative, underwent endoscopy under local anesthesia with 10% Lidocaine spray, following decongestion of the nasal mucosa by applying vasoconstritive gauze packing (as Naftizine 0.05% or Xylometazoline 0.05%) on the surface of the nasal mucosa. In these cases, the examination was performed in a sitting position of the patient with the head in slight deflection position. The examination was performed 4 times consecutively, which provided a systematic visualization of the nasal fossae and their lateral wall. The nasal endoscopy used Karl Storz set, which included a 4 mm rigid endoscope with a 0.30 and 70-degree view angle, a 2.7 mm rigid endoscope with a 30-degree view angle and a flexible endoscope, thus ensuring an accurate visualization of the internal surface of the nasal fossae.

ATMOS PC 2000 (Germany) guided by a simple standard menu, has a built-in printer and display. Rhinomanometry was performed in 60% of cases, viz. 33 children. The examination results were displayed on the screen of the rhinomanometer, as a Y / t graph or a rhinogram, as shown in fig. 1. The rhinomanometer was used to assess nasal permeability.

The difference in pressure between the choanae and inside the mask was measured, the values being converted into electrical signals via a differential pressure converter. The microprocessors further carry out the electrical signals

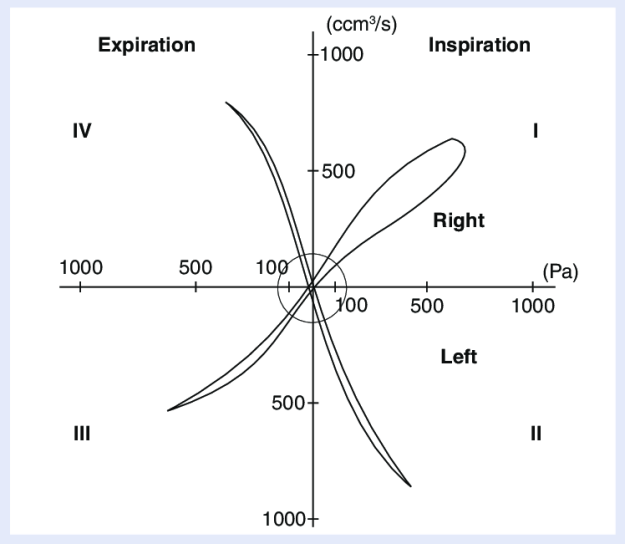

Fig. 1. Graphical representation of the RMM exam.

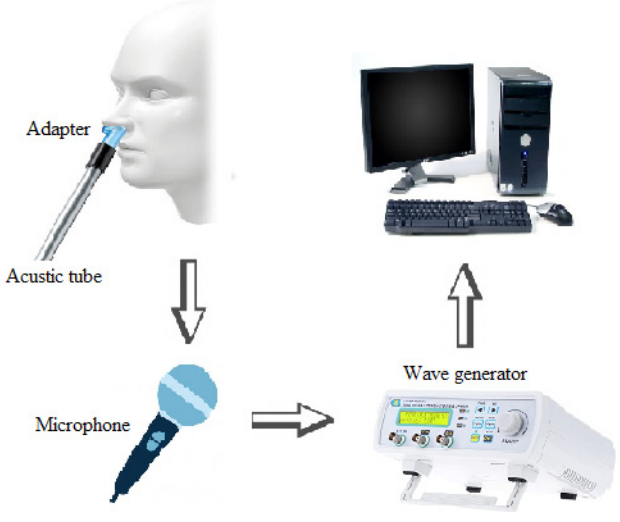

Fig. 2. Diagram of acoustic rhinometry devices.

processing. The concomitant measurement of the differential pressure and flow result in a flow-pressure curve, namely the nasal resistance curve. The examination was performed in a special room isolated from external noise, under constant temperature and humidity, the air temperature being 20-22°C. The patients spent about 20 minutes in the room in order to accommodate. The investigation was carried out in child's sitting position.

The device was mandatorily calibtared, prior to choosing the mask, the adapter depending on the nostril size and further being connected to the rhinomanometer tube. This technique uses a tube for measuring the choanae pressure that was fixed tightly to the nostril using a sponge nasal dressing. It is important to connect the mask, adapter and nostril airtight in order to avoid sound loss. The patient was examined during the apnea phase, at short intervals, as not to distort the obtained results by the changes in the pituitary mucosa caused by the nasal cycle. No rigid compression of the nostril was applied, as to prevent deformation of the nasal vestibule and changes in study results. The sound generator was turned on and the examination was carried out twice for each nostril. The investigation lasted about 3-4 minutes. A display of the mean curve for each nostril was shown on the rhinomanometer screen.

GM instrument 2000 was used to perform the acoustic rhinometry and study nasal cavity geometry. Computer assisted data analysis was carried out. The equipment includes an acoustic pulse generator with a frequency of 150 to 10000 Hz, an acoustic tube of 15 mm diameter and 580 mm long, a nasal adapter to connect the acoustic tube and the nostril, a microphone and computer (fig. 2).

30 children, namely 55% of cases underwent testing. They were tested under constant humidity and temperature conditions, whereas the noise did not exceed 60 dB. Children were assessed in the sitting position. The acoustic tube was at a 45-degree angle relative to the nostril. The patient spent about 20 min. to accommodate to the room environment prior to being tested. Each anatomical adapter was selected individually depending on the nostril size. The device was calibrated before the assessment. The child was instructed to hold his breath during the test. Display of the mean curve for each nostril was shown on the rhinometer screen. The study result was considered true, if the coefficient of variation of the curves did not exceed 1.15%.

The minimum cross-sectional (MCS) area and distance (D) at which these constrictions were located, and the nasal cavity volume (V) were studied. All data were processed on a computer and printed.

The mucociliary clearance was assessed in children from the main group via the saccharin test in 75% – 41 children. The standard procedure of this test is to place saccharin powder in the inferior nasal concha (saccharin can be colored blue), until the patient feels the taste of saccharin as time passes. The patient's cooperation is required, since he/she should report the sweet taste. It is forbidden for the patient to sneeze and blow the nose, as these might affect the position of the particles. Patients were instructed to swallow at least once per minute. It could be checked by blue staining of the pharynx. This method shows a mean mucociliary transport time (MTT) of about 10 minutes, though MTT up to 30 min is still considered a normal one.

CT examination was performed in 30 children – 50% of cases. It is essential in determining damages of the sinus structures, which are quite difficult to diagnose at rhinoscopy and endoscopy , as well as at X-ray. CT has a high diagnostic value for detecting impairment of the ostiomeatal complex and of the ethmoidal, sphenoidal sinus, which is impossible to assess via simple X-ray examination. The imaging study was carried out on axial and coronal planes, on a Siemens Somatom Emotion Duo computer tomography system. The fineness of the nasal bony structures and the paranasal sinuses require accurate and high-resolution cups. The CT assessment is essential for a precise study of sinus opacity and sinus wall integrity (normal, thin, and densified). In case of pediatric rhinosinusitis, CT imaging might reveal factors contributing to or maintaining the inflamma-

tory response of the rhinosinusal mucosa such as various abnormalities and anatomical endonasal variations: septal deviations at different segments, concha bullosa, paradoxically curved cornett and various deviations, excessive pneumatization of ethmoid bulla, etc. [22].

Results and discussion

Nasal endoscopy

The endoscopic examination, performed in children from the study group, was mostly aimed to examine the middle nasal meatus by visualizing pathognomonic changes of rhinosinusitis, thus mucosal edema was found in 90.9% of cases, congestion – in 84.09% of cases, presence of mucopurulent secretions – in 95.45% of cases. Another parameter to be monitored in patients with chronic rhinosinusitis was the presence of adenoid vegetation, found in 10 children, viz. 22.73% of cases.

Endoscopy enables to assess the presence of anatomical variations within the nasal fossae, which exhibit a pathogenic role in chronic rhinosinus: nasal septum deviation was recorded in 50% of patients, concha bullosa – 25%, paradoxically curved cornett / concha – 20.45%. The data are shown in fig. 3.

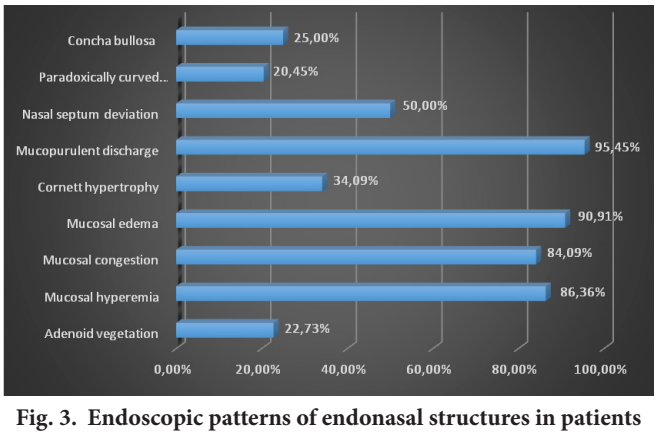

Fig. 3. Endoscopic patterns of endonasal structures in patients from the study group.

CT scan

CT scan revealed the following paranasal sinus impairment: edema of the sinus mucosa was found in 26 cases (86.67%), hydro-aerial level – in 14 cases (46.67%), opacification of ethmoid cells – in 14 cases (46.67%).

The study and analysis of computed tomography images was mainly aimed at detection and describing septal deviations in children from the study groups, since it may block the structures of the ostiomeatal complex, thus contributing to the inflammatory response. CT can determine the particular deviation site and its patterns (deformation, thickening or pneumatization of the nasal septum). The results of the CT scan showed a frequent deviation of the nasal septum in children from the study groups with an incidence of 22 cases (73.33%). The studies on the physiology and pathophysiology of the nose and paranasal sinuses regarding the occurrence and subsequent development of the chronic inflammatory process of the paranasal sinuses pay special attention to the anatomical factor, namely the presence of abnormalities and anatomical variations, particularly those that occur in the ostiomeatal complex. Thus, concha bullosa, was found in 9 (30%) cases, paradoxically curved middle cornett – in 7 (23.33%) cases, Haller cells – in 5 (16.67%), pneumatization of the ethmoid bulla – in 15 (50%) and accessory maxillary sinus ostium – in 3 (10.00%) cases. The data are shown in fig. 4.

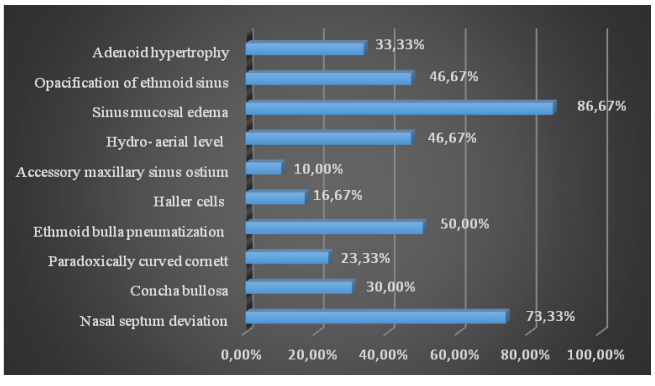

Fig. 4. Structural changes and variations of endonasal anatomy in patients from the study group.

Rhinomanometry

An objective study of nasal resistance revealed signs of nasal congestion in children from the main group. According to specialized literature, the indices of nasal structures in healthy children are as following: total volume at 150 Pa – 564 cm / sec. and total resistance at 150 Pa – 0.37 Pa / cm^3 / sec. The study revealed mild nasal obstruction in 5 (15.15%) cases, moderate obstruction in 19 (57.58%) cases and severe obstruction was reported in 9 (27.27%) cases. Data analysis of Figure 5 shows that children with chronic and recurrent rhinosinusitis from the study groups exhibited a reduced total volume of the nasal fossa, compared to the normal ranges. There is an increase of total resistance at 150 Pa in the main group, compared to the control one.

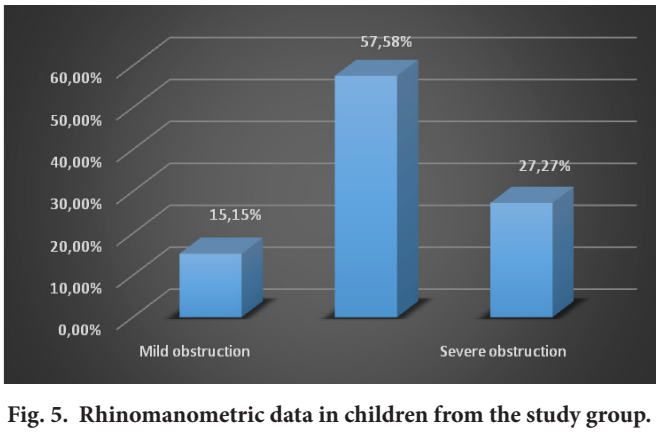

Fig. 5. Rhinomanometric data in children from the study group.

Nasal permeability was studied by acoustic rhinometry. The obtained data revealed that patients from the study group exhibited changes in the endonasal geometry, characterized by a decrease of the nasal fossae volume and a re-

duced minimum cross-sectional area. The results are shown in tab. 1.

Table 1

Acoustic rhinometric data in patients from the study group

Indices	Study group	Normal ranges
V (cm³)	2.76	5.7
A1(cm²)	0.382	0.790
D1 (cm)	1.29	0.940
A2(cm²)	0.420	0.780
D2 (cm)	2.81	2.68

Mucociliary clearance

The mucociliary clearance of the children included within the study was assessed by saccharin test. The normal mucociliary transport time (MTT) is about 10 min. MTT up to 30 min is also considered normal. The test results showed a mucociliary clearance time of ≤30 min in 6 (14.63%) patients, of 31-60 min – in 23 (56.10%) patients and > 60 min – in 12 (29.27%) patients. The results are shown in fig. 6.

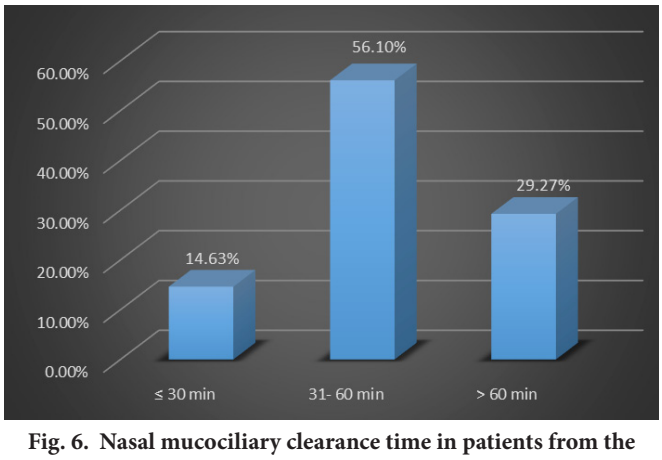

Fig. 6. Nasal mucociliary clearance time in patients from the study group.

Conclusions

1. Optical endoscopy provides a comprehensive and atraumatic assessment of the paranasal sinus structures and ostiomeatal complex, as well as helps to determine the anatomical variations that show a major impact in the development and maintenance of chronic rhinosinusitis.

2. CT scan enables an accurate visualization of the ostiomeatal complex, which is responsible for maintaining the recurrent and chronic rhinosinusitis, thus being considered important in surgical treatment.

3. Rhinomanometry provides objective evidence in studying nasal permeability and can be used to assess the total volume and resistance in patients with inflammatory rhinosinusitis.

4. Acoustic rhinometry opens up new perspectives and opportunities in the study of nasal passages and their pathological changes. Its non-invasive features, simplicity and speed are particularly important in pediatric practice.

5. The mucociliary clearance test is an essential diagnostic tool in assessing the functional status of the pituitary nasal mucosa and performing the protective function.

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Authors' contributions
MT designed the trial and drafted the first manuscript; MM interpreted the data and revised the manuscript critically. Both authors revised and approved the final version of the manuscript.

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Ethics approval and consent to participate
The study was approved by Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (protocol No 5 of April 15, 2020). It was obtained an informed consent from all participants in the study.

Conflict of Interests
No competing interests were disclosed.

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Obtaining the Wild bergamot essential oil with high content of thymoquinone

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Abstract

Background: The Wild bergamot (*Monarda fistulosa* L.) is mentioned among plants with high content of thymol and carvacrol in the composition of essential oil. At the same time, it also produces significant amounts of thymoquinone – a substance with antimycotic, anticancerous and antituberculous activity. The objective of this study was to evaluate the factors which affect the thymoquinone formation during the processing of plant material, and to create a technological procedure for obtaining the Wild bergamot essential oil with maximum content of this active compound.
Material and methods: Aerial parts of the Wild bergamot have been harvested in the budding-flowering phase from the collection of the Scientific Centre for Medicinal Plants Cultivation of *Nicolae Testemitanu* State Uiversity of Medicine and Pharmacy. Essential oil isolation has been performed by hydrodistillation. Analysis of plant material and the obtained samples of essential oil have been performed by high performance liquid chromatography method with diode-array UV detection.
Results: The content of thymoquinone increases considerably during the process of fermentation of humidified plant material in the air stream prior to the essential oil distillation. Additionally, the content of thymoquinone in the essential oil can be increased by performing the distillation with a mixture of steam and air.
Conclusions: A technological procedure was created, which allowed obtaining the Wild bergamot essential oil, that contains 20-32% of thymoquinone and 23-32% of thymol and carvacrol, with a yield of 12.5-14.5 g from 1 kg of dried plant material.
Key words: *Monarda fistulosa* L., essential oil, thymoquinone, hydrodistillation.

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Introduction

Bacterial resistance to the majority of traditionally used antibiotics requires searching for new products with antimicrobial action [1, 2]. One of the directions of this searching are aromatic plants, which have a broad spectrum of actions, including antimicrobial ones [3, 4]. The genus *Monarda*, family *Lamiaceae*, is mentioned among these plants. Their species have been introduced into culture in many countries [5, 6], including the Republic of Moldova [7]. Phyto-chemical studies of *Monarda fistulosa* L. and *Monarda didyma* L. indicate the presence, in the essential oil, of high amounts of phenolic compounds (thymol and carvacrol) – up to 60-70% [8, 9].

Some sources indicate that the Wild bergamot essential oil also contains thymoquinone and thymohydroquinone [10, 11]. Their antimycotic, anticancerous and antituberculous actions were demonstrated both *in vitro* and *in vivo* [12-15].

However, according to our data, the thymoquinone content in the Wild bergamot essential oil, obtained by the traditional methods, is relatively low (not more then 3-4%). This depends on plant material drying and storage conditions, as well as technological parameters of the essential oil isolation process. Prior studies have described a method for intensification of the thymoquinone accumulation, based on some agrotechnical procedures [16]. However, we could not find any indications about the possibility to increase thymoquinone formation after the plant material harvesting. As a consequence, the objective of this study was to evaluate the factors which affect the thymoquinone formation during the processing of plant material, and to create a technological procedure for obtaining the Wild bergamot essential oil with maximum content of this active compound.

Material and methods

Plant material. Aerial parts of the Wild bergamot have been harvested in the budding-flowering phase from the collection of the Scientific Centre for Medicinal Plants Cultivation of *Nicolae Testemitanu* State University of Medicine and Pharmacy.

Apparatus. Essential oil isolation has been performed by hydrodistillation, using a laboratory-made installation, as-

sembled from peristaltic pump with adjustable speed of 0.5-1.0 L/h, flow evaporator with maximal electric power of 0.35 kW, cylindrical extractor with internal volume of 0.5 L, 2.8 L or 4.5 L and flow cooler. Analysis of plant material, as well as of the obtained samples of the Wild bergamot essential oil has been performed using *Agilent 1260* liquid chromatograph with diode-array UV detector. The following chromatographic conditions were used: analytical column *Zorbax Eclipse Plus C18*, 3.5 mm, 4.6 x 100 mm at 30°C; mobile phase: acetonitrile – 0.1% acetic acid solution (50:50), debit 1.2 mL/min; detection at 256 nm (thymoquinone) and 276 nm (thymol, carvacrol and thymohydroquinone).

Chemicals. In the study have been used reference substances: Thymol, Carvacrol and Thymoquinone (*Sigma-Aldrich*). Thymohydroquinone was obtained *in situ* as a product of thymoquinone reduction by ascorbic acid. Other reagents of analytical grade have been purchased from *Sigma-Aldrich Chemie GmbH* and *Merck* (Germany).

Results and discussion

As a "starting point", we have obtained the essential oil from fresh herb of Wild bergamot, using the traditional method of hydrodistillation. Ideally, the time interval from plant material harvesting and the essential oil distillation should be as short as possible, because the enzymatic oxidation of volatile phenols takes place at the storage of collected aerial parts. From each kilogram of plant material, loaded into the extractor of 4.5 L volume, we have obtained 6-7 g of essential oil. This product contains 40-60% of phenolic compounds (sum of thymol and carvacrol) and not more than 0.3% of thymoquinone. At storage the thymoquinone content decreases to about 0.05%. If initial value is overstated due to notable degree of fermentation, may appear an inconsiderable crystalline precipitate. This needs to be separated by decanting and filtration.

Obtained product presents an oily liquid of light-yellow to yellow colour, with a strong odour and spicy taste, specific for the Wild bergamot. It is soluble in 3-18 parts of 70% ethanol. Relative density is 0.909 to 0.929, refraction index – 1.490 to 1.505, dry residue – not more than 1%.

At drying of the Wild bergamot herb a significant part of thymol and carvacrol transforms into thymoquinone. It is due to high activity of oxidative enzymes. But at the essential oil distillation from dried plant material, a major part of thymoquinone reduces to thymohydroqiunone by different reducing substances present in the raw material. As a result, the thymoquinone content in essential oil, obtained from dried plant material, usually does not exceed 3-4% and continues to decrease during storage, sometimes practically to disappearance. Simultaneously, a considerable amount of thymohydroquinone forms as a crystalline precipitate.

To increase the thymoquinone yield at obtaining of the Wild bergamot essential oil, we have introduced two modifications into technological procedure:

1. *Additional fermentation of plant material prior to distillation.* With the purpose to reactivate enzymes, the dried plant material was humectated with water, then exposed to air fermentation. This process may be carried out on opened trays with periodical mixing, but more technological is to perform the fermentation with a continuous air stream, blowing through the humidified plant material, loaded into the distillation extractor. The main advantage of this approach is the use of one apparatus at two technological stages without reloading the plant material. In this phase of the study were optimized such parameters as amount of water, debit of air, temperature and duration of fermentation.

2. *Distillation of the essential oil with a mixture of air and steam.* This idea is based on the fact that oxygen protects the thymoquinone from reduction during the distillation. The air was pumped together with water into the mixer, connected to the inlet of the flow evaporator (fig. 1). The volume of the extractor was decreased to 2.8 L, because it was found, that high duration of the distillation process leads to decreasing of the thymoquinone yield due to its reduction to thymohydroquinone.

This approach has allowed obtaining the essential oil with thymoquinone content of 20-32% vs. 9-17% at steam distillation without air adding. We have obtained samples of essential oil with high thymoquinone content (up to 38%), using an extractor of small volume (0.5 L). This content was not depending significantly on the presence of air in steam, due to fast increasing of temperature in the extractor and short time of distillation process. However, both small amounts of raw material used (about 75 g) and essential oil obtained (1.1-1.5 g) in each load, have made this variant of low efficiency.

As a result of the study, the following laboratory procedure has been elaborated to obtain the Wild bergamot essential oil with high content of thymoquinone:

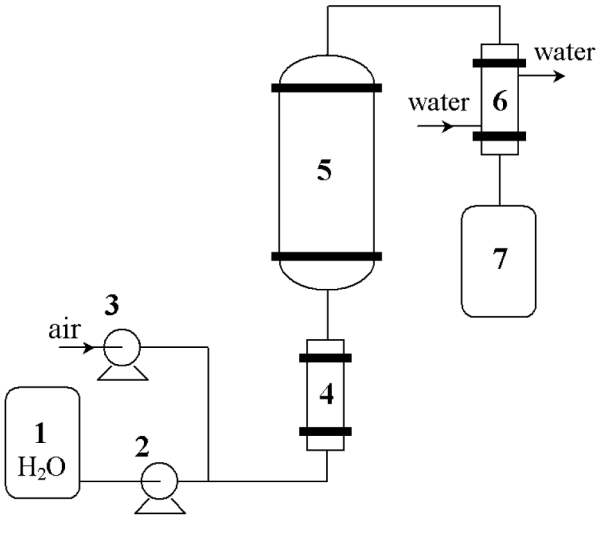

Fig. 1. Scheme of the laboratory installation for isolation of essential oil.
1 – feeding vial with water, 2 – water pump, 3 – air pump, 4 – electric flow evaporator, 5 – extractor with plant material, 6 – flow cooler, 7 – recipient for condensate.

Humectate 0.35 kg of the Wild bergamot herb, dried and fragmented, with 0.7 L purified water and mix thoroughly then load into a cylindrical extractor of 2.8 L volume. Keep the extractor at 35°C ± 2°C and blow with air, using a membrane pump, at 1.0-1.2 L/min for 6 h. Connect the extractor with a flow evaporator of 0.35 kW power and pump to the inlet of the evaporator water at 500-550 mL/h together with atmospheric air at 1.5 L/min at the start of process and 1.0 L/min after appearance of distillate from the flow cooler, connected to the outlet of the extractor. Upon termination of the distillation blow the extractor with steam only, to wash down the stratum of essential oil, deposited on the walls of the cooler. Collect about 250 mL of distillate into a narrow-necked flask, and then separate the layer of essential oil.

The yield of essential oil, obtained by the described method, was 4.4-5.1 g from a load of the extractor, or 12.5-14.5 g from one kilogram of dried raw material.

The product presents an oily liquid of dark orange-brown colour, with strong odour and spicy taste specific for the Wild bergamot. It is soluble in 4-10 parts of 70% ethanol. Relative density is 0.940 to 0.970, refraction index – 1.498 to 1.514. The essential oil contains 23-32% sum of thymol and carvacrol and 20-32% of thymoquinone. During storage the thymoquinone concentration decreases by 3-4%, and then stabilizes. At storage on light the colour becomes darker, and the thymoquinone content decreases.

At the initial period of storage of the Wild bergamot essential oil, independently of the technological procedure's parameters, thymoquinone reacts with reducing compounds presented in the product. This results into the thymohydroquinone formation. This process has a diphasic character: the thymoquinone concentration quickly decreases during the first week, and stabilization of the composition takes place after about a month of storage. The process kinetics can be formalized with the biexponential model type: $C = C_0 + A_1 \cdot e^{(-t/k_1)} + A_2 \cdot e^{(-t/k_2)}$. For this reason we suggest storing the obtained oil for a month in quarantine before use. After expiration of this period the product composition remains stable for at least 2 years at storage in hermetic glass containers.

Conclusions

A laboratory technological procedure has been elaborated for obtaining the Wild bergamot essential oil with high thymoquinone content. This procedure is based on the air-stream fermentation of plant material, humectated with water, prior to distillation, and on distillation of essential oil with a mixture of steam and air. The thymoquinone content was 20-32% with the essential oil yield of 12.5-14.5 g from 1 kg of plant material.

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Authors' contributions
IC designed the study, conducted the laboratory work and performed its technological part, interpreted the data, drafted the first manuscript. AC collected and processed the plant material, performed the analytical part of the laboratory work. VV interpreted the data, revised the manuscript. All the authors revised and approved the final version of the manuscript.

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

Conflict of Interests
No competing interests were disclosed.

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Typical and variant anatomy of the palmar arteries during perinatal period of ontogenesis

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Abstract

Background: The value of the study is finding typical and variant anatomy of the palmar arteries during fetal and early neonatal periods of ontogenesis, which is important while performing amputations, osteosynthesis, resection of bones followed by further prosthetics, as well as during catheterization of the arterial branches in order to deliver diagnostic and therapeutic substances in fetuses, neonates and infants.
Material and methods: The study was performed on 51 specimens of dead fetuses (from 4 to 10 months) and 10 neonates of both sexes died due to the causes not related to diseases of the muscular-skeletal system and without external signs of anatomical defects, without visual macroscopic deviations or anomalies from the normal structure of the upper limbs.
Results: During the perinatal period of ontogenesis the typical anatomy of the palmar arteries (formation of the superficial palm arch) is found in 62% of cases, the variant one – in 38%. The variant structure of the palmar artery during the perinatal period of ontogenesis is divided into closed and open-ended (continuous superficial palmar arch is absent) arteries. In their turn, closed palmar arteries include anastomosis formation in the form of an arch and without arch formation. In case of open-ended arteries the palm is supplied with blood through the branches from two or three arteries. The older the fetus is, the greater percentage ratio of the typical structure of the palmar artery becomes (9% of cases more) in comparison with other forms. Moreover, the open-ended artery system increases when the palmar surface is supplied with blood from the three arteries (28% of observations more) in comparison with those cases when the palmar surface is supplied through the two arteries. During the perinatal period of ontogenesis the amount of the variant arterial structure is more found on the left palm (25%) compared to the right one (17.5%).
Conclusions: Detection of the typical and variant anatomy of the palmar arteries is a morphological precondition to introduce new methods of performing radical and reconstructive-restorative surgeries on the hand.
Key words: arteries, palm, anatomy, fetus, neonate.

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Introduction

Both external and internal factors influencing the processes of growth, development and formation of the body, its parts and organs, stipulate certain human development produce certain effect on the formation of the circulatory system. Disorders during morphogenesis in general and angiogenesis in particular, can be caused by heredity, hormonal changes, xenobiotic effect etc. [1-5]. Modification of the structure and location of the arterial system on the upper limb demonstrates a great amount of variants. Complicated and numerous processes of the embryonic development transformations make up their etiological basis [6-9]. Different variants of arterial origin in the upper limb, relocation of the point of its beginning, variants of location, size, branching in the number of abductor branches or complete lack of vessels are found concerning certain arteries [6].

Considering the fact that arteries and veins are different vessels in their morphology and physiology, they have been recently believed to be formed from the primitive blood vessels according to the changes of hemodynamics and physiological factors. Though, original studies indicate that arterial-venous identity is stipulated genetically [10, 11].

Anatomical evidences concerning blood supply of the hand are numerous and do not differ one from another substantially. General recent clinical studies deal with the issues of the variant anatomy of the arterial bed of the hand mainly, and less – of its veins [12-17].

More comprehensive investigation of the blood vessels of the upper limbs will help to avoid errors in surgical and traumatological practice essentially during amputations, osteosynthesis, resection of the bones on the limbs followed by their prosthetics, and during catheterization of the arterial branches in order to deliver diagnostic and therapeutic substances etc. [14, 18, 19]. All the above stipulates the necessity to specify the current data and to find new ones concerning anatomical structure of the vascular network of the human hand during the perinatal period of ontogenesis

[20-22]. Our objective was to find anatomical peculiarities of the typical and variant structure of the palmar arteries during fetal and early neonatal periods of ontogenesis.

Material and methods

The study was performed on 51 specimens of dead fetuses (from 4 to 10 months) and 10 neonates of both sexes died due to the causes not related to diseases of the muscular-skeletal system and without external signs of anatomical defects, without visual macroscopic deviations or anomalies from the normal structure of the upper limbs. The adequate anatomical methods were applied during research: macrodissection, injection of the blood vessels, radiography, making topographic-anatomical sections, morphometry, and statistical analysis. The vessels were injected with a special mixture on the basis of red lead. After the specimens of dead fetuses and neonates were preserved, macro- and microdissection of the palmar arteries was performed by means of a forceps and scissors. By means of the pen «AIHFIO ZENTEL» red gel was applied on the arteries in order to examine syntopy of the arterial and adjacent structures better. It enabled to get accurate differentiation of the major arteries and their branches from other palm structures.

The study is performed keeping to the main regulations of the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects (1964-2000) and the Order of the Ministry of Health of Ukraine No 690, dated 23.09.2009. It is a fragment of a comprehensively planned initiated scientific-research study conducted by *M. G. Turkevych* Department of Human Anatomy, and Department of Anatomy, Clinical Anatomy and Operative Surgery, Bukovinian State Medical University: "Regularities of the Genital-Age Structure and Topographic-Anatomical Transformations of the Body Organs and Structures during the Ante- and Postnatal Periods of Ontogenesis".

Results and discussion

During the perinatal period of ontogenesis the final portion of the ulnar artery accompanied by two veins is found to pass along the ulnar sulcus moving to the wrist channel of the elbow side. The ulnar artery passing to the palm of the hand joins the lateral side of the pisiform bone, is located on the short palmar muscle and covered with the fascial thickening of the flexor retinaculum. It goes directly along the lateral side of the short flexor muscle of the little finger included into the tenor muscles of the little finger. The ulnar artery on the hand is located in the loose cellular tissue of the hand middle receptacle on the tendons of the long flexors, and it is covered by the palmar aponeurosis superiorly. When the fourth palmar digital artery emerges from the final portion of the ulnar artery, the latter becomes of a radial direction joining the final part of the tenor muscles of the thumb forming the arch, that is, superficial palmar arch. The latter one forms anastomosis with the superficial palmar branch of the radial artery. Three general palmar digital arteries pass from the convex part of the arch. The final portion of the superficial palmar branch of the radial artery extends the major artery of the thumb and the radial artery of the forefinger. Such kind of structure assuming the formation of the superficial palmar arch is called typical one (fig. 1). The typical structure of the palmar arteries with the formation of the superficial palmar arch is found in 62% of cases during the perinatal period [4, 9, 12, 13]. At the same time, the arch is found to be closed in all the observations (anastomosis formation). It is significant that with the term of fetuses the number of cases of the superficial palmar arch formation increases. Thus, it is found in 61% of observations during the second trimester of the intrauterine development, but in the third trimester and during the neonatal period it is found in 70%.

The variant structure of the palmar arteries is found in 38% of cases during the perinatal period of ontogenesis. The structural variants include both closed and open-ended arches, and blood supply of the palm through the ulnar and additional arteries.

Table 1

Variant structure of the palmar arteries during the perinatal period of ontogenesis

Variant structure of the palmar arteries (38%)			
Formation of the closed palmar arteries (13%)		Formation of the open-ended palmar arteries (25%)	
Anastomosis in the form of arch (5%)	Anastomosis without arch formation (8%)	Blood supply of the palm from the two sources (17%)	Blood supply of the palm from the three sources (8%)

In 3% of cases the palmar arteries form anastomosis in the form of an arch between the ulnar artery and the major artery of the thumb or the radial artery of the forefinger (fig. 2). At the same time, on the level of the final tenor part the ulnar artery divides into two general palmar arteries, one of which passes in the direction to the elbow edge of the palmar portion of the little finger, and another one – in the fourth interdigital space and to the anastomosis arch. The superficial palmar branch of the radial artery divided into the three final branches on the level of the final tenor portion: two general palmar digital arteries passing in the direction to the second and third interdigital spaces, and the major artery of the thumb, from which a branch emerges to the radial edge of the palmar surface of the forefinger.

In 8% of cases during the perinatal period of ontogenesis the palm is supplied with blood by means of the two arteries forming anastomosis without arch formation (fig. 3). The ulnar artery close to the final edge of the holder of the flexor muscles divides into three branches: two general palmar digital arteries and anastomosing branch. One general palmar digital artery passes along the palmar surface of the hypotenor to the elbow edge of the palmar surface of the little finger, and another one – into the fourth interdigital space. Anastomosing branch forms anastomosis between the third and fourth general palmar digital arteries. The radial part of the palm is supplied with blood from the system of the radial artery. The superficial palmar branch of the ra-

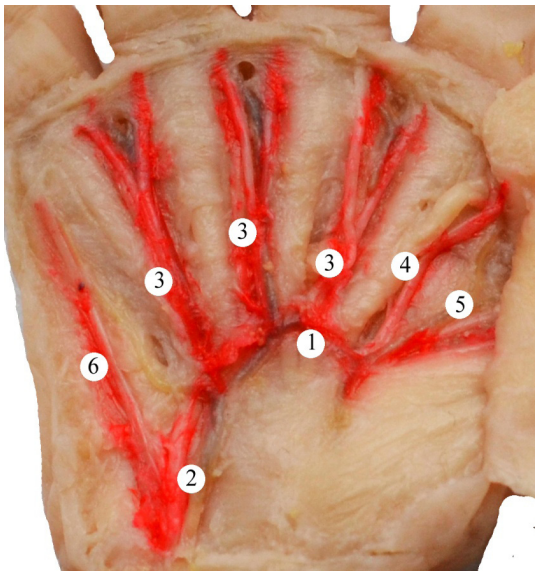

Fig. 1. Palmar surface of the right hand of a neonate (skin, subcutaneous tissue, palmar aponeurosis are removed). Macrospecimen. Magnified by 3.5ˣ: 1 – superficial palmar arch, 2 – ulnar artery, – general palmar digital arteries, 4 – radial artery of the forefinger, 5 – major artery of the thumb, – fourth general palmar digital artery.

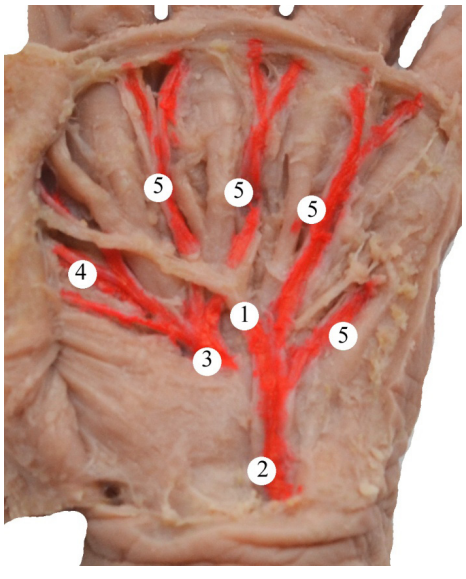

Fig. 2. Palmar surface of the left hand of a fetus, 310.0 mm of the parietal-calcaneal length skin, subcutaneous tissue, palmar aponeurosis are removed). Macrospecimen. Magnified by 3.8ˣ: 1 – arch-anastomosis between the ulnar artery and the major artery of the thumb, 2 – ulnar artery, 3 – superficial palmar branch of the radial artery, 4 – major artery of the thumb, 5 – general palmar digital arteries.

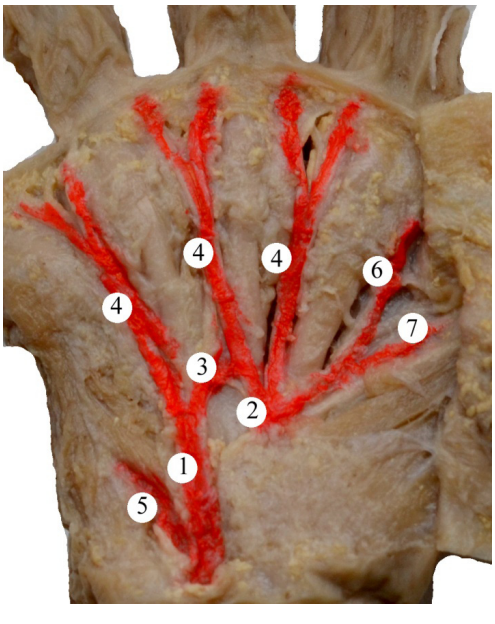

Fig. 3. Palmar surface of the right hand of a neonate (skin, subcutaneous tissue, palmar aponeurosis are removed). Macrospecimen. Magnified by 2.8ˣ: 1 – ulnar artery, 2 – superficial palmar branch of the radial artery, 3 – branch-anastomosis between the second and third general palmar digital arteries, 4 – general palmar digital arteries, 5 – fourth general palmar digital artery, 6 – radial artery of the forefinger, 7 – major artery of the thumb.

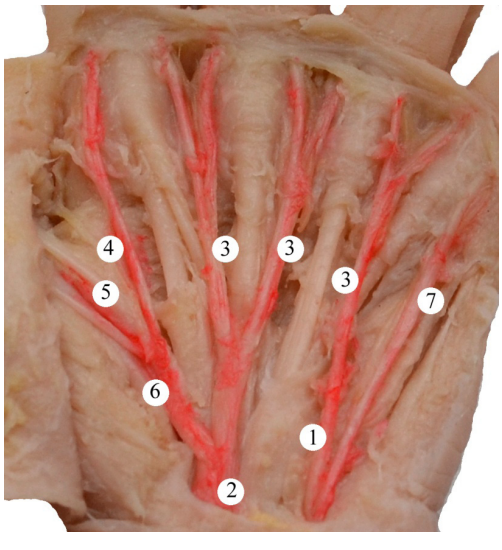

Fig. 4. Palmar surface of the left hand of a fetus, 320.0 mm of the parietal-calcaneal length (skin, subcutaneous tissue, palmar aponeurosis are removed). Macrospecimen. Magnified by 3.8ˣ: 1 – ulnar artery, 2 – superficial palmar branch of the radial artery, 3 – general palmar digital arteries, 4 – radial artery of the forefinger, 5 and 6 – major artery of the thumb, 7 – fourth general palmar digital artery.

dial artery close to the final end of the tenor divides into two general palmar digital arteries passing in the direction to the second and third interdigital spaces and the major artery of the thumb.

During the perinatal period the system of open-ended arteries (continuous superficial palmar arch is absent) is found in 25% of cases. The palm is supplied with blood through the two or three arteries. In 17% of cases the pal-

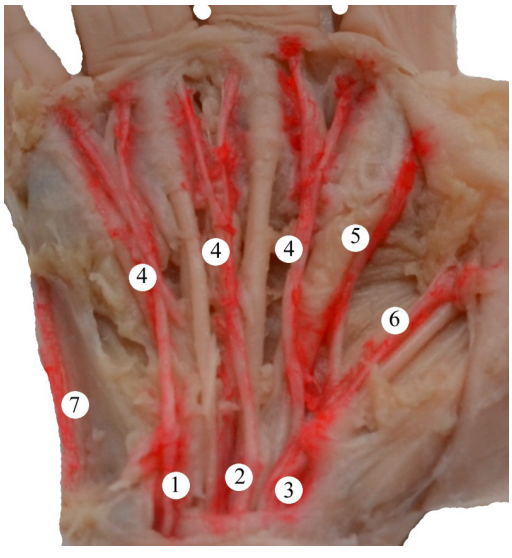

Fig. 5. Palmar surface of the right hand of a fetus, 250.0 mm of the parietal-calcaneal length (skin, subcutaneous tissue, palmar aponeurosis are removed). Macrospecimen. Magnified by 4.5ˣ: 1 – ulnar artery, 2 – accompanying artery of the median nerve, 3 – superficial palmar branch of the radial artery, 4 – general palmar digital arteries, 5 – radial artery of the forefinger, 6 – major artery of the thumb, 7 – fourth general palmar digital artery.

mar surface of the hand is supplied with blood through the branches of the ulnar artery and superficial palmar branch of the radial artery (fig. 4). In these cases the final portion of the ulnar artery divides into two general palmar digital arteries. One of them passes in the direction to the elbow edge of the palmar surface of the little finger, and the other one – to the fourth interdigital space. The superficial palmar branch of the radial artery gives two general palmar digital arteries and the major artery of the thumb. General palmar digital arteries pass in the direction to the second and third interdigital spaces. The major artery of the thumb branches to the radial edge of the palmar surface of the forefinger.

In 8% of cases the palm is supplied with blood through the branches from the three arteries: ulnar, accompanying artery of the median nerve, superficial palmar branch of the radial artery (fig. 5) [15, 16]. The final portion of the ulnar artery divides into two general palmar digital arteries. One of them passes in the direction to the elbow edge of the palmar surface of the little finger, and the other one – to the fourth interdigital space. The accompanying artery of the median nerve is located on the palm between the final portion of the ulnar artery and superficial palmar branch of the radial artery. It extends under the holder of the flexor muscles together with the median nerve. It passes into the general palmar digital artery on the palmar surface of the hand in the direction to the third interdigital space. The superficial palmar branch of the radial artery close to the final portion of the tenor muscles of the thumb divides into the general palmar digital artery passing in the direction to the second interdigital space, and the major artery of the thumb from which the branch to the radial edge of the palmar surface of the forefinger emerges.

With the term of fetuses the percentage ratio of the typical structure of the closed superficial palmar arches increases (9% of cases more) in comparison with the variant forms. In the third trimester of the intrauterine development and during the neonatal period the cases of formation of the superficial palmar arches in the form of arcades convex in the direction of fingers increase (6% of cases more) compared to the second trimester. Though, the number of cases when there is anastomosing branch between the third and fourth general palmar digital arteries decreases with the term from 10.5% of cases (the second trimester of the intrauterine development) to 4 % (the third trimester of the intrauterine development and during the neonatal period). The number of cases when the palm is supplied with blood from the system of the open-ended arteries (continuous superficial palmar arch is absent) during the perinatal period of ontogenesis is practically similar.

In the second trimester of the intrauterine development the variant structure of the open-ended arteries when the palmar surface of the hand is supplied with blood from the system of two arteries is found in 78% of cases, in the third trimester and during the neonatal period – 50%. That is, the percentage decreases contrary to the open-ended arteries when the palmar surface is supplied with blood from the system of the three arteries – it increases from 22% of cases to 50% respectively [17].

During the perinatal period of ontogenesis the greater amount of variant structure of the arteries is found on the left palm than on the right one – 25% and 17.5 % respectively. Although during the perinatal period of ontogenesis this peculiarity changes: the greater number of structural variants of the arteries on the left palm is found in the second trimester, but in the third trimester and during the neonatal period – on the right one.

Conclusions

1. During the perinatal period of ontogenesis the typical anatomy of the palmar arteries (formation of the superficial palm arch) is found in 62% of cases, the variant one – in 38%.

2. The variant structure of the palmar artery during the perinatal period of ontogenesis is divided into closed and open-ended (continuous superficial palmar arch is absent) arteries. In their turn, closed palmar arteries include anastomosis formation in the form of an arch and without arch formation. In case of open-ended arteries the palm is supplied with blood through the branches from two or three arteries.

3. With the term of fetuses the percentage ratio of the typical structure of the palmar artery increases (9% of cases more) in comparison with other forms. Moreover, the open-ended artery system increases when the palmar surface is supplied with blood from the three arteries (28% of observations more) in comparison with those cases when the palmar surface is supplied through the two arteries.

4. During the perinatal period of ontogenesis the amount of the variant arterial structure is more found on the left palm (25%) compared to the right one (17.5%).

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Authors' contribution

OS designed the trial and revised the manuscript critically; VG collected the data, drafted the first manuscript. Both authors revised and approved the final version of the manuscript.

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Ethics approval and consent to participate

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REVIEW ARTICLE

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Management of pharmaceutical risk factors – warranty of patient's safety

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Abstract

Background: The dispensing process is an integral part of the quality use of medicines and together with patient counseling forms the core of professional activities of a pharmacist. The process of dispensing and counseling is composed of a succession of steps, which if interrupted or completed incorrectly, could result in poor quality outcomes for the patient and less than desirable consequences for the pharmacist. The sequelae to serious dispensing errors may be far-reaching, including patient morbidity and mortality, increased health expenditure due to hospitalization and treatment, and loss of credibility and professional standing for the pharmacist, along with the risk of litigation and financial loss. This study is concerned with the process of drug delivery, including the risk factors that increase the likelihood of medication errors and the measures that can be implemented to achieve a quality process. The goal is evaluation risk factors in community pharmacies that have a negative impact in providing pharmaceutical assistance to patients and developing quality criteria for working conditions.
Material and methods: This research is a systematic evaluation where selected studies data were combined quantitatively, to obtain a summary value.
Results: The most important risk factors affecting the pharmaceutical assistance to the patients were identified and criteria of quality working conditions were developed.
Conclusions: Effective risk management in the community pharmacy contributes to quality assurance; anticipation and optimization of the means of control; establishing increased confidence in the management system; implementation of the concept of responsibility for all staff.
Key words: community pharmacy, risk factors, working conditions.

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Introduction

In community pharmacies, the release process is an integral part of the rational use of medicines and, together with the patient's counseling, forms the basic professional activities of a pharmacist. These activities allow the efficient and safe administration of medicines by patients. The release and counseling process is a succession of steps that, if not interpreted correctly, could lead to negative consequences for the patient and undesirable situations for the pharmacist [1]. Some of the most serious consequences can be major medication errors, including trauma and in some cases patient's death, increased health expenses due to hospitalization and treatment, and loss of credibility in the pharmacist's professional skills, along with the risk of litigation, and financial loss [2].

With the increasing emphasis on providing high quality results based on services in pharmaceutical care, it is appropriate for the pharmacist to self-evaluate in all activities to ensure that they provide the highest quality services, both in terms of consumers, as well as from a professional point of view. This study is concerned with the process of drug delivery, including the risk factors that increase the likelihood of medication errors and the measures that can be implemented to achieve a quality process [3].

Purpose – evaluation of risk factors in community pharmacies that have a negative impact in providing pharmaceutical assistance to patients and developing quality criteria for working conditions.

Material and methods

This research is a systematic evaluation in which the data from the selected studies were combined quantitatively, to obtain a summary value. The research was carried out in 4 stages:

1. Formulation of the question to be investigated and the criteria for including the studies;
2. Selection of studies through databases;
3. Identification of eligible studies (elimination of ineligible studies);
4. Filling in the missing and necessary information, analyzing it and drawing conclusions.

Results and discussion

Studying the specialized literature we have identified few studies that analyze the impact of risk factors in providing pharmaceutical assistance to the population and in the current knowledge of the best methods to reduce medication errors. Quality research is needed to determine the effectiveness of the following interventions, which would contribute to the prevention of risk factors and their consequences:

- Identifying situations where medication errors may occur in order to improve safety and confidence in the system through continuous remedial actions;
- Studying each error to determine the factors that made its appearance possible;
- Evaluation of errors produced for applying the necessary corrections or preventing them in the future;
- Installing a computerized alarm system, which warns of the potential risk that may have a negative impact on the provision of pharmaceutical assistance;
- Organizing thematic trainings related to the topic of the management of the factors that contribute to the appearance of medication errors;
- Involvement of pharmacists in reporting the factors that determine them when making mistakes.

The risk is the possibility of being hurt or of having caused various damages. It is a phenomenon commonly encountered in every day life, often associated with the term "insecurity". ISO (International Organizaton for Standards), in the document entitled "ISO/IEC Guide 73 Risk Management – Vocabulary – Guidelines for use in standards" defines risk as "A combination of the probability of an event and its consequences". Other definitions for risk are: chance to lose; the possibility of losing; uncertainty affecting the outcome; dispersion of expected results.

Risk is an uncertain event that, if it occurs, adversely affects the objectives of an activity or a group of activities. And this happens either because an unplanned thing happened in the course of an activity, or because something that was planned did not happen as planned [4].

Most of the time, when planning an initiative, we try to establish the level of our expectations in relation to that initiative. Any deviation from these expectations that evolve adversely to our interests is a form of risk manifestation. Events recorded in case of risk manifestations are often consequences of the interdependence of some risk factors [5].

Risk factors are the factors that contribute to the formation of the risk situation or its aggravation, in certain contexts. The main consequence of the risk factors present in the community pharmacy are medication errors.

Medication errors generally refer to errors in the release process (e.g., wrong drug delivery or improper concentration, incorrect indications, the medicine distributed to the wrong patient, etc.) which are not detected and corrected before the patient leaves the pharmacy and which can lead to undesirable results [6]. The specialized literature indicates little information about the current statistics of medication errors. In the Republic of Moldova, according to the information of the Agency of Medicines and Medical Devices, it is difficult to obtain concrete data regarding the rate of medication errors, because pharmacists tend not to report errors unless the consequences have been particularly serious and / or it is possible to make a claim for compensation for the harm caused to health.

According to Lloyd N. Sansom, PhD in Pharmaceutical Sciences, University of Australia, statistics maintained by the Pharmaceutical Council of Victoria (Australia) in May 1985 revealed 78 errors reported in 156 weeks. This is equivalent to a bi-weekly report or a single error for every 1.7 million prescriptions issued – an incredibly low rate.

An analysis of prescriptions in the United States of America suggested that 5% of prescriptions contain at least one type of medication error.

Researchers Allan EL., Barker K.N. and Malloy M.J. from the Department of Pharmaceutical Care System at Auburn University in the United States of America used a sophisticated technique to study the nature and frequency of medication errors and the quality of patient counseling in 100 randomly selected community pharmacies. In 100 orders issued based on prescriptions, 24 medication errors were detected, 4 of which were significantly serious. It was concluded that the problems related to counseling and assistance in the community pharmacy require attention to the application of quality management [7].

The studies carried out so far have primarily involved the prevention of errors in pharmacies and the most important steps have been identified to improve the medication process, but the factors that can cause medication errors and their consequences have been neglected. The factors contributing to these errors were declared by the researchers, insignificant, with no evidence on them, however, they still affect the pharmaceutical assistance provided to patients [8].

The risk factors present in the community pharmacy are disorders of the three elements that make up the work system (pharmacist, workload and work environment). The main categories of risk factors present in the community pharmacy are:

1. Risk factors specific to the pharmacist, which can be manifested as:

- His wrong actions (choosing a wrong concentration or product);
- Omission of the verification procedure;
- Reduced communication with the patient;
- Lack of experience or knowledge;
- Misinterpretation of a prescription;
- The patient's misunderstanding of the problem and treatment.

2. Risk factors specific to the workload, which can be manifested as a result:

- An oversized task in relation to the pharmacist's capacity or lack of personnel;
- Additional work, fatigue and frequent interruptions;
- Pressure of responsibilities;
- Overload.

3. Risk factors specific to the work environment, which can be manifested as:

- *Factors of the technical environment:* endowment with information systems, the pharmacist's position, the correct arrangement and endowment of the workplace;
- *Factors of the physical environment:* luminous atmosphere, sound environment and microclimate;
- *Factors of the psychosocial environment:* stress, overwork, psycho-emotional state [9].

To analyze the pharmacist's own risk factors that have a negative impact on the occurrence of medication errors, an analysis of each type of error is required. The main reason for choosing an incorrect concentration or product is described as a result of thinking like: "looks the same" or "sounds about the same", respectively the similarity in sounds or letters of the drug name. Common sources of errors are due to too long names, including capital letters within the drug name, difficulties in differentiating drug concentrations and forms due to soils indicating prolonged effects, etc. Some packages or labels contribute to errors, because the identification of the drug is unclear as to its safety. Sometimes the information on the label is not readable and easily understood by all users.

The pharmacist, in addition to exercising his obligations to deliver the appropriate medicines, will need to apply his communication skills wisely. Good communication and counseling is a condition, without which the performance of the quality pharmaceutical act is an unattainable project. It is through the successful communication with the patients that it will be possible to provide psychoemotional assistance and counseling, with the training of rational medication administration skills. The correct communication assumes the pharmacist's ability to identify the way of predisposing the patient to listen and fulfill those recommended by the specialist.

Sometimes, the pharmacist does not have enough time or skills to listen to the patient and to take into account his emotional state. However, the experience of a good relationship means understanding and trust between the pharmacist and the patient, which can be achieved through communication and counseling [10].

The risk factors inherent to the workload exist in two forms of manifestation:

1. Under / oversize the requirements imposed on the pharmacist, respectively requirements not appropriate to his possibilities.

2. The inappropriate content or structure of the workload in relation to the purpose of the work system or to the requirements imposed by the risk situations.

This form of manifestation of risk factors is based on disregarding the physical and mental possibilities of the pharmacist. As a result, the degree of difficulty and complexity of the workload can lead to either under-solicitation or over-applying of the pharmacist.

The conditions that can lead to the appearance of risk factors are:

- The physical request produced by:
  - Static effort;
  - Forced or vicious work positions;
  - Dynamic effort.
- The psychic request produced by:
  - High volume of work;
  - Difficulty of decisions to be made in a short time;
  - Repetitive operations;
  - The monotony of work [11].

For a proper adaptation of each pharmacist to the work process, to the demands of each patient, and also to ensure a major efficiency in the activity, the aspects related to the working environment, the conditions under which the pharmacist carries out his activity are also taken into consideration. According to A. Tabachiu, we appreciate the most important elements of the environment, which must be taken into account when organizing the workplace:

1. Technical environment;
2. Physical environment;
3. Psychosocial work environment [12].

The study of the technical environment of the workplace, of the physical environment and human activity in modern production is ergonomics. In the design of the technical equipment, in its location in the workspaces and in the preparation of the personal protection equipment, the anthropometric data are taken into account.

Another objective of ergonomics is the arrangement of workplaces, which are realized taking into account some essential conditions:

- The pharmacist must be in a proper, natural position;
- Ensuring freedom of movement;
- The working table, the information and control devices must be in the optimal visual and normal area;
- Standardizing the placement of information devices in a suitable grouping and placement, for similar places;
- The pharmacist's working position in accordance with the plan and direction of the objects he works with and the technological flow;
- Efficient placement of technical equipment, technical documentation.

The position of the pharmacists influences the way of performing the work operations and the resting state. An inadequate posture leads to decreased work productivity, overwork, illness and stress [13].

The elements that can be taken into account to evaluate the *physical environment* are: the ambient light, the sound environment and the microclimate (temperature, humidity, air movement). It is considered that these factors can exert their influence on the physiological and psychological processes, having consequences on labor productivity and security, regardless of the specific activity.

The indicators of the physical environment are evaluated starting from the consideration of the following aspects: productivity (labor efficiency); physiological processes (physiological measurements); psychosociological processes (boredom, routine).

The light environment is particularly important, being a basic condition of the normal conduct of the work process. Lighting conditions the proper reception of information visually.

It is necessary, for the efficiency of the work, to ensure an adequate light environment, not too weak, because it would overload through the effort of accommodation, but not too strong because it overloads the prolonged muscular contraction of the pupil. In the pharmacy service room there must be both natural and artificial lighting. The coefficient of natural lighting must be 1-1.5 [14].

The sound work environment is a consequence of secondary and direct sources. Z. Bogathy believes that the most harmful factor in the environment is noise, as it can cause fatigue, and implicitly, the decrease in productivity in work, both from a quantitative and qualitative point of view. Noise is a consequence of the disordered overlapping of sounds with different frequencies and intensities.

For the study of the sound environment, a study was conducted in the community pharmacies in the USA, regarding the association between ambient noise and correctness of drug delivery from pharmacies. Pharmacists were monitored video for 23 days. Video data analysis was used to detect unpredictable, predictable, uncontrollable and controllable sounds.

The study aimed to determine the significance of the sounds of the environment in detecting errors in drug delivery. Noise was found to have a significant effect on pharmacists, increasing the incidence of medication errors by 3.23% [15].

For efficient work performance it is important to consider the microclimate in which the human body feels in thermal equilibrium. The parameters in which the temperature must be kept in a room to ensure comfort are: 18°- 24° C in summer and 17°- 22° C in winter; the optimum humidity is 30-70% and the air speed is 4-8 m / sec.

The psychosocial phenomena that develop in the work process and which designate the psychosocial environment influence, in their turnefficiency and job satisfaction, through the existence of cooperative or, on the contrary, conflictual relations and stress situations.

Studies on stress and professional burnout reported that pharmacists in community pharmacies are exposed to higher levels of stress.

Stress and professional burnout should be exploited regularly in each pharmacy and it is recommended to develop individual and organizational stress management in them. Since pharmacist studies are mainly based on the pharmaceutical sciences, further training in stress and emotion management needs to be promoted [16].

Satisfactory working conditions contribute to both the physical and mental well-being and professional performance of pharmacists. That is why it is important to promote the creation and implementation of an adequate and comfortable working environment – a working environment that favors the quality of the pharmaceutical assistance provided to patients. Other factors that generate risks in the community pharmacy are shown in figure 1.

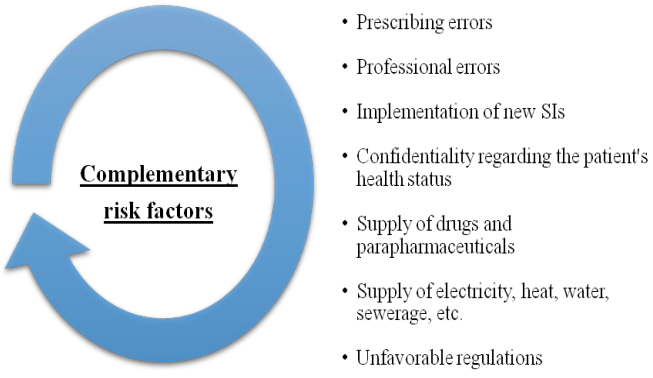

Fig. 1. Complementary risk factors [17].

Any risk factor, once identified and estimated from the perspective of the magnitude of its effects, creates a new "picture", different from the current one. The new perspective viewed from the perspective of risk gives the opportunity to identify new situations, which can be positive or neutral but easily transformed into opportunities.

Most of the risk factors present, at least theoretically, equal chances to materialize or remain at the potential level, so to generate or not the risk situation.

Management of pharmaceutical risk factors. Managing pharmaceutical risk factors (Figure 2) means identifying and evaluating risks, as well as establishing how to respond to risks, that is, to put in place means of internal control that would mitigate the possibility of their occurrence or the consequences they would have if they materialized

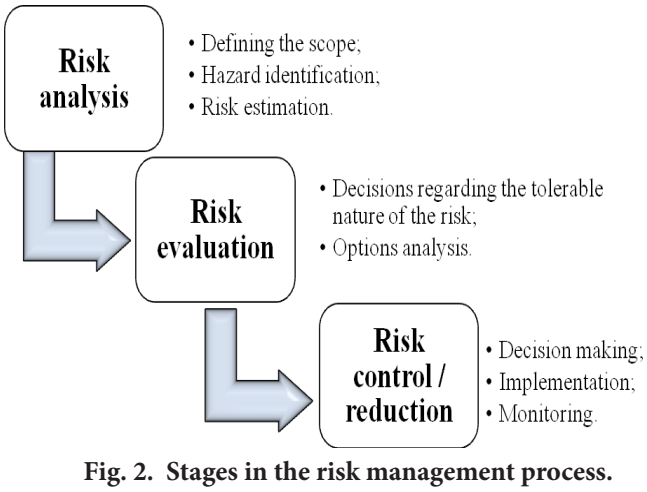

Fig. 2. Stages in the risk management process.

Risk factors can be identified using different methods:

a) Elaboration of checklists that include potential sources of risk, the members of the activity implementation team, the deadline, etc. ;

b) Synthesizing the documentation of the activities identical to the one in progress;

c) Promoting the experience of the personnel involved in activities to identify risk factors;

d) Establishing the risks arising from the outside [18,19].

But this is not enough. In most cases it is impossible to notice all the specific features of an activity. For example, the human factor has long been declared as the most uncontrol-

lable element of any system. It is possible to speak of risks arising from arrogance, ignorance or even fear, voluntary or involuntary actions, psychological particularities, etc.

Conclusions

Risk management is a cyclical process that involves monitoring and solving the risk factors of the pharmaceutical activity.

Effective risk management in community pharmacies contributes to:

a) Quality assurance:
   – Offers reasonable assurances that the objectives will be achieved;
b) Anticipation and optimization of the means of control:
   – It allows the identification of the main risks faced by pharmacists;
   – Mobilizes available resources to address risk factors in an appropriate manner;
c) Establishing the increased confidence in the management system:
   – Risk factor management is an essential part of the managerial and control environment;
d) Implementation of the concept of responsibility for all staff employed:
   – Each manager is responsible for the identification and effective management of risk factors in the field;
   – Pharmacists realize the purpose and roles regarding risk factor management.

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NCB conceptualized the idea, conducted literature review, wrote the manuscript, revised and approved the final text.

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Actualities of disproportionate affection of women vs men in Alzheimer's disease

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Abstract

Background: Alzheimer's disease (AD) is a neurodegenerative disease of the elderly, being recognized worldwide as the most common cause of dementia. However, the harm generated by this disease to women and men is disproportionate, in women the disease is recorded twice as much. Numerous research studies have tried to find an answer regarding the causes of this disproportionality. So far, some fundamental differences between macroscopic, microscopic and biochemical structures of female vs. male brain have been investigated. First of all, emphasis was placed on macroscopic structural differences. In this study, a particular role was attributed to APOE4 gene which was shown to be an increased risk factor of AD in women who possess this allele. Hormonal changes in women, such as decreased postmenopausal estrogen, greatly influence disease incidence and prevalence. All these factors tell about the increased susceptibility of women to this disease. However, the definite mechanisms of this disease are incompletely elucidated and further studies are needed.
Conclusions: The identification of pathobiochemical mechanisms based on gender, that influence the incidence and prevalence of Alzheimer's disease is essential. Thus, it could be a target in the development of effective preventive therapeutic strategies from the prodromal phase of the disease. In this context, the development of personalized treatment according to gender specifics should be considered in future.
Key words: Alzheimer's disease, women, APOE4 gene, mitochondria, oestrogen, depression.

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Introduction

Dementia is a clinical syndrome characterized by a progressive and irreversible cognitive decline, which mainly affects the memory, functionality and person's behaviour, producing major personality changes. Alzheimer's disease (AD) is recognized as the most common cause of dementia worldwide, accounting for approximately 80% of all dementia cases [1].

Research study has shown that the diagnosis of Alzheimer's disease is defined by the presence of amyloid beta (Aβ), neurofibrillary glands (NFTs), neurodegeneration and not by the presence of specific symptomatology [2].

Statistics has shown that women are more affected by AD compared with men. In the United States, 2/3 of those affected by AD are women [3]. Initially, female longevity was considered the main reason of AD. Although, subsequent studies proved that the involved mechanisms are much more complex and include intrinsic factors related to genetic, endocrine, inflammatory, and structural characteristics of the brain. All these factors have a well-defined role in this imbalance. [4].

Brain structural differences

First and foremost, women's and men's brains differ structurally. In various studies, with the help of MRI, was shown that men have larger tonsil and thalamus, while women have a larger hippocampus [5]. Women's cerebral volume is smaller, but richer in grey matter as compared with men's one, which is larger in size and has bigger volume of white matter [6]. As well, men have greater interhemispheric connectivity. However, when we talk about metabolism, in women the blood flow is more abundant in the parietal cortex, and in men it is richer in motor, visual cortex, and in cerebellum [7, 8].

Implications of APOE4 gene

APOE gene encodes APOE protein, which binds to lipids, forming lipoproteins. Their role is essential in the cholesterol transport through the blood stream [9]. At least 3 alleles of APOE gene are known: APOE2, APOE3 and APOE4 [10]. ε4 allele of APOE4 gene represents a genetic risk factor for sporadic AD with late onset [11]. APOE4 allele was associated with mitochondrial dysfunction and glucose hypometabolism in the brain [12]. There are major differences between female and male APOE4 allele carriers. Women who possess E4 allele have a higher genetic risk of AD developing, which is manifested by a more rapid evolution with a more severe cognitive decline, compared with men that have the same allele [10, 13]. Concurrently, the female E4 allele carriers are more likely to develop AD because they are more susceptible to protein accumulation. This condition effects directly beta amyloid aggregation and promotes pro-

teolytic cleavage that generates toxic fragments in the brain [6, 14]. Women are endowed with a larger neural network that serves as brain reserve and allows the compensation of amyloid deposition, that results in a significantly higher verbal memory [15].

Hormonal imbalance and menopause

AD pathobiochemical changes manifest slowly, progressively and may last for decades. Studies suggest that the increased risk for women to develop the disease between 65 and 75 years is due to hormonal changes and menopause, which begin 15-20 years earlier [16]. Menopause is considered to be a neuroendocrine transition and ends with reproductive senescence. It presents a set of neurological symptoms, and causes estrogen regulation dysfunctions, such as thermoregulation, sleep and circadian rhythms, depression and impairment of multiple cognitive domains. It has been shown that the menopause onset is directly influenced by the uncoupling of brain energy metabolism, which in turn is mediated by estrogen. Both men and women produce estrogen, only that women produce a much higher amount of estrogen, which is also called the female hormone. With menopause onset, hormone production decreases. On the other hand, men continue to produce testosterone – the male sex hormone, which when reaches the brain cells, is converted into estrogen. In conclusion, at the same age, women have less estrogen in the brain compared with men [17, 18].

Oestrogen actions in the brain

Oestrogen plays an essential role and has multiple functions in the reproductive, cardiovascular, skeletal, and central nervous systems physiology. Oestrogen is vital for prefrontal cortex and hippocampus functioning and initiates spinogenesis and synaptogenesis [19, 20]. Three physiological estrogens are known, including estradiol (E2). During menopause, it decreases producing a series of changes in cognition, sleep, and mood. Studies have shown that estradiol also interacts with cholinergic, dopaminergic and mitochondrial functions [21]. Oestrogen has a neuroprotective role against dementia, and its dysfunction aggravates the neurodegenerative process, especially in women [22, 23]. For many years, epidemiological studies have shown that the prevalence of Alzheimer's disease was significantly lower in women taking postmenopausal hormone therapy, or the disease was milder compared with those who did not [24]. However, a case-control study in Finland, conducted between 1999-2013 and attended by 84739 women diagnosed with postmenopausal AD, has shown surprizing results. The results of the study demonstrated that long-term systemic hormone therapy may be accompanied by an increased risk of AD. Only vaginal estradiol presents no risk [25].

Mitochondria

In cellular metabolism, mitochondria play an essential role, not only for ATP production, but also in the production of reactive oxygen species (ROS) following the discharge of electrons from the respiratory chain [26]. In the presence of beta amyloid, mitochondria increase the formation of reactive oxygen species which on the one hand are harmful, but on the other hand act as signalling molecules [27]. Mitochondria are an essential source of ROS in cells that have high oxidative capacity, such as neurons, skeletal muscle cells and cardiomyocytes. Upon prolonged exposure, ROS can produce mutations in mtDNA, which accumulate over years. Primordial impairment occurs on the mitochondrial cells of the brain. However, in mouse experiments, in the mitochondrial cells of the female mouse brain, lower oxidative stress was found when compared with the male ones [28]. The liver and brain mitochondria of the female mouse produce less peroxides than the male ones. Nevertheless, ovarectomy reversed this process, equalling female/male peroxides production. Estrogen replacement therapy produced surprising results, namely the peroxides production was smaller in female than in male (F<M). Thus, the lack of postmenopausal oestrogen administration may be the reason for the increased AD prevalence among women [29].

Depression prevalence in women

Depression is an important risk factor for developing Alzheimer's disease [30]. The hippocampus represents a brain structure composed of dentate gyrus, and hippocampus itself which is composed of three fields – CA1, CA2, CA3 and subiculum. It is a structure responsible for learning and memory, which is affected in AD [31]. Gender and sex differences exist and are related mainly to hippocampal plasticity and cognition, women being more prone to cognitive decline in Alzheimer's disease [32]. Numerous studies have shown that the increased prevalence of depression in women correlates with hormonal changes and is predominantly manifested during puberty, before menstruation, after pregnancy and perimenopause. Estrogen administration decreases the risk of depression. But why do men who possess testosterone have lower rates of depression? Research has shown that in the male brain there are many receptors for estrogen, and through endogenous aromatase, testosterone turns into estrogen. Apart from the hormonal implications, the presence of different brain circuits in men is suspected [33]. In women with mild cognitive impairment, proteins spread earlier and faster through the brain than in men's brain [34, 35].

Conclusions

Gender in Alzheimer's disease could be a potential risk factor, as studies have shown that 2/3 of women are affected by this disease. Although, it was initially thought that this is due to the female longevity. Later, various research studies have shown the involvement of several macroscopic and biochemical factors in this disproportionality.

The identification of pathobiochemical mechanisms based on gender, that influence the incidence and prevalence of Alzheimer's disease is essential. Thus, it could be a target in the development of effective preventive therapeutic strategies from the prodromal phase of the disease. In this context, the development of personalized treatment according to gender specifics should be considered in future.

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AO designed the trial and drafted the first manuscript; LL interpreted the data and revised the manuscript critically. Both authors revised and approved the final version of the manuscript.

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Interaction between SARS-CoV-2 and human organism

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Abstract

Background: COVID-19 is a part of the betacoronaviridae group, sabercoviridae subgroup. At the moment we are confronting a pandemy, which has a completely new pathologic pattern for the whole world. Considering the highly contagious characteristics of the virus, which is the cause of death for many people, and also the fact that many people continue to be infected with this virus there is a stringing necesity of combating this pandemy. Because of that we need the knowledge that regards the physiopathologic mechanisms, particularities of the host-invader interactions, relevance of asymptomatic forms, explanations of the evolving divergencies, posibility of recurrent infections, clinical signs, comorbidities that harshen this pathology, pharmacologic agents that are effective to fight the infection and immune mechanisms of defense in the organism. A great importance is given for the identification of the intial criteria needed for a prognostic, to prevent the critical forms of pathology and to set the parameters for the severity indicators.
Conclusions: The research in the field of SARS-CoV-2 requires the gathering of the databases that are related to the investigated persons, to establish the clinico-evolutive differences for the COVID-19 patients according to many factors that could influence the course of the disease. An important difference is the identification of early signs and prevention of the critical disease forms, by extending the laboratory investigations, establishing the parameters for severity indicators through determining the degree of the immune response.
Key words: COVID-19, SARS-CoV-2, Coronavirus.

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Introduction

Starting from 2020, the international medical community directed its attention towards the new coronavirus, COVID-19 and the pathology that it determines – SARS-CoV-2. This virus appeared for the first time in the city of Wuhan, Hubei province, China and has spread very fast at a global level. The hastened spread of this virus puts the world into the evidence of the high transmissibility of this family of viruses with a significant mortality and morbidity. As this virus appeared recently, it needs a continuous research. We are in a continuous pandemy with COVID-19, scientists are fighting to understand the similarities and differences between COVID-19 and SARS-CoV-1 at genomic and transcriptomic level [1]. The main targets of the virus are the lungs, but it has a serious effect on heart especially in case of a cronically affected one that needs a supplementary effort in order to deliver the oxygenated blood to the tissues. Patients with persistent cardiac affections have a weaker immune system. The immune response is decreased when advancing in age and in people with chronic comorbidities. One of the objectives of this study was to present the most recent data in order to determine the immune response in patients with COVID-19.

To realize this objective we used the Google Search results and the PubMed database. For advanced search of the bibliographic sources we applied the following filters: articles with integral texts, articles in the English language published before 28.04.2020. After a preliminary analysis of the titles we selected the original, editorial articles of narrative synthesis, systematic and meta-analysis that contained relevant information and contemporary concepts about COVID-19. The information of the publications included in the bibliography was collected, classified, evaluated and synthesized putting into evidence the main aspects of the contemporary visions about the infection and immunity in COVID-19.

Results and discussion

The SARS-CoV-2 virus is entering the organism via the ACE2 [2-10] that is intensely expressed in the cells of the nasal mucosa, bronchi, lungs (type I and type II alveolocytes), heart, esophagus, kidneys, stomach, billiar sac, ileon, and neurons [5, 7, 8, 10]. After the invasions, the celullar proteases (TMPRSS2 and Furing) will lead to the final internalisation of the virion [11].

The anterior situses are the most affected in case of SARS-CoV-2, the epithelial cells being distructed after the invasion [12]. The supposed enter gates are – the respiratory epithelia at pulmonary level [7], through the olfactory fillia that are penetrating the lamina cribrosa [5, 13]

or through the nervous fibers of the pulmonary mechano- and chemo-receptors via the nucleus *tracti solitarii* and the neuroinvasion can also take place through the limphatics [13]. The specificity for this receptor was conferred by the spike glycoprotein that has the S1 and S2 subunits that are attached to the viral envelope (capsid) [1, 4, 5, 14, 15]. There are numerous polimorphisms for this protein in the same viral species, thing also characteristic for COVID-19 [1], the spike protein is expressed 10-20 times more intensely by COVID-19 comparatively with SARS-COVID-1, the fact argumented by the enhanced viral permeability [5, 16]. Another way of cell invasion is through the CD147 receptor (Basigin/EMMPRIN) via the spike-CD147 protein [17], another membranary receptor that could stimulate the matrix metalloproteases (MMP) – MMP1, MMP2, MMP3, MMP9 [18, 19], because of this COVID-19 could affect the T lymphocytes [20]. Once entered in the cell, the virus starts the translation of the own ARN for the pp1a and pp1ab proteins codified by the ORF1a and ORF1b genes – that play a key role in the viral replication, that are cleaved by the nsp 1-16 proteins [14, 21], respectively the inhibition of this chain could signifcantly decrease the infecting potential in the host. The antigens characteristic for COVID-19 can be recognized by the following receptors in human body – Toll-like receptor (TLR), Nod-like receptor (NLR), pattern recognition receptors (PRR), RIG-1 like receptor (RLR) and the cytolosic receptor for melanoma differentiation-associated gene 5 (MDA5) [4, 14] that are on the surface of the antigen presenting cells and also interact with the CD26, cyclophilin enzime [15]. This leads to the release of the pathogen-associated molecullar patterns (PAMP) [14], that could interact with the cells of the human organism in order to unleash the formation of inflammasomes that lead to the apoptosys through the NF-κβ pathway intracellulary and will determine the local inflammation of tissues that in the most severe cases will determine the systemic inflammatory response syndrome (SIRS) [22], along with the acute respiratory distress syndrome (ARDS), trombosys and pulmonary embolism [12]. In the infection with SARS-COVID-1 we observe a hiperglicemy, reffering to the infection with SARS-CoV-2 we haven't observed such modification at the moment [11]. During the febrile syndrome manifestion eczematous eruptions may appear [23, 24] or vesicles surrounded by erythematous halos that could form crusts – that are located preponderentely in the thoracic region [25], [42]. Lesions characteristic for frostbites were identified for the most patients with SARS-CoV-2 without confirming their association with the respective infection [26]. More recentely we have a report of the acral cutaneous lesions [27].

An incidence of 1% of conjunctivitis was reported in a meta-analysis [28]. Along with the spike protein, COVID-19 presents the hemaglutinin-esterase, membranary protein M, nucleocapsidic protein, the small capsidal protein, the internal protein and specific group protein – potential targets for vaccines [29].

In SARS-CoV-2 the activation of the CD8+ lymphocytes and CD4+ lymphocytes is being observed along with the C3a and C5a components of the complement that play a major role here, the worst scenario being the systemic inflammatory response syndrome (SIRS) with multiple organ dysfunction syndrome (MODS) [1, 2, 4]. In SARS-CoV-2 was observed the cytokine storm [10, 30]. In the plasma of the patients with SARS-CoV-2 we identified the following cytokines – IL-1, IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, IL-13, IL-17 and the colony stimulating factor of granulocytes (G-CSF), the macrophage colony stimulating factor (M-CSF), IP-10, MCP-1, MIP-1α, the hepatocyte growth factor (HGF), IFN-γ and TNF-α [13, 14], particularily we see the presence of the interferon 1 (IFN1) that plays a major role in the inhibition of the Th1 differentiation and the amplification of the Th2 cells, chemokines like IP-10 and MCP-1 that are intensely expressed in the COVID-19 [13, 30] will determine the chemotaxis of the numerous cells of the immune system, especially the neutrophils that will cause diffuse alveolary lesions and ARDS [1-3, 10, 13, 30]. The severe cases have a basal lymphopenia, leucocytosis an increased neutrophil/lymphocyte level and less monocites, eosynophils and basophils. The number of Th lymphocytes is decreased, the number of Ts lymphocytes is decreased, the number of naive Th circulant levels is increased, the number of Th lymphocytes is decreased [9, 31] this is explainted by the fact that COVID-19 could enter these cells via the CD147 [20]. Th lymphocytes express the Notch1 gene [32], having a high fraction of fatigued T lymphocytes [12]. The serin proteases and cathepsins are released during the immune response and could cleave the spike proteins – reducing the viral permeability [14]. The levels of Angiotensin II is positively correlated with the viral dose and the severity of lesions because it denotes the high levels of ACE receptors expression and hastens the ARDS installation [3]. The ORF1ab, ORF10 and ORF3a proteins could coordinate the hem attack in the β1 chain of the hemoglobin and "steal" the Fe ions from the hem, also the capsidal glycoproteins could bind to the porphirinic chain [33], a meta-analysis has shown that patients with SARS-CoV-2 have a decreased total hemoglobin [34]. An increased level of C reactive protein (PCR) is observed because of the systemic inflammation, an increased level of feritin because of the hem attack and an incresed level of procalcitonin due to the response to the infection in the organism [35]. At the cardiac level an acute cardiac lesions could appear [36], that could evolve in a cardiogenic shock having an elevated concentration of troponin. The mechanisms of proposed lesions are – myocardial hypoxy, the distruction of the microcirculation vessels, endothelial descuamation and cytokine mediated lesions – there are no proofs at the moment that the myocardium could be infiltrated with lymphocytes, the global effects are represented by the cardiac failure, arrythmia and cardiac decompensation [29]. Taking into consideration that the dopamin decarboxylase gene is positively correlated with the ACE2 gene, we could suppose that alterations in the synthesis of the dopamine are correlated with the physiopathologic mechanisms of the COVID-19 [37]. At the initial stages it could cause anosmia, ataxia and convulsions – in the most severe cases it could lead to respiratory stop [38].

The infection starts with the activation of the dendritic cells that reside in the pulmonary tissue, that will present further the antigens to the naive T cells that will secrete different chemokines and cytokines [29], the CD8+ cells have the tendency to secrete high quantities of IL-6 and the CD4+ have the tendency to secrete high quantities of IFN-γ and GM-CSF [12, 29] but they could secrete the chemokine ligand 9,10 and 11 (CXCL) and cytolytic molecules just as granzyme B [29]. A series of comorbidites could make the infection with SARS-CoV-2 worse, like arterial hypertensions, determined by the amplification of the renin-angiotensin-aldosteron system (RAAS) by inducing endothelial lesions that will lead to cardiac, pulmonary and renal complications [3, 14, 39]. It can induce the distruction of the hemato-encephalic barrier; the cerebrovascular pathologies will worsen the SARS-CoV-2 flow [5, 39]. The pulmonary pathologies (Chronic obstructive pulmonary diseases – COPD), diabetes mellitus and advanced age were recognized as risk factors [7, 11, 14], in diabetes mellitus an increased expression of ACE2, furin was observed along with the dysfunction of the CD4+ cells and increased concentration of IL-6 [11], furin being codified by the Notch1 gene [32]. The patients with respiratory insuficiency need on-stage intubation [40]. The infection has weaker manifestions in children because they are temporary adapted in order to combat the viral infections, also the ACE2 receptors are less pronounced in their organism [9, 12], the male gender has a more severe symptomatic because of increased ACE2 expression [12]. Patients with pulmonary cancer are more susceptible to COVID-19 infection [41]. We have to mention that along with the generic symptoms of SARS-CoV, although we mentioned before about dermatological findings, we can observe chicken-pox like vesicles [42] and an increased level of lactate-dehidrogenase (LDH) and creatinfosfokinase (CPK) [3]. We reported a case in which an asymptomatic woman that presented an infection with COVID-19 in the tardive partum period hasn't transmited the virus vertically [43].

The long-term consequences after SARS-CoV-2 infection at the moment are not known in details, but it was reported that in 40% of the patients with SARS-COVID-1 were observed cardiovacular anomalies and dysregulations of the lipidic metabolism [44].

A series of pharmacological agents are potentially efficient for combating SARS-CoV-2 infection, we will expose a part of them: Baricitinib [30, 45], Ruxolitinib and Fedratinib are inhibitors of JAK-STAT signaling and respectively it wouldn't diminish the systemic inflammatory effects in SARS-CoV-2 [45]. Chloroquine is supposed to be a strong antiviral agent, useful in the treatment of pneumonia caused by COVID-19, being very economic from the financial point of view [9, 21, 30, 46, 47], it is very effective to inhbit the distruction of hem by the ORF1ab, ORF3a and ORFa proteins along with the capsidal glycoproteins [33]. Hidroxychloroquine (HCQ) could glycosilate the ACE2 receptors, it could induce the cleavage of the spike proteins and block the cytokine storm, but the Ivermectin – an antiparasitary agent could interfere with the nuclear transport of the viral proteins dependent on the α/β importin, a combination of HCQ/Ivermectin is recognized to be used in order to combat SARS-CoV-2 but we haven't conducted a study about its effect [48]. The efficiency of Ivermectin in SARS-CoV-2 was tested only *in vitro* at the moment (14.04.2020) [49]. Erythropoietin has shown its efficiency in order to combat the enemy caused by SARS-CoV-2 in case study [50], also in a theoretic review there are proposed the pharmacological agents used in case of pulmonary edema caused by high altitudes (EPAM) – acetazolamide, nifedipine and inhibitors of phosphodiesterase without being clinically tested at the moment [51]. The inhibitors of the viral proteases (nsp inhibitors) like lopinavir and ritonavir are potential antiviral agents that could combat this infection [21], a combination of lopinavir-ritonavir was used to combat SARS-COVID-1 and MERS-COVID [52]. Favipiravir could inhibit the binding of the spike and ORF7a proteins to the porphirin ring [33]. Corticosteroids are potential antiinflammatory agents [30], but the corticosteroids determine an increase in the spread of the virus into the organism [13]. The inhibitors of the interleukin-6 could be useful in order to combat SARS-CoV-2 [9], the results of a clinical study show that the administration of tocilizumab (an inhibitor of IL-6) could be useful in order to combat COVID-19 if it is administered many times [53]. At the moment the inhibition of Notch1 gene is discussed in order to prevent the cytokine storm [32]. A study made *in vitro* proves that the multiplication of COVID-19 is inhibited via the blockage of CD147 with agents like Meplazumab [17, 54]. Azithromycin can inhibit the action of some matrix metallproteinases. AS the STEM cells present a CD147 receptor, they could also serve as a target for SARS-CoV-2 [18], being proved to be a good adjuvant agent along with hydrocholoroquine in a non-randomised clinical study [55].

The identification of the viral dose from the salivary prelevate taken from the posterior oropharyngeal mucosa could be an efficient method to determine COVID-19 at an incipient stage of manifesting SARS-CoV-2, but it is necessary to combine this inspection with an antibody analysis because they present an inverse correlation with the viral dose [52]. In the blood plasma consequently there are antibodies with a median seroconversion on the 11th day, IgM with a median seroconversion on the 12th day and IgG with a median seroconversion on the 14th day ($p<0.05$), the tests for the antibodies could be useful in order to assess clinical suspections in the symptomatic patients that don't have a viral ARN detectable in plasma [56]. The administration of convalescent plasma for the patients with SARS-CoV-2 was proven to be efficient to attenuate the infection manifestations, according to a theoretic review in a series of cases, at the moment there are no avaible randomised clinical trials (RCT) [57]. The antibodies in SARS-CoV-2 infection could be detected only in the medium and late stage [58]. The patients that could recover after an infection of SARS-CoV-2 could have high viral doses in the fecal masses [59].

At the moment the histopathological aspects of SARS-CoV-2 are not yet known in details, according to a theoretic review, the patients with pulmonary cancer presented histopatological patterns- macroscopic (pleurisy, pericarditis, pulmonary consolidations and edema) and microscopic (alveolary proteic exsudates, focal reactive hiperplasia of the pneumocytes with grouped celullar infiltrations and gigantic polynucleated cells without hyalin membranes). The patients that presented the ground glass image on the pulmonary radiography, had diffuse alveolar lesions with exsudates (characteristic for the distant lesions of immune systems in SIRS). No viral inclusions were detected in the histopathological findings [60]. Diffuse alveolar lesions were observed in another study, along with infiltrates of CD8+ lymphocytes and CD4+ lymphocytes around the high caliber bronchioli, in the majorty of cases being present hemoragic foci and intracelular trombi, type II pneumocytes with cytopatic effect (desquamed), the alveolar capillaries were hypertrophiated with perivascular edema, in this case there was no hyaline membrane but were identified CD61+ megacariocytes. In the heart we identified disseminated celullar necrosis, without focal accumulations [61]. The ground glass image in the pulmonary radiopgraphy identified diffuse alveolary lesions in the organizatoric phase, fibrinous alveolar proteic exsudate, hyperplasy of the reactive pneumocytes type II along with interstitial fibrosis and chronic inflammatory infiltrations [62]. At the renal level there were acute lesions of the proximal tubule manifested with vacuolar degeneration and epithelium desquamation. For the patients with pathologic pulmonary manifestation we identified the acute pyelonephritis, more rarely – erytrocitary aggregation in the tubes with consequent obstruction, ocasionally we observed the hemosiderin inclusions. The distal tubules didn't present serious pathological modifications [63].

Conclusions

SARS-CoV-2 became a global threat for health, with the number of patients increasing gradually. An increased incidence of diverse comorbidities was observed along with those that presented severe infection. The mechanism of cardiac lesions is not clear but it probably implies a combination of direct viral lesions and immunised damage mediated by the inflammatory cytokines/chemokines and cytotoxic response of the immune cells in the further infection stages. The immune response of the host and the contributors to the cytokine storm in the infection with SARS-CoV-2 is complex. The exhaustion and significant deregulation of the T lymphocites could contribute to the immune degradation and hyperactivity. The treatments for COVID-19 are bimodal with a treatment group oriented towards the temporary infection and viral replication, there is also another group that takes into consideration the modulation of the immunity at the systemic inflammatory stage. It is important to make complete immune searches for the patients with diverse comorbidites in order to understand the systemic deregulations better. It is necessary to acquire more data about the immune response according to the severity of disease and to take the needed measures in the managerial acts in the infection control.

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GP conceptualized the project and designed the research; VP revised the manuscript critically. LC interpreted the data; VC performed the laboratory work; LP drafted the manuscript; DC drafted the manuscript and designed the research. All authors revised and approved the final version of the manuscript.

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Identification and clinical significance of folliculo-stellate cells in normal hypophysis and adenomas

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Abstract

Background: Folliculo-stellate cells were first identified with electron microscopy as a non-hormone secreting accessory cells, star-shaped and follicle-forming cells. They represent about 4-20% of pituitary cells from the anterior pituitary lobe. The folliculo-stellate cells control several anterior pituitary activities. However, they do not produce hormones, but they produce growth factors, cytokines, vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), leukemia inhibitory factor (LIF), IL-6, and macrophage migration inhibitory factor (MIF), IL-1β and TNF-α. Regardless of a long period of pituitary research and many morphological, cytophysiological studies, it has been reported that a precise understanding of the major functions of folliculo-stellate in the pituitary gland remains unknown. Consequently, there are still many unsolved issues.

Conclusions: This article intends to review the characteristics of folliculo-stellate cells and their uncertain functions in the adenohypophysis, such as their importance as stem cells, in the process of maturation and aging. New researches about the origin and differentiation of folliculo-stellate cells may provide many relevant answers about physiopathology of the pituitary gland and the pathogenesis of pituitary tumors, as well as their influence on the quality of life. Immunohistochemical profile studies of folliculo-stellate cells in pituitary gland, can be useful in elucidation of morphological features and may have a predictive role for the early identification of pituitary microadenomas, prognosis of pituitary tumors and treatment.

Key words: folliculo-stellate cells, S-100 protein, hypophysis, pituitary tumor.

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Introduction

The pituitary gland is a small endocrine regulator complex, essential organ to the human body. It represents an intermediary body between the signal exchanges among the hypothalamus and peripheral organs. It also contains substantial functions in a lot of processes such as growth, reproduction, metabolism and immune response. The anterior pituitary gland includes granular hormone-producing cells, which act by controlling the growth (growth hormone GH), lactation (prolactin PRL), thyroid function (triiodothyronineT3 and thyroxineT4), adrenal function (adrenocorti-cotropic hormone-ACTH-), gonadal function (follicle-stimulating hormone-FSH- and luteinizing hormone-LH-) [1, 2]. The posterior pituitary secretes vasopressin (antidiuretic hormone-ADH-), which is the hormone responsible for maintaining water balance, and oxytocin, that plays a role in uterine contraction and lactation [3, 4].

Along with the discovery of folliculo-stellate cells, a multitude of their important functions have been identified. This literary review focuses on presenting the characteristics of folliculo-stellate cells and their importance as stem cells, in the process of maturation, aging, and in the pathogenesis of pituitary tumors.

This research is a meta-analysis of contemporary scientific literature reported in PubMed and Springer Link database, with reference to folliculo-stellate cells, by the following key words: "folliculo-stellate cells", "S-100 protein", "hypophysis", "pituitary tumor" and selected articles published in the 2000-2019, as well as several earlier articles and books, available in English. During the first stage, we have identified all the articles corresponding to the subject under investigation. After collecting the articles, we proceeded to selecting the valid information from them and extracting relevant data. Finally, we analyzed all the data and drew up the conclusions.

Results and discussion

Folliculo-stellate (FS) cells of the adenohypophysis represent a population of nongranulated cells described in a large number of species [1]. They show typical morphological features including a star shape, with thin cytoplasmic projections extending between granulated cells and well-developed junctional complexes [5]. Joining together, FS cells surround irregular microcavities and project microvilli into the lumina. The immunocytochemical localization of S-100 protein, glial fibrillary acidic protein, and vimentin constitutes an easy method for investigating their presence and distribution in the normal pituitary gland and in pituitary adenomas. FS cells have immunohistoche-mical specificity and express positivity for the S-100 protein [6]. Also it has been reported, that they tend to be immunoreactive to

other markers such as glial fibrillary acidic protein (GFAP), major histocompatibility (MHC) class II surface antigens, cytokeratins, and vimentin [6]. Due to the variable expression levels of these markers, FS cells are currently considered to be phenotypically and functionally heterogeneous, with three main subtypes being proposed: astrocyte-like (also expressing GFAP), dendritic cell-like (also expressing MHC class II), and epithelial cell-like (also expressing cytokeratins) [7].

Although the expression of glial cell markers raised the hypothesis of a neuroectodermal origin of FS cells, most evidence supports that they derive from the epithelium of the Rathke's pouch, as granulated adenohypophyseal cells do [4].

Besides the roles FS cells have in the normal anterior pituitary, their identification in pituitary tumors suggests FS cells may also have major implications in these tumors. However, the available knowledge concerning FS cells in human pituitary tumors comes mainly from IHC studies assuming their presence by means of S-100 immunoreactivity. Their role in the initiation, maintenance, and progression of pituitary tumors or in the functioning phenotype of these tumors remains largely undetermined [3, 8].

In one of the biggest studies, Voit et al., studied FS cells. 286 cases of pituitary tumors have been researched in patients presenting acromegaly [9]. The authors reported that FS cells were found to be either isolated or grouped, forming network-like structures. These structures were frequently in close relationship with the tumor cells. Their results showed that 198 out of 286 tumors (69%) contained FS cells. The number and distribution of FS cells were varying: 35% of the tumors (100 cases) presented few widely sparse FS cells, 15% of the tumors (43 cases) presented FS cells scattered throughout the tumor, and 19% of the tumors (55 cases) showed abundant FS cells. When they examined clinical correlations, the researchers noticed the existence of a negative correlation between the density of FS cells and the preoperative mean prolactin levels. It is an observation rather contradictory to what was published *in vitro* work that generally showed that the interaction between prolactin-secreting cells and FS cells, and/or their secreted molecules, results in an increased prolactin level. The same researchers found that preoperative mean growth hormone levels were higher in patients with tumors containing few widely sparse (64.5-8.1 g/L) or scattered FS cells (83.1-17.1 g/L) than in those patients with tumors lacking FS cells (44.9-4.1 g/L). This observation supports the possibility that part of the role of FS cells in pituitary tumors may be similar to the function of FS cells in the normal pituitary, where one of their roles is to regulate the secretory capacity of normal endocrine pituitary cells [3]. However, it is interesting that the difference observed with the widely sparse or scattered FS cells was not noticed in the case of abundant FS cells. Indeed, the preoperative mean growth hormone level was lower in patients with tumors containing abundant FS cells (41.0-5.7 g/L) than in patients with tumors without FS cells (44.9-4.1 g/L). This may suggest that during tumor microenvironment remodeling, the function of these cells is modified or there are different types of FS cells [9, 10].

Another study, Vajtai et al. started by analyzing three cases of pituitary tumors, two prolactinomas, and one gonadotroph tumor. These tumors presented with an inflammatory reaction mediated by T lymphocytes that selectively involved the tumoral tissue [9]. The authors observed that perivascular T lymphocytes (predominantly CD4+) tended to mix with cells immunopositive for the S-100 protein. In order to differentiate the FS cells from inflammatory foci presented monocytic/dendritic properties, researchers performed double immunohistochemical staining for S-100 protein and the MHC class II antigen HLA-DR and they found that in these 3 cases, many of the FS cells co-expressed both epitopes. These cells that could not be morphologically distinguished from the FS cells negative for the MHC class II antigen HLA-DR were distributed both between tumoral acini and alongside intratumoral vessels, mingling with the T lymphocytes [9]. CD1a (as a marker for Langerhans' cells) and CD21 (as a marker for follicular dendritic cells of lymphoid type), as well as cytokeratins, tested negative. Moreover, no FS cell co-expressing S-100 protein and HLA-DR in the peritumoral tissue in these 3 cases, neither in the tumoral tissue of other 48 cases of pituitary tumors lacking an inflammatory reaction. The scientists postulated that an appropriate inflammatory TME may induce an FS cell subset to adopt a dendritic cell-like phenotype and that these cells may have an antigen presentation function in pituitary tumors [11].

To understand the mutual interactions between the tumor and the peritumoral tissue during tumor progression, Farnoud et al. looked at the boundary between the tumor and the adjacent normal anterior pituitary tissue in a series of 18 pituitary tumors [12]. Researchers reported the presence of a transition zone between the tumor and the peritumoral tissue, with a modified architecture. Intriguing, they observed that density of the FS cells tends to be higher in the transition zone and its vicinity compared to the density observed in the tumor center or in the normal pituitary tissue distant from the tumor. In addition, alterations of the basement membrane were observed in the peritumoral tissue adjacent to this transition zone [13].

In 2000, the first FS cell line derived from a human gonadotroph pituitary tumor was established and named PDFS, for pituitary-derived FS cells. PDFS were demonstrated to show an epithelial-like morphology and to express S-100 protein and vimentin. The presence of FS cells in the TME of pituitary tumors and their association with clinical traits support their potential role in tumor genesis related processes, but a better understanding of their heterogeneity and their functions in tumors is still needed before we can assess their potential use as a therapeutic target [9].

Another study is focusing on the development of the folliculo-stellate cells in human fetal pituitaries [5]. They also have been investigated by immunohistochemical methods for S-100 protein and glial fibrillary acid protein. S-100 positivity was first observed in pars intermedia cells in a 13-

week fetus. Staining with this antiserum is seen in cells of the pars distalis after 15 weeks. Glial fibrillary acid protein was not apparent until 18 weeks, when only cells in the pars intermedia were stained. These cells were not seen in the pars distalis before 28 weeks' gestation, but were present in a 39-week specimen and in a 5-day old baby. In most pituitaries examined, cells staining for S-100 and glial fibrillary acid protein were more concentrated in the pars intermedia than the pars distalis. These results suggest that folliculo-stellate cells in the human pituitary originate in the neurally associated facet of the pars intermedia and pass through this lobe to reach the pars distalis. Since these cells stain for glial related antigens, they may be a modified form of glial cells and arise in the neuroectoderm. Evidence for this hypothesis is given by a lack of both S-100 and glial fibrillary acid protein in the pituitaries of 3 anencephalic pituitaries. Differences in the timing of S-100 and glial fibrillary acid protein immunoreactivity may be related to developmental aspects of the folliculo-stellate cell, or to the presence of two distinct cell types [14].

Conclusions

New studies about the origin and diferentiation of FSCs will provide answers about physiopathology of the pituitary gland. The immunohistochemical localization of S-100 protein, glial fibrillary acidic protein, and vimentin constitutes represent an easy method for investigating follicular-stelated cells presence and distribution in the normal pituitary gland and in pituitary adenomas. Expression of glial cell markers supports the hypothesis of a neuroectodermal origin of FS cells, still, most evidence supports the idea that they derive from the epithelium of the Rathke's pouch. The elucidation of morphological features of folliculo-stellate cells will have a predictive role for the early identification of pituitary microadenomas. Further research using immunohistochemical markers may clarify the origin and the role of this mysterious cell type in the normal and pathological pituitary glands.

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Author's contributions
EP designed the trial and drafted the first manuscript, interpreted the data and revised the manuscript critically. The author revised and approved the final version of the manuscript.

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