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Vol. 58, No 5
October, 2015**Welcome to the scientific and medical journal
Curierul Medical!**

From its debut in 1958 the journal has striven to support the interests of Moldovan medicine concerning the new concepts of its development. The Editorial Board warmly welcomes both the readers of and the authors for the journal, all those who are enthusiastic in searching the new and more effective ways of solving numerous medicine problems. We hope that those who want to make their contribution into the science of medicine will find our journal helpful and encouraging.

The journal is accredited by the National Council for Accreditation and Attestation. The journal publishes official papers, scientific articles, editorials, clinical studies and cases, lectures, methodological guides, reviews, brief reports and correspondence. The journal welcomes articles in English, Romanian and Russian. The journal editorial policy provides the prompt publication of papers within 12 weeks after receiving them.

**Bine ați venit la revista științifică medicală
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De la prima apariție în 1958, revista susține și dezvoltă noile idei în domeniul medicinei, în Republica Moldova. Colegiul de redacție agreează cu multă considerație atât cititorii cât și autorii articolelor, pe toți acei care cu mult entuziasm caută noi și mult mai efective metode de soluționare a multiplelor probleme ale medicinei. Sperăm, că toți acei care doresc să-și aducă aportul la dezvoltarea științelor medicale, vor găsi revista noastră utilă și atractivă.

Revista este acreditată de către Consiliul Național de Acreditare și Atestare. Revista publică comunicări oficiale și, totodată, sunt editate diverse publicații, inclusiv independente: articole științifice, editoriale, cercetări și prezentări de cazuri clinice, prelegeri, îndrumări metodice, articole de sinteză, relatări scurte, corespondențe și recenzii. Revista publică articole în limba engleză, română și rusă. Politica de editare a revistei prevede examinarea operativă și publicarea articolelor timp de 12 săptămâni după înaintare.

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Curierul Medical!**

С первого дня своего выпуска в 1958 году журнал стремится поддерживать и развивать новые идеи в области медицины в Молдове. Редакционная коллегия всегда рада как читателям, так и авторам статей, всем тем, кто с энтузиазмом ищет новые, более эффективные способы решения многочисленных задач медицины. Мы надеемся, что все те, кто хотят внести свой вклад в медицинскую науку, найдут наш журнал полезным и вдохновляющим.

Журнал аккредитован Высшей Аттестационной Комиссией Республики Молдова. В журнале печатаются официальные материалы, научные статьи, наблюдения из клинической практики, обобщающие статьи, краткие сообщения, методические указания, рецензии и корреспонденция. В журнале публикуются статьи на английском, румынском и русском языках. Издательская политика журнала предусматривает оперативное рассмотрение и публикацию статей в среднем в течение 12 недель после поступления.

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RESEARCH STUDIES

The treatment of donor areas in patients with burns using preparations based on azo compounds

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Abstract

Background: The purpose of this work is to develop a new method of treatment of the skin donor areas without using a gauze dressing and, at the same time, isolating the affected area to prevent the infections and reduce to minimum the pain-causing factor.

Material and methods: For this purpose, there have been conducted studies using the preparations based on azo compounds as such azo compounds have the azo-group (-N = N-) in their molecular structure and have the property to react with the proteins forming azoproteins which further transform into a condensed substance. On the surface of skin they transformed into a membrane that covers tightly the area and isolates it from the environment. The studies were conducted in 222 patients with 1548 donor areas. Among them, 84 patients were children under the age of 14 and 138 adults.

Results: The results obtained have shown that the preparations based on azo compounds by entering into reaction with the proteins, contained in the wound, absorb all the discharges from the wound, become film rapidly, cement the microflora, avoiding its action, and fix onto the wound, isolating it from the surrounding environment.

Conclusions: 1. The preparations based on azo compounds are of great importance for the treatment of donor areas. 2. The membrane formed on the surface of the wound completely isolates the wound from the surrounding environment. 3. Following the application of the preparations based on azo compounds the pain reduces and this is extremely important for the treatment of burnt children, for whom the common treatment is affective.

Key words: preparations based on azo compounds, azoproteins, microflora, pain-causing factor.

Introduction

It is well known that the basic skin layer represents the source of epithelisation of the donor area. But, in case of any complications such as the action of microflora or resulting from the surgical technique, the epithelisation of the donor area is slow and extended. The dressing applied on the donor area causes much pain every time when changed and it is unendurable, especially for children. Therewith, we bear considerable costs for the dressing material.

The purpose of this work was to replace the gauze dressing with another type of dressing that would close tightly the wound, isolate the wound from the surrounding environment, block the action of microflora and exclude the pain.

With this in view, we used the preparations based on azo compounds for the treatment of donor areas.

Material and methods

Azo compounds are chemical substances included in the group of dyes having the azo group (-N = N-) in their molecular structure. They have the property to react with the proteins forming azoproteins which in turn quickly contract into a compact state.

The treatment was performed on 1548 donor areas of 222 patients (tab.1).

Results and discussions

In 7 (3.2%) cases out of the total number of patients complications appeared on the donor areas. The main cause of such complications was the fact that the patients had shower and washed off a part of preparation from the donor area.

After removing the skin graft, a gauze layer is applied on the surface of the donor area serving as a framing for the membrane formation on this surface. The azo compound 5% solution is then applied to the entire surface of the donor area using the forceps with a pad (fig. 1, 2).

The membrane on the surface of the donor area becomes firm, compact and well-fixed on the wound during 16-24 hours, depending on the temperature of the surrounding environment. The optimal temperature for membrane formation is 26°. The formed membrane causes no discomfort to the patient. In such conditions, children play and have a normal behaviour. The membrane formed on the surface of the donor area is firm and has a grey colour (fig. 3).

On the 9th-12th day after the surgery, when the surface of the donor area is epithelised, a layer of Vaseline is applied (fig. 4).

On the second day, the membrane is easily removed with a forceps (fig. 5). It becomes elastic due to the ointment action.

Knowing the length of time when the donor area epithelises, depending on the thickness of the graft, we can easily schedule the day of the membrane removal. In our

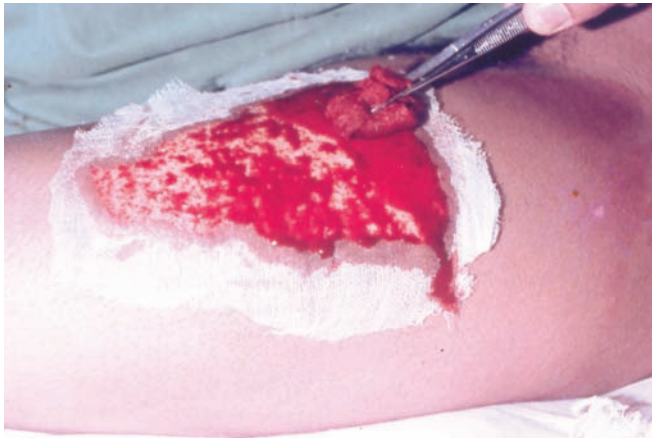


Fig. 1. Application of the azo compound solution.

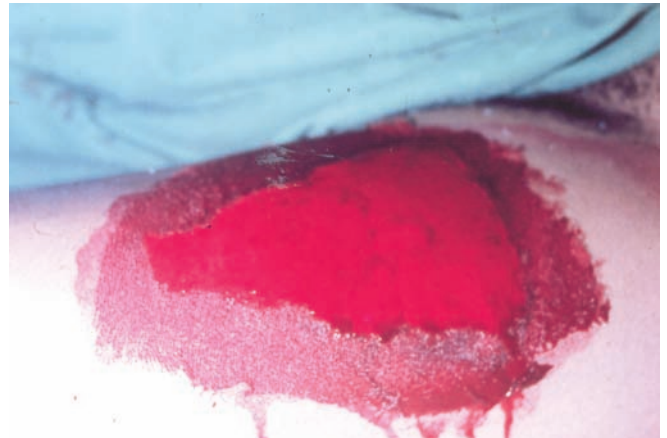


Fig. 2. Image of the complete coverage of the donor area.



Fig. 3. The azo compound membrane on the surface of the donor area.



Fig. 4. Membrane surface covered with Vaseline.



Fig. 5. Membrane removal.



Fig. 6. The patient Maria P. on the 8th day after the trauma.

Table 1

Results of the treatment of donor areas using preparations based on azo compounds

Affected surface	No. of patients	No. of surgeries	No. of donor areas	Mean epithelisation time	No. of donor areas with complication
up to 5% >	39	39	87	9	-
6-15	87	89	348	11	-
16-25	47	78	468	13	-
26-35	22	48	240	13	2 0.8%
36-45	11	34	217	15	3 (1.4%)
46-60	13	20	116	14	1 0.9%
60 and > %	3	15	72	15	1 1.3%
	222	323	1548	13	7 (3.2%)/(0.4%)

Note: 3.2% - as related to the number of patients, 0.4% - as related to the number of donor areas.

observations, 151 (68,4%) patients had been removed the membrane on the 9th-11th day after the surgery and only 71 (31,6%) patients had been removed the membrane after the 15th day after the surgery.

Example

The patient Maria P., 26 years old, medical record no. 373923, was admitted with I-II-IIIAB and IV degree burns on the face, neck, thorax, and abdomen and on the upper limbs. The affected surface constituted 46% of the body surface (IIIB-IV – 35%). The first stage of skin plasty was performed on the 15th day after the trauma, after 7 days – the second and, soon, the third stage of skin plasty were performed. She was discharged 59 days after the trauma (fig. 6).

The membrane formed on the surface of the donor area reduces the plasmorhea, reduces the pain to minimum and makes it possible to reduce the time interval between the surgeries. It also offers the possibility not to apply the dressing on the donor area of the patient during the treatment.

This method offers a great advantage for the treatment of burnt children.

Conclusions

1. Azo compounds have a very important role in the treatment of donor areas.
2. Using azo compounds in burns treatment of donor areas allows avoiding pain.

3. Preparations based on azo compounds used for the treatment of donor areas block the action of microflora in the wound.

4. Preparations based on azo compounds have a great advantage for the treatment of donor areas of the children.

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Evaluation of consumption in defined daily doses of antimicrobial drugs for systemic use in hospitals

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Abstract

Background: Defined daily doses remain as the most objective factor and can be considered as the cornerstone for making decision on determining the needs and organization of rational use of medicines, as well as for providing in practices suggestion of WHO Global Strategy that defines the use of antimicrobials in hospital treatment of patients.

Material and methods: For this study we used data of 2009-2014 period, in the Emergency Medicine Institute of the Republic of Moldova which shows the dynamics of consumption of medicinal remedies of pharmaco-therapeutic group J – Antibacterials for systemic use of the Anatomical Therapeutical Chemical World Health Organization's classification.

Results: Total annual consumption of drugs group J – Antibacterials for systemic use in the evaluated period records a decrease from 662.2 DDD/1000 in 2009 to 464.1 in 2014 or by 29.9%, of which for parenteral use from 568.9 in 2009 to 346.9 or by 39% and for oral use from 93.28 to 117.20 or an increase by 25.6%. From total consumption the ratio between parenteral and oral use in the evaluated period varied from 85.9/14.1 to 74.8/25.2 percentage.

Conclusions: The obtained data reveals that consumption of drugs group J – Antibacterials for systemic use in the evaluated period decreased significantly and in 2014 recorded a mean consumption of European hospitals.

Key words: antibiotics, consumption, defined daily doses, occupied-bed days, rational use.

Introduction

We can find more international, regional and national programs [1-8] aimed to survey the usage of antibiotics, involving dozens of countries which have thousands of hospitals and other public health institutions. The article aims at collating and evaluating data on antimicrobial or antibacterials (antibiotics) usage in the Emergency Medicine Institute over a certain time, enabling monitoring of trends in use of this group of drugs based on the Anatomical Therapeutical Chemical (ATC) World Health Organization's (WHO) classification system standards. Present research covers antibiotics utilization data, as a rate based on defined daily doses, enables reporting and comparison of total-hospital usage for the period from January 2009 to January 2014. This report provides data which could be used to target particular areas of antibiotics' usage. At the hospital level the usage trends is a parameter for identifying overall changes in anti-infective treatment practices.

Emergency Medicine Institute of the Republic of Moldova (EMI), was founded in 1959. Clinical Services of EMI include: Orthopedic-Traumatology Clinic for 150 beds, Surgery Clinic for 140 beds, Neurosurgery Clinic for 80 beds, Neurology Clinic for 70 beds, Maxillo-facial clinic for 30 beds, Urology Clinic for 40 beds, Gynecology Clinic for 30 beds, Microsurgery Clinic for 30 beds, Municipal Center with 8 seats hemodialysis and 9 beds, Clinical intensive care units (CICU) for 30 beds, in total the above clinical services of the institution include 600 beds, also are included 5 emergency medical help substations and 4 out-patient departments of traumatology and orthopedics [9].

The primary aim of the study was to evaluate institutional representative data on antibiotics utilization for a period of six years (2009-2014), according to WHO requirements to

determine the value of defined daily doses (DDD) per 1000 occupied-bed days (OBD) and compare these data with the results of the use of antibiotics in hospitals from other countries. Based on obtained data it aimed to make conclusions on the consumption of antibiotics in hospitals for ensuring the optimization of planning needs and their rational use.

To determine DDD and compare the consumption of antibiotics for the period of 2009-2014, the statistics data concerning the number of treated patients, the number of occupied bed/days and total annual quantities of antibiotics were used. The total number of occupied bed/days in the institution was 188762 in 2009, 191556 in 2010, 186246 in 2011, 199816 in 2012, 193019 in 2013 and 187558 in 2014 [10, 11, 12].

Antimicrobial data are aggregated over the time period of interest at hospital level and converted to standardized usage route based on the WHO definition of DDD with 1000 OBD as the denominator [13, 14, 15].

Material and methods

For this study we used data on a six-year (2009-2014) period in EMI, which show the dynamics of consumption of medicinal remedies of pharmaco-therapeutic group J – Antibacterials for systemic use, as classified ATC, classification system of World Health Organization indicating the nature value. Statistical, analytical, mathematical, comparative, logical and descriptive methods of study were used.

Results and discussion

To determine DDD and compare the consumption of antibiotics for the period of 2009-2014, the statistics data concerning the number of treated patients (only patients with health insurance and other free treated by the state categories

of citizens), the number of occupied bed/days and total annual quantities of medicines were used.

In figure 1 are demonstrated the total (parenteral and oral forms) antibiotic groups use rates of DDD/1000 OBD in EMI. The average aggregate annual rate for total-hospital antibiotics utilization period decreased from 662.4 DDD/1000 OBD in 2009 to 464.1 DDD/1000 OBD in 2014, or by 29, 9%. Near every group of antibiotics is shown the usage rate for 2014 and the yearly trends for the evaluation period in comparison with 2009.

Annual usage rate data, aggregated by year and therapeutic group, for six years from January 2009 to January 2014, demonstrated the increase in usage rates for tetracyclines by 253.7% (from 8.2 to 20.8 DDD/1000), amphenicols by 160% (from 0.5 to 0.8 DDD/1000), other beta-lactam antibiotics by 100.7% (from 270.8 to 272.6 DDD/1000) and antimycotics for systemic use by 178.5% (from 12.1 to 21.6 DDD/1000). A decline in usage rates was recorded for beta-lactam antibiotics, penicillin by 75.6% (from 85.5 to 20.8 DDD/1000), sulfonamides and trimethoprim by 100% (from 5.7 to 0 DDD/1000), macrolides and lincosamides by 96.6% (from 44.4 to 1.5 DDD/1000), aminoglycoside antibiotics by 47.4% (from 83.1 to 43.7 DDD/1000), quinolone antibiotics by 49.2% (from 91.0 to 46.2 DDD/1000) and other antibiotics by 40.9% (from 61.1 to 36.1 DDD/1000).

In figure 2 are presented trends of usage rates DDD/1000 OBD of antibiotic groups (parenteral forms) in EMI. The average consumption annual rate in the evaluation period recorded a decline from 568.9 DDD/1000 OBD in 2009 to 346.9 DDD/1000 OBD in 2014 or by 39%. Near every grupe of antibiotics is shown usage rate for 2014 and the yearly trends for the evaluation period in comparison with 2009.

During the evaluated period was registered an increased usage of amphenicols by 160% (from 0.5 to 0.8 DDD/1000) and antimycotics for systemic usage by 180% (from 0.5 to 0.9 DDD/1000). A decline in usage rates was recorded for beta-lactam antibiotics by 95.1% (from 79.9 to 3.9 DDD/1000), other beta-lactam antibiotics by 7.9% (from 268.5 to 247.3 DDD/1000), macrolides, lincosamides and streptogramins by 98% (from 43.7 to 0.9 DDD/1000), aminoglycoside for systemic by 47.4% (from 83.1 to 43.7 DDD/1000), quinolone antibiotics by 65.7% (from 41.7 to 14.3 DDD/1000) and other antibiotics by 31% (from 50.9 to 35.1 DDD/1000).

In figure 3 are presented trends in groups of antibiotics for oral use in EMI in the evaluation period. The average consumption annual rate of antibiotics for oral usage increased from 93.28 DDD/1000 OBD in 2009 to 117.2 DDD/1000 OBD in 2014 or by 125.7%. Near every group of antibiotics is shown usage rate for 2014 and the yearly trends for the evaluation period in comparison with 2009.

As we can see from fig. 3 in the consumption of oral forms of antibiotics in the evaluated period was registered an increased usage for tetracyclines by 253.7% (from 8.2 to 20.8 DDD/1000), beta-lactam antibiotics by 301.8% (from 5.6 to 16.9 DDD/1000), other beta-lactam antibiotics by 11.5 times (from 2.2 to 25.3 DDD/1000) and antimycotics for systemic use by 180% (from 11.5 to 20.7 DDD/1000). A decline in

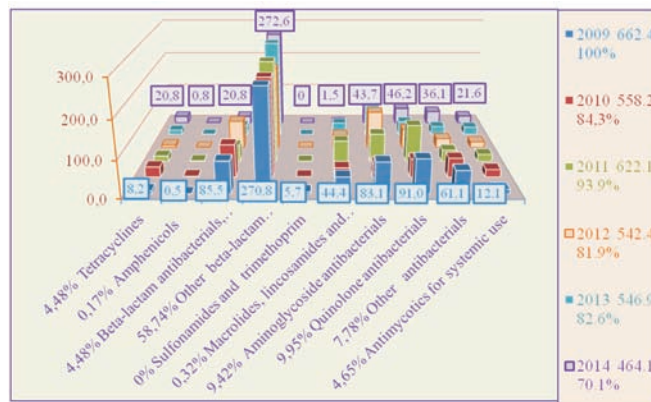


Fig. 1. Total antibiotics usage rates DDD/1000 OBD in 2009-2014 (parenteral and oral forms).

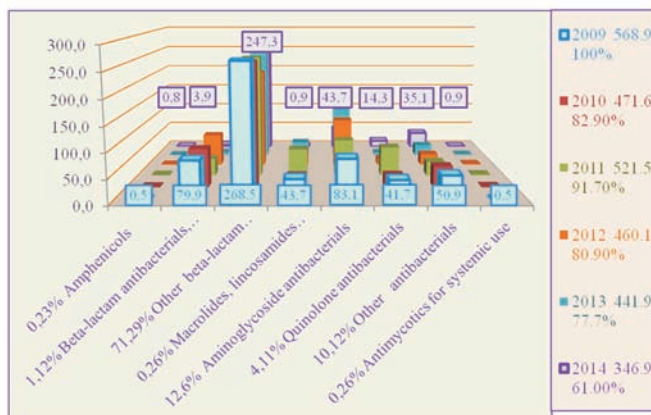


Fig. 2. Total antibiotics usage rates DDD/1000 OBD in 2009-2014 (parenteral forms).

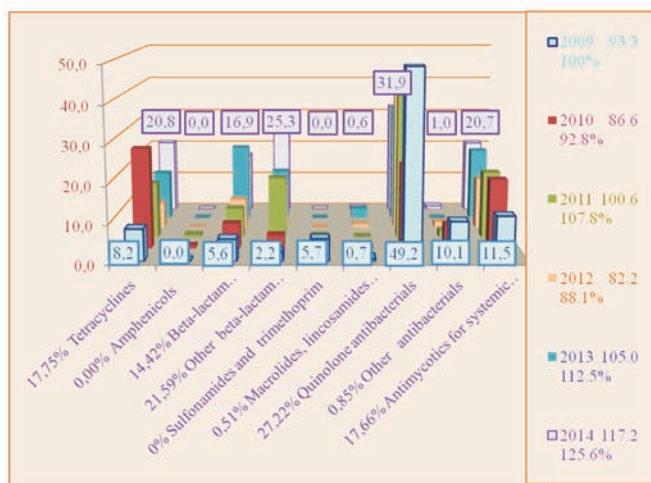


Fig. 3. Total antibiotics usage rates DDD/1000 OBD in 2009-2014 (oral usage).

usage rates was recorded for sulfonamides and trimetoprim by 100% (from 5.0 to 0 DDD/1000), macrolides, lincosamides and streptogramins by 14.2% (from 0.7 to 0.6 DDD/1000), quinolone antibiotics by 35.2% (from 49.2 to 31.9 DDD/1000), other antibiotics by 90.1% (from 10.1 to 1.0 DDD/1000). The annual trends of consumption of antibiotics for parenteral and oral use are presented in table 1.

From the data presented in table 1 we can state that the usage trends of antibiotics for parenteral and oral use during

Table 1

Trends of antibiotics for parenteral and oral usage in NSPCEM in 2009-2014

Route/years	2009		2010		2011		2012		2013		2014	
TOTAL parenteral	568.9	85.9%	471.6	84.5%	521.5	78.7%	460.1	84.8%	441.9	80.8%	346.9	74.8%
TOTAL oral	93.28	14.1%	86.6	15.5%	100.6	21.3%	82.25	15.2%	105.0	19.2%	117.2	25.2%
TOTAL	662.2	100%	558.2	84.3%	622.1	93.9%	542.4	81.9%	546.9	82.6%	464.1	70.1%

Table 2

Percentage usage trends of DDD/1000 OBD per day of antibiotics group ATC J01 between the EMI of the Republic of Moldova and seven European countries

Country/ Antibacterial groups	EMI of RM	Bulgaria	Ireland	Estonia	Lithuania	Latvia	Sweden	Finland
Tetracyclines	4.37	1.42	1.12	4.44	2.5	3.0	12.01	7.5
Beta-lactam Penicilins	4.48	20.0	9.44	32.77	24.16	30.33	50.66	18.57
Other Beta-lactam antibiotics	58.74	51.44	49.44	26.11	22.	37.66	13.33	32.5
Macrolides Lincosamides and streptogramins	0.32	7.85	14.44	10.55	2.5	4.33	4.0	5.35
Quinolone antibiotics	9.95	7.85	7.77	10.55	6.25	11.66	10.66	12.14
Other antibiotics	7.78	14.28	14.44	14.15	40.41	10.66	7.33	20.71

the evaluated period, recorded for parenteral use a decline by 11.1 % (85.9% - 74.8%), and for oral use respectively an increase by the same 11.1% (25.2%-14.1%).

The percentage usage trends of DDD/1000 OBD per day of antibiotics group J – Antibacterials (ATC J01) for systemic use of the EMI of Republic of Moldova and other seven countries from the European Union, such as: Bulgaria, Ireland, Estonia, Lithuania, Latvia, Sweden and Finland are presented in table 2.

Utilizing the DDD/1000 OBD per day data [15, 16], we have calculated the percentage usage trends for the large antibiotics groups ATC J01 of the EMI and seven countries from European Union. The results demonstrate that the average proportion of consumption in seven countries from

European Union and EMI are for tetracyclines 4.37:4.6, beta-lactam antibiotics 26.6:4.48, other beta-antibiotics 58.47:33.3, macrolides, lincosamides and streptogramins 7:0.32, quinolone antibiotics 9.6:9.95, other antibiotics 15.4:7.78.

In table 3 is presented total-hospital usage rates of DDD/1000 OBD of antimicrobials between the EMI and ten international researches with the data from more than 2000 hospitals of European countries.

The results show that in ten international researches [4, 20, 21, 22, 23, 24] the medium minimal consumption of DDD/1000 is 433.77 and medium maximal – 499.31 units. So, recorded consumption in EMI of 464.1 DDD/1000 units of antibiotics in 2014 in comparison with the presented below results is intermediate.

Table 3

Comparison of total-hospital usage rates of DDD/1000 OBD of antibiotics between the EMI and ten international researches

Hospitals	DDD/1000	% consumption
EMI of the Republic of Moldova	464.1	464.1 = 100%
34 public/43 private hospitals in France [17]	395 - 422	85.11% - 90.93%
Antibiotic use in 530 French hospitals [18]	62.3 - 557.7	13.42%-120.17%
University Hospital of Geneva [19]	400	86.62%
Besancon University Hospital in France [20]	535.4	115.36%
74 south-western French hospitals [21]	400 - 450	86.19% - 96.96%
University Medical Center Rotterdam The Netherlands [22]	547	117.86%
1115 hospitals in France [23]	370 - 393	79.72% - 84.68%
University Hospital Huddinge, Sweden [24]	430	92.65%
139 hospitals from 30 European countries [25]	496	106.87%
All hospitals in Netherlands [4]	702	151.26%

Conclusions

1. The average aggregate annual rate for total-institutional antibiotics utilization decreased from 662.4 in 2009 to 464.1 DDD/1000 OBD in 2014, or by 29.9%. Of which a significant decline in usage rates was recorded for beta-lactam antibiotics, penicillin by 75.6% (from 85.5 to 20.8 DDD/1000), macrolides and lincosamides by 96.6% (from 44.4 to 1.5 DDD/1000), aminoglycoside antibiotics by 47.4% (from 83.1 to 43.7 DDD/1000), quinolone antibiotics by 49.2% (from 91.0 to 46.2 DDD/1000) and other antibiotics by 40.9% (from 61.1 to 36.1 DDD/1000). At the same time an increase in usage rates was recorded for tetracyclines by 253.7% (from 8.2 to 20.8 DDD/1000) and antimycotics for systemic use by 178.5% (from 12.1 to 21.6 DDD/1000).

2. For parenteral form of use the consumption annual rate in the evaluation period recorded a decline from 568.9 DDD/1000 OBD in 2009 to 346.9 DDD/1000 OBD in 2014 or by 39%. Of which a significant decline in usage rates was recorded for beta-lactam antibiotics by 95.1% (from 79.9 to 3.9 DDD/1000) lincosamides and streptogramins by 98% (from 43.7 to 0.9 DDD/1000), aminoglycoside for systemic use by 47.4% (from 83.1 to 43.7 DDD/1000), quinolone antibiotics by 65.7% (from 41.7 to 14.3 DDD/1000) and other antibiotics by 31% (from 50.9 to 35.1 DDD/1000).

3. For oral form of use the consumption annual rate recorded an increase from 93.28 DDD/1000 OBD in 2009 to 117.2 DDD/1000 OBD in 2014 or by 125.7%, of which more significantly for tetracyclines by 253.7% (from 8.2 to 20.8 DDD/1000), beta-lactam antibiotics by 301.8% (from 5.6 to 16.9 DDD/1000), other beta-lactam antibiotics by 11.5 times (from 2.2 to 25.3 DDD/1000) and antimycotics for systemic use by 180% (from 11.5 to 20.7 DDD/1000). At the same time a decline in usage rates was recorded for sulfonamides and trimetoprim by 100% (from 5.0 to 0 DDD/1000), quinolone antibiotics by 35.2% (from 49.2 to 31.9 DDD/1000) and other antibiotics by 90.1% (from 10.1 to 1.0 DDD/1000).

4. The average proportions of consumption of main groups of antibiotics in seven countries from the European Union and EMI are for tetracyclines 4.37:4.6, beta-lactam antibiotics 26.6:4.48, other beta-antibiotics 58.47:33.3, macrolides, lincosamides and streptogramins 7:0.32, quinolone antibiotics 9.6:9.95, other antibiotics 15.4:7.78.

5. The recorded consumption of 464.1 DDD/1000 units of antibiotics in EMI in 2014 in comparison with medium minimal consumption of 433.77 and medium maximal of 499.31 units of DDD/1000 from ten international researches is intermediate. This is a result of six years efforts for rational use of antibiotics since 2009 when consumption of antimicrobials DDD/1000 in EMI was 662.2 units or by 132.7% more than medium maximal in European countries.

6. One of the most evident results of the present research is a clear demonstration that today implementation of DDD program consumption study of antibiotics in all hospitals of health system of the Republic of Moldova is indubitable necessity for qualitative determination of annual planning of necessary drugs and their rational use organization.

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Optimization of orthodontic treatment for children with Angle class III malocclusion by determining the influence of blink-reflex indices

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Abstract

Background: The actual problem is developing the neurophysiological criteria for the diagnosis and monitoring of children with Angle class III malocclusion, because in this pathology there is a wide range of preclinical disorders with adverse influences upon various functions of the body. The interdisciplinary approach is supplemented by neurophysiological diagnosis, which facilitates a correct monitoring of orthodontic treatment during the mixed dentition stage. In recent years, neurophysiological methods gained a wider spread in the diagnosis and monitoring of children with Angle class III malocclusion. Aim of the study: assessing the correlations between the blink reflex indices and the degree of anxiety or anticipated pain.

Material and methods: Fifty eight children aged from 9-12 years, who were divided into 2 groups, statistically equivalent by age, gender, facial development degree, presence of a balanced psycho-emotional state. Patients in both groups were examined using traditional clinical exams and complementary exams were completed through neuro-physiological examination of the blink reflex. In our studies, we performed a correlative analysis between the R3 indices (presence/absence), the degree of dental anxiety, assessed on the Dental Anxiety Scale according to N. Corah and pain self-assessment indices based on the Visual Analogue Scale (VAS).

Results: In children with Angle class III malocclusion, in the blink reflex complex, in 31% of cases the R3 wave is present, which is associated with psycho-emotional mechanisms of inhibition/activation for anticipated pain.

Conclusions: Perspective future research in children with Angle class III malocclusion needs to be focused on uncovering the correlation between the expression degrees of trigeminal reflexes with the functional disorders in the stomatognathic and extra-stomatognathic systems (vestibular functions, pain syndromes, etc.)

Key words: malocclusion, blink reflex, interdisciplinary approach.

Introduction

The diverse etiopathogenesis of Angle class III malocclusion requires an interdisciplinary approach in the diagnosis and treatment of this orthodontic disorder. From recent scientific sources, it can be observed that the interdisciplinary approach is supplemented by neurophysiological diagnosis, which facilitates a correct monitoring of orthodontic treatment during the mixed dentition stage. In recent years, neurophysiological methods gained a wider spread in the diagnosis and monitoring of children with Angle class III malocclusion. It has been demonstrated that many of the clinical manifestations of this pathology are associated with the activity of the trigeminal system [15], but it is still unknown how the activity of this system changes in accordance with disease severity, applied therapeutic methods, etc. Currently, research is being conducted on trigeminal reflexes in order to individualize the orthodontic treatment [13]. This aspect of the problem is perspective because clinical manifestations in children with Angle class III malocclusion (orthodontic pain, disadaptive functional disorders in the muscle activity, etc.) are associated with the trigeminal system activity [6]. It is essential to develop comprehensive diagnostic methods (clinical and neurophysiological), due to the fact that an efficient orthodontic treatment of Angle class III malocclusion will not be limited only to the clinical manifestations at the occlusion level. Therefore, as a working hypothesis for this study, we conceived that the realization of a complex diagnosis in children with Angle Class III malocclusion, by associating orthodontic and neurophysiological methods may lead to an increased efficiency of occlusal rehabilitation, by individual-

lizing the effect on the stomatognathic system.

Aim of the study: assessing the correlations between the blink reflex indices and the degree of anxiety or anticipated pain.

In order to accomplish the proposed objectives there were applied modern scientific methods of neurophysiological research with corresponding statistical processing. There were applied neurophysiological investigation methods of the brainstem structures, trigeminal reflexes and of the muscle adaptive potential for the stomatognathic system muscles [11].

Specific for the orthodontic pathology is that it creates different pathogenic conditions for the initiation of muscle dysfunction; tooth movement during orthodontic treatment process is causing not only pain, but also significant changes in the muscles of the stomatognathic system. Different variations of occlusal pathology are associated with specific changes not only in the muscles of the stomatognathic system, but also in different muscle groups at distant sites (muscles of the neck, trunk, arms and legs) [2,8].

The start of neuromuscular era in orthodontics is based on extending the knowledge about the interaction of the trigeminal somatosensory afferentation with the cerebral structures involved in the motor activities, including the stomatognathic system (chewing, etc.) [15].

It has been shown that there are two trigeminal nerve areas of greater importance in the brainstem area for the afferentation of oral and temporomandibular joint areas: the first area is situated between Vi/Vc (*nucleus interpolaris* and *nucleus caudalis*); the second one, Vc/C_{1,2} – between *nucleus caudalis* and upper cervical dorsal horns [3,4]. It was assumed

that these areas have different functional roles in the painful and inflammatory phenomena, associated with the trigeminal nerve and the oromaxillofacial area. In experiments on animals, it has been shown that during temporomandibular joint inflammation, the neurons from Vc/C_{1,2} activate, subsequently the impulses ascend with a modulatory effect on the intermediate zone of Vi/Vc [17]. In other experiments, it was also demonstrated that morphine injection in the Vc/C₁ area has altered the evoked potentials by 30% from the Vi/Vc area [14]. Important for orthodontics is that the nociceptive afferentation in the oromaxillofacial area, activates mainly the Vi/Vc area.

Trigeminal system is quite complex in its structure and function. This system is involved in the pathogenesis of various diseases (dental, neurological, neurosurgical, vertebroneurological, etc.). In each of these domains, different aspects of the system are being studied. One of the trigeminal reflexes that has a wider application is the blink reflex. The blink reflex indices are used to differentiate the treatment for pain syndromes related to the oral cavity. Blink reflex, through the stimulation of *n. alveolaris inferior*, has a predictive importance for various sensory disorders of the jaw region or for sensory disturbances related to the orthodontic treatment [10].

The R1 component of blink reflex is achieved through the A_β fibers that activate low threshold mechanoreceptive (LTM) neurons, probably located in the main trigeminal sensory nucleus. The R2 component appears to stimulate both A_β fibers and A_δ fibers with the input being in the wide-dynamic range (WDR) neurons, located in the spinal trigeminal nucleus. Interneurons of the R1 wave are located in the trigeminal sensory nucleus and the interneurons for R2 wave in the spinal trigeminal nucleus [11]. Nociceptive stimulation activates A_δ fibers and/or C fibers, then excites *subnucleus reticularis dorsalis* (SRD) neurons with the occurrence of the R3 component. This mechanism is partially involved the R2 component generation. The R3 component has been less studied, its generating mechanisms being still unclear. Unlike R3, the R2 component of the blink-reflex has been quite well researched, currently the neural networks involved in its generation are known; it can be reproduced and can be generated by different stimuli (electrical, thermal, etc.). The R2 component is being used widely in various experimental and clinical studies. It is important that the R1 and R2 indices make it possible to distinguish the non-nociceptive components from the nociceptive ones in the trigeminal system. Facilitation of the R2 component can be observed in migraine attacks, pain related to damage of the trigeminal system, orthodontic pain. Promising results were obtained by applying the blink reflex to differentiate atypical odontalgia.

Temporomandibular joint dysfunction in association with painful syndrome manifests itself by a decrease of the latencies for the R1 component ($p < 0.05$) and for the ipsilateral and contralateral R2 component ($p < 0.0001$) [6,7]. The Wallenberg syndrome changes the R2 wave in 90% of the cases, while wave R1 remains unmodified.

The trigeminal system and the trigeminal reflexes are involved in the pathogenesis of many orofacial pain syndromes. The pain syndrome from the occlusal and temporomandibular disorders, leads to more significant changes in the brainstem

area, in comparison with the somatosensory and motor cortical areas [4].

The study of the trigeminal system and features of the trigeminal reflexes will make the development of new diagnostic and treatment technologies possible for patients with orthodontic disorders, that will include both the influence on peripheral components (peripheral electrical stimulation, etc.) and also direct stimulation of brain structures involved in processing the trigeminal reflexes (magnetic or electrical transcranial stimulation, etc.) The current issue is being important for children especially during the process of development and consolidation of various intra- and extra-stomatognathic systems, regarding the possible impact of the orthodontic pathologies.

Material and methods

In this study, there were included 58 children, aged from 9-12 years, who were divided into 2 groups, statistically equivalent by age, gender, facial development degree, presence of a balanced psycho-emotional state. Patients in both groups were examined using traditional clinical exams and complementary exams were completed through neuro-physiological examination of the blink reflex. Clinical exams were performed at the Department of Pediatric Oromaxillofacial Surgery, Pedodontics and Orthodontics at the "Emilian Coțaga" Clinic and at the dental clinic "Orto-Dental" SRL. Electroneurophysiological investigations were executed at the university clinic Neuronova, under the supervision of university professor, academician V. Lacusta. Modern specialized software was applied [20] in conjunction with the neurophysiological equipment – Neuro-MVP (*Neurosoft*).

The neuro-physiological examination methodology for the blink reflex

Blink reflex is a defense reflex that may be caused by applying various stimuli (mechanical, chemical, photostimulation etc.). To obtain this reflex and to conduct the electro-physiological analysis, the supraorbital region is stimulated, at the points of emergence of the first trigeminal nerve branch (supraorbital foramen). The stimulation parameters were: pulse duration of 0.1-1ms, current intensity of 5-15mA, frequency of 0.1 to 0.4 Hz. The reflex was recorded by placing electrodes in the region of the eye muscles. Blink reflex includes the afferent fibers of the trigeminal nerve (the first branch), the efferent fibers of the facial nerve, the nuclei of these nerves, the neurons from the reticular formation of the brainstem [7].

The reflexive response contains three components (fig. 1): R1 - oligosynaptic ipsilateral response with a latency of 10-14ms (mainly generated in the pons); R2 - polysynaptic bilateral response with a latency of 25-40ms (*tractus spinalis n. trigemini*, reticulate formation in the lower brainstem areas); R3 - polysynaptic bilateral response with a 70-100ms latency (intercalary neurons of the brainstem, the neural structures involved in nociceptive control of the gray matter in the area of the midbrain and of the raphe nuclei 5) (fig. 1).

In our studies, we performed a correlative analysis between the R3 indices (presence/absence), the degree of dental anxiety, assessed on the Dental Anxiety Scale according to N. Corah [4] and pain self-assessment indices based on the Visual Analogue Scale (VAS).

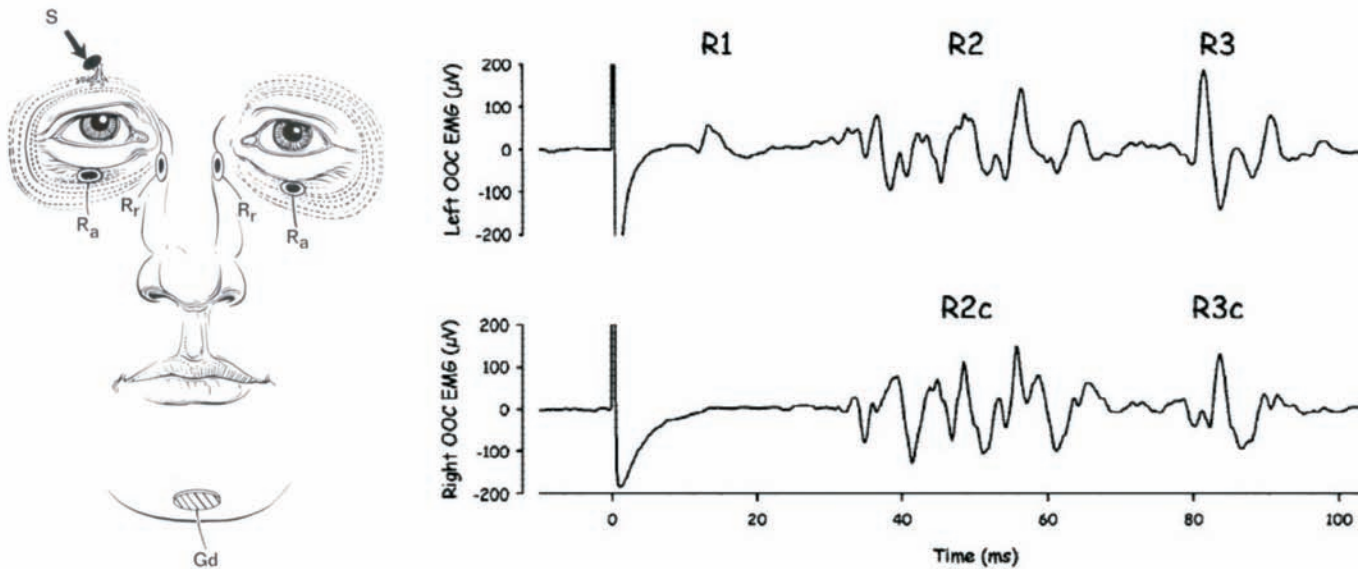


Fig. 1. The R1, R2 and R3 waves of the blink reflex.

Results and discussion

For the analysis of the blink reflex, we have distinguished the R2 component depending on the latency and duration, with a general assessment of the excitability of the involved cerebral structures (tab. 1).

Processing of the blink reflex indices was achieved by assessing the velocity of the nerve impulse conduction in the trigemino-facial system, the analysis of the segmental brainstem apparatus functional status and by revealing the regulatory influences from the suprasegmental brain structures. Latent wave periods are considered pathological when they are higher than 10.82 ± 1.2 ms for R1, 35.25 ± 3.7 ms for ipsilateral R2, 35.36 ± 3.27 ms for contralateral R2 [1].

Different combinations of the blink reflex indices give the possibility of highlighting the affected brain areas [1,6]: superolateral portion of the pons with the impaired sensory fibers of the trigeminal nerve; anterolateral region of the pons with the impaired facial nerve nucleus and its fibers; the dorsolateral/mediolateral/paramedian/medial regions of medulla oblongata, with the affected region located in the trigeminal nerve fibers at the level of pons (*nucleus sensorius principalis*); location in neurons that connect the ipsilateral and contralateral structures.

These dysfunction variants may be revealed in the process of neurological diagnosis, but their activity is closely associated with the stomatognathic system and nowadays new technologies have been developed that make it possible to use the functional diagnosis for neuro-stomatological pathologies.

The analysis of the blink reflex indices (R1 and R2 waves) has shown that in 34 children with Angle Class III maloc-

clusion (tab. 2), there were significant deviations in comparison with healthy children, which constitutes 58.6% of all investigated patients and that in the post-treatment period, pathological variants of these reflexes were found in 20.7% of cases (12 children).

The diagnostic value of pathological changes of the blink reflex in children with Angle class III malocclusion is not high (specificity 58.3%, sensitivity 49.2%), thus as a single diagnostic method (mono-test), it cannot be used for the studied pathology.

The decrease of the latent period in the R1 wave in children with Angle Class III malocclusion may be explained by the functional disorders at the level of the main trigeminal sensory nucleus and in the neurons of pons. On the other hand, the reduction of the latency of R1 and R2, both ipsilateral and contralateral, may be explained by a more pronounced dysfunction of the trigeminal system, which is present in children with Angle class III malocclusion. This dysfunction according to the literature leads to pronounced changes in the brainstem (trigeminal structures, reticulate formation) the disorders being more severe in comparison with those at the level of cortical somatosensory areas [9,19].

Of all investigated children with Angle class III malocclusion, in 31% of cases (18 patients) the R3 wave was present before treatment, while at three years post-treatment, the R3 wave was found only in 3 children (5.2%). This data reflects the neurophysiological nociceptive/antinociceptive mechanisms at the level of the brainstem and of other suprasegmental structures that had notably improved. The latent period of R3 was 84.61 ± 1.67 ms. It needs to be considered that in the literature

Table 1

General assessment of the excitability

Index	Normoexcitability	Hipoexcitability	Hiperexcitability
Latency, ms	$36,9 \pm 0,6$	$39,7 \pm 0,5$	$31,4 \pm 0,7$
Duration, ms	$35,1 \pm 0,5$	$31,2 \pm 0,4$	$49,9 \pm 0,8$

Table 2

Dynamics of electrophysiological indices of the blink reflex in children with Angle class III malocclusion under the influence of treatment

Parameters/Stimulation/ Values in healthy children (n = 18)	Children with Angle Class III malocclusion (n = 58)	
	Investigation period	Latent period, ms
R1 sinistra stimulation 12,13 ± 0,31	Before treatment	9,49 ± 0,23***
	3 years after-treatment	10,8 ± 0,21**xxx
R2, ipsilateral sinistra stimulation 36,85 ± 0,93	Before treatment	32,53 ± 0,84**
	3 years after-treatment	34,60 ± 1,05
R2, contralateral sinistra stimulation 37,21 ± 1,44	Before treatment	33,94 ± 1,52
	3 years after-treatment	38,88 ± 1,46x
R1 dextra stimulation 12,48 ± 0,29	Before treatment	9,58 ± 0,24***
	3 years after-treatment	11,10 ± 0,80x
R2, ipsilateral dextra stimulation 36,72 ± 0,61	Before treatment	33,08 ± 0,82*
	3 years after-treatment	37,55 ± 0,81xxx
R2, contralateral dextra stimulation 37,43 ± 0,71	Before treatment	33,85 ± 1,07
	3 years after-treatment	38,24 ± 0,78xxx

Note: conclusive statistical differences: for patients with Angle Class III malocclusion vs. healthy children: * - p <0.05; ** - P <0.01; *** - P <0.001; before vs. after the treatment: x - p <0.05; xxx - p <0.001.

there is evidence of the link between the intensity of the pain syndrome and the presence of the R3 wave – disappearance of pain is associated with the disappearance of the R3 wave.

The analysis of the blink reflex waves has been conducted based on the patient's age – with an increase in age, an inhibition deficit appears in the brainstem structures, as well as an insufficient activity of the antinociceptive systems, that leads to the reduction or disappearance of the R3 component. The results of the comparative analysis show a higher frequency of the masseter reflex inhibitor (MIR) disorders in comparison with blink reflex disorders. Nevertheless, this argument is not the main one, as each of these reflexes has specific neural mechanisms and they possess diagnostic value in the context of the applied diagnosis or treatment.

From the literature it is known that patients with occlusal disorders are experiencing increased emotionality during the orthodontic treatment (61.6% of cases), due to the lowering of the pain threshold in the oral mucosa region (71.4%).

In our investigations, for children with the R3 wave of the blink reflex (31% of cases) we analyzed self-assessment of anticipated pain (from a few days before the visit at the

doctor until the visit for adjusting orthodontic devices) in comparison with actual pain during the visit and pain experienced in the past (during the last visit at the doctor). The level of anticipated pain (Visual Analogue Scale - VAS) was elevated, which demonstrates a stressful state of anticipation of future visits at the doctor, a fear of possible pain (tab. 3). Some children have rather large pain self-assessment values reaching as high as 7-9 points on the VAS scale. In comparison with the anticipated pain, the real pain showed a statistically significant lower level (p <0.05). The self-assessment of previously experienced pain had lower average values than the self-assessment of anticipated pain (p <0.05).

We performed a comparative analysis of the dental anxiety level, assessed with the aid of Corah Dental Anxiety Scale. In children with R3 wave, the anxiety level has constituted 3.31 ± 0.19 points, and in children without the R3 wave, anxiety had values of 2.37 ± 0.23 – the difference is statistically significant (p <0.01) with greater expression of dental anxiety in the first group. These results demonstrate a connection between the psycho-emotional mechanisms with the processes of R3 wave generation and modulation. In this aspect, of great importance

Table 3

Pain self-assessment by Visual Analogue Scale (VAS) in children with Angle class III malocclusion depending on the presence/absence of the R3 wave of the blink reflex

Patients with Angle Class III malocclusion	Pain self-assessment (VAS)		
	Pain experienced before	Anticipated pain	Real pain
Blink reflex with R3 wave (n = 18)	4,12 ± 0,75+	6,82 ± 1,10*	3,52 ± 1,20+
Blink reflex without R3 wave (n = 40)	4,13 ± 1,96	3,42 ± 0,98+x	3,48 ± 1,81

Note: Statistically significant differences compared to the real pain: * - p <0.05; difference in the group with presence of R3 vs. group without R3 wave: x - p <0.05; difference compared to anticipated pain: + - p <0.05.

is the correlation analysis between the blink reflex waves and the dental anxiety level (tab. 4).

Table 4

Correlation analysis between the blink reflex waves and the dental anxiety level

Rxy: dental anxiety-blink reflex	Rxy	p
R1	0,34	>0,05
R2	0,48	<0,05
R3	0,69	<0,01

The strongest correlations ($p < 0.01$) were found between wave 3 and the level of dental anxiety determined according to Corah Dental Anxiety Scale (fear and feelings of uneasiness, anxiety during the waiting period for the visit at the doctor – for a few days, a few hours, minutes before, anticipation of orthodontic maneuvers while in the dental chair).

As a result of our investigations, it can be concluded that in children with Angle Class III malocclusion, a disorder occurs in the balance of nociceptive/antinociceptive systems with an impact on psycho-emotional processes and on the values of pain self-assessment. According to the literature, the R3 wave is associated with attention focus - if during the investigation of the blink reflex, the patient is prior informed about the stimuli that will be applied, and then the R3 wave is vastly diminishing or even disappears [16]. Williams et al. [21] have shown that for people who have viewed pleasant images (positive emotions) the blink reflex was inhibited and under the influence of unpleasant images (negative emotions); the reflex was facilitated/enhanced. The authors concluded that emotional expression control could influence the blink reflex expression by the intensifying (positive emotions) or diminishing (negative emotions) of the supraspinal inhibition. Serogl et al. [18] observed that orthodontic pain was minimal in people who had self-control of the psycho-emotional state. It has been shown that regarding the experience related to pain, the psychic cerebral matrix is stronger than the pain matrix. Preparing the children with Angle Class III malocclusion before the treatment requires explaining the safeness of the procedures and of the devices to be applied, cultivating a positive emotional attitude and motivation such as to ensure an appropriate patient-physician collaboration. It was shown that such pain-preventing instructions considerably influence the pain intensity.

Our results and literature data make it possible to conclude that in children with Angle class III malocclusion, the fact of identifying the R3 wave in the blink reflex complex will require a special attention concerning assessment of the psycho-emotional state in order to ensure an adequate psychological preparation.

In conclusion, it appears that in the composition of the blink reflex in children with Angle Class III malocclusion, the R3 wave is present in 31% of cases, being associated with psycho-emotional mechanisms of pain inhibition/activation.

Another trigeminal reflex, investigated by us, in children with Angle class III malocclusion was the trigemino-cervical

reflex (TCR). In recent years, particular attention is drawn upon the interaction between the stomatognathic systems with other systems (stomatognathic-extra-stomatognathic interaction). A special role in this aspect has the interaction of the stomatognathic system with the cervical muscles, because the pathologic trigemino-cervical reflexes are present in various neurological and neuro-stomatological disorders. Analyzing the recent literature, we found that the current investigations regarding the trigemino-cervical reflex (TCR) under physiological conditions are performed mainly experimentally on animals without any clinical data. There is a necessity of investigations of this reflex, in particular for children with Angle Class III malocclusion, because in this pathology, various polymorphic disorders occur in the stomatognathic system, as well as in the cervical region (trigemino-vascular disorders, etc.). It is known that disorders of trigemino-cervical system, along with the trigemino-vascular system are one of the main causes for various headaches.

Disorders of the trigemino-cervical neuronal system lead to headaches and migraines [1]. Accordingly revealing the multidirectional pathogenic pathways between the trigeminal structures and the cervical region is a current problem, the solution to which will contribute significantly to optimization of the differential diagnosis and will ensure an adequate treatment, not only of malocclusions, but also for associated pain syndromes. It was established that not only the pathogenic influence from the stomatognathic system is important, but also vice versa – disorders of the cervical region give rise to pain in the jaws and jaw muscles. Some of latency periods of the trigemino-cervical reflex resemble the ones from the masseter inhibitory and blink reflex. This aspect of the problem is being studied experimentally, due to the fact that when there are essential differences between latency periods of the TCR and that of the R1 blink reflex wave, more severe disorders are occurring [12]. Based on experimental studies, trigemino-cervical reflex is considered a sensitive test for neuromuscular, trigemino-cervical disorders. Moreover, using TCR can reveal pathologies that are not diagnosed on EMG.

Conclusions

1. The manifestation frequency of pathological trigeminal reflexes in children with Angle class III malocclusion increases in this order: trigemino-cervical reflex (41.4%) → blink reflex (58.6%) → masseter inhibitory reflex (93.1%); masseter inhibitory reflex has the diagnostic value for children with Angle class III malocclusion of 85.5% (specificity) and 81.2% (sensitivity).

2. Under the influence of the treatment, in children with Angle class III malocclusion, the frequency of manifestation for pathological trigeminal reflexes decreased by 46.7% for the masseter inhibitory reflex, 37.9% for the blink reflex and 8.6% for reflex- trigemino-cervical reflex.

3. In children with Angle class III malocclusion, in the blink reflex complex, in 31% of cases the R3 wave is present, which is associated with psycho-emotional mechanisms of inhibition/activation for anticipated pain.

4. Perspective future research in children with Angle class III malocclusion needs to be focused on uncovering the correlation between the expression degrees of trigeminal

reflexes with the functional disorders in the stomatognathic and extra-stomatognathic systems (vestibular functions, pain syndromes, etc.)

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Duplex ultrasonography in evaluation of complications of portal hypertension in liver cirrhosis

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Abstract

Background: Portal hypertension is a serious complication in liver cirrhosis and it is responsible for most of the deaths at patients with the respective disease. Determination of the pressure gradient by the catheterisation of hepatic veins is a laborious method and often associated with complications. There are necessary accessible methods to evaluate portal hypertension with an acceptable degree of confidence. This study aims to determine Doppler hemodynamic indicators with important predictable goal in case of complications of the portal hypertension associated with liver cirrhosis.

Material and methods: The study group included 111 patients diagnosed clinically, biologically and imagining with liver cirrhosis. The cytolytic, cholestatic, hepatoprive syndromes and the gravity of hypersplenism were evaluated. Esophageal varices were identified by upper endoscopy. The hepatic morphology, including the personalisation of the vascular tree, was evaluated by conventional and duplex ultrasonography.

Results: The increase of the vascular resistance at the level of lienal artery ($r = 0.454$), and the Splenoportal Index had an important correlation with the severity of the Splenomegalic Syndrome ($r = 0.516$). The prediction of the esophageal varices with high risk for haemorrhage was set at the value of 7-10 cm/sec of the time-weighted average velocity measured in the hepatic portal vein (RP+3.13). Ascites and portal thrombosis were more frequent at an index of the portal hypertension of (IHPT) >2.1 (RP+3.5).

Conclusions: Evaluation of the hemodynamic indicators by duplex ultrasonography allowed to highlight the predictive parameters in case of complications of the portal hypertension associated with liver cirrhosis.

Key words: liver cirrhosis, hemodynamic indicators, esophageal varices, duplex ultrasonography.

Introduction

The portal hypertension associated with liver cirrhosis is a clinical hemodynamic syndrome, defined by increased gradient of portal venous pressure above 5 mm/Hg [1,2,4]. The clinical description of this syndrome includes the presence of esophageal varices and bleedings from them, ascites, spontaneous bacterial peritonitis and hepatorenal syndrome, which also are the main causes of death or indications for a liver transplantation at patients with liver cirrhosis [6,11]. The development of the liver cirrhosis gradually leads to increase of the portal pressure and to depression of the hepatic function, which often are associated with major complications in stages of decompensation [8,9,17].

Variceal haemorrhage significantly reduces the survival rate in cirrhotic patients both by consequences of the haemorrhagic shock and by hepatic impairment, thus resulting in polyorganism insufficiency [6,7,10,14,15]. Severe circulatory deficiencies in hepatic circulation, caused by advanced morphological changes, as a result create premises for thrombosis at different levels on the splenoportal axis which alter even more the hepatic function [11,12,16].

At present, ascites continues to be another important problem in the behaviour of patient with liver cirrhosis. The prospect of a successful treatment is when the ascetic syndrome occurred reduces. Complicated hemodynamic changes, which inevitably occur, lead to the irreversible challenged hepatic function [3,4,9,11].

The splenomegaly associated with severe hypersplenism continues to be subject for controversial discussions in aim to correct the haematological disorders, which occur within this syndrome present in the cirrhotic portal hypertension.

Duplex ultrasonography is an accessible, non-radiating imaging method, with an acceptable accuracy as to morphologically evaluate the liver and provides important data regarding the disorders of vascular flow in the hepatolienal system [3,5,13].

The study's goal is to estimate the importance of hemodynamic parameters assessed through the duplex ultrasonography, as well as the connection with the complications associated with the cirrhotic portal hypertension and the determination of velocimetry indicators with predictable importance in order to earlier discover the respective complications.

Material and methods

In 2012-2014, the Hepatology Department of the Clinical Republican University Hospital studied 111 patients diagnosed with cirrhosis of the liver with various aetiologies (mainly of viral origin 97.8%). The average age of selected patients was of 48.4±1.66 years old. From the viewpoint of sex distribution, men 46 (41.0%), women - 65 (59.0%).

The patients had an enhanced clinical and biological evaluation. The cytolytic, cholestatic, hepatoprive syndromes were assessed. Depending on the severity of cytopenia, the hypersplenism was divided into 3 degrees (mild, moderate, and severe). The esophageal varices were diagnosed by upper

endoscopy. According to Shertsinger, they were classified as absent or present, and the severity of varices described in degrees. The evaluation of severity of hemodynamic disorders was studied by duplex ultrasonography, which included the features of the arterial and venous flows in the hepatolienal basin, by assessing the indices of impedance, congestion (CI), spleno-portal (SPI), vascular portal (PVI), portal hypertension index (IPH). According to N. Anghelescu the quantification of the severity of portal hypertension (PH) was assessed in correlation to the PH hemodynamic classification into: stage I- splenomegaly (SPL) with or without hypersplenism (HPL), stage IIA- SPL+HPL+VE (esophageal varices), stage IIB- SPL+HPL+VE+gastrointestinal bleeding (HD) - 1 episode, stage IIIA- SPL+HPL+VE+ HD+reducible ascites, stage IIIB- - SPL+HPL+VE+ HD+irreducible ascites, precoma, hepatic encephalopathy .

The data analysis was carried out by using Excel Microsoft Office 2003 and EpiInfo 7.1. programme, based on their functions and modules. When statistically processing, a set of operations was used, by making specific procedures and techniques: estimation of parameters and hypothesis testing was performed by calculating errors using Student t-test of significance and the materiality threshold "p", to highlight the connection between two characteristics correlation coefficient was calculated. In the analysis of applied diagnostic tests there were used Se (sensitivity), Sp (specificity), positive and negative predictive values.

Results and discussions

After a complex examination based on laboratory and endoscopic investigations of patients included in the study, we found that the hypersplenism syndrome was recorded in most cases with the following distribution: mild 10 (9.0%) cases, moderate 25 (22.5%), severe 35 (31.5%) cases, no syndrome noted in 25 (22.5%) cases. The degree of esophageal varices, set by upper endoscopy, was distributed as follows: grade I - 35 cases (31.5%), grade II - 24 (21.6%), grade III - 13 (11.7%); no one in 32 cases (28.8%). No esophageal varices were found in 3 patients (2.7%) due to previously made endoscopic endoligature. Pursuant to PH hemodynamic classification, the patients were divided by stages: I- 25 (22.5%), IIA- 39 (35.1%), IIB- 11 (9.9%), IIIA- 16 (14.4%), IIIB- 4 cases (3.6%).

The duplex ultrasonography showed the morphological aspect of the liver, severity of splenomegaly, presence of portosystemic collaterals. The hepatolienal circuit was assessed both on venous side: hepatic portal vein, splenic vein, as well as on the arterial one: hepatic artery, lienal artery. The study included velocimetry indicators, blood flow rate, pulsatility and resistance indicators. The indicators used to estimate the portal hypertension were: congestion indicator, splenoportal indicator, vascular portal indicator, portal hypertension indicator. The ascetic syndrome was noted in 25 cases (22.5%), no syndrome in 86 cases (77.5%), with distribution per grade: grade 1-11 (9.9%), grade 2- 10 (9%), grade 3 - respectively 4 cases (3.6%). Within the study, thrombotic complications were also observed in 11 cases (11.8%). Out of these, full thrombosis

was found in a single case (0.9%), partial thrombosis in 8 cases (7.2%), post-thrombotic changes 2 (1.8%). By assessing more quantitative indicators used by duplex ultrasonography, we have set those with more important correlation depending on the size of esophageal varices. Thus, the resistance indicator (RI) at the level of lienal artery had a correlation coefficient of $r=0.412$ ($p<0.01$), whereas the relevance of spleen dimensions increased once the degree of variceal severity increased $r=0.399$ ($p<0.05$). The relevance of time-weighted average velocity (TWAV) in the hepatic portal vein ($r = -0.377$, $p<0.05$) diminished once the varices grew in diameter.

Within the study, we noted a significant statistical correlation of the timely-weighted average speed in the hepatic portal vein, congestion indicator ($r=0.316$, $p<0.05$), splenoportal indicator ($r=0.409$, $p<0.01$), and vascular portal one ($r=-0.293$, $p<0.05$), portal hypertension indicator ($r=0.397$, $p<0.05$) depending on the variceal dimensions (tab. 1).

Thus, we found a significant difference in indicators used once, the level of esophageal varices advanced. It is well known that the varices' dimensions represent a major risk factor for

upper gastrointestinal haemorrhage. During the research, we have analysed the forecast of variceal levels depending on TWAV in the hepatic portal vein. Thus, we have set the important predictable indicators in order to forecast esophageal varices of grade III, the relevance of which increases once the speed in the hepatic portal vein gradually diminishes (tab. 2).

By analysing the correlation between the Doppler indicators and presence of the ascetic syndrome, we have set that a value under 2.1 of IPH ascites is more rarely noted compared to patients with ascites of different degrees with an IPH over 2.1 ($r=0.468$, $p<0.01$) (tab.3). The vascular portal indicator decreased proportionally with the increase of the volume of ascetic fluid ($r=-0.472$, $p<0.01$).

Thrombosis in the portal system is one of the vascular complications which progressively disturb the hemodynamic already compromised within the cirrhosis of the liver. Analysing in whole a string of vascular parameters, there was found a significant statistical difference of PVI and IPH depending on the presence of thrombotic modifications in the venous system ($p<0.001$). Thus, the respective indicators represent an acceptable forecast for thrombotic complications (tab. 4).

Table 1

Hemodynamic indicators depending on staging of esophageal varices

Indicators	Absent	Grade I	Grade II	Grade III	P Abs-II P Abs-III P I-III
TWAV (cm/sec)	14.2±0.30	13.8±0.41	12.5±0.54	11.7±0.44	<0.01 <0.001 <0.001
CI	0.09±0.001	0.11±0.001	0.14±0.01	0.12±0.01	<0.001 <0.01 >0.05
SPI (%)	0.47±0.02	0.49±0.02	0.57±0.03	0.62±0.02	<0.01 <.001 <0.001
PVI	10.0±0.35	10.6±0.50	8.4±0.53	8.2±0.48	<0.05 <0.01 <0.001
IPH	1.9±0.07	2.0±0.12	2.4±0.15	2.5±0.15	<0.01 <0.001 <0.01

Table 2

Forecasts of big esophageal varices depending on the TWAV variations in hepatic portal vein

TWAV (cm/sec)	Se(%)	Sp(%)	PPV(%)	NPV(%)	LR+	LR-
7-10	75	76	80	96	3.13	0.33
11-14	44	82	70	61	2.44	0.68
15-18	0	50	0	43	0	2.0

Legend: Se – sensitivity, Sp – specificity, PPV - positive predictive value , NPV - negative.

Table 3

Indicator of portal hypertension in forecasting ascites

Parameters	Se%	Sp%	PPV%	NPV%	LR+	LR-
Coefficient IPH>2.1	88	80	58	96	4.4	0.15

Table 4

Forecasts for thrombosis by assessing hemodynamic parameters (PVI, IPH)

Parameters	Se%	Sp%	PPV%	NPV%	LR+	LR-
PVI <9.6	91	60	22	98	2.2	0.15
IPH >2.1	91	74	30	98	3.5	0.12

Table 5

Hypersplenism depending on the flow in the lienal artery

Parameter	Se%	Sp%	PPV%	NPV%	LR+	LR-
Pathological flow in lienal artery	79	69	87	54	2.5	0.3

During the study, we were set to also establish the predictable values in order to forecast the hypersplenism syndrome based on assessed hemodynamic parameters. Thus, it was observed a significant correlation of the splenoportal index with degrees of severity of hypersplenism ($r=0.516, p<0.01$). Also, the impedance index increased at the level of lienal artery positively correlates with the presence of hypersplenism ($r=0.454, p<0.01$). This allowed us to calculate the forecasts indexes for this syndrome too (tab. 5).

The cirrhosis of the liver represents the final stage of most congestive hepatopathies, including in evolution of liver fibrosis, and is described by chronic cellular destruction. The irreversible structural changes lead to deterioration of the hepatic function and progressive changes of blood flow. The portal hypertension is a precocious consequence and a more important one in evolution of complications of liver cirrhosis. The gradual blood flow disorders lead to the opening of other draining pathways, one of them being the gastroesophageal reflux [10]. Advanced varices are important predictors of bleedings. The gotten results allow us to forecast the occurrence of big-sized varices, once the average speed in the hepatic portal vein gradually diminished (RP+3.13). The venous congestion in the portal splenopathy was observed by the increase of the splenoportal index simultaneously with the advancing of the hypersplenism syndrome ($r = 0.516, p<0.01$). The increase of vascular resistance at the level of lienal artery is directly

related to the advance of severity of hypersplenism Se 79%, and Sp 69%, which represent acceptable values for forecasting this complication. In order to highlight more complexly the blood flow disturbances in cirrhosis of the liver, which include the affection at venous level, as well as at arterial level, we were set to make an evaluation of the vascular portal index and portal hypertension index when forecasting ascites and venous thrombosis. Thus, we found that at a value of over 2.1 of IPH, RP+ in order to forecast ascites is of 4.4 higher than value below 2,1. The thrombotic complications were mainly noted in patients who had an PVI<9.6 (RP+ 2.2), and IPH>2.1 (RP+3.5), which allowed us to make a satisfactory forecast for these complications too. During this study was obtained ROC curves for IPH > 2.1 for predicting ascites and thrombotic complications, this way the area under the curve (AUC) for predicting ascites is 0.823, and for predicting thrombosis, this index had AUC of 0.808. The pathological flow on lien artery has the area under the curve of 0.678, which shows acceptable accuracy in the prognosis of this syndrome (fig.1).

Conclusions

1. Duplex ultrasonography is a modern and accessible imaging method, which allows complex assessment of severity of circulatory disorders in the portal hypertension associated with cirrhosis of the liver.

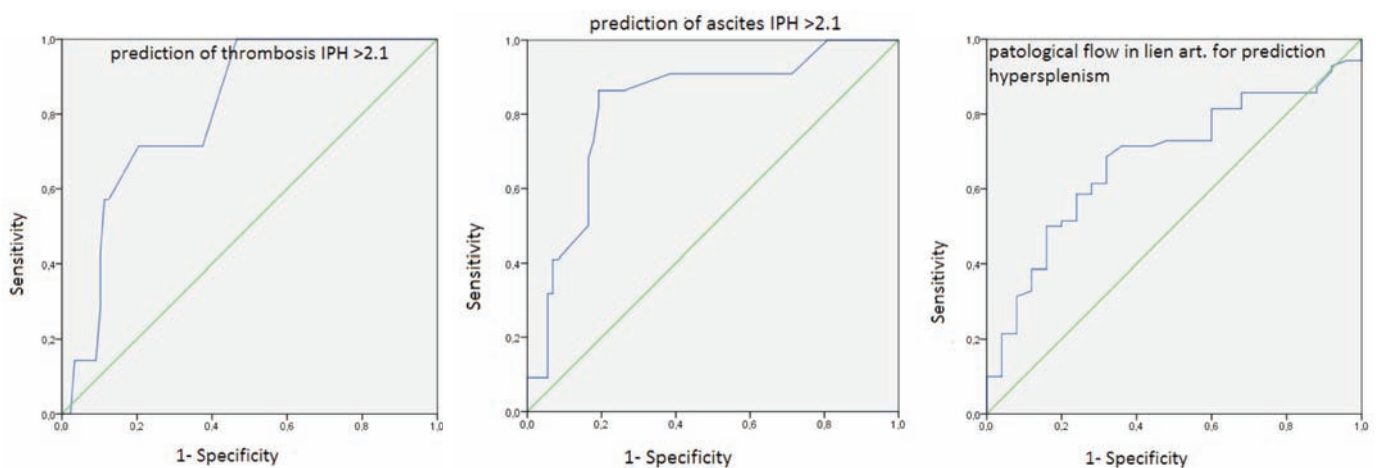


Fig. 1. ROC curves for predicting cirrhotic complications.

- Average speed in the hepatic portal vein is an important predictable indicator for big-sized variceal swellings with high risk of bleedings.
- Complex examination of hepatolienal circulation and estimation of vascular portal index, portal hypertension index provides an accurate forecast of important complications in cirrhosis of the liver, which allows an appropriate curative management.

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Expression of CK5 basal cytokeratin in primary breast carcinoma

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Abstract

Background: CK5 positive cells represent progenitors for glandular and myoepithelial lineages of mammary epithelium. During epithelial differentiation there is a gradual decrease of CK5 expression. In case of benign lesions the proliferating luminal cells show a high expression of CK5. Contrary, the majority of malignancies which are derived from differentiated glandular cells line do not reveal immunohistochemical staining with CK5 marker. The aim of this study was to compare the expression of basal cytokeratin CK5 vs hormone receptors, HER2, Ki67 and molecular subtype's immunohistochemically defined in the primary breast carcinomas of NST type.

Material and methods: We processed 108 invasive breast carcinomas of NST type. The specimens were formalin-fixed and paraffin-embedded as traditionally. Sections were immunostained (ER, PR, HER2, CK5 and Ki67) automatically with Leica Bond-Max autostainer.

Results: Breast carcinoma of NST type was in majority of cases CK5 negative (94 cases/87%). The positive CK5 cases had a high grade of differentiation. CK5 negative tumors were usually hormone positive, but in 8 cases/6.5% a combined simultaneous CK5-ER (PR) positive expression was determined. From 22 HER2 positive cases, 16 were CK5 negative. CK5 value correlated statistically significant with all used markers, except grade of differentiation: a positive Pearson coefficient was determined in relation to HER2 and Ki67, and a negative one compared to hormone receptors and molecular subtype.

Conclusions: We support CK5 potential value in molecular subtype's differentiation. Breast carcinoma of NST type is usually CK5 negative and hormone positive. The presence of cases with simultaneous expression of CK5 and hormone receptors is an open field to debate the existence of other, transient molecular subtypes and we expect a further confirmation in larger study groups.

Key words: molecular subtypes, invasive carcinoma of NST type, basal cytokeratin CK5.

Introduction

The secretory portion of the normal breast consists of the following five distinct cell populations: committed stem (progenitor) cells which are CK5 positive, glandular precursor cells which express all spectrum of cytokeratins (CK5⁺/CK8/18/19⁺), glandular end cells, positive for luminal cytokeratins (CK8/18/19⁺), myoepithelial precursor cells positive for CK 5/6⁺ and SMA⁺ (smooth muscle actin), and myoepithelial mature cells, SMA positive. The CK5 positive cells represent progenitors for both glandular and myoepithelial lineages of mammary epithelium. During epithelial differentiation there is a gradual decrease of CK5 expression. In case of benign lesions the proliferating luminal cells express in excess CK5 protein and opposite, in case of the majority of malignancies glandular cells line do not reveal immunohistochemical staining with CK5 marker [1; 2].

Breast cancer is a heterogeneous disease with different clinical outcomes. It is one of the most common cancers in females worldwide. The modern classification purposes to divide it into at least five molecular subtypes which are: two hormone positive types (Luminal A and Luminal B) and three hormone negative types (HER-2 expressing, Basal-like, and Normal breast-like), each with distinct clinical features, and different prognosis [3]. Nielsen et al. purposed to differentiate immunohistochemically these subtypes by a panel of four antibodies (ER, HER1, HER2, and cytokeratin 5), point of view sustained also by Goldhirsch et al. (2013) that supplemented this panel with Ki67 as a marker of proliferation [4; 5].

The aim of this study was to compare the expression of basal cytokeratin CK5 vs hormone receptors, HER2, Ki67 and molecular subtypes immunohistochemically defined in the primary breast carcinomas of NST type.

Material and methods

Patients. There were investigated primary breast carcinomas of 108 patients, 33-86 years old from the Oncological Institute, the Republic of Moldova during 2012-2013 years. No drug therapy preceded and all patients underwent radical mastectomy and lymph nodes dissection.

Tissue processing and immunohistochemistry. The specimens were fixed in 10% phosphate buffered formalin for 24-48h and paraffin (Paraplast High Melt, Leica Biosystems)

embedded as usual. For histopathological assessment 4-6 µm sections were cut and stained with hematoxylin and eosin. Two independent histologists reviewed the cases. Discrepancies in diagnoses were solved by consensus with simultaneous viewing. Histological grade was scored by the Scarff-Bloom-Richardson grading system. The immunohistochemical assessment included 5 markers: for ER (clone Er/6F11), PR (clone Pr16), and human epidermal growth factor receptor 2 (HER2/polyclonal), marker of proliferation Ki67 (clone Ki67/K2) and basal cytokeratin CK5 (clone CK5/ XM26) (tab. 1). Specimens were processed automatically on Leica Bond-Max autostainer (Leica Microsystems GmbH, Wetzlar, Germany). The hematoxylin solution, Harris modified (HHS32, Sigma-Aldrich) was used for counterstaining.

Microscopic evaluation. Ki67 marker, as well as hormone receptors were counted using a semi-quantitative method performed by Suci et al. (2014) [6]. For Ki67 marker we used a 14% threshold as a limit to distinguish positive/negative cases [5].

The anti-ER and anti-PR markers were scored as a percentage of nuclear positive stained cells at least to 1000 cells. We followed the guidelines of ER and PR assessment purposed by Allred, which are combining the percentage of positive cells with intensity of nuclear staining [7]. The cases scored as +1 – +3 were considered positive. The threshold of positivity was 10%.

The HER2 status was interpreted in accordance with ASCO (American Society of Clinical Oncology) recommendations [8]: *HER2⁰* – if no staining observed or weak, barely perceptible membrane staining until 10% of cells; *HER2¹* – in case of a weak membrane staining of >10%; *HER2²* – in case of incomplete, weak/moderate circumferential membrane staining of more than 10% of tumor cells or complete circumferential intense staining less than 10% of cells; *HER2³* – in case of intense, circumferential staining of more than 10% of tumor cells. Cases with HER2 scored as +2 and +3 were considered positive. The positive cells of normal ducts served as internal control.

The CK5 expression was interpreted as Azoulay et al. previously defined: **0** – no tumor cells stained; **+1** – less of 10% of tumor cells stained; **+2** – 10-50% of positive tumor cells; **+3** – more than 50% of tumor cells stained [9]. Expression was scored positive (>0) if any cytoplasmic and/or membranous staining tumor cells were observed.

Table 1

The surrogate markers: source, dilution, systems of detection and retrieval, time of incubation

Antibody/Clone	Source/incubation time /dilution	Detection/time	Retrieval system/time
ER/6F11	Leica Biosystem Newcastle Ltd, Newcastle Upon Tyne, UK/15 min/RTU	Bond Polymer Refine Detection System (Leica Biosystems, Newcastle Upon Tyne, UK), 15 min	Bond Epitope Retrieval Solution 1, (Leica Biosystems, Newcastle Upon Tyne, UK)/20 min
PR/16			
HER2 /polyclonal	Dako Glostrup Denmark/30 min/RTU	EnVision-HER/30 min	Dako Target Retrieval Solution, pH6/20 min
Ki67/K2	Leica Biosystem Newcastle Ltd, Newcastle Upon Tyne, UK/15 min/RTU	Bond Polymer Refine Detection System (Leica Biosystems, Newcastle Upon Tyne, UK), 15 min	Bond Epitope Retrieval Solution2, (Leica Biosystems, Newcastle Upon Tyne, UK)/20 min
CK5/ XM26			

RTU: ready to use

The results were grouped in 5 subgroups: 1. ER⁺ (and/or PR⁺), HER2⁻, CK5⁻, Ki67<14 as Luminal A; 2. ER⁺ (and/or PR⁺), HER2⁺, CK5⁻ or ER⁺ (and/or PR⁺), HER2⁻, CK5⁻, Ki67>14 as Luminal B; 3. ER⁻, PR⁻, HER2⁺, CK5⁻ as HER2 over-expressed; 4. ER⁻, PR⁻ and HER2⁻, CK5⁺ as Basal-like; 5. ER⁻, PR⁻, HER2⁻ and CK5⁻ as 5NP (5 negative phenotype).

Image acquisition and data processing. Slides were examined on Nikon Eclipse 80i microscope with Nikon DS-Fi1 installed camera by using Nis-elements 2.30 imaging software (Nikon Instruments Europe BV). A MS Access 2007 database was used to store and group the data.

Statistical analysis. The WINSTAT 2012.1 (R. Fitch Software, Bad Krozingen, Germany) software was used for a descriptive statistics, the mean value, standard error of mean and median were determined for Ki67. For all the tests a P ≤ 0.05 was considered significant. A Pearson's correlation (r) was used to determine the relationship between different variables for a P ≤ 0.05. The strength of the correlation was appreciated as: .00-.19 – “very weak”; .20-.39 – “weak”; .40-.59 – “moderate”; .60-.79 – “strong”; .80-1.0 as “very strong”.

Ethics. This study has been approved by the Ethics Committee of the “Nicolae Testemitanu” University of Medicine and Pharmacy from Chisinau, the Republic of Moldova (approval number 21/13/31.03.2014).

Results

The CK5 was evaluated as negative in 94 cases/87%. The ER marker was positive in 88 cases/81,5%, the PR in 77 cases/71,3% and HER2 in 22 cases/20,4%. The Ki67 was encountered at high level (more than 14% of positive cells) in 58 cases/53,7% with a mean of 21,46±1,94 and median 15.

The G2 grade of differentiation was the most frequent registered (60 cases/55.6%), followed by G3 (40 cases/37%) and G1 (8 cases/7.4%). In relation to CK5 expression we realized that most of CK5 negative cases showed G2 and G3 grade. We have to mention that majority of G1 cases (7 from 8) were CK5 negative too (tab. 2).

By comparing the scores of markers expression we realized that majority of hormonal positive and HER2 negative cases had a lack of CK5 expression (tab. 3).

Table 2

CK5 expression in relation to grade of differentiation

CK5 expression	Grade of differentiation	No	%	
0	G1	7	6,5	87%
0	G2	53	49,1	
0	G3	34	31,5	
1	G2	5	4,6	13%
1	G3	2	1,9	
2	G1	1	0,9	
2	G2	1	0,9	
2	G3	2	1,9	
3	G2	1	0,9	
3	G3	2	1,9	

Table 4

CK5 expression grade vs molecular subtype

CK5 expression	Molecular subtype	No	%	
0	5NP	4	3,7	87%
0	Her2	6	5,6	
0	Luminal A	36	33,3	
0	Luminal B	48	44,4	13%
1	Basal-like	1	0,9	
1	Her2	1	0,9	
1	Luminal A	1	0,9	
1	Luminal B	4	3,7	
2	Her2	1	0,9	
2	Luminal A	1	0,9	
2	Luminal B	3	2,8	
3	Basal-like	1	0,9	
3	Her2	1	0,9	
Total		108	100,0	

Table 3

CK5 expression grade in relation to hormone receptors and HER2 scores

CK5	ER	No	%	CK5	PR	No	%	CK5	HER2	No	%
0	0	13	12,0	0	0	24	22,2	0	0	74	68,5
0	1	4	3,7	0	1	8	7,4	0	1	4	3,7
0	2	11	10,2	0	2	14	13,0	0	2	6	5,6
0	3	66	61,1	0	3	48	44,4	0	3	10	9,3
1	0	3	2,8	1	0	3	2,8	1	0	5	4,6
1	1	1	0,9	1	1	1	0,9	1	3	2	1,9
1	3	3	2,8	1	2	1	0,9	2	0	2	1,9
2	0	2	1,9	1	3	2	1,9	2	3	2	1,9
2	3	2	1,9	2	0	2	1,9	3	0	1	0,9
3	0	2	1,9	2	2	2	1,9	3	2	2	1,9
3	1	1	0,9	3	0	2	1,9				
				3	3	1	0,9				
Total		108 cases/100%									

Table 5

Pearson's correlation between surrogate markers, molecular subtype and grade of differentiation

	CK5	ER	PR	HER2	Ki67	Subtype	Grade
CK5							
r		-0,35	-0,20	0,22	0,41	-0,27	0,07
p		0,0001	0,0212	0,0124	0,0000	0,0023	0,2332
Er							
r	-0,35		0,52	-0,39	-0,30	0,67	-0,08
p	0,0001		0,0000	0,0000	0,0009	0,0000	0,2056
Pr							
r	-0,20	0,52		-0,24	-0,17	0,47	-0,18
p	0,0212	0,0000		0,0071	0,0363	0,0000	0,0360
HER2							
r	0,22	-0,39	-0,24		0,11	-0,54	0,00
p	0,0124	0,0000	0,0071		0,1195	0,0000	0,4836
Ki67							
r	0,41	-0,30	-0,17	0,11		-0,38	0,25
p	0,0000	0,0009	0,0363	0,1195		0,0000	0,0047
Subtype							
r	-0,27	0,67	0,47	-0,54	-0,38		-0,15
p	0,0023	0,0000	0,0000	0,0000	0,0000		0,0618
Grade							
r	0,07	-0,08	-0,18	0,00	0,25	-0,15	
p	0,2332	0,2056	0,0360	0,4836	0,0047	0,0618	

Note: Grade – grade of differentiation; Subtype – molecular subtype; r – Pearson correlation coefficient. Statistical significant values are given in Bold.

The most commonly determined molecular profile in our research was Luminal B (55 cases/50,9%), followed in regression by Luminal A (38 cases/35,2%), HER2⁺ (9 cases/8,3%), 5NP (4 cases/3,7%) and Basal-like (2 cases/1,9%). Three molecular subtypes, Luminal A, Luminal B and HER2 were determined in both groups, CK5 positive and CK5 negative, while 5NP was registered only in CK5 negative group and Basal-like in CK5 positive group only (tab. 4).

The CK5 expression correlated weakly, but statistically significantly with all markers, except grade of differentiation (tab. 5).

Discussion

Abd El Rehim et al. (2004) consider that the secretory portion of the normal breast consists of the following five distinct cell populations: committed stem (progenitor) cells which are CK5 positive, glandular precursor cells which express all spectrum of cytokeratins (CK 5⁺/CK8/18/19⁺), glandular end cells, positive for luminal cytokeratins (CK8/18/19⁺), myoepithelial precursor cells positive for CK5/6⁺ and SMA⁺ (smooth muscle actin), and myoepithelial mature cells, SMA positive

[10]. By this, CK5 positive cells in fact represent progenitors for both glandular and myoepithelial lineages of mammary epithelium. During epithelial differentiation there is a gradual decrease of CK5 expression, associated with an increase in expression of CK8/18/19 in the glandular cells, and smooth muscle actin in the myoepithelial cells along the pathways of differentiation. By the Bocker W et al. (2002) data, in the lactating breast, there is a segregation of epithelial structures into CK8/18 expressing secretory zone and the proliferative zone which harbors cells of both glandular (CK8/18⁺) and basal/myoepithelial (CK 5/6⁺) type [11].

In case of benign lesions the proliferating luminal tumors show a high number of CK5/6 positive cells because of proliferation of both glandular and basal cells [12]. In Heatley M et al. (1995) opinion, the majority of malignancies which are derived from differentiated glandular cells line do not reveal immunohistochemical staining with CK5/6 leading, by this explaining both CK5 negativity in most lesions of atypical hyperplasia and ductal carcinoma in situ [13].

Genes expression profiling has identified various breast carcinoma classes with prognostic significance [3; 14].The

authors purposed initially to distinct 5 subtypes, as Luminal A, Luminal B, HER2+, Normal breast-like, and Basal-like. The luminal tumors were defined as hormone receptor-positive and negative for HER2 and usually tend to have a good prognosis. In contrast, HER2+ tumors are negative for hormone receptors and positive for HER2 and have been shown to have poor prognosis. The existence of normal breast-like tumors is still debated, but majority of researches consider that it expresses genes characteristic of adipose tissue and other non-epithelial breast cells, with a relatively poor prognosis.

Among all the molecular classes, Basal-like breast carcinoma seems to have the worst prognosis [15]. These tumors are negative for hormone receptors and HER2, and positive for CK5 and/or HER1 [16]. Basal-like tumors are also the most common type of tumors in patients with germline BRCA1 mutations. Such tumors are considered as high proliferative ones, with a low cellular differentiation [17; 18]. Such results are in line with our data, in which from 14 CK5 positive cases only one was evaluated with G1. In accordance with Sood et al. (2014) the CK5 did not show statistically significant correlation with age, tumor size and stage, histological type, the state of tumor margins, presence of lymphoid infiltrate and necrosis, lymph node status, and Ki67 positivity [18]. Authors reported a single significant correlation, CK5 vs tumors' grade, result debated by Rao et al. (2013) and confirmed by us: CK5 correlated significantly with all studied markers, except grade of differentiation [17] (tab. 5).

Some authors debate in the literature whether triple-negative tumors (negative for hormone receptors and HER2, CK5 positive) are synonym with Basal-like carcinoma [19]. Cheang et al. (2008) by using additional markers identified a cohort of patients with a significantly worse outcome in the group of triple-negative tumors, it means that breast carcinoma is not homogenous even inside the molecular subtypes [20]. This is in line with present research, where positive CK5 was determined in all molecular subtypes, defined by surrogate markers in accordance with Goldhirsch et al. (2013) criteria [5] (tab. 4). Moreover, in 9 of 14 cases CK5 positive marker was associated with Luminal phenotype.

Conclusions

We support CK5 potential value in molecular subtype's differentiation. Breast carcinoma of NST type is usually CK5 negative and hormone positive. The presence of cases with simultaneous expression of CK5 and hormone receptors is an open field to debate the existence of other, transient molecular subtypes and we expect a further confirmation in larger study groups.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Clinical application of perfusion computed tomography in the early diagnosis of acute ischemic stroke and hemorrhagic transformation prediction

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Abstract

Background: Our study was designed to evaluate the efficacy of perfusion computed tomography (PCT) in patients with acute phase of stroke for the early diagnosis of this pathology and prediction of hemorrhagic transformation in the ischemic area.

Material and methods: We studied the functional PCT maps obtained at admission from 23 patients with acute ischemic stroke, compared to follow-up computer tomography or magnetic resonance imaging.

Results: Mean Transit Time (MTT) map showed that the highest sensitivity (80.3%) and parameters of relative Cerebral Blood Flow (rCBF) and Cerebral Blood Volume (rCBV) were the most specific (95.0% and 96.9%, respectively) in the early diagnosis of ischemic stroke. Automatic technique "Tissue Classification" showed the highest value of the overall accuracy (91.7%), a significant correlation with the final stroke extension and differentiation of potentially salvageable regions from the irreversibly damaged, which plays an important role in the treatment management. Evaluation of permeability function of the blood-brain barrier with a Permeability Surface area product (PS) showed high values of specificity, sensitivity and overall accuracy (89.5%, 75.0% and 87.0%) in the prediction ability of hemorrhagic transformation.

Conclusions: Quantitative analysis of functional parameters of dynamic cerebral perfusion computed tomography has significant efficacy in emergency diagnosis of acute ischemic stroke and hemorrhagic transformation prediction in tissue exposed to ischemia.

Key words: Computer tomography perfusion, acute ischemic stroke, hemorrhagic transformation, blood-brain barrier permeability.

Introduction

Stroke is the third leading cause of death in industrialized countries and the most frequent cause of permanent disability in adults worldwide [1, 2]. According to the American Stroke Association, every 45 seconds someone has a stroke, every 3 minutes someone dies of a stroke. According to this association 85 % constitutes ischemic stroke, which is the third leading cause of death (behind heart disease and all forms of cancer), leading cause of serious, long-term disability in the USA, where every year 700000 people experience a new or recurrent stroke, about 500000 are primary and 200000 are recurrent attacks and 40000 more women than men will have a stroke. One of the most undesirable complication of ischemic stroke is hemorrhagic transformation (HT), which may further complicate an already devastating clinical condition. HT after acute ischemic stroke is known to associate with poor outcome and delays the initiation of proper anticoagulation treatment, especially for stroke with cardioembolism [3]. Historically, hemorrhagic transformation, initially designated as "red softening," has long been recognized by neuropathologists to occur as a natural consequence of ischemic brain injury. To search for new treatments as well as intervention measures for HT, it is important to understand its underlying mechanism and identify its specific predictors [4].

CT is considered the gold standard in diagnostic imaging for the patients with acute stroke and the cerebral perfusion (perfusion computed tomography - PCT) is the most promising CT method in this regard. PCT is performed by the dynamic image acquisition and generates information, where the image intensity represents tissue density and changes depending on the time after intravenous injection of contrast agent. These data are used for calculating of the various per-

fusion-related parameters: cerebral blood flow - CBF, cerebral blood volume - CBV, mean transit time - MTT, time of local peak enhancement (TTP - time to peak) and capillary permeability (permeability surface - PS). Results are displayed in a graphic format (parametric images) as functional brain maps. These standard perfusion metrics are used to detect an acute ischemic stroke and the presence of "penumbra," the hypoperfused but potentially salvageable tissue at risk of infarction [5, 6, 7].

Tissue at risk of infarction (where the synaptic transmission stops with abolition somatosensory evoked potentials and installation of the electrical failure, but ischemic impairment is reversible even if lower paralysis starts) will have decreased CBF, normal or higher than normal CBV and increased MTT. On the other hand, infarcted tissue (synthesis of adenosine triphosphate is stripped out by demand and cell membrane pumps fail, causing efflux of K⁺, influx of Ca²⁺, Na⁺ and H₂O into neurons, causing membrane depolarization - at this point the damage is irreversible), or dying tissue (i.e. loss of auto-regulation) will show a different pattern: decreased CBF, decreased CBV and normal or slightly elevated MTT [8].

CTP, can not only provide the diagnostic information about ischemic region, but and the functional brain data about blood-brain barrier (BBB) physiology, especially its permeability. Microvascular permeability (expressed as the transendothelial transfer of constant or permeability surface area product [PS]) is a metric of BBB integrity.

Increased blood-brain barrier permeability, one of the pathological reactions following ischemic stroke, is believed to predispose to complications such as hemorrhagic transformation [9], massive vasogenic oedema, infarct expansion [10] and unfavourable clinical outcome [11, 12]. Like the standard

perfusion metrics, PS can also be calculated using dynamic imaging by measuring the leakage of an intravascular tracer (contrast agent) into the extravascular (interstitial) space [13, 14]. BBB permeability imaging provides a physiologic individualized measurement intimately connected to the underlying pathophysiology of hemorrhagic transformation (ischemia-induced vascular damage followed by reperfusion) and may, therefore, offer excellent sensitivity and specificity.

Although there are multiple multicenter studies in the world literature, demonstrating the effectiveness of CT perfusion in early diagnosis of ischemic stroke, cerebral perfusion in Moldova is not a part of the urgent investigation's list for these patients, causing the delayed diagnosis and initiation of specific treatment, such as thrombolysis. Our study is characterized by scientific novelty in the application of CT perfusion in assessment of BBB permeability in patients with hemorrhagic transformation phenomenon. It is not yet discovered, which imaging method has the highest predictive value in forecasting the hemorrhagic transformation of ischemic stroke. This research is the first neuroimaging study in Moldova with PCT application in patients with acute and hyperacute ischemic cerebral infarction with stratified analysis of sensitivity, specificity and accuracy for each functional map in correct stroke diagnosis and hemorrhagic transformation prediction.

Material and methods

Imaging data were obtained in emergency mode after anamnesis and clinical data collection at the admission to the Institute of Neurology and Neurosurgery (INN) (Chisinau, the Republic of Moldova); investigations were analyzed retrospectively. Informed consent was obtained from each patient or relatives or legal representative before the investigation with information about the benefits of the procedure, risks of introducing the contrast agent and the dose of ionizing radiation. The study included patients who underwent PCT and who met the following criteria: 1) age 18 years and more, 2) women of childbearing age who are not pregnant and not breastfeeding, 3) signs and symptoms suggestive of cerebral infarction (e.g. hemisensory disorders, hemiparesis, aphasia or visual field abnormalities) lasting up to 12 hours 4) no evidence of intracerebral hemorrhage, and 5) the patient underwent follow-up CT or MR imaging to confirm or rule out ischemic stroke.

Scanning Protocol. Brain PCT was performed at the Radiology Department of INN with the use of multidetector tomography equipment (64 slices) - VCT select (producer - General Electric Healthcare, USA) by dynamic scanning method (Cine Mode) with the administration of the contrast agent after non-contrast axial scan for the hemorrhagic stroke exclusion. Scanning parameters: tube rotation time - 1.0 seconds; the full length of rotation - 360 degrees; 5 mm slice thickness; the length of region covered by the dynamic scan - 40 mm; the overlap interval = 0.0 mm; total scan time - 40 seconds; active detector - central (20 mm); Gentry tilt - parallel to the orbital-meatal line, small field of view (25 cm); X-ray current voltage = 80 kV; current intensity - constant 150 mA; the total obtained number of primary images - 792. The scanning process started with 60 ml nonionic iodinated con-

trast Visipaque (iodixanol 320 mg/ml iodine concentration) automatic injection (Power Injector Nemoto with 2 syringes) in cubital vein at 4 ml/s flow rate, followed by administration of 40 ml saline at a rate of 4 ml/s. Multidetector CT technology allowed acquisition of four adjacent 5 mm thickness sections for each location. In most cases, the first section was localized on the basal nuclei and III ventricle's level (position above the orbits for the lens protection) with caudo-cranial data acquisition. The time before the contrast agent reached brain parenchyma allowed the base non-contrast images acquisition.

Data postprocessing was carried out after images transfer to the Advantage Workstation 4.6 (General Electric, Milwaukee, USA), using CT Perfusion 4 software - image analysis software package for dynamic stroke images assessment, protocol "Brain Stroke". Perfusion CT algorithm included image intensity quantification in CT image sets and the absolute values calculation in the following functional color maps: CBF - cerebral blood flow, mean transit time - MTT, CBV - cerebral blood volume, time to peak - TTP, and permeability surface area product - PS. General Electric CT Perfusion deconvolution algorithm is based on the convolution model. One of the inherent advantages of the convolution model is that it makes no assumption that the injection rate of the contrast agent, and subsequently the time it takes to reach its peak concentration in the vasculature, are nearly instantaneous or at least shorter than the minimum transit time of tissue, as other models do. Conversely, the convolution model takes into account the actual injection rate of the contrast agent, as determined from the time series data from a reference Region of Interest (ROI) located in an artery (arterial tissue density curve), when computing the quantitative values of perfusion parameters. Specifically, the algorithm "deconvolves" the arterial tissue density curve from the tissue density curve in each tissue voxel to compute an impulse residue function (IRF) from which the perfusion parameters are calculated. The perfusion parameters such as IRF T0, MTT, CBF, CBV and PS are derived from the tissue IRF, and, where applicable, are normalized to the pixel value associated with 100% of blood in a large vessel. The parameters are further normalized to the average tissue density, and hence, are expressed in their actual units of measurement per unit mass (e.g. BV is expressed in ml/100 g of wet tissue).

Cerebral blood flow (CBF) represents flow of blood through vasculature including conductance vessels (arteries, arterioles, capillaries, venules, veins, sinuses), being measured in ml/100g wet tissue/min. **Cerebral blood volume (CBV)** represents volume of blood in cerebral vasculature, being measured in ml/100 g wet tissue. **Mean Transit Time (MTT)** is the average residence time of contrast agent in a given tissue location. Mathematically, MTT is computed as the first moment of the IRF from IRF T0, and is displayed in seconds. In CT Brain Stroke, the calculated MTT is the sum of both the intravascular and extravascular contrast residence times (i.e. it incorporates the time interval required for the IRF to reach post-enhancement baseline). **Time-to-peak (TTP)** is defined as the time interval between the onset of the tissue enhancement and the peak of the tissue density curve. In practice, it is computed as the time interval between the last pre-enhancement image and the image with the maximum

intensity value. TTP is displayed in seconds. **Permeability Surface area product (PS)** is computed from the IRF, and is displayed in ml per 100g of wet tissue per minute (ml/100 g/min). The PS has the same units as CBF, as it quantifies the diffusion of some of the contrast agent into the interstitial space. It is used to assess the permeability of blood vessels. Permeability is related to the diffusion coefficient of the contrast agent through the pores of the capillary endothelium into the interstitial space due to the deficient or leaky blood brain barrier. It has also been linked to the diffusion of contrast agent through the large holes of the sinusoidal capillaries. In the tissue IRF, the contrast agent diffusion is related to the extraction fraction, or the fraction of contrast agent, which remains in the intravascular space after the initial IRF response and which then, diffuses exponentially into the interstitial space. **Tissue Classification** software analyzes the tissues affected by a stroke and is measured in ml/100 g of tissue. The input for this protocol is the computed maps of Average CBF and CBV. The tissue with blood volume below the threshold will be displayed in purple on blood volume map. The tissue with normal blood volume will be displayed in yellow on blood volume map.

The formal definitions of Blood Flow and Mean Transit Time assume that the injection of the contrast agent is instantaneous and there is no recirculation, which is not the case in clinical practice. This means that the algorithm must take into account the actual injection rate of the contrast agent to obtain *quantitative* results for CBF and MTT. For this purpose, the algorithm uses data from a reference ROI located in an artery to deconvolve the time course data and compute an *impulse residue function* (IRF) for each pixel location.

The statistical analysis included maps with absolute values - TTP, MTT, PS and relative values expressed in % of the affected region, compared with the healthy contralateral region (absolute value of healthy region is considered = 100%) - rCBF and rCBV and automatic computerized map Tissue Classification created by the software that reveals the penumbra regions and stroke by applying rCBF and rCBV thresholds [15, 16]. By this method, ischemic cerebral area (penumbra and infarction) has been defined to include cerebral pixel with decreased CBF > 34%, as compared to the corresponding region of healthy brain hemisphere, defined on the basis of clinical symptoms. In this selected region, pixels with values higher or less than 2.5 ml / 100 g were highlighted to differentiate penumbra and cerebral infarction [15, 16].

Data analysis was performed retrospectively individually for each functional map from 23 PCT investigations, performed on admission in INN. RCBV, rCBF, MTT, TTP, PS and Tissue Classification maps were analyzed, with registration of visual abnormalities in perfusion indices in four anatomical regions of the brain, according to Alteplase Thrombolysis for Acute Noninterventional Therapy in Ischemic Stroke study (ATLANTIS) [17], plus 10 anatomic regions defined by the Alberta Stroke Program Early CT Score (ASPECTS) [18] and brain territories corresponding the vascularization of anterior cerebral artery (ACA) and posterior cerebral artery (PCA) in right and left cerebral hemisphere, with total of 32 territories. For ATLANTIS system pathological changes were assessed in

four areas of the brain: 1) frontal, 2) temporal, 3) parietal and 4) basal nuclei area and insular cortex; were recorded with a score of 0-4. For ASPECTS system were assessed 10 areas of brain tissue, perfused by the middle cerebral artery (MCA), including the caudate nucleus, putamen, internal capsule, the insular region, M1, M2, M3, M4, M5, M6, were recorded with a score of 0-10 points (10 points maximum score indicating normal tissue).

The PCT maps evaluation consisted of their assessment by subjective visual comparison with the healthy hemisphere. Data were also analyzed using automated prototype software PCT (Tissue Classification, General Electric). As mentioned above, this software automatically builds a functional map of irreversible cerebral infarction and penumbra areas, based on reference values of rCBF and rCBV. In this automatic computerized map was evaluated possible presence of penumbra/infarction in four ATLANTIS anatomical regions of the brain, 10 ASPECTS regions and ACP / ACA territories.

Blood-brain barrier permeability was analyzed on functional PS map from the "tumor perfusion" module of PCT 4 (General Electric) software, which calculates microvascular permeability and fractional blood volume based on the Patlak method [19]. PS color maps were not generated in time of initial assessment (on admission) and, therefore, had no importance in the treatment strategy selection (the fibrinolytic therapy administration) or subsequent clinical management. Patlak method is a two unidirectional compartments model which calculates PS by the linear regression. Each voxel of brain tissue contains 3 spaces: intracellular, intravascular (plasma) and extravascular (interstitial). Since iodinated contrast hydrophilic molecules (tracer) do not cross hydrophobic cell membrane and intracellular contrast agent enhancement can be ignored; only 2 compartments are considered (intravascular and extravascular).

The region of interest was traced individually ("freehand region"), for delineation of total tissue at risk of cerebral ischemia, based on the functional parameters abnormalities: increased MTT and decreased CBF. A copy region was reflected in the healthy hemisphere, being generated automatically by the PCT software for blood-brain barrier permeability assessment in contralateral normal brain tissue.

Absolute values of microvascular permeability were recorded, with the subsequent retrospective comparison of mean values in patient's group showed hemorrhagic transformation on follow-up brain CT and in group without this complication. To appreciate the true/false positive and negative results and calculating the sensitivity, specificity and accuracy, was used reference level = 2.3 ml/min/(100g), previously published [20] and validated to differentiate patients with possible hemorrhagic transformation (HT) of ischemic stroke.

According to the European Cooperative Acute Stroke Study "HT" was defined on non-contrast CT imaging as increased density area inside a hypodense region of typical vascular distribution [21]. Hemorrhagic transformation has been classified in two types: hemorrhagic infarction - HI and parenchymal hematoma - PH [21, 22, 23]. These forms have been subdivided into two types: HI 1 and HI 2, PH 1 and PH 2. HI1 are small peripheral petechiae in the ischemic region; HI2 - are confluent petechiae in the ischemic area, but without

mass effect. PH1 has been defined as intracerebral hematoma with mass-effect, occupying less than 30% of the ischemic region and the PH2 – hematoma, occupying more than 30% compared to the initial stroke volume.

CT or MRI follow-up images of each patient were analyzed according to the algorithm described above. In the follow-up investigation, performed a few days after onset of symptoms, final ischemic stroke region was recorded. The results were considered standard criterion for calculating the sensitivity, specificity and accuracy of PCT maps: rCBV, rCBF, MTT, TTP, and PS Tissue Clasification.

Results

We included in our study all the patients who underwent PCT investigation procedure described above in the INN radiology department with symptoms of acute stroke between January 2010 and January 2015. 23 patients met the inclusion criteria. Nine patients (9) were men and 14 women, with a mean age of 63.95 years (minimal age – 42 years and maximal age - 85 years). Clinical manifestations of stroke were recorded on the left side of the body in 16 patients and on the right side - in 7 patients. The average time from the onset of symptoms and the moment of PCT protocol was 6.6 hours, with a minimum of 1.0 and maximum of 12.0 hours.

A follow-up CT was performed in 21 patients (mean time from onset of symptoms to the follow-up imaging = 5,6 days, varying from 3 to 10 days). One patient was investigated by MRI (on the third day from the disease onset) including DWI (diffusion weighted imaging) and fluid-attenuated inversion recovery (FLAIR). In a patient with acute ischemic stroke with early extensive hemorrhagic transformation, follow-up investigation was not carried out, because of the extremely serious concomitant complications with consecutive death, which occurred 5 hours after admission. The final diagnosis was established as: ischemic stroke - in 21 patients (19 cases of non-lacunar stroke and two lacunar cases); transient ischemic attack (TIA) – in 2 patients. The region of the ischemic strokes had the following location: vascular region of anterior cerebral artery (ACA) - 1 patient, middle cerebral artery (MCA) territory - 14 patients, and posterior cerebral artery (PCA) region - 4 patients. Among the patients included in our study,

6 had suffered an ischemic stroke in the past, before the actual PCT procedure. Our control imagistic investigations revealed 4 patients with hemorrhagic transformation (HT) of the ischemic stroke. In 3 patients HT was detected 4-7 days after onset of clinical symptoms; in a patient early haemorrhagic transformation was registered (at hospital admission - 1 hour after onset of symptoms). According to ECASS II classification of HT, 1 patient presented HI type 2 of HT; 2 patients – PH type 2 and 1 patient – PH type 1. Male / female ratio was 3/1, but the small number of patients with hemorrhagic transformation determined impossibility of epidemiological data appreciation in this research group.

The sensitivity, specificity and precision of the PCT data in the ischemic stroke assessing are displayed in **Table 1**. The general precision of the PCT maps in the infarct region detecting in acute ischemic stroke was up to 86.6%, and overall accuracy of PS in predicting hemorrhagic transformation of ischemic strokes - up to 87%. The TTP and MTT maps (79.4% and 80,3%, respectively) were significantly more sensitive than rCBF and rCBV (67.3% and 57,1%, respectively). The sensitivity of the automatic classification of the lesion tissue (Tissue Classification) was 72.3%, which is close to average sensitivity of PCT maps (71.3%). rCBF and rCBV (95.0% and 96,9%, respectively) showed significantly higher specificity than the TTP and MTT (81.3% and 82.3%, respectively).

The automatic method for tissues classification showed very high specificity (96.6%), broadly similar with the rCBV map specificity (96.9%). The blood brain barrier permeability PS map showed both high sensitivity and specificity (75.0% and 89.5%, respectively) for hemorrhagic transformation prediction. The overall PCT maps accuracy in the early diagnosis of ischemic stroke presented a range of values between 81.0% and 91.7%. rCBF and rCBV showed an important overall accuracy (89.5% and 89,0%, respectively), significantly higher compared with TTP and MTT parameters (81.0% and 81.9%, respectively). The automatic algorithm Tissue Classification for cerebral ischemia region delimitation showed the highest value of the overall accuracy (91.7%).

False-negative PCT results were generally related to the lack of spatial coverage. PCT failed to fully reveal non-lacunar stroke only in one case from 23, which was identified by

Table 1

Sensitivity, specificity and overall accuracy of CT perfusion in patients with acute ischemic stroke

	True-positve (TP)	Fals-positve (FP)	Fals-negative (FN)	True-negative (TN)	sensibility TP/(TP+FN)	specificity FN/(TN+FP)	overall accuracy (TP+TN)/(TP+TN+FP+FN)
TTP	116	110	30	480	79,4%	81,3%	81,0%
MTT	118	104	29	485	80,3%	82,3%	81,9%
rCBF	99	29	48	560	67,3%	95,0%	89,5%
rCBV	84	18	63	571	57,1%	96,9%	89,0%
tissue classification	107	20	41	568	72,3%	96,6%	91,7%
average					71,3%	90,4%	86,6%
PS (HT)	3	2	1	17	75,0%	89,5%	87,0%
736							

control MRI in the brainstem region. This anatomic region, usually, is not included in the PCT scanning surface because it requires the inclusion in the irradiation region of the lens and may cause artifacts produced by the bones from the skull base. In 2 cases, follow-up imaging showed lacunar strokes that were too small to be detected by the PCT maps. The other false-negative cases were false negative regions in patients with true-positive pathologies in which PCT has identified most, but not all ischemic areas. In one case, surprisingly, in the region in which control imaging showed cerebral infarction, PCT revealed hyperemia (high rCBF and rCBV, low MTT and TTP), but not oligemia. This result was included in the list of false-negative cases in accordance with the used evaluation criteria. Similar situation was observed in 2 patients with transient ischemic attack (TIA) like final diagnosis. This fact should be considered in future studies. Finally, in 2 patients, PCT maps were of poor quality due to motion artifacts which created difficulties in detecting oligemia in some areas. We included these results in false-negative category.

A case of arterial recanalization was also documented. In this case, the rCBF, MTT and TTP maps showed abnormalities in some areas, which were not confirmed as ischemic region in the control investigation. The automated computerized map described these areas as "penumbra". PCT false-positive results were recorded in 2 cases of TIA, where TTP and MTT maps showed a pattern suggestive of ischemia. Old infarcts also gave false-positive results because they can be differentiated from acute stroke only when PCT maps are interpreted in comparison with non-contrast CT in the corresponding region.

PS showed an increased permeability of the blood-brain barrier (BBB) in 5 patients from the total study group (23 patients), only in 3 of them hemorrhagic transformation (HT) was recorded. According the HT ECASS II classification, 2 patients presented "parenchymal hematoma (PH)" type 2 and 1 patient - PH type 1. In 2 other patients with increased permeability of the BBB, the control investigation (false-positive results) revealed massive stroke development occupying an entire cerebral hemisphere with extensive cerebral edema, complicated with the opposite part shift of middle cerebral structures, brain tissue herniation through the foramen magnum with subsequent significant deterioration of the neurologic state and vital parameters and death. The permeability of the BBB in ischemic stroke region on the PS map in patients with hemorrhagic transformation HI type 2 did not exceed the given reference level (false-negative results), but the native CT control investigation, realized 4 days after the symptoms onset, showed hyperdense regions of HT. In 17 patients we detected lower values than the given reference level for the BBB permeability, but the control CT didn't identify HT. This group of patients formed the true-negative group for the PS map.

Discussion

After analyzing the obtained data, we found that the dynamic PCT examination had high sensitivity, specificity and general accuracy in detecting acute ischemic stroke within less than 12 hours from symptoms onset. TTP and MTT maps (79.4% and 80.3%, respectively) were significantly more sensi-

tive than rCBF and rCBV (67.3% and 57.1%, respectively), but rCBF and rCBV were significantly more specific (95.0% and 96.9%, respectively). The automatic method Tissue classification showed the highest overall accuracy (91.7%), very high specificity (96.6%) and average sensitivity (72.3%). Example of PCT investigation of acute ischemic stroke with 2 follow-up non-contrast CT is shown in Figures 1 and 2.

The role of PCT maps in the assessment of cerebral perfusion in comparison with other imaging methods. Realization of accurate quantitative PCT maps, through deconvolution method, was validated in a number of studies, which evidenced the high specificity and sensitivity of this imaging method [24-32]. Validation was performed by comparison with Xenon Computer Tomography (XeCT) [28, 33], Positron Emission Tomography (PET) [34] and Magnetic Resonance Perfusion (MRP) [35-38], both in experimental and clinical studies [24,25,27].

According to other study results [39], the correlation between MRP and PET perfusion values was not as suggestive as it was expected. Perfusion CT has a higher spatial resolution and it is easier to be quantified than MR perfusion. Also, MRP may be more sensitive to contamination by large vessels artifacts. These factors may contribute to the possibility that visual assessment of ischemic core / penumbra region to be more accurate using PCT than MRP [40, 41]. Moreover, if to exclude the vascular pixels from the CT-CBF calculation, quantification of the mean CBF is very accurate, compared to the obtained values by $H_2^{15}O$ positrons in PET investigation [42].

When compared with MRP, PCT has the advantages on increased speed of realization, lower cost, and, the most important, the wider availability. PCT functional parameters (CBV, CBF and MTT) can be easier quantified than their similar MRP values, explained, partially, by the linear relationship between the concentration of iodinated contrast agent and the CT image density (Hounsfield units), a relationship which can't be attributed to gadolinium concentration and MRI signal intensity. However, as other techniques with the use of the bolus of contrast, the quantification depends on the deconvolution method, used for CBF calculation, which compares the curve based on the contrast enhancement in the tissue and the intra-arterial appearance of the contrast agent. Due to its availability, simple methodology and quantitative results, CTP has the potential to extend patients access to new treatment strategies and clinical studies based on imaging methods. A current disadvantage of the CTP method is the limited scanning region, which depends on the manufacturer and the CT scanner generation. Many contraindications for MRP examination, including difficulty of scanning of patients with installed monitors or artificial ventilation, the presence of cardiac pacemaker or implantable defibrillators, the risk of aspiration during the long supine position and the difficulty to obtain historical data to exclude metal implants, are absent in case of CTP examination.

In our study the highest sensitivity was registered for MTT map due to the presence of 118 true-positive areas identified on this map.

PCT false-negative results were related, mostly, to the lack of spatial coverage, which has been reported for other

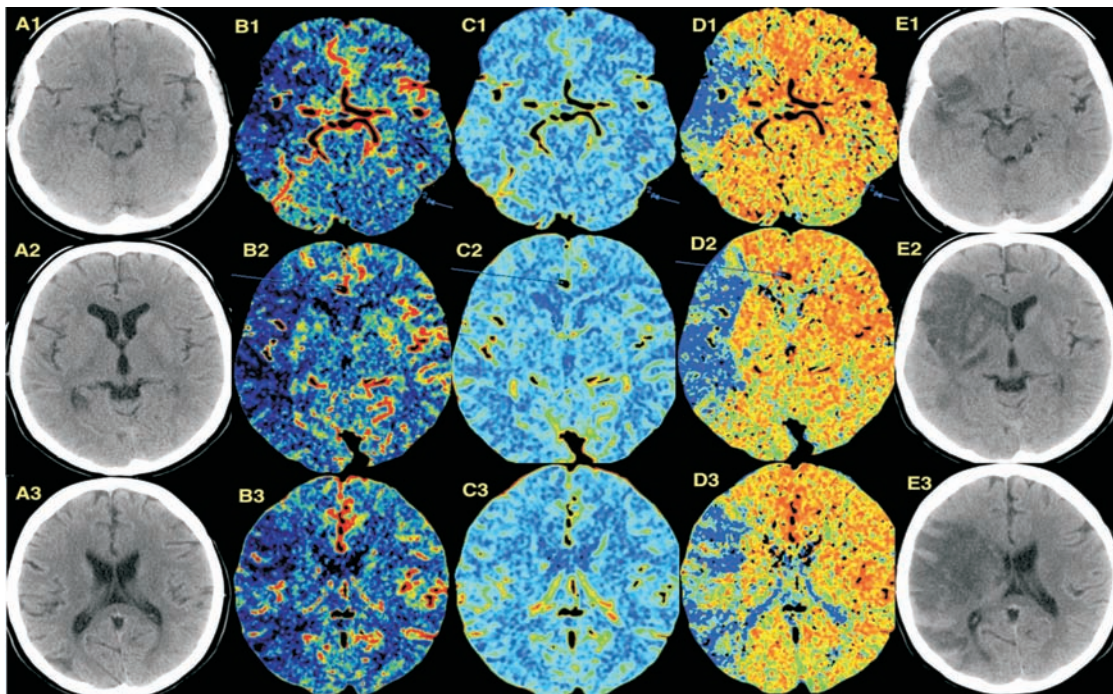


Fig. 1. Female patient, 40 years old with known history of arterial hypertension (grade III) and diabetes type 2, presented in emergency department of INN with left hemiparesis. A1, 2, 3 – non contrast CT, 2 hours after symptoms onset – there are no signs of abnormalities. B, C, D – PCT investigation (2 hours after symptoms onset). An extensive area of cerebral hypoperfusion in the right middle cerebral artery vascularization territory. B1, 2, 3 – rCBF map. C1, 2, 3 – rCBV map. D1, 2, 3 – MTT map. E1, 2, 3 – non contrast CT follow-up investigation on 4th day after symptoms onset. An extensive area of cerebral ischemic infarction in the right middle cerebral artery vascularization territory, diffuse right hemisphere edema with compression of the right lateral ventricle.

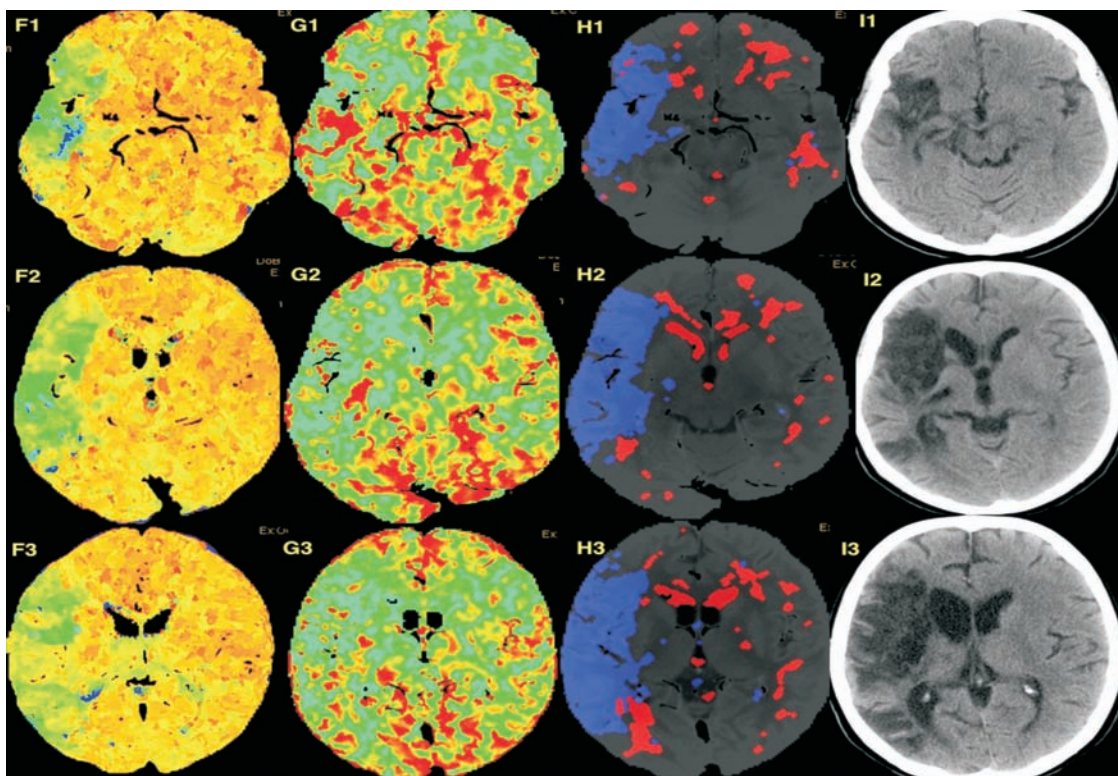


Fig. 2. The same patient. F, G, H – continuation of PCT investigation. F1, 2, 3 – TTP map. G1, 2, 3 – PS map. H1, 2, 3 – automatic Tissue Classification map, blue zones correspond the irreversible infarcted tissue, red zones correspond to “penumbra”. I1, 2, 3 – non contrast CT follow-up investigation after 6 months, chronic stage of ischemic stroke, massive cystic-gliotic changes in the right middle cerebral artery vascularization territory with retraction of the right lateral ventricle.

PCT dynamic techniques [43, 44]. We also identified false-negative areas in patients with true-positive pathology (e.g., PCT showed majority of ischemic areas, but not all). Some lacunar strokes were not identified on the PCT maps, due to the lack of spatial resolution. In 1 patient, we identified the pattern that was included in the group of false-negative results based on the classification algorithm used in our study. This case showed hyperemia (high rCBV and rCBF, low MTTs and TTP), but not oligemia in a territory where control investigation showed the formation of cerebral infarction. This situation was probably related to the so-called "luxury perfusion" and should be considered in future studies.

The majority of false-positive PCT results were associated with TIA. In case of TIA, MTT is prolonged but rCBV is also increased, probably due to insufficient circulation and cerebral vascular autoregulation with subsequent preservation of rCBF. PCT false-positive results were also recorded in case of subacute and chronic infarcts, which usually have the same PCT characteristics as acute strokes (extended TTP and MTT, reduced rCBF and rCBV). Subacute and chronic infarcts are easily distinguished from the acute one (less than 12 hours from symptoms onset), based on CT scan without contrast. In this study, non-contrast CT was not assessed, which increased the number of false-positive PCT results. We couldn't appreciate the impact of combinative evaluation of non-contrast CT investigations and PCT maps, but it is likely to reduce the number of false-positive cases.

Clinical interpretation of PCT data should include evaluation of sensitive and specific maps for screening of patients with acute cerebral ischemia. MTT and TTP maps are extremely sensitive for detecting ischemia. TTP map is also sensitive in the detection of intracranial or extracranial vascular stenosis, independently of cerebral ischemia. That is why MTT map is more useful in ischemia screening, considering that it doesn't highlight stroke only, but TIA also. rCBF and rCBV maps are the PCT maps with the highest specificity and their combined interpretation by the automatic computerized method has an accuracy of 91.7%. In our study, not all patients were investigated by the follow-up CT-angiography; therefore it was impossible to systematically assess the correlation of recanalization or persistent arterial occlusion and also the resolution of penumbra or the increase of ischemia region over penumbra with PCT maps precision.

We have not performed an interpretation which combines various PCT maps, but we can assume that the sensitivity and specificity of PCT maps associated with non-contrast CT investigation analysis can be considerably higher when using MTT to identify ischemia and rCBF and rCBV maps to confirm the diagnosis in areas with prolonged MTT. In addition, the integration of CT angiography results (CTA) could increase this accuracy and could be the subject of further studies. Most patients in our study had clinical suspicion of hemispheric stroke, limiting the number of patients with lacunar stroke or other small strokes and lesions in the posterior fossa. The PCT technique is limited in the diagnosis of posterior fossa lacunas and bigger strokes because of the limited spatial resolution and artifacts from this region. Another limitation of our study is the requirement of control scanning for enrollment. This criterion excludes a large proportion of

patients with TIA and perhaps the assessment of accuracy by reducing non-stroke patients.

In our study of PCT maps analysis in the acute stage of ischemic stroke, four patients developed hemorrhagic transformation in the evolution of the pathology, which allowed the retrospective assessment of the blood-brain barrier permeability through analysis of Permeability Surface area product (PS). PS showed high specificity, sensitivity and overall accuracy (89.5%, 75.0% and 87%, respectively) in prediction of hemorrhagic transformation. Cerebral microvascular permeability research was considerably limited because of the very small group of patients (4 persons). Our results require validation in larger groups of patients, with the whole spectrum of HT forms (4 types of HT). However, despite the small group of patients, our results correspond to previously published studies.

A recent study [45] has demonstrated a difference between PS values in HT and non-HT groups. The authors confirmed that any PS values from 6.0 to 9.8 ml / 100 mg per minute can be used as a threshold value for HT prediction with 100% sensitivity and specificity. The authors showed that elevated PS may be evidenced in the hyper-acute period of the ischemia using dynamic PCT data of the contrast first-passage. Patients with high PS and who haven't been treated with recombinant tissue plasminogen activator (rtPA) developed asymptomatic, small infarcts (hemorrhagic petechia). Christopher D. d'Esterre (2013) has showed that patients with HT presented significantly higher PS values than the group without hemorrhagic transformation. A reference PS value of $0.23 \text{ ml}^{-1} \cdot \text{min}^{-1} \cdot (100\text{g})^{-1}$ differentiated HT group from the non-HT group [46]. Their results showed that, in addition to the PS parameter and the ASPECTS score, other factors (age, gender, or NIHSS score at baseline) were not associated with HT. Hom J. et al. investigated the CT perfusion possibilities in predicting HT and malignant edema in patients with acute ischemic stroke [47]. Researchers have reported that the use of perfusion BBB values above the mentioned threshold, like single predictor of HT, has a sensitivity of 100% and a specificity of 79% (5 false-positive results from 32).

Other functional CT perfusion parameters have been also studied to determine the correlation with HT, but the results showed no statistically significant difference, excepting rCBV map. Patients with HT presented a larger mean total volume of cerebral tissue at risk of ischemia, a prolonged rMTT and a reduced rCBF, when compared to the control group, although these parameters showed no significant statistical difference. The persons with HT had a mean rCBV significantly lower than the control group ($p = 0.01$). Consecutively, with every 0.1-U decrease in rCBV, the risk of HT increases by 14% [48]. Prediction of HT, by reduced pretreatment CBE, was first proposed by Ueda et al. with the use of SPECT [49]. A 50% reduction from the normal value of the CBF was considered as critical value for the HT development [50]. Gupta et al., in their study including 23 patients with stroke or symptomatic carotid stenosis, have concluded that ipsilateral CBF average of 13 ml / 100 g per minute was an indicator for the development of HT [51]. In another cohort, they found that rCBV, but not rCBF, was a stronger predictor of HT. These results are similar with other studies where rCBV (but not rCBF) is a strong predictor of the penumbra viability in acute ischemic

stroke patients [52], with similar values [53] or even lower [54] than those reported by the authors for patients with HT. Therefore, the penumbra viability indicators can indirectly predict the HT, which occurs more often in the infarct core region than in the irreversible penumbra part. Jain et al., found that rCBV level of at least 0.98 can predict the development of HT in stroke patients, with 72% specificity [48].

Our study's design did not include analysis of other PCT parameters (excepting PS) as predictive factors for potential hemorrhagic transformation, remaining the subject for further studies.

Conclusions

Quantitative analysis of functional parameters in dynamic cerebral perfusion computed tomography is very effective in the emergency diagnosis of acute ischemic stroke and hemorrhagic transformation prediction. Mean Transit Time map showed the highest sensitivity, relative Cerebral Blood Flow and relative Cerebral Blood Volume parameters were the most specific in the early diagnostic of ischemic stroke. The automatic method Tissue Classification showed the highest overall accuracy, significant correlation with final ischemic region extension, stratification of recoverable regions (penumbra) and constantly affected areas – facts, which are very important in treatment strategy selection. Assessment of blood-brain barrier permeability functions by analyzing the Permeability Surface area product, showed high specificity, sensitivity and overall precision in the hemorrhagic transformation predictive ability.

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A comparative study of rehabilitation methods of patients with edentulous arches associated with insufficient bone volume

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Abstract

Background: The rehabilitation of patients with insufficient bone support may be difficult because of multiple disturbances from stomatognathic system and the necessity to reestablish the lost bone volume.

Material and methods: In this study were included 24 patients who were treated with conventional prosthesis (15 patients) and fixed implanting supported prosthesis (9 patients).

Results: Implant-prosthetic rehabilitation with preliminary bone augmentation has esthetic, functional and biomechanical advantages over conventional prosthesis but it is more costly, traumatic and requires a long rehabilitation period with multiple surgical procedures. However, because of their disadvantages and hard conditions these prostheses are not always functional may not fully restore the lost functions of stomatognathic system. The necessity of additional surgical procedures, a higher cost and a longer rehabilitation time limit the applicability of this method.

Conclusions: Implant supported prostheses provide a psychological comfort and prevent the progression of bone atrophy. Rehabilitation with conventional prostheses permits to restore the lost soft and hard tissue volume and patients’ integration into the society. Bone grafting allows restoring of lost soft and hard tissue volume which allows inserting of implants of standard size and manufacture of functional and aesthetical restorations. The questionnaire analysis has shown that patients that wore conventional prostheses for many years are usually unsatisfied by their performance and require implant prosthetic rehabilitation with fixed restorations. The last ones are well tolerated by patients and provide a psychological comfort that cannot be achieved with conventional prostheses.

Key words: edentulous arches, insufficient bone volume.

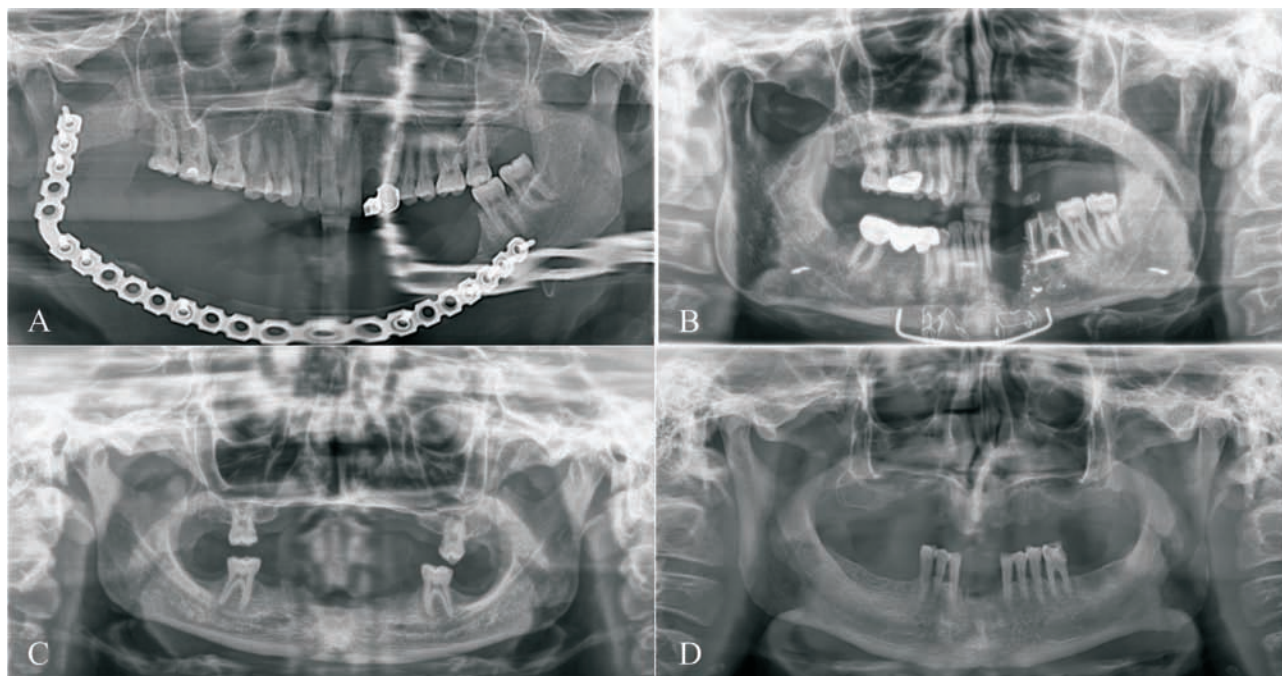
Background

The increasing demand of patients for an aesthetic and functional rehabilitation implies the necessity of a complex treatment in order to obtain the expected result. Often, the lack of bone support makes the rehabilitation difficult or even impossible.

There are many causes of insufficient bone support depending on the etiology (fig. 1) (bone atrophy, trauma, infection, tumors, traumatic extractions, periodontitis, congenital malformations, etc.). According to Atwood, teeth loss leads to bone crest loss which is manifested mainly on buccal side [1]. The rehabilitation of patients after tumor resection is often difficult to realize [2]. There have been proposed many methods for the rehabilitation of patients with insufficient bone support which have their cons and pros. According to the data of a study realized in 1990 based on 32 articles, the functional rehabilitation of patients with different forms of mandibular resections was barely obtained. In the same study it is mentioned that the expected functional and aesthetic results were obtained only in 4% of patients [3]. Even now, conventional acrylic prostheses are used to replace the extensive defects of dental arches. These prostheses are easy to be made, cheap and restore the lost bone volume. Their use leads to bone atrophy, loss of prostheses mobility and their fracture. Nowadays, implant-prosthetic rehabilitation gains popularity due to the poor performance of the conventional prostheses. According to Carl E. Misch, implant-supported prostheses provide good aesthetics; function and distribution of force, moreover, fixed implant-supported prostheses provide a psychological comfort due to their natural perception [4, 5]. In order to obtain a good aesthetic and functional result, there are some methods of implant placement in cases

of insufficient bone support: alternative methods of implant placement (short/narrow implants, tilted implants, zygomatic pterygomaxillary implants) and methods of bone grafting with implant insertion of standard sizes [6].

Alternative methods of implant placement in lateral areas with insufficient bone volume are widely used because of their advantages (low cost, minimum of surgeries, short rehabilitation time, etc.). According to the literature, short implants have a less success rate (Misch 16%, Goodacre 10%, Naert 19%) than standard or big size implants [7]. Some authors consider better to create sufficient bone volume for standard implant insertion thus avoiding overloading of the implants providing a better force distribution [8, 9]. The method is chosen by the prosthodontist together with surgeon, after a thorough analysis of parameters that can influence the rehabilitation results. According to Misch's data, it is necessary to analyze more than 60 parameters during implant treatment planning (prafunctions, crown height, masticatory dynamics, abutment position, force direction, opposing arch type, etc.) in order to obtain good functional and aesthetic results [10]. In case of insufficient bone volume, all these conditions can amplify the force applied to the prostheses and implants thus leading to their breakage, peri-implant resorption, TMJ disorders, porcelain fracture etc. [4]. Not least, patient's aesthetics influences to some extent the treatment plan, the wish to have a perfect smile especially in cases with gummy smile. Often, the lack of bone support compromises the aesthetics and the close insertion of muscles leads to mucosal dehiscence with further implant exposure. Thus, the prosthodontist's role is not only to determine the shape, position color of future prosthesis but also the position of the implants in dental arch. The adequate bone volume is necessary not only for placement of longer and



B - x-ray. Fig. 1. Insufficient bone volume in the upper and lower jaws caused by:
A) tumor resection; B) trauma; C) lack of tooth germs; D) traumatic extraction.

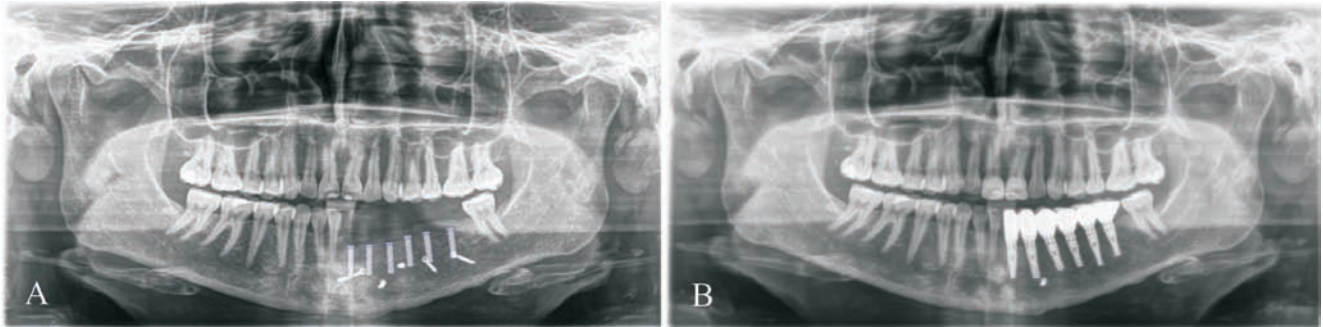


Fig. 2. Patient M.O. Implant insertion planning on OPG (A) and implant evaluation after 9 months from prosthesis delivery and 17 months postop (B).

wider implants but also for a good gingival contour and force distribution which requires 1-2 mm of cortical [11].

Aim: Evaluation of methods for patients' rehabilitation with insufficient bone support by comparative analysis of conventional and implant prosthetic methods with preliminary bone augmentation.

Material and methods

Study was based on clinical examination and treatment of 24 patients aged between 28-67 years with insufficient bone volume in the jaws of different etiology. The patients were divided in two groups according to the treatment plan. This was done in order to compare the groups and determine the advantages of each method in case of insufficient bone volume.

The first group consisted of 9 patients (mean age 38±2.77) with insufficient bone volume of the jaws. They were rehabilitated with implant supported prostheses after preliminary bone augmentation by osteodistraction (2 patients), iliac crest bone grafting (5 patients) and adjacent sites bone grafts (2 patients). 52 two stage dental implants with conventional implant loading have been inserted. Forty-five dental implants have been inserted in the lower jaw and 9 implants in the upper jaw.

The second group included 15 patients mean age 54.8±2.3, out of them 10 patients with insufficient bone support on one jaw and 5 patients on both jaws. In this group 1 patient underwent a mandibular resection. All patients from the second group have been rehabilitated with conventional acrylic prostheses. The patients came for prostheses adjustment after 2 weeks. They have been supervised for 12 months after prostheses delivery. Satisfaction degree and prostheses stability have been evaluated during the study period.

In order to choose the treatment method it is necessary to take into account the factors that might influence the long term results: the volume and shape of the bone defect, patient's motivation, concomitant disease, angulation and implant dimensions, crown height, the shape of bone crest, the soft tissue volume have been evaluated. The following criteria for group comparison have been taken: rehabilitation time, prostheses appearance and the adaptation time, prosthesis stability, bone and soft tissue status, price, patient's satisfaction degree. For

both groups have been taken panoramic X-rays (Orthophos XG3) and if necessary CBCT (Plabmecca ProMax 3D). The bone volume, before and after augmentation/implant insertion, implant angulation and their dimensions (fig. 2) have been determined on these images.

Implant stability has been determined by Periotestometry, Mombelli bleeding index has also been evaluated. Impressions have been obtained using open and closed tray technique with A or C silicone. Centric relation has been recorded with wax rims and transferred in the laboratory together with facebow records. The casts were mounted on semi-adjustable articulator (Amann Girrbach, Artex) (fig. 3a).

The mesostrucutre consisted of standard straight and angulated abutments. Plastic or metal frames of the future


<p>CLINICA STOMATOLOGICA</p>  <p style="text-align: right;">Questioner</p>	
<p>This questionnaire is anonymous, the data collected by the private clinic "Omnicent" will serve for better understanding of patients' needs and will improve the treatment quality.</p> <p>Questionnaire of patients' satisfaction:</p> <p><i>Answers to questions 1-10 must be given in numbers: 1 – the most negative, 4 – the most positive. To questions 11-13 answer only the wearers of conventional removable prostheses. To questions 11-13 answer only the wearers of implant supported prostheses.</i></p>	
1.	Do you eat with your prosthesis?
2.	Do you feel any discomfort when you chew?
3.	How well can you chew?
4.	Do you feel any changes in the food taste?
5.	Do you feel comfortable when you speak?
6.	Do you feel any unpleasant smell from your mouth?
7.	Can you easily clean your prostheses?
8.	Are you satisfied by the aesthetics of your prostheses?
9.	Do you feel that the prostheses affect your social life?
10.	How does the prostheses wearing influences your social integration?
<p>Conventional prostheses wearers</p>	
1.	Do you want to have implants in the closest future? If not, why?
2.	Did you have previously other removable prostheses?
3.	How many did you have your removable prostheses repaired?
<p>Implant supported prostheses</p>	
4.	Why did you accept implant-prosthetic rehabilitation?
5.	Why don't you want conventional removable prostheses?
6.	Did you previously wear any removable prostheses? Why do you refuse to wear them again?

Fig. 5. The sample of anonymous questionnaire used for determination of satisfaction degree.

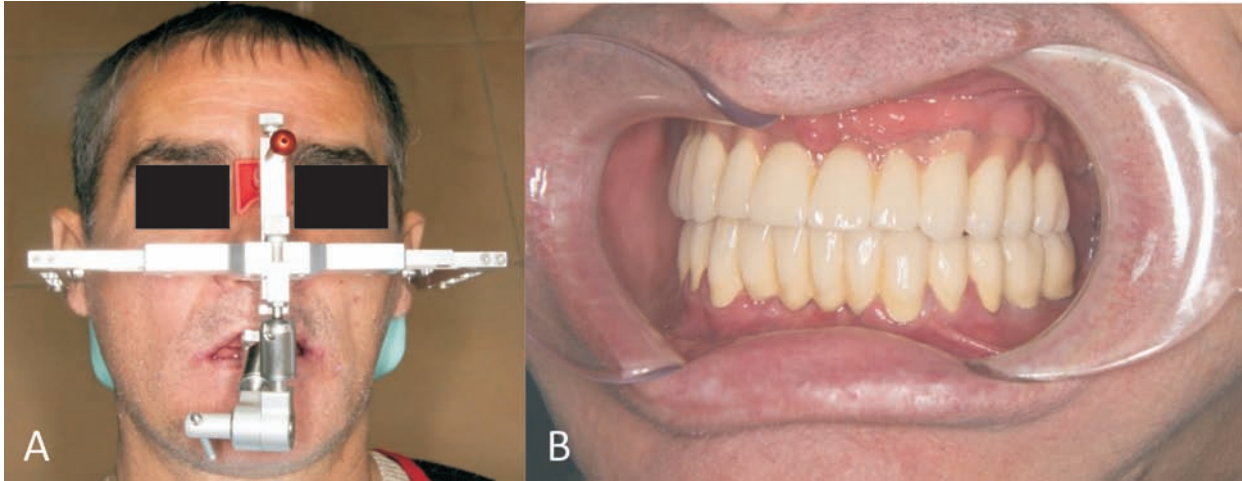


Fig. 3. A - Face-bow transfer (AmannGirrbach). B - Final prostheses.

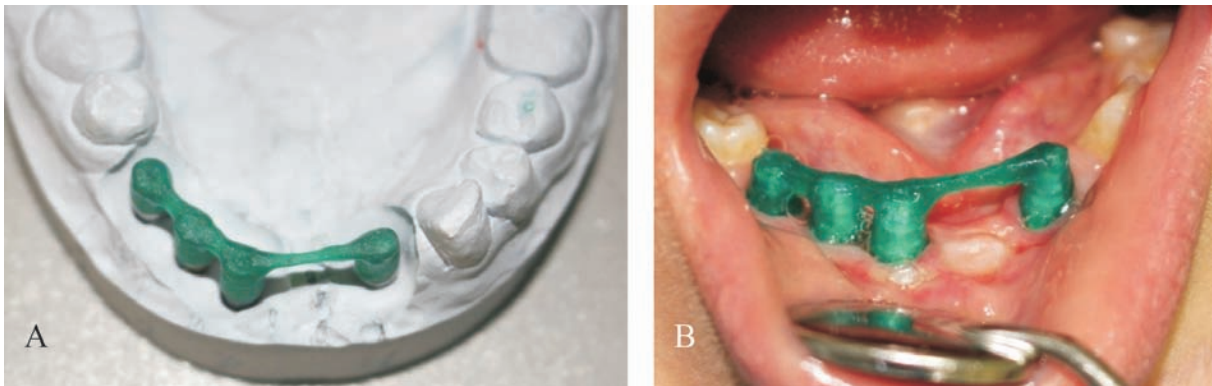


Fig. 4. Patient B. O. The aspect of plastic frame on the cast (A) and in the oral cavity (B).

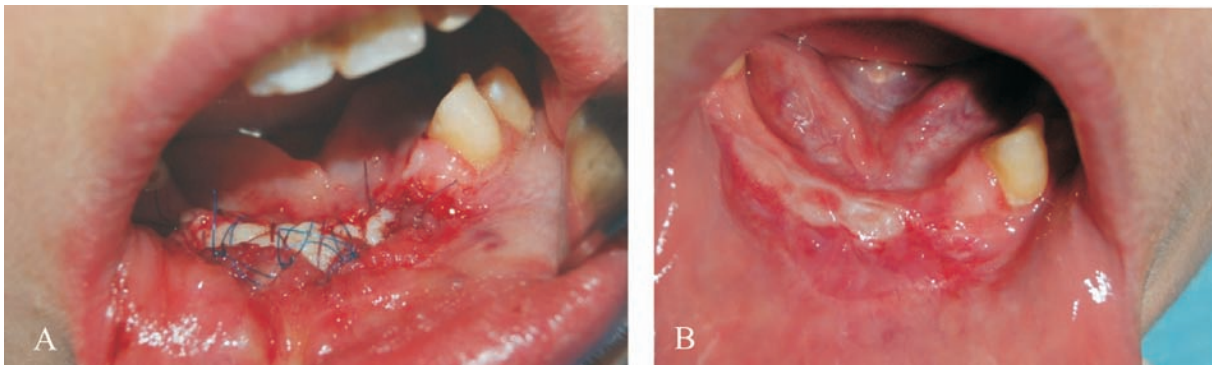


Fig. 6. Patient B. O. Free-tissue graft from hard palate: A - Intraoperator view; B - Graft aspect after 4 months.

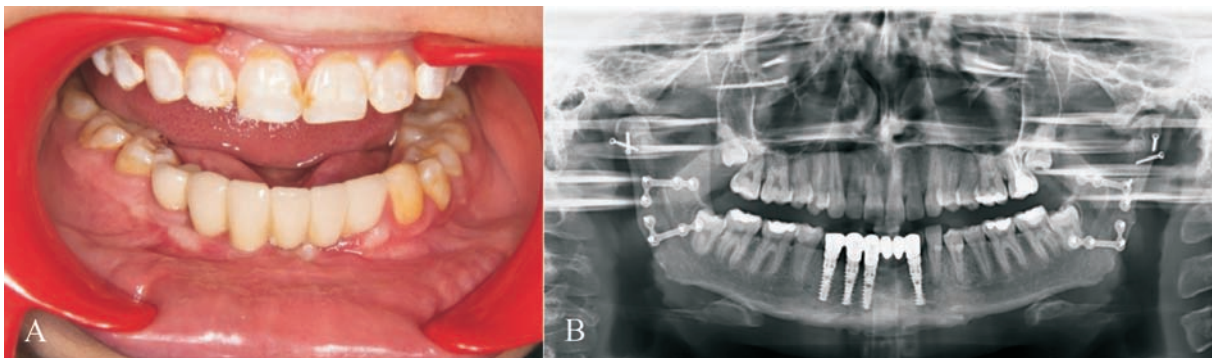


Fig. 7. Patient B. O. B - radiologic imaging. Prosthesis appearance 10 months after its delivery and 15 months after implant insertion: A - intraoral view.

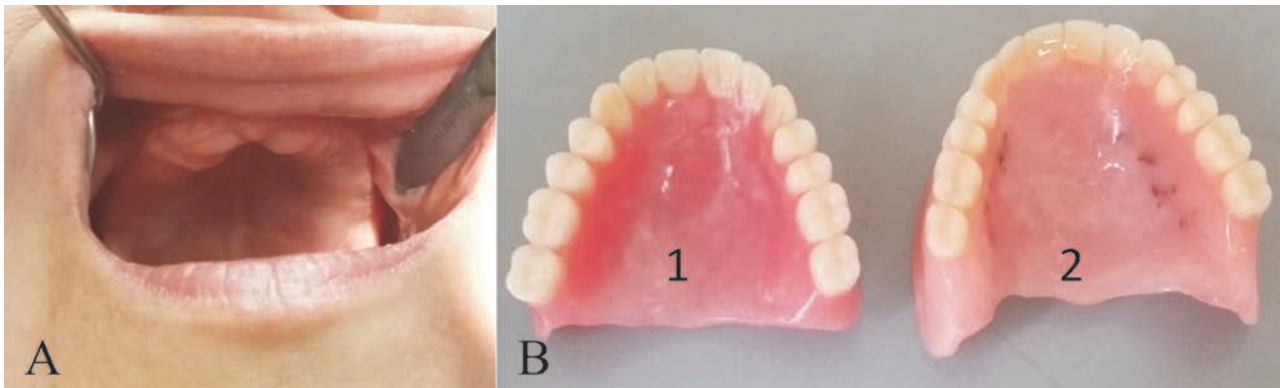


Fig. 8. Patient C. L. A – Severe atrophy in the posterior regions of maxilla. B – conventional prostheses 1- the newly made acrylic prosthesis, 2- The old prosthesis that had already been broken once and now has lost its stability.

prostheses were firstly tried in the buccal cavity with the determination of the teeth color (fig. 4).

The necessary bone volume for augmentation was calculated depending on the defect so it would allow the insertion of standard sized implants and will provide a crown length more than 8 mm and less than 15mm. All the defects implied more than 4 teeth and the inserted implant were connected together to allow a better stability and distribution of force. The manufacturing procedures of conventional prostheses is well-known and will not be described in this paper. A questionnaire consisting of 16 questions was made to determine the satisfaction degree of patients (fig. 5). The answer to first 10 questions was in form of numbers from 1 to 4 according to Linkert (1- the most negative, 4 the most positive). Questions 11-13 were for patients from group I and questions 14-16 were for patients from group II.

Results

In cases of patients from the first group, special attention was given to the necessary bone volume that would provide the possibility of standard sized implant insertion and manufacturing of fused-to-metal dental prostheses type FP1, FP2, FP3. The rehabilitation time varied depending on the chosen method and the case complexity. In 2 patients from the second group, additional procedures such as vestibuloplasty and free-tissue graft from hard palate were necessary (fig. 6).

Due to a sufficient number of implants of standard sizes, this method allows the manufacturing of functional and comfortable prostheses which are easily accepted because of their natural aspect. There has not been noticed any changes in peri-implant soft and hard tissues after 6 months (fig. 7). In one case has been noticed the exposure of implants at their crestal aspect.

The follow-up period was 12 months after prostheses delivery. At this point the bone resorption was 0.5 ± 0.08 mm mesial and 0.41 ± 0.06 mm distal. With this method it is possible to obtain good esthetic, functional and aesthetic results, also it allows to create an appropriate crown space, crown-implant ratio and insertion of implants in places which will facilitate force distribution. However, fixed implant-prosthetic reha-

bilitation with preliminary bone augmentation has some disadvantages as: long-term rehabilitation period (12.6 months in this study), the necessity of additional surgical procedures, higher price, bone graft resorption. All these limit to some extent the applicability of this method. In one case the graft was lost due to its exposure and the patient refused additional surgical procedures. In two cases it was necessary to perform additional grafting procedures to increase the volume.

Patients that refused implant-prosthetic treatment were rehabilitated with partial removable dentures that have some disadvantages as: bad prosthesis stability, trophy acceleration, low masticatory performance etc. [12, 13]. However there are many patients that choose conventional prostheses because they don't require surgeries, are cheaper and the rehabilitation time is shorter. But, according to our questionnaire, patients that wore dentures for many years are unsatisfied by their prostheses, the necessity to make a new one after a year or so (fig. 8) and usually, they accept implant prosthetic rehabilitation. Four patients from our study solicited implant prosthetic rehabilitation after wearing the conventional prostheses for many years despite the surgical risks and long rehabilitation time. Similar data have been found in a study realized by Agnieszka Koszuta et al. on 464 partially or completely edentulous patients [14].

Conclusions

1. The development of implantology permits to realize efficient and predictable results. Implant supported prostheses provide a psychological comfort and prevent the progression of bone atrophy.

2. Rehabilitation with conventional prostheses permits to restore the lost soft and hard tissue volume and patients' integration into the society. However, because of their disadvantages and hard conditions these prostheses are not always functional may not fully restore the lost functions of stomatognathic system.

3. Bone grafting allows restoring of lost soft and hard tissue volume which allows inserting of implants of standard size and manufacture of functional and aesthetic restorations. However, the necessity of additional surgical procedures, a

higher cost and a longer rehabilitation time limit the applicability of this method.

4. The questionnaire analysis has shown that patients that wore conventional prostheses for many years are usually unsatisfied by their performance and require implant prosthetic rehabilitation with fixed restorations. The last is well tolerated by patients and provide a psychological comfort that cannot be achieved with conventional prostheses.

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Morphology of the spleen and its ligamentous apparatus

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Abstract

Background: Knowing structural aspects of spleen is of interest, especially in surgical interventions, both urgent and elective ones, particularly in modern times when surgical interventions wider take place with maximal preserving injured organs, including in isolated or concomitant abdominal trauma.

Material and methods: Spleen capsule histoarchitectonics was studied by histotopograms with hematoxylin and eosin, van Gieson's, Weigert's resorcinol and fuchsin staining. The following parameters were studied depending on age and gender by morphometric method: spleen form, linear dimensions of spleen and its ligaments. Descriptive statistics allowed presenting results in tables.

Results: In teenagers (age Group VII), the most frequent form of the spleen is the intermediate one, while in adults (Groups VIII1 and VIII2) and people of the senile age – the prolate form. Difference in spleen length is statistically significant in age Groups VII and VIII1 – 121.5 ± 3.12 mm and 125.1 ± 3.08 mm respectively in men; 94.7 ± 2.09 mm and 85.8 ± 2.11 mm – in women ($p < 0.001$). Difference in the spleen width depending on gender is statistically significant for Group VII – 77.2 ± 2.11 mm in men and 61.6 ± 2.25 mm in women; in age Group VIII1 – 78.1 ± 2.07 mm in men and 59.2 ± 2.16 mm in women. For the examined age group the mean height of the gastrolial ligament is 51.8 ± 0.81 mm for men, and 45.9 ± 1.00 mm for women.

Conclusions: The capsule of spleen is formed by collagen fiber fascicles, elastic fibers as well as relatively few myocytes. The height of the gastrolial ligament is bigger in men than in women. Spleen dimensions decrease with aging.

Key words: spleen, capsule, ligaments, form of spleen.

Introduction

Industrial development, agricultural mechanization, sports promotion in population, increasing number of tourists lead to growth of injury cases that in recent years hold

an important place in mortality. According to WHO data, each year 8-10 million patients are admitted to hospitals with various injuries [1].

In the Republic of Moldova, injury incidence constitutes 4946.3 cases per 100 000 people, and in mortality structure

injuries constantly occupy the 3rd place, along with cardiovascular and oncologic diseases [2]. Serious concomitant chest and abdominal trauma is one of the severest conditions, both during military conflicts and peaceful daily life. According to literature data, its rate ranges between 4 - 5% [3] and 10% [4]. In recent years, there is a tendency of this rate to increase up to 18% [5, 6, 7].

Frequency of spleen injuries is increasing in recent times [8]. According to reports, their rate is growing, the incidence varying from 15.5% [3] in closed injuries to 58.17% in open abdominal traumas among all concomitant thoracoabdominal lesions [9].

Knowing structural aspects of spleen is of interest, especially in surgical interventions, both urgent and elective ones, particularly in modern times when surgical interventions wider take place with maximal preserving injured organs, including in isolated or concomitant abdominal trauma. The present research objective is elucidation of some peculiarities of spleen structure and ligaments.

Material and methods

Spleen capsule histoarchitectonics was studied by histopograms with hematoxylin and eosin, van Gieson's, Weigert's resorcinol and fuchsine staining.

The following parameters were studied depending on age and gender by morphometric method:

- Linear dimensions of spleen ligaments (in 65 cadavers);
- Spleen form in humans (in 273 cadavers: 154 men and 119 women);
- Spleen dimensions (184 gross specimens from adults (106 men and 78 women)).

Descriptive statistics allowed presenting results in tables.

Results and discussion

In the abdominal cavity the spleen is situated in the superior part and contacts with the diaphragm, stomach, tail of the pancreas, left angle of the colon, left adrenal gland, and left kidney.

The spleen is covered with a fibrous capsule that coalesces to its exterior with the peritoneum. The structure of the conjunctive layers of the spleen is based on some morpho-functional particularities that ensure, on the one hand, its tenacity, and on the other hand offer to the organ possibilities of extension. The parenchyma and conjunctive stroma of the spleen are integrated components. The conjunctive skeleton represents a conjunctivoelastical bioconstruction that executes the trophic function, is used as a support for the organ, maintains the specific form of the spleen, ensuring the pulp cells an intimate contact, creating an optimal biological environment based on distribution of nutritive substances and metabolism products elimination through the vascular tree.

According to publications [10, 11], the stroma represents a complex structure, formed from the organ capsule, intrasplenic perivascular layers, trabecules made of conjunctive tissue and a reticulate matrix for the red pulp and lymphoid follicles that form the white pulp.

The authors [12, 13, 14] describe outside the spleen a serous layer, under which can be found the fibrous layer, called classically splenic capsule. The capsule is very brittle, well defined, composed of dense connective tissue. It contains elastic fibers and myocytes among rare collagen fibers. From the capsule conjunctive trabecules leave into its inside, gradually branching and intersecting, forming incompletely demarcated spaces that communicate and house the splenic parenchyma.

M. Raica et al. (2009) believe that in humans, smooth muscle cells almost miss in the capsule structure, and therefore the spleen does not act as a blood reservoir. The stroma of red and white pulp, in turn, is formed by reticular tissue, representing a three-dimensional network forming the supporting structure and providing a functional microenvironment for the parenchyma.

In our study, the spleen exterior is defined by a well-shaped capsule with rectilinear and, more rarely, sinuous trajectory. The capsule introsusceptions in most cases do not fall deeply in the organ, but when forming deep fissures their walls are formed by fibrous tissue.

The thickness of the capsule varies from one sector to another of the spleen, and this is observed in all age groups. The course and thickness of the capsule do not depend on the organ shape or gender. Sometimes on the same organ, along the straight path of the capsule, thickenings are observed with changes in the trajectory path and fibrous layer thickness.

The visceral peritoneum covers entirely the spleen, excluding the hilum of the organ; it accretes intimately with organ's fibrous tunic. It should be noted that the peritoneum is characterized by a less pronounced development of networks of elastic fibers in relation to the well-developed collagen fibers, which is a basic structure of this anatomic formation, with a specific elasticity. They, to the extent of their physical and mechanical properties, cause quite high tenacity of serous tunic of the organ. In the visceral peritoneum spleen ligaments are inserted possessing a number of important functions for the organ [16, 17, 18, 19].

On the whole length of ligaments insertion on the splenic capsule, regardless of age, in the peritoneum there are nerves, blood vessels of different caliber, which link them with the organ. Very thin collagen fibers of the splenic ligaments and external tunic of vessels participate in the formation of the outer layer of the capsule. In the sectors of transition from the peritoneum to the splenic capsule surface, where an obvious tension force influences the organ, histotopography of the colagenoelastical elements modifies, the fascicles become thicker. Collagen fibers are well developed, wavy, forming fascicles composed of connective elements arranged free, easily expandable. In the hilum region, the capsule is strengthened with collagen fibers oriented in all directions, leaving from the pancreaticocolic ligament, accompanying blood vessels.

The visceral peritoneum on the diaphragmatic surface accretes intimately with the thin outer layer of the capsule, with collagen fibers stacked compact, almost without gaps, oriented parallel to the organ surface.

There is not a single opinion in the literature on the number of layers of tissue capsule and their composition. Ac-

ording to some data [20, 21], connective tissue membrane of the spleen presents a heterogeneous formation that consists of two slides with different origin. The external lamella is the peritoneum's visceral tunic and the internal one - a thin sheet of connective tissue, strong and low-stretching - fibrous tunica that accretes outward with the peritoneum. It consists of a network of collagen fibers, elastic fibers, and smooth muscle cells, the last ones giving the possibility of contraction

A. P. Sorokin et al. (2009) suggest that the splenic capsule consists of three layers: external, medium, and internal - which is adjacent to the splenic pulp. The outer layer thickness is 18-45.15 mkm. It consists of thin collagen fibers (1.5x1.5 - 14x25 mkm) with diverse orientation. The medium spleen layer is the thickest (38.7 - 85.5 mkm), its fibers become thicker and bed compact spaces. The profound layer is at the border with the organ parenchyma, having a thickness of 19.9-57 mkm. Collagen fibers of 1.5 to 8.5 mkm lie freely having various orientations.

According to own data, taking into account size and character of fibrous fascicles, we distinguish only 2 layers of the capsule: external and internal. The outer layer of the capsule is composed of well-developed collagen bundles, wavy, stacked compact with parallel orientation on the surface of the organ. The thickness and orientation of collagen bundles are very individual. They pass into free spaces and interweave with collagen fibers from the inner layer of the capsule, increasing the thickness of this portion.

On the visceral surface of the spleen, the external layer of the capsule becomes more compact. The collagen fibers are situated freely, with evident spaces between them. The fascicle trajectory can be oblique or perpendicular to the longitudinal axis of the organ. At the border with the visceral peritoneum, in the outer layer of the capsule, blood and lymphatic vessels are present. Perivascular collagen fibers are well-developed and represented by conjunctive fascicles.

The internal layer of the capsule situated at the border with the pulp of the organ has thin collagen fibers, situated freely with different space orientation. In the center of the layer their direction is parallel with the surface. At the periphery of the layer, the fibers associate in bundles, their density rises around the blood vessels. The direction of the collagen fibers of the internal part when passing into the parenchyma of the spleen is obliquely perpendicular.

In the capsule elastic fibers are present, arranged compactly, sometimes with free spaces in the peripheric portion, filled in with fundamental substance and blood vessels in the peripheric zone. The central part of the capsule is formed of corrugated fascicles, arranged freely, although sometimes compactly, passing to the perpendicular direction to the splenic surface.

The collagen fibers from the capsule spread into the depth of the spleen in the form of conjunctive tracts named *splenic trabecules*. The trabecules contain more contractile elements than the capsule, go through the organ, representing not only attachment structures of the reticular stroma and parenchyma, but also the storage of the blood vessels.

In some cases the splenic trabecules are formed by the confluence of two conjunctive branches with their origin in the internal layer of the capsule. The branches unite near the capsule, forming a thick and long trabecule, or they penetrate deeply into the parenchyma and confluent there. In other cases, the internal layer of the capsule is well-developed, formed of corrugated thick collagen fibers that unite in bundles, and change the orientation into transversal forming incipient trabecules. Sometimes the trabecules start from a thickening of the internal layer of the capsule, modifying the thickness and architectonics of the internal layer.

Dimensions, form and direction of the trabecules in the parenchyma of the spleen vary a lot. In some cases, a trabecule begins with 2-3 thin fascicles that do not change evidently the architectonics of the internal layer. In the depth of the organ, the trabecule forks and thickens. The trabecules are oriented into the parenchyma of the spleen in different directions and contain 9-12 vessels in their structure. They go through the organ representing not only attachment structures of the reticular stroma but also serve as storage for blood, lymphatic vessels and nerves.

Along with collagen fascicles, in the structure of the trabecules a well-developed net of elastic fibers exists. The elastic fibers form circular perivascular fascicles in the vessel tunics where they strengthen the trabecular structure.

According to authors [22, 23, 24], in some animals the spleen is encapsulated in a musculofiber membrane. In the hilum, on the medial surface of the spleen, the origin capsule branches into trabecules that accompany nerves and vessels in the interior of the spleen. These trabecules are formed of collagen, elastic fibers, doubled by the smooth muscular fibers, thereby forming a skeleton that offers contractility to the spleen. In humans, in the structure of the internal layer of the capsule and in trabecules, number of myocytes is low [25, 26].

The spleen is somehow a mobile organ, especially when performing respiratory movements. The organ is held in place by the peritoneal contacts, by the thorax aspiration and partially by the abdominal pressure, established by the tonic contraction of the abdominal muscles. Its mobility is conditioned concurrently by the stomach and transverse colon mobility. The visceral peritoneum covers the spleen on all its sides, except the hilum and the ligament fixation line, growing intimately with its fibrous tunica, forming the ligaments of the organ.

C. Enculescu (2006) determined that the development of the spleen causes several plicae at the level of the dorsal mesogastrium, that will further participate in the formation of the greater omentum by their transformation into ligaments: phrenicosplenic plica with the development of the phrenicosplenic ligament, pancreaticosplenic, spleno-renal and gastrosplenic plicae, the latter developing into the gastrosplenic ligament.

G. I. Vind (1999), examining the spleen ligaments in the context of their embryonic development, considers that the first formation to appear is the gastrolial ligament. This ligament starts from the greater curvature of the stomach, has a trapezoid form, narrows in the cardiac portion of the

stomach and largens at its inferior portion. The fascia that appeared because of blending the left surface of the dorsal mesogastrium and the posterior parietal peritoneum, passes between the pancreas and the renal fascia. The lateral margin of this blended fascia passes immediately into the posterior margin of the spleen and is called *splenorenal ligament*. The superior part of the blended structure is directed upper than the kidney, and unites the spleen with the diaphragm. This part is called the *phrenicolienal ligament*. Usually, there is a junction between the inferior pole of the spleen and the left flexure of the colon, called the *splenocolic ligament*.

The dorsal embryonic mesogastrium is responsible for the production of the splenic ligaments [12, 32, 33, 34, 35]. After the separation into two plicae, the mesogastrium surrounds the organ, forming 2 main ligaments: gastrosplenic and splenorenal. The following ligaments differentiate from them: splenophrenic, splenocolic, pancreaticosplenic, phrenicocolic, pancreaticocolic, and the presplenic plica. The last ones are named minor ligaments, but they can cause problems in surgery. According to author [36], in 70% of 3-month embryos, the pancreaticosplenic ligament is already formed.

N. P. Bisenkov et al. (1978), A. P. Sorokin et al. (1989) considered that the permanent ligaments of the spleen, well-contoured are the gastrolial, phrenicolienal, phrenicocolic. From the embryologic point of view, the phrenicolienal ligament is formed as a secondary accreting of the dorsal mesogastrium with the posterior layer of the parietal peritoneum, and from the surgical point of view the ligament represents the mesentery of the spleen, represented by two layers of peritoneum. In case of a high number of ligaments, besides those named anteriorly, the authors distinguish the colicolienal, supplementary gastrolial, and splenorenal ligaments.

The number of splenic ligaments may vary between 3 and 7 [38]. The minimal number is found in 37% of cases (as a rule, in people with a wider thoracic cavity), and the maximal number – in 12% of cases (as a rule, in people with a narrower thoracic cavity).

The anatomy of the splenic ligaments was also studied by authors [39]. The authors describe 4 main ligaments of the organ: gastrolial, colicolienal, phrenicocolic and phrenicolienal. The gastrolial ligament has 2 parts. In the superior

part, where the spleen is close to the stomach, the collagen fibers are denser. The authors consider that the term "splenorenal ligament" is uncertain, because besides the peritoneal coverage, there are 3 structures between the spleen and the left kidney: collagen tissue with adipose perirenal tissue, prerenal fascia and an adipose capsule (perirenal adipose tissue). It is important for the surgeon to know the direction of the collagen fibers in the splenic ligaments. In all the ligaments, most fibers come from 2 directions: laterocranial – mediocaudal and mediocranial – laterocaudal.

According to [40], the left phrenicocolic ligament, unlike the right one, is better contoured and can be met in all cases. The phrenicocolic ligament unites with the splenocolic one, and their separation can only be conventional. If the left phrenicocolic ligament is well-developed, the splenocolic ligament can also be noticed, both having a common area of fixation on the transverse colon.

Any kind of abnormal ontogenesis of the main splenic ligaments manifests as their diminution, elongation or absence. The authors [16, 41, 42, 43, 44, 45, 47, 52] consider that the gastrolial, splenorenal, phrenicolienal and splenocolic ligaments maintain the spleen in the normal position. The main ligaments that prevent ptosis are gastrolial and splenorenal. The phrenicolienal ligament only limits moving the spleen down [46, 47, 48, 49, 50, 51].

Gastrolial ligament represents in our study a duplicature of the peritoneum. Its anterior layer leaves from the vertical portion of the greater curvature of the stomach (that forms the bottom of the stomach) to the left and posteriorly, reaching the hilum of the spleen. The uniting of the anterior layer of this ligament with the spleen takes place from near the posterior pole of the organ till the anterior margin of the hilum, and sometimes till the anterior pole, alongside the anterior margin of the spleen. The posterior layer covers the hilum on the gastric surface and is a part of the omental bursa peritoneum. Inferiorly, the gastrolial ligament transforms into the gastrocolic ligament. The dimensions of the gastrolial ligament depend on age and gender. Linear dimensions of the ligament have been determined in 3 points: 1) from the stomach to the posterior pole of the spleen; 2) in the splenic hilum region; 3) from the stomach to the anterior pole of the spleen.

Table 1

Height of the gastrolial ligament in age Group VII, mm

Gender	Group VII									Mean value in the group
	Height			Height			Height			
	At the posterior pole			At the hilum			At the anterior pole			
	min	max	mean	min	max	mean	min	max	mean	
M	28	46	36.8±0.96	42	62	51.7±0.84	52	82	66.9±0.82	51.8±0.81
F	22	45	33.6±1.02	33	60	46.8±1.00	50	65	57.5±0.99	45.9±1.00
t			2.2845			3.7519			7.3124	4.5847
p			<0.05			<0.01			<0.001	<0.01
Total	22	46	35.2±0.99	33	62	49.3±0.92	50	82	62.2±0.81	48.9±0.91

Table 2

Height of the gastrolial ligament in age Group VIII₁, mm

Gender	Group VIII ₁									Mean value in the group
	Height			Height			Height			
	At the posterior pole			At the hilum			At the anterior pole			
	min	max	mean	min	max	mean	min	max	mean	
B	17	26	21.4±1.08	22	42	31.8±1.12	26	61	46.4±1.14	33.2±1.11
F	23	62	42.2±2.32	28	73	50.3±2.16	36	86	60.6±2.18	51.0±2.22
t			8.1279			7.6035			5.7722	7.1715
p			<0.001			<0.001			<0.001	<0.001
Total	17	62	31.8±1.72	22	73	41.1±1.64	26	86	53.5±1.66	42.1±1.67

Table 3

Height of the gastrolial ligament in age Group VIII₂, mm

Gender	Group VIII ₂									Mean value in the group
	Height			Height			Height			
	At the posterior pole			At the hilum			At the anterior pole			
	min	max	mean	min	max	mean	min	max	mean	
B	8	45	26.2±1.09	12	60	35.6±1.18	14	75	44.1±1.04	35.3±1.10
F	12	42	26.8±1.24	18	56	36.8±1.22	20	78	48.8±1.13	37.5±1.19
t			0.3634			0.7070			3.0604	1.3576
p			>0.05			>0.05			<0.01	>0.05
Total	8	45	26.5±1.17	12	60	36.2±1.20	14	78	46.5±1.09	36.4±1.15

Table 4

Height of the gastrolial ligament in age Group IX, mm

Gender	Group IX									Mean value in the group
	Height			Height			Height			
	At the posterior pole			At the hilum			At the anterior pole			
	min	max	mean	min	max	mean	min	max	mean	
B	15	40	27.7±1.06	20	62	40.6±1.02	26	78	51.8±1.06	40.0±1.05
F	14	50	31.8±1.20	18	75	45.5±1.14	20	80	48.6±1.12	41.9±1.15
t			2.5607			3.2032			2.0751	1.2201
p			<0.05			<0.01			<0.05	>0.05
Total	14	50	29.8±1.12	18	75	43.1±1.08	20	80	50.2±1.09	43.0±1.09

The information on mean values of the ligament dimensions depending on gender in age Group VII is presented in table 1.

Based on the information presented in Table 1, we can conclude that for the examined age group the mean height of the gastrolial ligament is 51.8±0.81 mm for men and 45.9±1.00 mm for women. The difference depending on gender is statistically significant (p<0.01).

Table 2 reflects the information on mean values of the ligament height depending on gender in age Group VIII₁.

Based on the information in the table, we can state that the mean height of the gastrolial ligament in men is smaller (33.2±1.11 mm) than in women (51.0±2.22mm). The difference depending on gender is statistically significant for the age group analyzed (p <0.001).

The examination results for the second period of maturity (age Group VIII₂) are presented in table 3. According to the results, we can conclude that for this age group the mean height of the gastrolial ligament is 35.2±1.10 mm for men,

Table 5

Height of the gastrolial ligament, mm

Gender	Group X									Mean value in the group
	Height			Height			Height			
	At the posterior pole			At the hilum			At the anterior pole			
	min	max	mean	min	max	mean	min	max	mean	
B	12	38	24.1±1.08	16	60	37.7±1.16	20	75	47.2±1.04	36.3±1.09
F	12	40	25.4±1.11	20	55	37.2±1.20	24	69	46.8±1.22	36.5±1.18
t			0.8394			0.296			0.2495	0.124
p			>0.05			>0.05			>0.05	>0.05
Total	12	40	24.8±1.09	16	60	37.5±1.14	20	75	47.0±1.02	36.4±1.08

and 37.5±1.19 mm for women. The difference depending on gender is not statistically significant ($p>0.05$).

Information on mean values of the gastrolial ligament height depending on gender for advanced age is presented in table 4. The results prove that the mean value of the gastrolial ligament height in the lot is 40.0±1.05 mm in men, being higher in women – 41.9±1.15 mm. The difference depending on gender is not statistically significant ($p>0.05$) in Group IX.

Table 5 presents the results of morphometric study of the gastrolial ligament depending on gender in people of the senile age (75-90 years). Based on the information presented in table 5, we can state that the mean value for both genders is almost equal: 36.3±1.09 mm in men and 36.5±1.18 mm in women. The difference depending on gender is not statistically significant ($p>0.05$) in Group X.

The phrenicolial ligament is composed of 2 layers of peritoneum that begin from the lumbar part of the diaphragm. The dorsal layer passes from the left to the right and anteriorly, getting close to the diaphragmatic surface or the posterior margin of the spleen, where it passes on its renal surface. This ligament covers the tail of pancreas and all splenic vessels, including the root of the left gastroepiploic artery. The ligament with its dorsal layer continues down covering a part of the left kidney and forming the splenorenal ligament. The ventral layer of the ligament from the lumbar part of the diaphragm continues to the left, covering the left adrenal gland, comes close to the splenic hilum, where it grows together with the dorsal layer of the mentioned ligament.

The inferior portion of the anterior layer of the phrenicolial ligament that spreads from the tail of pancreas till the splenic hilum is named the pancreaticosplenic ligament.

The size of the pancreaticolial ligament depends on the pancreas localization. According to [53], the tail of pancreas touches the spleen in 27% of cases and is situated at a distance of 1 cm from the spleen in 73% of cases.

A. Rosen et al. (1988), having analyzed results of 32 CTs and 37 autopsies, noticed that in 29,7% of autopsies and 25% of CTs, the tail of pancreas was adjacent to the splenic hilum.

If the tail of pancreas is shorter and further from the sple-

en, posterior layer of the peritoneum forms phrenicolial or renolial ligament [55].

The splenorenal ligament is formed of 2 peritoneal layers. The anterior layer that belongs to the omental bursa comes from the posterior wall of the bursa and reaches the hilum of the spleen where it is attached. The posterior layer passes from the anterior surface of the left kidney and reaches the splenic hilum.

Linear parameters and forms of the ligaments are various: long, short, narrow, wide. All these peculiarities determined in the embryonic period can be quite insignificant or may cause splenic ptosis, torsion or deviation. Somewhat, motility of the spleen depends on elasticity of the splenic ligaments and length of the splenic vessels.

The splenorenal ligament is 2.5-5.5 cm long, it contains between its layers the main splenic vessels (splenic pedicle) and their branches [56].

To avoid surgical complications, we should take into account the variety of forms, surfaces, limits and sizes of the spleen, as well as its relations with adjacent organs [57].

During ontogenesis and ulterior development, the form and dimensions of the spleen modify. Dimensions and pressure of the adjacent organs play an important role in the stabilization of spleen form.

In the special literature different forms of the spleen are described. Author [58], having studied 100 cadavers, describes 3 forms of the spleen: cuneate, tetrahedral and triangular. In the opinion of [37], the organ can have 2 marginal forms: short and wide (oval or almost circular on the diaphragmatic surface) spleen and the other form is narrow and long. Other authors [57] describe 3 forms of the spleen: cuneate (44%), tetrahedral (42%) and triangular (14%).

According to the results of studies [10], the basic forms of the spleen are: discoid, ellipsoid and mixed. The semilunar, unsymmetrical disc, triangular, oval with a sharp anterior pole, oval with a sharp posterior pole forms are named *transitional*. More often (25%) the oval form with a sharp anterior pole is observed, in 16% of cases – unsymmetrical disc form, in 14,5% – ellipsoid form, in 11,5% – oval form with a sharp posterior pole. And just in 10.5% of cases the semilunar form was

Table 6

Frequency of spleen form types depending on gender

Spleen form	Men, n=154		Women, n=119		t	p
	Abs.	P±ES (%)	Abs.	P±ES (%)		
Prolate <63.0%	79	51.3±4.03	45	37.8±4.44	2.2506	<0.05
Intermediate 63.0-75.0%	57	37.0±3.89	51	42.9±4.54	0.9872	>0.05
Round 76.0% >	18	11.7±2.59	23	19.3±3.62	1.7081	>0.05
Total	154	56.4±3.99	119	43.6±4.55	2.1149	<0.05

Table 7

Frequency of spleen form types in age Group VII

Spleen form	Men, n=16		Women, n=10		t	p
	Abs.	P±ES (%)	Abs.	P±ES (%)		
Prolate <63.0%	7	4.5±1.67	3	2.5±1.43	0.9092	>0.05
Intermediate 63.0-75.0%	8	5.2±1.79	7	5.9±2.16	0.2496	>0.05
Round 76.0% >	1	0.6±0.62	0		0.9641	>0.05
Total	16	10.3±2.45	10	8.4±2.54	0.5381	>0.05

observed, in 8,5% – discoid form, more rarely the mixed and triangular forms are seen – 8% and 6% respectively.

The forms of the spleen are variable because this is a soft parenchymatous organ, changed by the influence of dimensions and form of the adjacent organs; usually it has the form of a grain of coffee [33, 14].

Thus, studying diversity of the spleen forms has a long history. In recent years, timeliness of the problem increases due to progress of surgical techniques related with the parenchymatous organs, in tomographic investigations for diagnostics and treatment.

The study object has become the spleen form in humans and its individual variations studied in different periods of the ontogenesis and depending on gender. Analyzing the results obtained in this chapter, we stated the prolate, round and intermediate form of the spleen.

Based on the information presented in table 6, we can conclude that the most frequent form of spleen in men is the prolate one. It is in 79 (51.3±4.03%) observations of 154 included in study group I; this nr is almost a half of the analyzed cases. In women the most frequent form is the intermediate one. It is in 51 (42.9±4.54 %) observations of 119 included in study group II. The difference depending on gender for the prolate form is statistically significant – $p < 0.05$ (women – 37.8±4.44 % of cases, men – 51.3±4.03%). For the intermediate form the difference depending on gender is not statistically significant – 42.9±4.54% of cases in women and 37.0±3.89% in men ($p > 0.05$). The frequency of the round form was higher in women than in men – 23 (19.3±3.62%) and 18 (11.7±2.59%) observations respectively.

Based on the analysis of the results obtained regarding diversity of the spleen forms depending on gender, the material was studied depending on age of the deceased people.

According to the information in table 7, in the teen age (age Group VII) the most frequent form of spleen is the intermediate one. It encounters 8 (5.2±1.79 %) observations in men and 7 (5.9±2.16 %) in women. The prolate form had a much higher frequency in men than in women – 7 (4.5±1.67 %) and 3 (2.5±1.43 %) observations respectively. The difference depending on gender is not statistically significant ($p > 0.05$) for both forms. In the analyzed group, the round form is rare – 1 (0.6±0.62 %) observation in men and no cases in women.

Analyzing the obtained results; we concluded that in adults (Groups VIII₁ and VIII₂) the prolate form of the spleen has the highest frequency (tables 8, 9). According to the information in Table 8 (age Group VIII₁), the prolate form is in 18 (11.7±2.59%) observations in men and 2 (1.7±1.19%) in women. The difference depending on gender is statistically significant ($p < 0.001$). In age Group VIII₂, the number of observations of the prolate form is much higher than in the previous age group. It is in 29 (18.8±3.15%) observations in men and 10 (8.4±2.54%) in women. For the prolate form of the spleen, the difference depending on gender is statistically significant ($p < 0.05$) in age Group VIII₂. The second form by frequency is the intermediate form. In age Group VIII₁, it is in 10 (6.5±1.99 %) observations in men and much less in women – 2 (1.7±1.19%). In age Group VIII₂ the intermediate form was seen more often: 22 (14.3±2.82%) observations in men and much less – 8 (6.7±2.29%) in women. In both age groups, the differences depending on gender is statistically significant ($p < 0.05$). The round form was more rarely observed than the previous 2 forms. In women of age Group VIII₁ 4 (3.4±1.66%) observations were registered and only one case (0.6±0.62 %) in men.

In the second period of maturity (age Group VIII₂) the round form was observed in 8 women (6.7±2.29 %) and just

Table 8

Frequency of spleen form types in the 1st period of adulthood (Group VIII₁)

Spleen form	Men, n=29		Women, n=8		t	p
	Abs.	P±ES (%)	Abs.	P±ES (%)		
Prolate <63.0%	18	11.7±2.59	2	1.7±1.19	3.5109	<0.001
Intermediate 63.0-75.0%	10	6.5±1.99	2	1.7±1.19	2.0751	<0.05
Round 76.0% >	1	0.6±0.62	4	3.4±1.66	1.5783	>0.05
Total	29	18.8±3.15	8	6.7±2.29	3.1071	<0.01

Table 9

Frequency of spleen form types in the 2nd period of adulthood (Group VIII₂)

Spleen form	Men, n=154		Women, n=119		t	p
	Abs.	P±ES (%)	Abs.	P±ES (%)		
Prolate <63.0%	29	18.8±3.15	10	8.4±2.54	2.5698	<0.05
Intermediate 63.0-75.0%	22	14.3±2.82	8	6.7±2.29	2.0909	<0.05
Round 76.0% >	6	3.9±1.56	8	6.7±2.29	1.0099	>0.05
Total	57	37.0±3.89	26	21.8±3.78	2.8003	<0.01

Table 10

Frequency of spleen form types in the elderly

Spleen form	Men, n=28		Women, n=42		t	p
	Abs.	P±ES (%)	Abs.	P±ES (%)		
Prolate <63.0%	13	8.4±2.24	14	11.8±2.96	0.9172	>0.05
Intermediate 63.0-75.0%	9	5.8±1.88	23	19.3±3.62	3.3098	<0.001
Round 76.0% >	6	3.9±1.56	5	4.2±1.84	0.1244	>0.05
Total	28	18.1±3.10	42	35.3±4.38	3.2040	<0.01

Table 11

Frequency of spleen form types in the senile age

Spleen form	Men, n=24		Women, n=33		t	p
	Abs.	P±ES (%)	Abs.	P±ES (%)		
Prolate <63.0%	12	7.8±2.16	16	13.4±3.12	1.4746	>0.05
Intermediate 63.0-75.0%	8	5.2±1.79	11	9.2±2.65	1.2512	<0.05
Round 76.0% >	4	2.6±1.28	6	5.0±1.99	1.0109	>0.05
Total	24	15.6±2.92	33	27.7±4.10	2.4019	<0.05

Note: the organ form types were studied by the spleen index: SI = (spleen width / spleen length)*100%. When SI is < 63.0%, spleen form is prolate, 63.0 – 75.0% - intermediate, > 76.0% - round form (A. K. Inakov, 1985).

6 (3.9±1.56%) in men. For the round form of the spleen, the difference depending on gender is not statistically significant (p>0.05) in both age groups.

According to the information in table 10, in the advanced age group, in men the prolate form of the spleen is observed in 13 (8.4±2.24%) cases. In women this form was seen in 14 (11.8±2.96%) cases. The difference depending on gender is not statistically significant (p>0.05). The intermediate form of the organ was seen more often in women – 23 (19.3±3.62%) observations and more rarely in men – 9 (5.8±1.88%) obser-

vations. The difference depending on gender is statistically significant (p<0.001). As for the round form, in age Group IX, more observations were registered in men – 6 (3.9±1.56%) cases and 5 (4.2±1.84%) – in women. For the round form, the difference depending on gender is not statistically significant (p>0.05).

The results obtained in age Group IX are presented in table 11. Based on the obtained information, we can conclude that the most frequent form of spleen was the prolate one. It is in 16 (13.4±3.12%) observations in women and 12 (7.8±2.16%)

Table 12

Length of spleen depending on gender

Age groups*	Men, n = 106		Women, n = 78		t	p
	Abs.	M1 ± ES1 (mm)	Abs.	M2 ± ES2 (mm)		
VII	16	121.5 ± 3.12	10	94.7 ± 2.09	7.1365	< 0.001
VIII1	20	125.1 ± 3.08	8	85.8 ± 2.11	10.527	<0.001
VIII2	30	119.2 ± 3.16	20	113.9 ± 2.14	1.3887	> 0.05
IX	20	124.1 ± 3.41	20	122.2 ± 2.12	0.4732	> 0.05
X	20	105.5 ± 2.54	20	109.1 ± 2.08	1.0966	>0.05
Total	106	119.2 ± 1.26	78	105.1 ± 2.07	5.8185	<0.001

Table 13

Width of spleen depending on gender

Age groups*	Men, n = 106		Women, n = 78		t	p
	Abs.	X1 ± ES1 (mm)	Abs.	X2 ± ES2 (mm)		
VII	16	77.2 ± 2.11	10	61.6 ± 2.25	5.0574	<0.001
VIII1	20	78.1 ± 2.07	8	59.2 ± 2.16	6.3174	<0.001
VIII2	30	79.0 ± 2.08	20	79.1 ± 2.17	0.0333	>0.05
IX	20	84.5 ± 2.19	20	80.0 ± 2.19	1.4971	>0.05
X	20	71.4 ± 2.14	20	69.8 ± 2.35	0.5034	>0.05
Total	106	78.2 ± 2.22	78	69.9 ± 2.15	2.6857	< 0.01

Note*: The periods of ontogenesis are classified on the basis of periodization approved by Institute of Age Physiology of the Academy of Sciences of USSR (by A. A. Markosyan (1969), S. B. Tikhvinskiy, S. V. Khrushchev (1991)), as well as the one proposed by R. Robacki (quoted by M. Ștefanț et al., 2000). Women: VII – 16-20 years, VIII₁ – 21-35 years, VIII₂ – 36-55 years, IX – 56-74 years, X – 75-90 years. Men: VII – 17-21 years, VIII₁ – 22-35 years, VIII₂ – 36-60 years, IX – 61-74 years, X – 75-90 years.

in men. The difference depending on gender is not statistically significant ($p > 0.05$). The 2nd most frequent form in age Group IX was the intermediate one: 11 (9.2±2.65%) cases in men and 8 (5.2±1.79%) in women. The difference depending on gender is statistically significant ($p < 0.05$). The round form is rarely seen in the elderly: 6 (5.0±1.99%) observations in women and 4 (2.6±1.28 %) in men. The difference depending on gender is not statistically significant ($p > 0.05$).

When describing the structural particularities of every organ a special place is occupied by the linear parameters. They become one of the main criteria when determining the presence of pathological processes, determining at the same time clinical symptoms of a disease, because structure and functional condition of the tissular substrate – morphophysiological assembly – is a unitary process that ensures the vitality of each organism [59].

Linear dimensions and weight of the spleen may vary a lot, especially in pathological processes: it is bigger in tuberculosis, malaria, typhoid fever, syphilis [60, 27]. In terms of individual variability of the spleen its linear dimensions (length, width) are understood that have a variability range. The spleen dimensions were studied by gross specimens from adults depending on age and gender. The information concluded

on mean values of the spleen dimensions depending on age, gender and number of case studies is presented in tables 12, 13.

Based on the obtained information (table 12), we can conclude that the difference depending on gender is statistically significant, the mean value – 119.2±1.26 mm in men included in study group I, and 105.1±2.07 mm in women included in study group II ($p < 0.001$). The difference is also statistically significant for the spleen length in age Groups VII and VIII₁ – 121.5±3.12 mm and 125.1±3.08 mm respectively in men; 94.7±2.09 mm and 85.8±2.11 mm in women ($p < 0.001$). In Groups VIII₂, IX, the difference depending on gender is not statistically significant ($p > 0.05$). The length of the organ in men is a little bit larger than in women (119.2±3.16 mm and 113.9±2.14 mm; 124.1±3.41 mm and 122.2±2.12 mm).

The analysis of results presented in Table 13 shows the difference in the spleen width depending on gender that is statistically significant for Group VII – 77.2±2.11 mm in men and 61.6±2.25 mm in women; in age Group VIII₁ – 78.1±2.07 mm in men and 59.2±2.16 mm in women. The difference depending on gender is statistically significant ($p < 0.001$). In Groups VIII₂, IX and X the difference is not statistically significant ($p < 0.05$). The width values in men are almost equal to those in women.

Conclusions

The capsule of spleen is formed by collagen fiber fascicles, elastic fibers as well as relatively few myocytes.

In teenagers (age Group VII), the most frequent form of the spleen is the intermediate one, while in adults (Groups VIII₁ and VIII₂) and people of the senile age – the prolate form of the spleen.

As for spleen length, the difference is statistically significant in age Groups VII and VIII₁ – 121.5±3.12 mm and 125.1±3.08 mm respectively in men; 94.7±2.09 mm and 85.8±2.11 mm – in women (p<0.001). Difference in the spleen width depending on gender is statistically significant for Group VII – 77.2±2.11 mm in men and 61.6±2.25 mm in women; in age Group VIII₁ – 78.1±2.07 mm in men and 59.2±2.16 mm in women.

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Old and new problems of combatant posttraumatic stress disorder

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Abstract

Background: Almost all studies describe post-traumatic stress disorder (PTSD), which is relatively new diagnostic category as accepted by ICD-10: trauma, re-experience avoidance and increased arousal. The purpose of this study was to determine the "pathos" of the disease (personality features), factors, forming in the "hot point" and the "nosos" of PTSD as a dynamical active pathological process, starting in the peaceful life.

Material and methods: We used a battery of methods of investigation, auditioning each other, including statistical methods. The patients were totally 308 male combatants aged 22-43 y. o. (main group – 174, who committed violent offence after returning from «hot point») and control group – 134 persons without such behavior, observed in the period 1993-2005 years.

Results: The quality of specific adaptation to the military environment usually increases during prolonged stay in it. This way, in the main group were found heightened frequency of readiness to vital emotionally-affective manifestations, unconscious fear with the feeling of increasing threat, objectless anxiety, insomnia and nightmares, depression with groundless worries or with monotonous non-expressed melancholy, non systematic self-accusation ideas, vacancy towards everything around, "tiredness of life". Asthenia together with vital tonus decreasing, hyperesthesia, hyper pathia, incontinence of emotions, psychic numbing dysmnnesia also were created, but previously in the second group. In the main group (with violence behavior) often was mentioned combat brutalization.

Conclusions: Our findings suggest that the "pathos" of the disease (new reactive abilities, negative personal changes) appears and only after coming back to the peaceful life, under an impact of additional insalubrities and the "nosos" of PTSD is formed as a dynamical active pathological process. Obtained data may indirectly indicate opened aggressive behavior's opportunity (prediction) in combatants.

Key words: posttraumatic stress disorder, inner pathological mechanisms, adaptation, criminal activity.

Introduction

Posttraumatic stress disorder (PTSD) is among the main disorders, which frequently begins after being in "hot points at war". But it is relatively new diagnostic category. In the Diagnostic and Statistical Manual of Mental Disorder (DSM-4) three clusters of PTSD symptoms are listed: trauma re-experience, avoidance and increased arousal. To fulfill the criteria for PTSD a person must have been exposed to life threatening stress (1, 15). According to published data inner mechanisms of PTSD

are insufficient to be taken into account in prevention strategies of deadaptation, criminal violence and for the development of disorder's investigations as a scientific problem (2, 3, 15).

According to ICD-10, only one diagnostic category – "reaction to hard stress and adaptation disturbance" – is identified as having explicit etiological connection with psycho-traumatic impact (only if this impact is extraordinary). In fact, it is identical to criteria of reactive states which do not always disappear without any impact on the personality.

Such sections as brief “acute reaction to stress” and “adaptation disturbance” do not cause any serious objections, but prolonged PTSD diagnosis causes certain doubts. For instance, we can suppose, that hard stresses act only as catalyst, but not as specific etiological factors; that PTSD would not necessarily be verified. The form of disease occurs on constitutional base, is identified by negative disorders, their typical co-existence with positive (reactive) symptomatic. Pathogenesis is for a long time considered to be a dynamical process, where cause unfortunately, main symptoms were not included into PTSD’s diagnostic criteria of ICD-10.

Entering of diagnostic description of PTSD into ICD-10, no doubt, had great social importance, opening the way for complex research of the problem of negative psychological and medical consequences of combat psychic trauma, natural and man-caused disasters, terrorism and violence. At the same time diagnostic criteria of PTSD in ICD-10 are not sufficient for understanding the mechanism of consequences of psychic trauma. In fact, they are identical to those, formulated by Karl Jaspers (1913) as well as to later specified criteria of reactive states. According to these criteria, such states manifest in case of impact of psychic traumas, which (directly or indirectly) are reflected in the symptoms of the disease and do not always disappear without any impact on the personality. The “postreactive” development of disease and even the development of steady organic changes are possible.

On the other hand, any mental disease occurs on certain constitutional base, but does not lose its nosologic independence. The form of disease is identified by typical negative disorders, their typical co-existence with positive (reactive) symptomatic. Etiology only limits the range of possible consequences, but does not close it completely. As it is well-known, pathogenesis is for a long time considered to be a dynamical process, where cause and consequence may change places. Unfortunately, main symptoms were not included into PTSD’s diagnostic criteria. In the description if ICD-10, PTSD is nothing more, than simple syndrome.

The aim of the investigation. Our paper considers a part of the problem under investigation - determination of different clinical-dynamic characteristics of combat-related PTSD, as well as personality features and prerequisites and mental disturbances for better understanding etiological and pathogenesis bases in mentioned category.

In the present paper we try to verify the concept which differs from the one accepted by ICD-10: in our view, firstly the “pathos” of the disease (new reactive abilities, negative personal changes) appears and only after coming back to the peaceful life, under an impact of additional insalubrities the “nosos” of PTSD is formed as a dynamical active pathological process [4, 5].

Material and methods

Totally 308 male combatants, aged 22-43 y. o. (main group – 174, who committed violent offence after returning from «hot point») and control group – 134 persons without such behaviour, were observed in the period from 1993 to 2005

years. Target participants were identified according to results of forensic psychiatric examination (1st group) and examination at Rehabilitation Center and Hospital for War Veterans in Moscow. The data collections (questionnaire’s points) were previously got at self-report; but additional data about personality feature’s dynamics were got in face-to-face interview.

All the procedures followed in accord with standards of the ethic Committee. Participants firstly were informed about confidentiality about described information and were explained the purpose of the investigation in details.

Methods of investigation: clinico-psychopathologic; quality of life self-evaluating scales, pathopsychological (MMPI, projective “Hand test” and Lusher, test Bassa-Darka, Impact of Event Scale-Revised by Horowitz M. J. (1979) in modification by Marmar C. R. et al. (1996) and statistical methods [8, 14].

We used special card for investigation, which unclouded biographical characteristics, personality forming factors, behavior in childhood and in adolescent period, accentuation type, psychological and social stressors during life, different traumas, especially subjective social psychotraumas, behavioral disturbances after being at “hot point”, coping-mechanisms after returning into normal life, quality of life objective evaluating, criminal anamnesis, mental disturbances before committing crime, mental state at the period of committing crime, results of traumas, wounds, confusions diagnosis at the last investigation and at the period of committing crime, forensic qualification of the mental state.

Psychiatric Assessments. Using ICD-10 two independent psychiatrists carried out the assessments. The agreement rate between the assessors was 94%. The duration of the illness in both groups was not less than 2 years prior to study.

Results and discussion

War combatants do differ from others in the style of their emotional reactions, affects’ contents and worldview – in considerable, rather special and quite one-type way. This specific of the psychic is shown in everyday life and in hospital (especially when one watches the combatants when they meet together).

In the main group’s combatants significantly dominated dysphoric conditions (in comparison with control group) – 11.1% and 6.7%, $p > 0.05$. But in the control group significantly main was impressive type of emotional reactions, according to passive-defensive tendencies – 27.6% and 17.1%, $p > 0,0025$.

Negative symptomatology of mental disorders, observed in combatants, is reflected in position of ICD-10, as “chronical personality changes after catastrophe”. But contrary to this position diagnostic criteria of PTSD show, that personality change may be its chronic outcome. At the same time, the diagnosis mentioned above should be stated only after 2 years from the moment of PTSD verification. That’s why the period of urgent adaptation is characterized by regressive, ontogenetically earlier forms of reacting. The defense of the organism is revealed in conscience disintegration.

Combat stress, being a consequence of extreme impact, appears in every person and obligatory raises the risk of pa-

thology formation. Previous individual experience turns out to be insufficient and even discordant with the reality.

Acute stress reactions are of "out personal" character; with minimal individual differences, and their constitutional "basis" is almost not visible. At the same time, the degree of mental activity's disintegration, the predominance of sano- or pathogenetic tendencies in the dynamics of disease depend on premorbid vulnerability. We observed prevalence of comorbide explosive features in the main group in comparison with the control persons (39.7% and 12.0%, $p > 0,001$) in comparison with frequency of asthenic disturbances in control combatants (15.0% and 19.4%, $p > 0,0002$).

After the acute period is over, the cognitive processing of psychotrauma experienced and its consequences starts. The adaptation to unexpected changes of life conditions is often complicated by emotional (more seldom – obsessive-depressive) disorders. As a rule these disorders are reversible during several days, weeks or (more seldom) months. As a rule, appearance of more prolonged and more specific mental disorders is connected with the factor of personal predisposition. Specifically, PTSD symptomatology reflects decompensation of premorbid personality features. We regard this symptomatology as a pathoplastic later development, the specificity of which (relative to the source of decompensation) is gradually lost.

The pathogenetic mechanism in the condition of prolonged traumatic combat stress is formed in a different way. At the same time, combatant PTSD can be understood as a transforming reactive process of heightening specific steadiness of organism against military stressors, fixating in memory traces of new behavioral skills and stereotypes, in order to save life and fulfill the necessary tasks. The quality of specific adaptation to the military environment usually increases during prolonged stay in it. Certain compensatory features become more specific and steady. Out of them we should specially highlight those, which are important for readaptation in subsequent peaceful life: the perception of environment as a hostile one; hyperactivity of attention, watchfulness, automatic actions; readiness for impulsive defensive reaction at threatening factor in the form of hiding ($p > 0,0001$), running away or aggression and physical destruction of the sources of threat; decreasing of susceptibility to suffering and death; "going away" from moral problems solving; ability to momentarily mobilize all the forces and for quick relaxation later.

In our view, described specific adaptation in military conditions happens because of cortical behavior control's depression and deep-undercortical phylogenetically old vital affects releasing. In its turn, the following moments are the biological base of the above-mentioned reaction to stress: sensoric hyperafferentation, biological deprivation, psychosomataical exhaustion, protective inhibition of cortical neurons, which protects them from sensor damage, prolonged limitation of basic ("organical") needs, deficit of intrapsychic overexamination, impossibility to verbalize many military experiences.

In this way, heightened readiness to vital emotionally-affective manifestations, unconscious fear with the feeling of

increasing threat, objectless anxiety, insomnia (11,5 % cases in the main group and 25,6 % - in the control; $p > 0,001$) and nightmares (35,7 % in the main group and 13,5 % in the control; $p > 0,0001$), depression with groundless worries or with monotonous non-expressed melancholy, non systematic self-accusation ideas, vacancy towards everything around, "tiredness of life", asthenia together with vital tonus decreasing, hyperesthesia, hyperpathia, incontinence of emotions, psychic numbing (prevalent in the 1st group - $p > 0,0001$) and dismnesia are created. Simultaneously impulsive motives of behavior (often including brutal explosiveness, psychoactive substances abuse, and suicidal tendencies) are formed. In the main group (with violence behaviour) often was mentioned combat brutalization ($p > 0,0001$). Not only associations, connected to former military experience, but also any other negative life events may contribute to vital affects' exacerbation. Military and peaceful environments are completely contradictory, so after the war even usual events may become stressogenic factors.

Reorganization of psychological, neurohumoral and psychophysiology processes, aimed at long-term adaptation to extreme conditions involves not only deep "layers" of psychic, but also ontogenetically later (and so more vulnerable) layers – which means, the structure of personality itself. But, in contrast to deficit states of processual genesis, upper emotions suffer much less. Socially positive aims are kept (or often even enhanced) in initiative behavior of military combatants. In several cases compensatory-adaptational psychobiological and personal changes, acquired in military environment, become a steady emotional-behavioral stereotype. Outside military situation this adaptation is considered to be pathological. Positive PTSD symptomatology in combatants also has its peculiarities. Repeated feelings and memories about traumatic events often transform into obsessive-phobic complexes (77.2%).

For example, obsessive memories about war of one combatant reduced completely, but he suffered from obsessive fear for the safety of his small son and wife. We observed phenomena of involuntary-perceverative, organic character, similar to reminiscences: eidetic echomnesia in the form of "frozen", repeated, but at the same time bright, sensually full visualizations of the events of the past. In case of increased cortex inhibition (for instance, when eyes are closed, during falling asleep or sleeping), impulsive motives of behavior (often including brutal explosive outbursts, psychoactive substances abuse, suicidal tendencies) are formed. It seems that the appearance of echomnesia is connected with existence of dominant excitement point in structures of "sensory brain", which may appear as a consequence of experienced prolonged and intensive metabolic effects of stress reactions. In this contest we considered to be informative statistically significant correlations between panic attacks and paroxysmal activity's low threshold at brain EEG ($p > 0,005$). Another syndrome of PTSD ("avoiding conditions, which remind about stress") also has its own peculiarity. At the same time many combatants consider their military past as "the best years of life", choose

corresponding professions (for instance, work in police), dream to return to military environment and really feel themselves better in it. This is not an accident, because in the conditions, which contributed to the development of mechanism of full long-term adaptation, this state is compensated.

We got some statistically significant differences in pathopsychologic investigation of combatants, who committed violent offence, – in comparison with control group. For example, projective “Hand test” showed tendency towards aggressive behavior in cases of sanity (during forensic examination) – $p > 0,05$.

In the main combatant group personal deadaptation level was significantly greater (point MAL – $p < 0,05$) and tendency towards avoiding reality more expressed (point WITH – $p < 0,0001$).

This data correlates with the rates of executed Bassa-Darka test. According to Bassa-Darka test all investigated positions were significantly different in two groups. Correlations between animosity index and injury level and with feeling guilty were estimated ($r=+0,85$; $p=0,0001$ and $r=+0,74$; $p=0,0007$). The rate of possibility of aggressive behavior in comparative combatant group lets us suppose that those persons became aggressive only in especially important situations.

Special attention should be given to distinctly higher percentage of the answers in the main group on the Crip category ($n=7,5$ (main group) and $n=3,4$ (comparative group)), which reflects hypochondria and real health problems.

Distinctly higher level of irritability in combatants of the main group reflects their high readiness to show negative feelings after the smallest agitation (heightened hastiness, rudeness), which, combined with heightened susceptibility to offence ($p < 0,01$) easily leads to aggressive acts connected to the situation.

Patient’s sense of guilt is connected to his conviction that he is a bad person, and also points at remorse he feels. Indexes of aggressiveness and hostility in the main group were distinctly higher than in comparative one (where they differed from the indexes of aggressiveness and hostility in population as a whole).

Using modified 8-coloured test by Lusher showed significant greater anxiety level in the patient’s main group ($p=0,004$), what correlates with “Hand test” results. In main group of combatants additional colors on the first places in color range were found distinctly more often ($p=0,037$), than in comparative group. We should mention, that on the first place in the main group such colors as +5 ($p=0,001$), +7 ($p=0,05$) and +0 ($p=0,05$) were found distinctly more often, while in comparative group this place was mostly given to +2 ($p=0,02$) and +4 ($p=0,02$). In order to reveal the peculiar features of the phenomenological shape of PTSD and its intensity in groups being compared, we used Impact of Event Scale-Revised (IES-R) – Horowitz M. J., Wilner N. et al., 1979), modified in 1996 by Marmar C. R. (1996), using rank correlation (Spearman) in the main combatant’s group showed straight dependence between “opened” aggressive behavior (Hand test) and intrusion level – $r=+0,73$, $p > 0,003$; physi-

ologic excitement – $r=+0,55$, $p=0,01$. Thus, information got by IES-R method may indirectly indicate opened aggressive behavior opportunity.

Consequences of military traumatic stress – is one of the main inner barriers to the harmonious social adaptation of combatants in society. Initial stress, which they got on a war, is complicated by a secondary one – the necessity to adapt to usual conditions. It often becomes a basis for behavioral deviations, which may also include aggressive tendencies. That’s why we should consider the treatment and rehabilitation of such persons as one of the priority medical and social tasks in XXI century.

Conclusions

We consider this paper as a part of the problem under investigation, mentioned above – determination of different clinical-dynamic characteristics of combat-related PTSD, as well as personality features and prerequisites and mental disturbances for better understanding etiological and pathogenesis bases in mentioned category. In our view, acquired data points at correction of the scientific concept stated at the beginning of the article. They already may have certain independent practical meaning for the therapy and rehabilitation of combatants, but also serve as basis for development of pathogenesis planned for the future and for understanding clinical dynamic of PTSD.

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REVIEW ARTICLES

Analysis of the legal framework on donation and transplantation of organs and cells in the Republic of Moldova

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Abstract

Background: Due to rapid advances in transplantation medicine, the use of human organs, tissues and cells for transplantation has steadily increased during the past decades. Nevertheless, the success of organ transplantation has led to a huge imbalance between the scarce supply and the abundant demand for available organs for transplantation. The imbalance is one of the main reasons for illicit activities in this domain. The aim of this article was to review the most important legislative acts which regulate the activities in the field of donation and transplantation of organs, tissues and cells in the Republic of Moldova. The development of an appropriate transplant system in the Republic of Moldova represents a priority for the Government and specifically for the Ministry of Health to ensure high standards of quality and safety as to the procurement, testing, processing, preservation, storage and distribution of human organs, tissues and cells.

Conclusions: In the Republic of Moldova there is an ongoing development in law enforcement and implementation of quality assurance standards. The law on transplantation of organs, tissues and cells of human origin was adopted by the Parliament of the Republic of Moldova in March 2008. According to the new legislation the Transplant Agency was created as a public body in May 2010. Despite multiple efforts made by the Ministry of Health, Transplant Agency and other state institutions to improve the legal framework there are still some deficiencies to be overcome by adopting normative acts to prevent cases of trafficking in organs and tissues or other illicit activities in this area.

Key words: transplantation, donor, organs, legislation.

Introduction

Transplantation of organs, tissues and cells is one of the top areas of modern medical practices, which saves thousands of lives and contributes considerably to the improvement of the quality of life of hundreds of thousands patients around the world every year. During the past 5 decades, transplantation has become a well-developed medical procedure and in case of many illnesses it often represents the only possible treatment.

It's a paradox, but namely the transplant development has contributed to the extension of the waiting lists. According to the Global Observatory on Donation and Transplantation data, 112,631 solid organ transplants were performed in 2012 around the world [1]. This accounts to 10% of the global demand for organs. Simultaneously, each year approximately 13,961 patients pass away, while waiting for a transplant graft. The mortality rate on the waiting list for a heart, liver or lung transplant varies between 15 and 30%. The European

Union (EU) records significant differences with regards to the number of cadaveric and living donors. These differences cannot be explained easily. Even in the EU countries, where well-developed services are available, there are significant differences related to organ donation and transplantation and it seems that certain organizational systems are more efficient than others.

The transplantation includes the organ, tissue and cells donation and their subsequent implantation, the two processes being fully interdependent. Organ procurement does not concern only the transplantation teams. More and more health professionals, working in teams and international organizations are trying to strengthen the cooperation between doctors and management structures. It is vital for the entire medical community to acknowledge the problem and to be directly and indirectly involved in the donation and transplantation process. The medical staff can contribute

indirectly to the education of the general public, chase away the fears and encourage a positive attitude towards donation. All medical staff can directly support the identification of potential donors, with their subsequent evaluation and maintenance therapy. Like any medical activity, the overall success of the transplantation depends on the responsibility of all representatives of the health system, and on the whole society. The use of substances of human origin for treatment raises serious ethical concerns for the society [2,3]. Any transplantation program has the major goal to increase the number of transplant operations by optimizing the number of altruistic donors and by ensuring transparent and equitable, but also medically efficient allocation of organs and tissues. The donation and transplantation activity must be based on the rigorous compliance with the general ethical norms.

By Law No 261-XVI of 06.12.2007, the Republic of Moldova ratified the Additional Protocol to the Convention on Human Rights and Biomedicine concerning Transplantation of Organs and Tissues of Human Origin, adopted by the Council of Europe on 24 January 2002 [4,5]. The protocol urges states to provide equitable access to transplantation services for patients, to protect the rights and freedoms of donors and recipients of organs and tissues of human origin, and to eliminate the misuse of transplantation that may lead to acts endangering human life, well being and dignity. According to the Protocol, the states will cooperate by exchanging information on the demand for organs and tissues, and by offering mutual support for organ and tissues transportation to and from their territory.

The Republic of Moldova has committed to take the necessary measures in order to prevent and curb immediately any violation of rights in organ transplantation and to introduce adequate sanctions in its legislation.

Alignment of the Republic of Moldova to the international and European standards on human rights and biomedicine. National Regulatory Framework

The organ procurement and transplantation was regulated for the first time in the Republic of Moldova with the enforcement of Order of the Ministry of Health of USSR No 153 of 22 February 1982 on the "Right to procure and preserve kidneys for transplantation" and the decision of the Scientific Council Board of the Science Academy of USSR of 29 June 1982 on the "Right to perform kidney transplantation operations in the Republican Clinical Hospital".

Law No 473-XIV on Transplantation of Human Organs, Tissues and Cells of 25 June 1999 was the first law that regulated the donation and transplantation of organs and tissues in the Republic of Moldova [6].

The evaluation of the Moldovan system for transplantation of organs, tissues and cells of human origin, performed by the Council of Europe experts under the Joint Programmer between European Commission and Council of Europe for Moldova 2004-2006, confirmed the topicality of this issue and the need for immediate interventions. To overcome this situation various workshops and round tables were organized with the participation of Council of Europe experts. These meetings addressed the current situation related to organ

transplantation and the fight against organ trafficking in the Republic of Moldova. The Council of Europe experts drew the attention to the deficiencies of the current legislation - cadaveric procurement only in case of brain death, prohibition of organ exchange within an international cooperation, non-acceptance of spouses or other relatives, except first kinship, as living donors - which were the key factors preventing the development of this field. The Council of Europe experts recommended to adopt a new Law on Transplantation of Human Organs, Tissues and Cells, aligned to Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells [7,8,9]. Authorities of the Republic of Moldova have carried out a series of legislative and regulatory actions with the view to adjust the legal framework to the international legislative norms and EU requirements, facilitate the transplantation of human organs, tissues and cells, help save lives and improve significantly the quality of life, and prevent the trade in parts of human bodies [10,11,12,13]. Respectively, the following national legal framework on transplantation of human organs, tissues and cells is in place today:

- Law on Transplantation of Human Organs, Tissues and Cells No 42-XVI of 06.03.2008 (Official Gazette No 81/273 of 25.04.2008);
- Government Decision No 386 of 14.05.2010 establishing the Transplant Agency;
- Government Decision No 1207 of 27.12.2010 approving the Regulation on the Organization and Modus Operandi of the Independent Approval Commission under the Ministry of Health and authorization criteria for the performance of procurement and transplantation activities.

These laws and regulations were enforced, further developed and elaborated with the approval of a series of orders by the Ministry of Health. They regulate the requirements, procedures, and techniques for the authorization of healthcare facilities and medical staff in procurement and transplantation and the quality of organs, tissues and cells for transplantation [14,15].

Law No 42-XVI of 06.03.2008 on Transplantation of Human Organs, Tissues and Cells is the main internal law [10]. It regulates the transplantation of all human organs (a vital differentiated part of the human body, consisting of different tissues that maintain its structure, vascularization and physiological functions with an important level of autonomy), tissues (all parts (anatomical formations) of the human body, consisting of cells) and cells (individual cells or cell conglomerates, which are not linked by any form of tissue).

Article 2 of Law No 42 -XIV defines the transplantation as "a medical process intended to restore certain functions of the human body by transferring equivalent organ, tissues, and cells from a donor to a recipient. Transplantation might be from a person to another (allogenic) or from a person to himself (autologous)".

In order to perform the transplantation of organs, tissues or cells it is necessary to comply with certain clear rules and principles imposed by the law.

These are stated expressly in Article 3 of Law No 42-XVI of 06.03.2008 on Transplantation of Human Organs, Tissues and Cells, as follows:

a) protection of dignity and identity of human beings, without discrimination, and respecting the integrity and other fundamental rights and freedoms related to transplantation of organs, tissues and cells;

b) The therapeutic benefit for the recipient by timely transplantation of an organ, tissues and cells from a living or cadaveric donor, unless there aren't any therapeutic methods with a similar efficiency;

c) Ensuring quality, by observing the professional standards and obligations, during any intervention related to transplantation of organs, tissues and cells;

d) Traceability, by guaranteeing the identification of organs, tissues and cells intended for transplantation, during the procurement, storage and distribution, from the donor to the recipient and vice-versa;

e) Protection of human rights and freedoms and prevention of trade in parts of the human body;

f) Fair access of patients to transplant services.

Procurement of Human Organs, Tissues and Cells

Procurement represents the process by which the donated organs, tissues or cells become adequate for transplantation. A difference is made between the procurement of organs, tissues and cells from living donors (*ex vivo*) and procurement of organs, tissues and cells from cadaveric donors (*ex mortuo*). This procedure, both from living and cadaveric donor, can be performed only under certain conditions, set by the Additional Protocol to the Convention on Human Rights and Biomedicine concerning Transplantation of Organs and Tissues of Human Origin [15] and Law No 42 of 06.03.2008 on the Transplantation of Human Organs, Tissues and Cells.

Violation of any of the conditions set by the Law is an assault to the dignity and integrity of the human being.

Conditions for the Procurement of Organs, Tissues and Cells from Living Donors

Chapter III of the Additional Protocol to the Convention on Human Rights and Biomedicine concerning Transplantation of Organs and Tissues of Human Origin sets the conditions for organ and tissue removal from living persons.

In its turn, Article 15 of Law No 42-XVI of 06.03.2008 on the Transplantation of Human Organs, Tissues and Cells lists expressly the following conditions for procurement from a living donor. But these are not exhaustive, as a series of other conditions are stipulated in several other articles such as Article 9, 19 and 20 of the Law. Also, it is important to mention that both the Protocol and the Law stipulate additional conditions for organs procurement compared to the tissue and cells procurement from living donors, as well as for the tissues and cells procurement from persons, who do not have the capacity to express their consent.

Based on the above-mentioned documents, we can specify the following conditions:

1) Organs, tissues and cells procurement from a living donor is allowed only when compatible organs, tissues and

cells from a cadaveric donor are missing and only for the therapeutic benefit of the recipient;

2) Human organs, tissues and cells procurement, for therapeutic purposes, can be performed from living donors who have reached the age of majority, who have full legal capacity, after receiving in advance their written, unforced, preliminary and express consent. The donor may reverse the consent until the moment of the procurement;

Human organs, tissues and cells cannot be procured from persons, who lack capacity to express their consent. However, the law makes an exception to this: tissues and cells procurement is allowed in the case of regenerative tissues and cells, provided that the following conditions are met cumulatively:

a) To have the agreement of the legal representatives of the donor or of the guardianship. This requirement is applicable for minors as well;

b) The procurement must be authorized by the Independent Approval Commission;

c) The donor must be a relative of first kinship of the recipient (for the minor donor this has to be a sibling);

d) The procedure to cause a minimal risk for the donor;

e) Procurement from a minor donor cannot be performed if he/she has expressed his/her refusal in writing, verbally or in any other form.

The Ministry of Health Order No 885 of 18.11.2011 approving the documents for the Independent Approval Commission on the consent for or refusal from procurement of organs, tissues and cells from living donors, Annex 3, approved the informed consent form for regenerative tissues and cells in the case of minors or incapacitated persons.

3) The donation consent shall be signed only after the donor was informed by the physician on the potential risks and physical, psychic, family and professional consequences that could be caused by the procurement;

4) The consent to donate shall be expressed according to the legislation on the rights and responsibilities of patients and shall be prepared as an informed consent form. The informed consent form for living donor donation of organs, tissues and cells was approved by Government Decision No 1207 of 27.12.2010, in Annex 3.

5) In case of organ procurement from a living donor it is mandatory to have the approval of the Independent Approval Commission.

The Independent Approval Commission's approval of the donation from a living donor is not required for the procurement of stem cells, sperm, femoral head (after endoprosthesis), placenta, umbilical cord blood or amniotic membrane for therapeutic purposes. However, in this case the procurement must comply with the bioethics rules and patient's rights.

In addition, procurement and transplantation of organs, tissues and cells must meet these restrictive requirements:

a) Procurement and transplantation of human organs, tissues and cells as a result of any physical or moral coercion is forbidden;

b) Donation and transplantation of human organs, tissues and cells cannot be object of certain judicial actions or documents, aiming at obtaining material or any other type of gain.

Conditions for the procurement of organs, Tissues and Cells from Cadaveric Donors

Certification of the moment of death is an issue in the case of procurement of organs, tissues and cells from cadaveric donors. According to Article 10 of Law No 42 of 06.03.2008, the legal procedure of cadaveric donor procurement can be initiated only when the person's death was declared, after it was certified according to certain criteria set by the Ministry of Health. Currently these criteria are not stated expressly in a separate regulation.

According to Article 11 of Law No 42 of 06.03.2008 in the case of a potential donor, it is necessary to confirm the death of the donor before the launching the legal procedure of donation, following one of these procedures: after a respiratory cardiac arrest or as a consequence of brain death.

In case of irresuscitable and irreversible respiratory cardiac arrest, the death is confirmed in at least 5 minutes after the performance of all intensive care measures, with all tests proving unambiguously the lack of blood circulation towards the brain and vital organs.

In case of brain death, the death is confirmed after the occurrence of irreversible changes in the vital centers of the brain, confirmed by specific tests, while the cardio-respiratory function is maintained artificially.

The brain death diagnosis is set according to the Ministry of Health Order No 260 of 05.04.2011 approving the "Standardized Clinical Protocol on Brain Death". This Protocol presents the criteria of clinical and instrumental diagnosis of brain death in adults (persons over 18 years old) and the relevant tests in order to facilitate the recognition and diagnosis of brain death and justification of the actions taken by the medical staff in case of suspected brain death.

Brain death diagnosis shall be established by a medical council consisting of three specialists: neurologist/neurosurgeon, anesthesiologist/intensive care physician, and manager of the institution. The brain death shall be confirmed by determining the clinical signs and performing the mandatory paraclinical tests. Brain death diagnosis shall be recorded in the Brain Death Determination Statement, according to the form presented in Annex 2 to the above-mentioned Protocol. As an essential condition for the determination of brain death, the physicians that certify the death of the person should not be the physicians, who are involved directly in the procurement of organs or tissues from the deceased person, who are involved in the subsequent transplantation, or who are in charge of providing care to the potential recipient [10].

At the same time, organs, tissues and cells are procured from cadaveric donors only if the clinical and laboratory tests exclude any communicable diseases, a potential contamination or other affections that represent a risk to the recipient.

The consent to donate is an important element in the procurement of organs, tissues and cells from cadaveric donors.

Article 17 of the Protocol is quite laconic on this subject, stating that organs or tissues shall not be removed from the body of a deceased person unless consent or authorisation required by law has been obtained. The removal shall not be carried out if the deceased person had objected to it.

Based on the legal regulations we can conclude that there are two types of consent: the presumed consent and the informed consent.

More explicit regulations on this subject are provided in Article 13 of Law No 42 of 06.03.2008 on the Transplantation of Human Organs, Tissues and Cells.

Thus, procurement is possible only when the person has expressed the consent to donate before death or during his/her lifetime. This is the express manifestation of the agreement to donate, according to the law, i.e. the express consent of the donor to donate.

Also, the procurement cannot take place, if during her/his lifetime the deceased person has rejected the donation in writing or in any other legal form. In such a situation, the manifestation of refusal to donate organs or tissues can have the form of a written document, with the signature of the refusing person, or a notarized statement.

After the death of the person, if he/she did not manifest the agreement to donate during the lifetime, donation is possible only if there is no written refusal to donate from at least one member of the family, from other relatives of first kinship or from the legal representative of the deceased. This is an informed consent and the emerging issue consists in the time period when the refusal can be expressed. Considering that the procurement should be performed timely, the relatives or legal representative of the deceased shall express their refusal within a very limited time period.

Also, donation is possible if after the legal certification of death, none of the close relatives or the legal representative have expressed their position regarding donation, and the contact data of the close relatives or the legal representative of the deceased person are lacking.

In forensic cases, cells, tissues and organs shall be procured from cadaveric donors with the consent of the coroner, provided that it does not compromise the forensic autopsy results.

Prohibition of Financial Gains from the Donation of Cells, Tissues and Organs. Criminal Liability for Trafficking in Organs

The low number of organs available for transplantation raises various moral and bioethics issues concerning their procurement and distribution. All international human rights documents state that the human body is sacred, intangible, must be respected both during the lifetime and after death and cannot be the object of any trade [16,17,18,19]. In order to avoid violation of human rights, in the international legislation, the specialized bodies adopted norms that set the general principles for donation of organs, with the most important being: principle of respecting the human dignity and prohibition of any violations thereof, principle of donors' anonymity, of gratuity, etc. Any action of procurement of organs from a living human body, which affects severely the life and integrity of the human body, is sanctioned by the Criminal Code. The failure to comply with the legal requirements for the selection and follow up monitoring of organ donors can lead to severe consequences for their health. In order to avoid these consequences, the organ transplantation shall to be planned and implemented as a grand gesture of dedication of a hu-

man to another human, an action of humans who, though have inalienable rights over their bodies, are members of the society and thus can bring a service to their peers, under the conditions of a healthy and reasonable generosity.

A Declaration on Human Organ Transplantation was adopted in October 1987 by the 39th World Medical Assembly. This Document criticizes strongly the donation of organs for commercial purposes. Subsequently, The World Health Organization (WHO) developed a set of guiding principles in the field of transplantation. WHO approved these principles at the 44th session in 1991 and recommend to states to use them in the development of policies in this area. According to Guiding Principle no.5 „Purchasing, or offering to purchase, cells, tissues or organs for transplantation, or their sale by living persons or by the next of kin of deceased persons, should be banned.” A similar position was adopted also in the Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine: Convention on Human Rights and Biomedicine of 4 April 1997. By ratifying this Convention, the Republic of Moldova assumed the obligation to prevent any human rights violations, provided in this Convention. Law No 42 of 06.03.2008 is a necessary legal support in this sense, which details the principle of prohibiting trade in parts of the human body, and simultaneously incriminates the trafficking in human organs, tissues and cells.

Thus, Article 27 of the Law prohibits earning any financial profits from the donation of human cells, tissues and organs and stipulates that the donation and transplantation of cells, tissues and organs cannot be the object of a material transaction. In this sense, the Independent Approval Commission controls if the donation is made with a humanitarian purpose, if it has an altruistic character and if it does not represent the object of a material transaction. Upon the evaluation of the legal motivation and procurement procedure, the Commission verifies if the donor is informed that accepting any monetary compensation for donation is illegal.

The sui generis act of tissues/organs donation is free of charge and respectively, does not depend on any transaction that aims at selling the respective organ/tissues or cells or the acceptance of a material benefit or of a benefit of any other nature.

Gratuity is the essence of the tissues and organs donation. It should not be regarded as the lack of a patrimonial advantage, but as the lack of any advantage, regardless of its nature.

Also, the legislator prohibits any advertising of human organs, tissues and/or cells transplantation for purposes of obtaining financial gains or related advantages, these actions being subject to criminal liability.

Article 29 2) of Law No 42 of 06.03.2008 states that the cost of the transplantation, investigations, hospitalization, surgery, drugs, sanitary materials, post-surgical care and any other expenses linked to transplantation can be covered by:

- a. Funds from the National Health Insurance Company;
- b. The state budget;
- c. Payments (fees) incumbent on the patients, according to the law, for medical services;

d. Donations from charity organizations and other legal entities, as well as from private persons, that are not linked directly to a certain transplant activity.

However, donors can receive an indemnification that is strictly limited to covering the expenses and of the following inconveniences:

a. Compensation of living donors for the lost income and other justified expenses, caused by the donation and related medical check-ups;

b. Payment of justified expenses for the medical services or technical services related to donation. Living organ donors benefit of compulsory health insurance policy for life, funded from the state budget.

By these regulations, the legislator does not exclude the possibility to provide some compensation for the act of donation, but totally excludes the legal possibility to provide compensations that would generate sales relationships between the donor and recipient or another interested person.

The Government has the main role in this case and should assume all the responsibilities towards the donor by providing a benefit for the temporary incapacity to work and providing health services from the state budget, as well as offering certain facilities for the provision of health services.

Article 28 of Law No 42 of 06.03.2008 prohibits the trafficking in human organs, tissues and cells, and obtaining any financial profits or advantages from trafficking in human body and its parts.

Trafficking in human organs, tissues and cells is a form of trafficking in human beings, which violates the human dignity and physical integrity.

According to the law of the Republic of Moldova, the human body cannot be viewed as an asset and cannot be the object of a patrimonial legal relationship. This conclusion is a general principle, though it is not provided expressly in the current law, it can be deducted from Article 27(1) from Law No 42 of 06.03.2008. At the same time, Article 28(2) from Law No 42 stipulates expressly that trafficking in human organs, tissues or cells is a crime, punishable in accordance with the criminal law.

The incriminated criminal facts, contained in the Criminal Code of the Republic of Moldova on the trafficking in human organs, tissues or cells, are stated in Article 158 - Trafficking in Human Organs, Tissues and Cells, Article 165 - Trafficking in Human Beings, Article 206- Trafficking in Children and Article 213¹ - advertising for illegal procurement of human organs, tissues and cells or their illicit donation [21]. The objective side of trafficking in human organs, tissues or cells from Article 158 includes plenty of actions, such as illegal procurement of human organs, tissues or cells by removing them from the living or dead human body of another person by unauthorized persons and/or unauthorized institutions according to the current law or in non-compliance with the legal provisions on the person's consent to donate or in order to earn income from this, as well as their illegal selling, procurement, use, storage, possession, transmittal, receipt, import, export, or transportation. There are the following aggravating circumstances for these actions: a) committal by a person

who committed previously a similar act; b) by physical or psychic coercion; c) against two or more persons; d) against a pregnant woman or a child; e) by two or more persons; f) by a public person, by a person in position of liability, by a public dignitary, by a foreign public person or by an international official; g) committed by an organized criminal group or by a criminal organization; h) resulting in severe damage to the corporal integrity or health, with the death or suicide of the person. It is worth noting that prior to the adoption of Law No 270 of 07.11.2013 on Amendments and Addenda to some Legal Acts, Article 158 used to incriminate only coercion of a person into procurement of organs or tissues for transplantation or for other purposes, committed by applying violence or threatening to apply violence.

From this perspective, in the spirit of Article 19 of the Convention on Human Rights and Biomedicine and Articles 27-28 of Law No 42 of 06.03.2008, the legislator intervened in the regulation of such social relations by sanctioning those who do not comply with the principles of gratuity of donation of organs, tissues and cells, stipulating exhaustively in the new version of Article 158 the actions for trafficking in organs, tissues and cells that constitute crimes.

Actions of recruitment, transportation, transfer, housing or receipt of a person, with or without his/her consent, for purposes of organs or tissues procurement, are part of the crimes stated in Article 165- Trafficking in Human Beings.

The objective aspect of the crime stated in Article 206 - Trafficking in Children, incriminates the recruitment, transportation, transfers, housing or receipt of a child, and offering or receipt of some payment or benefits to obtain the consent of a person, who detains control over the child in order to procure human organs, tissues and/or cells as socially dangerous acts, punished by the criminal law. An aggravating circumstance is if these actions were accompanied by procurement of human organs, tissues and/or cells.

Article 27(4) of Law No 42 of 06.03.2008 on Transplantation of Human Organs, Tissues and Cells prohibits advertising the need for transplantation of human organs, tissues and/or cells for purposes of obtaining financial gains or related advantages. Or, incriminating it as a criminal action and the sanction that would come to support the respective regulation did not exist. The legislator eliminated the legal gap by amending the Criminal Code with Article 213¹, that foresees the advertising for illegal procurement of human organs and/or tissues and/or cells or on their illegal donation. Therefore, only the advertising for illegal procurement of human organs, tissues or cells and advertising for illegal donation for purposes of getting some financial benefits, are criminally sanctioned. The authors of the announcements, as well as the individuals or legal entities, who make this information public, are subject to criminal liability.

The European experts jointly with other international organizations, such as the Council of Europe and World Health Organization recommend close monitoring of the issue of trafficking in organs. In addition, it is important to ensure periodic update on this topic for press and general public. In order to prevent trafficking in organs, in May 2012

the Ministry of Health and the Ministry of Internal Affairs of the Republic of Moldova signed the Cooperation Agreement on combating trafficking in human beings for the purpose of organs and tissues procurement. At the same time, to monitor the donation and transplantation of human organs, tissues and cells and to develop the waiting lists, the Transplant Agency jointly with the Ministry of Health established the Information System TRANSPLANT in February 2013, which is part of the Integrated Medical Information System (IMIS) and a component of the "Automated registry of social and demographic resources" of the State Informational Resources of the Republic of Moldova. During the implementation of some components of the information system, organizational and financial difficulties have been encountered. Thus, the collection and storage of data about the patients with scheduled dialysis treatment was troublesome, due to the shortage of medical staff in dialysis centers, and shortage of computers or their Internet connection.

At the same time, so far, no method of registering data on persons who refuse to donate organs, tissues and cells in the Registry for Refusal was set, the register being thus not operational. The implementation of this register requires significant financial efforts.

With the enforcement of Law No 103 of 12.06.14, in order to ensure traceability for all organs, tissues and cells, the process of establishing a single encoding system that will provide information on the main characteristics and properties of organs, tissues and cells was initiated. The traceability requirement shall be also applied to all the pertinent data on products and materials that get in contact with these organs, tissues and cells.

According to the law in force, all the data on the transplantation shall be recorded, stored and archived in electronic form for 30 years in the information systems designated for transplant activities [10,13,22].

Conclusions

In the Republic of Moldova, the donation and transplantation of human organs, tissues and cells are regulated by a series of national legal acts and, also, by international conventions signed by Moldova, as well as the European Directives, which the national legislation shall be aligned to. Law No 42 of 06.03.2008 on the Transplantation of Human Organs, Tissues and Cells forms the basis of the national regulations on transplantations. Despite the plenty of efforts made by the Ministry of Health, Transplant Agency and other state institutions aimed at improving the legal framework, there are still some deficiencies that have to be eliminated by approving some regulatory documents that would allow preventing the trafficking in organs and tissues or other illicit activities in this field.

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BOOK REVIEW

Monograph ”Community-acquired pneumonia and children’s recurrent respiratory tract diseases”

The author – **Ala Donos**, MD, PhD, Associate Professor

Printing-house of the Academy of Sciences of Moldova, Chisinau, 2015, 288 pages



The monograph is dedicated to the problem of global interest being found in the long-termed Global Program to reduce morbidity and mortality from childhood pneumonia (2013-2035) on salvation from death of every sick with pneumonia child under five years old. The World Health Organization, UNICEF, the World Bank, etc. are involved in pursuing these noble objectives.

The main purpose of the work is to improve diagnostics, management, treatment and recuperation program for patients with community-acquired pneumonia and recurrent respiratory tract disease through pursuing the following objectives:

- Promotion and standardization of research of childhood community-acquired pneumonia as a notion in the context of specialized international protocols and guidance;

- Identification of antenatal and postnatal risk factors of recurrent respiratory infection in children with community-acquired pneumonia at tender age;

-Evaluation of extensive implementation of modern methods of diagnostics within recognition and evaluation of immune system imbalance in risk groups with recurrent respiratory infection;

-Implementation of management and international treatment practice of community-acquired pneumonia within national plan on initial and hospital levels;

-Elucidation of results obtained through recuperation programs for children with community-acquired pneumonia and recurrent respiratory tract diseases within different risk groups;

The monograph "Community-acquired pneumonia and children's recurrent respiratory tract diseases" issued by associate professor, Doctor of Medical Science, Mrs. Ala Donos, is presented in 288 pages and contains 14 chapters, conclusions and practical guidelines, with bibliography of 298 sources, illustrated with 32 tables, 30 figures, 14 appendixes and 3 algorithm diagrams reflecting management, diagnostics and medical strategy in case of childhood community-acquired pneumonia.

The first chapters (1-5) are dedicated to ontogenesis of respiratory tract, summary peculiarities of fetus physiology, evaluating tests of pulmonic maturity, peculiarities of anatomical, functional development, clinical physiology of childhood respiratory, peculiarities of development and maturing of immune system during childhood.

Chapter 6 contains generalities on community-acquired pneumonia as one of the main reasons of childhood morbidity and mortality in the world. Here are also presented standard international definitions of community-acquired pneumonia and its classification for children.

Chapter 7 considers actual epidemiology and risk factors of community-acquired pneumonia, which threaten health and life of children. Hereunder are also represented epidemiological varieties of acute viral infections which are often the prerequisite of childhood pneumonia.

Chapter 8 takes notes on acute viral respiratory infections and their role in developing and evolution of community-acquired pneumonia. A separate paragraph here describes bacterial infection as an essential etiological factor. Pathogenesis is shown in the function of etiological factor.

Clinical presentation is delivered in the function of determining factors. The evaluation of medical case severity, establishing of diagnosis according to international requirements are presented. Special place is taken by management of community-acquired pneumonia on initial and hospital level.

In the following chapters there is detailed information on follow-on therapy, principles of empiric treatment and indications of medical assistance quality in community-acquired pneumonia. Here are found also typical errors in antibacterial treatment and preventive measures of childhood community-acquired pneumonia.

The second part of the monograph is dedicated to a child with risk of poor health and evolution of recurrent respiratory infection exposing cases of formation of the contingent of children.

Chapter 12 emphasizes characteristics of functional illness of immune system of a child with recurrent respiratory diseases.

The following two chapters presented evaluation of childhood health under the influence of risk factors, peculiarities of immune system modifications, therapy on immunity enhancement for children with recurrent respiratory diseases are described in detail.

Chapter 14 reflects importance of treatment and prophylaxis of viral infections, taking of antiviral actions of Pacovirina – locally produced preparation, treatment of infections with Herpes simplex virus, of immunomodulatory and interferon inducing preparations.

Conclusions and practical recommendations are well exposed and reasoned, correspond to the actual international strict principles and represent an argument in pursuing goals and objectives proposed by the author in the work. In the monograph are also reflected own data on the method of objectivization of forming a group of children with recurrent respiratory infection, principles of differential diagnostics with initial immune deficiencies, use of treatment and recuperation programs, as well as of the locally produced preparation Pacovirina.

I highly value the monograph and find it useful for pediatricians, family medical doctors, especially for medical students, interns and other doctors interested in childhood respiratory pathology from the viewpoint of new tactics on diagnostics, treatment, prophylaxis and recuperation programs.

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DOZE ȘI MOD DE ADMINISTRARE

Tratamentul trebuie început și supravegheat de către un medic cu experiență în diagnosticarea și tratamentul demenței Alzheimer. Terapia trebuie începută doar dacă este disponibilă o persoană care îngrijește pacientul și care va monitoriza cu regularitate administrarea medicamentului de către pacient.

Toleranța la tratament și doza de memantină trebuie evaluate la intervale regulate de timp, de preferat în primele trei luni de la începerea tratamentului. În consecință, beneficiul terapeutic al tratamentului cu memantină și toleranța pacientului la tratament trebuie evaluate la intervale regulate de timp, în conformitate cu ghidurile clinice în vigoare. Tratamentul poate fi menținut atât timp cât se poate evidenția un beneficiu terapeutic și pacientul tolerează tratamentul cu memantină. Întreruperea tratamentului trebuie luată în considerare atunci când nu mai sunt dovezi ale rezeței unui efect terapeutic sau dacă pacientul nu tolerează tratamentul. Comprimatele Denigma trebuie administrat o dată pe zi, la aceeași oră în fiecare zi. Denigma poate fi administrată cu sau fără alimente.

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Săptămâna 2 (zilele 8-14): Pacientul trebuie să administreze o doză de 10 mg o dată pe zi, timp de 7 zile.

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Săptămâna 4 (zilele 22-28): Pacientul trebuie să administreze o doză de 20 mg o dată pe zi.

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Denigma nu este recomandată pentru utilizare la copii și adolescenți cu vârstă sub 18 ani datorită lipsei datelor privind siguranța și eficacitatea.

Pacienții cu insuficiență renală

La pacienții cu insuficiență renală ușoară (clearance-ul creatininei 50-80 ml/min) nu este necesară ajustarea dozei. La pacienții cu insuficiență renală moderată (clearance-ul creatininei între 30-49 ml/min) doza zilnică trebuie să fie de 10 mg memantină. Dacă în cursul a cel puțin 7 zile de tratament doza a fost bine tolerată, aceasta poate fi crescută până la 20 mg/zi în concordanță cu schema de ajustare standard. La pacienții cu insuficiență renală severă (clearance-ul creatininei 5-29 ml/min) doza zilnică trebuie să fie de 10 mg.

Pacienții cu insuficiență hepatică

La pacienții cu insuficiență hepatică ușoară până la moderată (Child-Pugh A și Child-Pugh B), nu este necesară ajustarea dozelor. Nu sunt disponibile date privind utilizarea memantinei la pacienții cu insuficiență hepatică severă. Nu se recomandă administrarea memantinei la pacienții cu insuficiență hepatică severă.

CONTRAINDICAȚII

Hipersensibilitate la substanța activă sau la oricare dintre excipienți.

REAȚII ADVERSE

Cele mai frecvente reacții adverse raportate au fost: hipersensibilitate la medicament, amețeli, hipertensiune arterială, dispnee, cefalee. Pentru informații complete a se vedea instrucțiunea pentru administrare.

PROPRIETĂȚI FARMACEUTICE

Descrierea medicamentului

Comprimat filmate de formă ovală, culoare roz, cu ambele suprafețe plane.

PREZENTARE, AMBALAJ. Comprimate filmate 5 mg sau 10 mg. Câte 14 comprimate filmate în blister. Câte 1 sau 10 blistere împreună cu instrucțiunea pentru administrare plasate în cutie de carton.

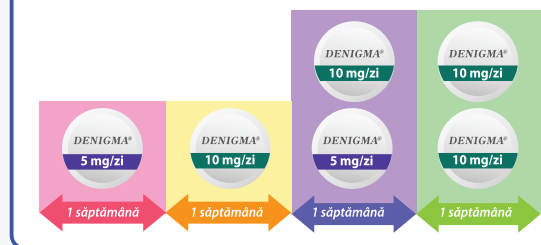
Termen de valabilitate. 3 ani. **STATUTUL LEGAL.** Cu prescripție medicală.

NUMELE ȘI ADRESA PRODUCĂTORULUI

Kusum Healthcare Pvt. Ltd.

SP 289(A), Riico Industrial Area, Chopanki, Bhiwadi (Raj.), India.

SCHEMA DE TRATAMENT ȘI ALEGE A DOZELOR



AMELIOREAZĂ PERFORMANȚELE INTELECTUALE!