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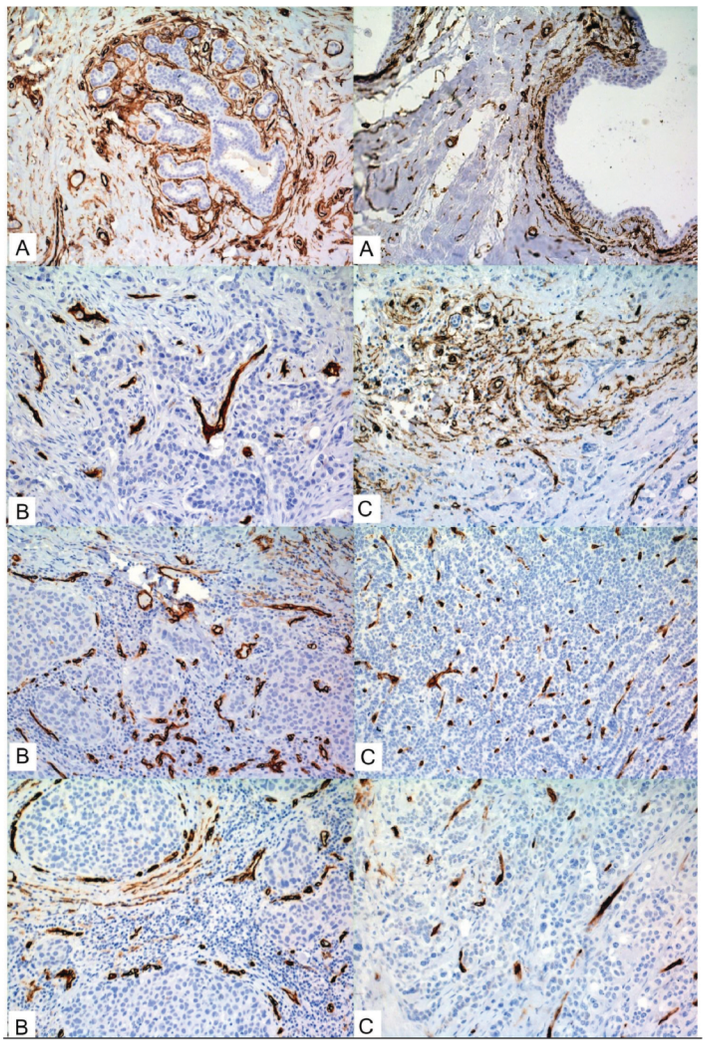
THE INFLUENCE OF DIABETES MELLITUS ON BLOOD VESSELS AMOUNT IN CASE OF BREAST CANCER

Ecaterina Foca, Dumitru Brinza, Elena Portnoi, Ecaterina Carpenco, Valeriu David, Lilian Saptefrati, Veaceslav Fulga



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RESEARCH ARTICLE

The influence of diabetes mellitus on blood vessels amount in case of breast cancer

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ABSTRACT

Introduction. Breast cancer is one of the most common cancers in females worldwide. There are evidences that women with diabetes mellitus have a 40% higher risk of mortality. CD34 is a cell surface glycoprotein, which functions as a cell-cell adhesion factor. Although its expression is traditionally related to hematopoietic cells, it is actually found on many other types of cells, endothelial too. Nowadays there are evidences that CD34 is a prognostic indicator by emphasizing its low expression in malignant tumors compared to benign ones. The aim of study was to determine the presence and numerical distribution of $CD34^+$ vessels in the normal mammary gland, as well as in NST breast carcinomas, with and without diabetes mellitus type 2.

Materials and methods. We processed immunohistochemically 58 invasive breast carcinomas of NST type. In 29 of cases, tumors were associated with diabetes.

Results. The present study did not reveal any statistical and morphological differences in CD34 expression between compared groups.

Conclusions. The expression of CD34 in breast cancer stroma is not homogenous, irrespective of association with diabetes mellitus type 2. The question if breast carcinoma and diabetes mellitus are concurrent or associated disorders remains open. Probably, the effect of carcinoma prevails in influencing the structure of the tumor microenvironment. We expect a further confirmation in larger study groups.

Keywords: CD34, breast cancer, diabetes mellitus type 2.

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Key messages

What is not yet known on the issue addressed in the submitted manuscript

Is the microvessels density influenced by associated diabetes mellitus?

The research hypothesis

The systemic metabolic action of diabetes can influence the angiogenesis at tumor site.

The novelty added by manuscript to the already published scientific literature

There are no data about microvessels density in case of breast cancer, alone or associated to diabetes mellitus type 2. For the first time was described the process of angiogenesis, at intra- and peritumoral sites in case of NST breast cancer, alone and associated with diabetes mellitus type 2.

Introduction

One of the leading disorders among females' population is considered to be breast cancer. It becomes younger in time and brings a serious threat to women's life as well as physical and mental health.

The pathogenesis of breast cancer is still unclear, which leads to poor outcomes of prevention and treatment of this disease [1]. In the past decades, the most of investigations were focused primarily on tumor cells. However, it is well known, that tumors are composed of parenchyma and stroma, two distinct but cooperative components that can influence malignant cells growth and spread. Many recent studies show that the tumor microenvironment plays an important role in tumor initiation, therefore it can be a fertile soil for disease progression [2]. The tumor microenvironment is made of cells and extracellular matrix, where the cellular component includes different types of cells, such as myofibroblasts, fibroblasts, myoepithelial cells, blood and lymphatic endothelial cells (and their precursors), pericytes, inflammatory cells and mesenchymal stem cells [3, 4]. Epithelial-mesenchymal correlations are crucial for normal mammary gland development and therefore they can play a critical role for breast cancer development and progression. The peritumoral environment interacts with parenchymal cancerous cells and can create favorable condition for tumor grow and invasion.

Cell-surface glycoprotein CD34 represents a glycosylated protein expressed by hematopoietic stem/progenitor cells, endothelial and mesenchymal cells at different sites, including breast [5]. Its function consists in the modulation of cell adhesion and signal transduction. This receptor is particularly sensitive to tumor angiogenesis, because it can clearly represent the state of neovascularization during the growth and progression of tumor [6]. Furthermore, Kaplan-Meier analysis showed that the survival time of patients with high CD34 expression was significantly shorter than that of patients with low CD34 expression. In another words, a high level of CD34 expression may be a potential indicator of a poor prognosis. The new made vessels provide nutrients for tumor cells and remove metabolic waste, thus promoting the growth rate of tumor [7].

Another raising life- and health-threatening chronic disease is diabetes mellitus (DM) type 2. When associated with cancer, it can worsen tumors' evolution and increase the mortality risk. Both of these disorders are the major causes of death worldwide [8]. However, there are limited evidences supporting this correlation that seems to be very complex and it needs further epidemiological, morphological, and molecular investigation. Insufficient care for either diabetes mellitus or breast cancer can affect the survival [9]. The wandering stuff is to investigate the impact of preexisting diabetes on the histopathological structure of breast cancer. It can give a reasonable explanation about breast cancer behavior on the diabetes mellitus background.

Thus, until now there are no evidences about how associated diabetes can influence the content of $CD34^+$ vessels inside of breast tumor and in the peritumoral area. The aim of this study was to clarify how diabetes mellitus, which affects many tissues, can influence the breast cancer environment. Therefore, we investigated the distribution of $CD34^+$ vessels inside of non-cancerous breast parenchyma, as well as in diabetic and non-diabetic breast cancer. As a result, we determined that diabetic status does not influence the amount of $CD34^+$ blood vessels at the intra- and peritumoral sites.

Materials and methods

Patients. We have investigated immunohistochemically 58 cases of invasive ductal breast carcinomas of NST type (during 2021-2022, Institute of Oncology, Republic of Moldova). In 29 cases, the tumor was associated with DM type 2. The mean age was 63.2±6.5 years in the group of patients with diabetes mellitus type 2 and 64.5±7.9 years in the non-diabetic group. All patients underwent a Madden modified radical mastectomy with lymph nodes dissection, without prior chemo- and radiotherapy. Pre-operative fasting blood sugar level was measured by colorimetric method.

Ten samples (breast) of women which died accidentally served as control group. The mean age was 64.2±6.2.

Tissue processing and immunohistochemistry. The specimens were fixed in 10% phosphate buffered formalin for 24-48h and paraffin (Tissue-Tek Paraffin Wax TEK III) embedded as traditionally (Tissue-Tek VIP 6 AI, Sakura Finetek, USA). 3-5 μm sections were cut for histopathological assessment (Accu-Cut® SRM™ 200 Rotary Microtome, Sakura Finetek, USA), stained with Carazzi hematoxylin (Bio-Optica, Italy) and eosin Y (05-M10007, Bio-Optica, Italy) and mounted (Tissue-Tek Glas g2 Glass Coverslipper, Sakura Finetek, USA). The histological grading of breast carcinoma was performed according to the Nottingham system, which takes into consideration tubules and glandular/acini formation, nuclear pleomorphism and mitosis [10]. The Ft of Nottingham score was evaluated by evaluating tubules and glandular/acini formation: score 1 – majority (>75%) form tubules and glandular/acini, score 2 – moderate (10-75%), score 3 – low (<10%) or none tubules and glandular/acini formation.

The evaluation of mitotic activity was made according to WHO (2019) recommendation, as follows (x400): score 1 – nuclei are very similar in size to the nuclei of benign pre-existing epithelial cells (< 1.5 times the size), with minimal pleomorphism, nucleoli are either not visible or very inconspicuous; score 2 – nuclei are 1.5-2 times bigger in size, with mild to moderate pleomorphism, with visible, small and inconspicuous nucleoli; score 3 – nuclei larger 2 times than the size of benign epithelial cell nuclei, with vesicular chromatin, with high variation in size and shape, often with prominent nucleoli.

In the present study we used pathological TNM staging, which is based on tumor size, the number of affected lymph nodes and the presence of distant metastases [11].

The histopathological diagnosis was assessed by two pathologists and suitable for immunohistochemistry cases were carefully selected. Heat-induced epitope retrieval (microwave, 900W, 95°C, 20 min, followed by cooling at RT for 20 min) was made in citrate buffer (10mM Citric Acid, 0.05% Tween 20, pH 6.0). Incubation with primary monoclonal antibody (CD34 Ab1, RTU, 30 min, clone QBEND/10, NeoMarkers, Fremont, CA) was followed by Novolink Max

Polymer Detection System (RE7280-K, 15 min, Leica Biosystems, DE). Specimens were processed automatically on Dako Autostainer Link 48 system. The Mayer's hematoxylin, Lille's modification (HMM500, ScyTek Laboratories) was used for counterstaining.

Microscopic evaluation. Microvessels amount was determined by hotspot approach [12]. Counts were made in ten fields (intra- and peritumoral or periductal/periacinar in case of control group, x200) containing the most abundant vascularity and the mean value (+/- SD) was determined. Any stained endothelial cells from adjacent vessels were counted as a single vessel, even in the absence of lumen.

Image acquisition and data processing. Slides were examined with Zeiss AxioImager 2.0 microscope with AxioCam Mrc5 installed camera by using ZEN core 3.5 imaging software.

Statistical analysis. Cases were grouped (MS Access 2007) into diabetic and non-diabetic groups by taking into account clinical and morphological data. The WINSTAT 2012.1 (R. Fitch Software, Bad Krozingen, Germany) software was used for descriptive statistics (M±SD, the median). The Spearman correlation (r_s) was used to determine the relationship between different variables. The CD34 values from 2 groups were compared by t-independent test. For all tests a value of $p \leq 0.05$ was considered statistically significant.

Ethics. This study has been approved by the Ethics Committee of the *Nicolae Testemitanu* State University of Medicine and Pharmacy (nr.7, 12.11.2021).

Results

Morphologically normal breast tissue contained dense concentric network of CD34 positive vessels in the intra- and interlobular area, around glandular ducts and acini (Fig.1). The extralobular stroma harbored few $CD34^+$ fibrocytes, which mainly surrounded thick-walled arteries. Slight CD34 staining was noted on small caliber blood vessels within the stroma. The mean of CD34 positive vessels in the periductal/periacinar region was estimated as 24.8±11.2 (ranging between 16 – 45, with 14 as median). No CD34 reactivity was observed inside of epithelial sheath.

In case of invasive ductal carcinoma, without associated diabetes, blood vessels with small, thin wall were haphazardly distributed within tumoral nests. The peritumoral areas contained blood vessels of medium caliber. Comparing the border between breast tumor and cancer-free zone, the abrupt loss of $CD34^+$ blood vessels was observed. The intratumoral content of $CD34^+$ vessels was evaluated as 10.5±8.3 (range 0 – 30, median equal to 9). At the peritumoral site, the number of $CD34^+$ cells was 14±8.5 (range 2 – 45, the median as 14). These values can be considered similar, because no differences were determined in case of t-independent (t=-1.6, p=0.12), as well as t-dependent tests (t=-1.58, p=0.13).

In case of carcinoma associated with diabetes the mean of $CD34^+$ vessels was 12.9±12.7 (0 – 59, median as 12) in the intratumoral site and 16±7.3 (0 – 27, 16 for median) in the peritumoral region. By comparing these areas we could not find statistically significant differences in case of t-independent (t=-1.17, p=0.25), as well as in case of t-dependent tests (t=-1.19, p=0.25).

Single statistically significant correlation was determined at the intratumoral site (table 1). In case of tumors with normal sugar level in the blood, intratumoral expression of CD34 correlated statistically significant with lymphovascular invasion (r_s=0.34, p=0.03) and pT stage (r_s=0.54, p=0.001).

Table 1. Spearman correlation between CD34 expression, patients' age, glucose level and tumor's features

	CD34it				**CD34pt**			
	Cancer		Cancer + Diabetes		Cancer		Cancer + Diabetes	
	r_s	p	r_s	p	r_s	p	r_s	p
Patients age	-0.08	0.34	0.07	0.36	-0.30	0.06	0.17	0.19
Sugar level	0.06	0.38	0.0	0.49	0.04	0.42	0.01	0.48
Nottingham grade	-0.04	0.42	**-0.34**	**0.04**	0.19	0.17	-0.17	0.19
Ft	-0.01	0.48	**-0.34**	**0.04**	0.23	0.11	0.16	0.20
Nuclear atypia	-0.09	0.32	0.0	0.49	0.04	0.42	-0.23	0.11
Mitotic activity	0.05	0.39	-0.22	0.13	0.12	0.27	-0.19	0.16
Lymphovascular invasion	**0.34**	**0.03**	-0.04	0.41	-0.06	0.38	-0.11	0.29
Perineural invasion	0.0	0.50	0.23	0.12	0.21	0.14	0.23	0.11
pT	**0.54**	**0.001**	-0.20	0.15	0.11	0.28	0.17	0.18
pN	0.30	0.06	0.18	0.18	-0.09	0.32	-0.07	0.36
CD34it					0.02	0.46	-0.16	0.20

Note: CD34it – CD34 positive vessels at the intratumoral site; CD34pt – at the peritumoral site; r_s – Spearman correlation; p – statistical significance; Ft – Nottingham's score Ft; pT – pathologic stage of tumor; pN – pathologic stage of lymph node metastases. With **Bold** are selected the statistically significant correlations.

In case of tumors associated with DM, $CD34^+$ vessels amount of intratumoral site negatively correlated with tumors grade and Ft (r_s=-0.34, p=0.04). No statistically significant correlations were determined in case of peritumoral expression of CD34 in both groups.

Comparing both groups, only sugar level (p=0.0001) and Ft (p=0.05) values were statistically different (table 2).

Table 2. The differences between breast cancers, alone or in association with DM type 2

	Student independent t-Test (p)
Patients age	0.51
Sugar level	**0.0001**
Nottingham grade	0.29
Nuclear atypia	0.79
Mitotic activity	0.48
Lymphovascular invasion	0.41
Perineural invasion	0.34
pT	0.79
pN	0.60
Ft	**0.05**
Nuclear atypia	0.81
CD34pt	0.34
CD34it	0.41

Note: p – statistical significance; pT – pathologic stage of tumor; pN – pathologic stage of lymph node metastases; Ft – Nottingham's score Ft; CD34it – CD34 positive vessels at the intratumoral site; CD34pt – at the peritumoral site. With **Bold** are selected the statistically significant differences.

Discussions

There are many experimental evidences to prove that tumor growth and future prognosis are dependent on angiogenesis [13]. The expansion of the tumor cells population requires the generation of new vessels, which in turn increase the opportunity of tumor to develop metastases. Thus, the intensity of angiogenesis can predict the future tumor's behavior and stimulate the expansion throughout body. Different studies have used diverse markers to highlight blood vessels in breast tumors in order to describe and measure angiogenesis.

CD34, also known as human hematopoietic progenitor cell antigen, is a constitutive stroma component of most tissues, including female breast [5]. It is a single-chain transmembrane glycoprotein with a molecular weight of 105-120 kDa, located on the long arm of chromosome, predominantly expressed on endothelial and hematopoietic progenitor cells, and closely associated with the process of angiogenesis [6, 12]. Nowadays, there are many studies, which have been conducted to investigate the distribution of $CD34^+$ stromal cells and vessels in neoplasms of various organs including salivary gland, stomach, colorectal tissue, breast, pancreas, uterine cervix and mammary gland [4, 13-15].

A total of 382 million people had diabetes in 2013 and this number is expected to rise to 592 million by 2035 [15]. About 16% of breast cancer patients suffer from diabetes and the last can be associated with 10-20% excess relative risk of breast cancer [16]. This opinion is in line with Giovannucci *et al.* (2010) data, which supports that diabetes can increase the risk of tumor development [17]. A well-designed meta-analysis provided by Bruijn *et al.* (2013) shows that women with diabetes had a 23% greater risk of subsequent breast cancer than those without diabetes [18].

CD34 has a diverse distribution in the tumoral stroma and it is evident that we have to consider carcinogenesis and tumor progression as a multicellular interaction within newly formed tissue, so called cancer tissue. Although the role of CD34, as a marker of angiogenesis in tumors is still unclear, there are many evidences that increase of $CD34^+$ vessels associates with a high risk of metastasis development [19]. Fathy *et al.* (2018) demonstrated that high CD34 expression is statistically significant associated with tumor size and stage, as well as lymph nodes metastasis development in cervical cancer [20]. Authors consider that high expression of CD34 signifies the presence of an anomalous vessel pattern and disturbing tumor vasculature is one of the primary strategies in cancer chemotherapy. Moreover, this marker has an important diagnostic and prognostic value – the decrease of CD34 expression after therapy correlates with its effectiveness [21].

The assumption before making this study was that DM affects blood vessels and this can alter the tumor's grow. DM is characterized by poor circulation and impaired angiogenesis, which appear to contribute to lesions and poor wound healing. Lega *et al.* (2018) consider that patients with associated DM have a poorer prognosis because of estrogenic effects of obesity or metabolic factors like growth-promoting influence of hyperinsulinemia and insulin resistance [22]. In case of present study, we couldn't find any numerical differences between vessels density, at intra- and peritumoral sites, caused by DM. It looks like, the tumor progression occurs in the similar way, in spite of all lesions caused by DM.

The effect of DM on normal tissues does not resume only on sugar level and atherosclerosis. D'Alessandra *et al.* (2021) determined that diabetes induces a transcriptional signature in bone marrow-derived $CD34^+$ hematopoietic stem cells [23]. Specifically, these cells displayed reduced expression of genes coding for proteins regulating antibacterial and antivirus host defense as well as macrophage differentiation and lymphocyte emigration, proliferation, and differentiation. Moreover, a consistent number of inflammatory genes coding for chemokines and cytokines were up-regulated.

The existing reports regarding the amount of vessels are still controversial, which led us to examine the distribution of $CD34^+$ vessels in the intra- and peritumoral stroma in diabetic and non-diabetic patients. Jarajapu *et al.* (2014) demonstrated that DM patients have microvascular complications, which exhibit severely limited capacity to generate ex-vivo expanded endothelial progenitor populations [24]. Moreover, vasoreparative dysfunction observed in diabetic $CD34^+$ cells is due to impaired autocrine/paracrine function and reduced sensitivity to hypoxia. In Durrani *et al.* (2021) opinion, association of DM type 2 worsen breast cancer prognosis and may decrease body defense capacity by changing tumoral microenvironment under disturbed hormonal activity by altering endogenous sex-hormone regulation and activation of the IGF and insulin-signaling pathways [25]. Rask-Madsen *et al.* (2013) consider that DM background alters blood vessels permeability at the level of microcirculation, statement, which let us initially to launch hypothesis that intra- and peritumoral environment of breast cancer, alone or in DM association, can be different [26].

This study has some limitations. First, it does not reflect the general population, because only women of 50 years and older have been taken. Secondly, we did not measure the sugar level in case of control group. Thirdly, we have no data about diabetes duration, a factor that in Zoungas et al. (2014) opinion is directly associated with macro- and microvascular changes [27]. Another limitation is the absence of a cut-off for CD34 expression, which splits the cases into the high/low expression [6]. Moreover, we did not take into consideration the status of lymph nodes.

Conclusions

The expression of CD34 in breast cancer stroma is not homogenous, irrespective of association with diabetes mellitus type 2. Question if breast carcinoma and diabetes mellitus are concurrent or associated disorders remains open. Probably, the effect of carcinoma prevails in influencing the structure of the tumor microenvironment. We expect a further confirmation in larger study groups.

Declaration of conflict of interest

Nothing to declare.

Authors' contribution

VF, VD and LS elaborated the hypothesis, design of the study and had a significant intellectual contribution in data interpretation and discussion of the results. DB, EP and EC gathered primary material, processed and described, counted blood vessels performed the statistical analysis. EF, DB and VF wrote the draft of the study. All authors have read and approved the final version of the manuscript.

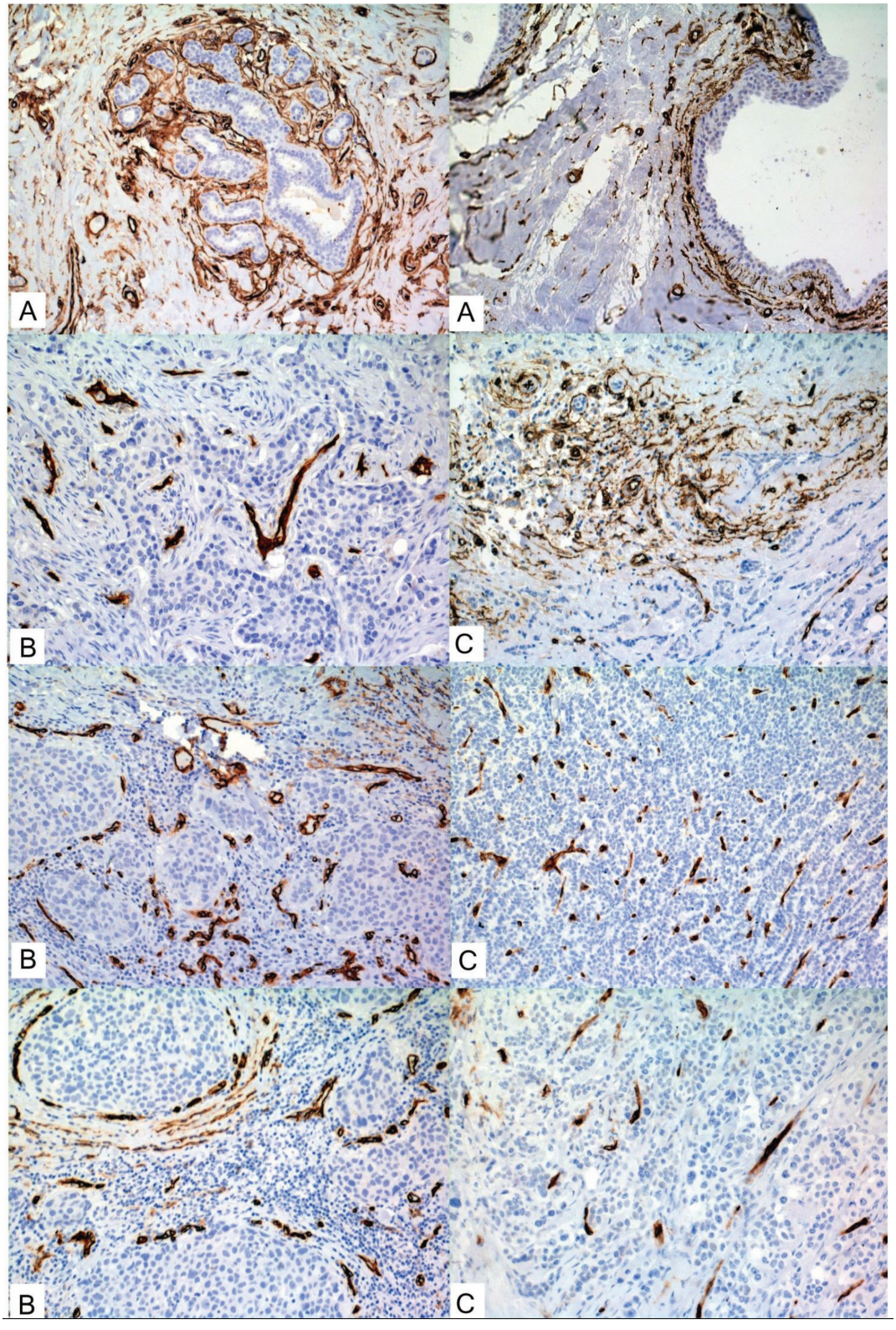

Fig.1
The representative image of CD34 (CD34 Ab1) expression in normal breast (A), breast cancer alone (B) and in association (C) with DM type 2 (x200). A – dense concentric network of CD34 positive vessels in the intra- and interlobular area, around glandular ducts and acini. The extralobular stroma harbors solitary $CD34^+$ fibrocytes, which mainly surround thick-walled arteries. B, C – blood vessels with small, thin wall are haphazardly distributed within tumoral nests. The peritumoral areas contain blood vessels of medium caliber. Between breast tumor and cancer-free zone, the abrupt loss of $CD34^+$ blood vessels is observed.

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RESEARCH ARTICLE

Clinical efficacy of midline lumbar interbody fusion arthrodesis with neuronavigation-guided cortical bone trajectory screws in the treatment of degenerative lumbar spondylolisthesis: a prospective randomized controlled trial

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ABSTRACT

Introduction. Currently, the standard treatment of degenerative spondylolisthesis involves pedicle screw fixation to enhance the success of intervertebral fusion. The traditional pedicle screw techniques require extensive lateral muscle dissection, resulting in significantly increased surgical-related morbidity. To address some of these shortcomings, the Midline Lumbar Interbody Fusion (MIDLIF®) technique has recently been developed. It involves the combination of the cortical bone trajectory screw fixation of the spine with intervertebral cage placement to achieve a solid interbody fusion. So far, the clinical efficacy of the MIDLIF technique in the treatment of low-grade degenerative spondylolisthesis is still unknown. All existing publications are studies with a low level of relevance or scientific evidence.

Materials and methods. A prospective randomized controlled trial was conducted between 2017 and 2022. The study analyzed the clinical and radiological effectiveness of the MIDLIF arthrodesis technique compared to the traditional lumbar interbody fusion techniques, used exclusively in the treatment of degenerative lumbar spondylolisthesis.

Results. The study enrolled 112 eligible patients with degenerative low-grade spondylolisthesis, randomly assigned into two groups. At 1 year post-operatively, MIDLIF provided a significantly better improvement in postoperative relief of low back pain and radiating pain, as well as a significantly better functional recovery. Additionally, MIDLIF resulted in lower surgical morbidity compared to traditional fusion techniques.

Conclusions. The success rate of MIDLIF arthrodesis is similar to that associated with traditional fusion techniques. At the same time, MIDLIF offers all the specific benefits of a minimally invasive approach, such as less postoperative pain, faster functional recovery, less bleeding, and fewer blood transfusions. Thus, MIDLIF might be a good alternative to the traditional intervertebral fusion techniques in the treatment of degenerative low-grade spondylolisthesis.

Keywords: MIDLIF, cortical bone trajectory, pedicle screws, degenerative spondylolisthesis.

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Key messages

What is not yet known on the issue addressed in the submitted manuscript

The efficacy of the midline lumbar interbody fusion technique (MIDLIF) in the treatment of degenerative spondylolisthesis is still unproven. The benefits and technical limitations of the neuronavigation guidance of the cortical bone trajectory (CBT) pedicle screw insertion are unclear and not fully studied.

The research hypothesis

MIDLIF arthrodesis with CBT screws can provide similar fusion

rates as the traditional surgical techniques. At the same time, the MIDLIF method should offer the advantages that are specific to minimally invasive surgical techniques, such as less intraoperative bleeding, less need for blood transfusions, lower postoperative pain intensity, faster functional recovery, etc.

The novelty added by the manuscript to the already published scientific literature

To the best of our knowledge, this is the first prospective, randomized controlled trial to investigate the clinical and radiological efficacy of the MIDLIF arthrodesis, which is used exclusively in the treatment of degenerative spondylolisthesis.

Introduction

Back pain has been called the „disease of the 21st century" because it has become a challenge for the public health system and is the leading cause of disability worldwide. Back pain is one of the dominant symptoms of ageing of the spine and affects up to 85% of the adult population at least once in their lifetime. Back pain is also one of the main causes of health care claims and sick leave in highly industrialized countries. Worldwide, back pain is the most common cause of work-related disability in people under 45 years of age and the third most common cause in people over 45 years of age.

In the 2010 and 2017 Global Burden of Disease studies, out of 291 pathologies analyzed, low back pain ranked as the largest contributor to global disability, being the leading cause of disability in 126 out of 195 countries.

Due to the intense process of demographic ageing, specific to the Republic of Moldova, the treatment of back pain is becoming a major priority for our medical system, which requires a systematic multidisciplinary approach.

The main causes of chronic back pain are the degenerative changes in the spine, such as yellow ligament and facet joints hypertrophy, the bulging of the posterior wall of the intervertebral disc, and marginal osteophyte formation, all of which lead to spinal canal stenosis, often associated with low-grade degenerative spondylolisthesis, which is an indicator of segmental instability of the spine. Lumbar spondylolisthesis is a condition of the spine involving the slippage of the upper vertebra in relation to the adjacent lower vertebra, resulting in narrowing of the neural foramina and compression of the emerging nerve roots.

With an annual incidence of 0.83% and a prevalence of 2.7% among men and 8.4% among women, we should now have around 15000 patients with lumbar degenerative spondylolisthesis in the Republic of Moldova, with a rate of 21612 new cases per year.

Currently, the standard intervertebral arthrodesis technique used in the treatment of degenerative spondylolisthesis involves osteosynthesis of the spine with transpedicular screws to enhance the success of bony fusion. The traditional techniques are very traumatic as they require extensive lateral dissection and tissue retraction to achieve an optimal screw placement angle, resulting in significantly increased operating time, considerable bleeding, increased morbidity, and a high rate of postoperative complications. To address some of these shortcomings of the traditional approach, the Midline Lumbar Interbody Fusion (MIDLIF®) technique has recently been developed. It involves the combination of the CBT screw fixation of the spine with intervertebral cage placement in order to achieve a solid interbody fusion (Fig. 1). The first report on this technique dates to 2009. Santoni et al. proposed the trajectory through the cortical bone and demonstrated that CBT screws have 30% higher pull-out strength resistance than traditional pedicle screws [1].

Subsequently, many morphometric and biomechanical studies have been carried out, demonstrating that CBT pedicle screws possess biomechanical properties equivalent to and sometimes superior to those of traditional pedicle

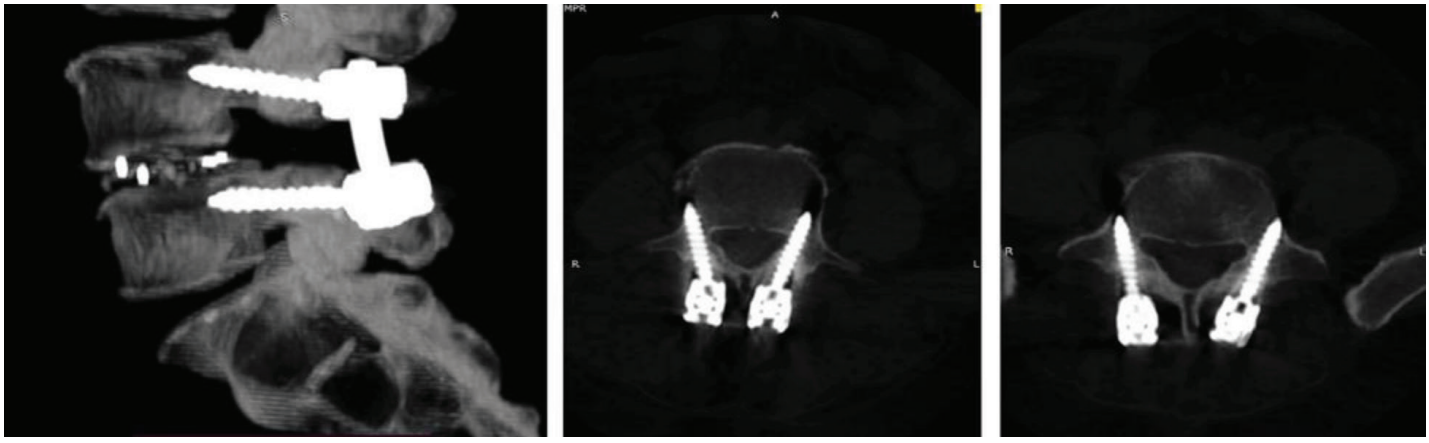

Fig. 1 Midline Lumbar Interbody Fusion (MIDLIF).
Three-dimensional CT reconstruction (Left), CBT screws in the L4 (Centre) and L5 (Right) vertebrae.

screws [2-9]. However, the clinical efficacy of the MIDLIF technique in the treatment of low-grade degenerative spondylolisthesis is still unknown. All existing publications are studies with a low level of scientific evidence relevance, with most of them being observational, retrospective, or cohort studies. The only randomized clinical trial, published by Lee et al. in 2015 [10, 11], examines the efficacy of CBT screw fixation applied in a wide variety of degenerative spinal pathologies (canal stenosis with or without spondylolisthesis, degenerative disc disease, repeated herniated disc recurrences). To date, there have been no prospective, randomized, controlled clinical trials comparing the efficacy of the MIDLIF technique with that of traditional lumbar arthrodesis techniques (PLIF, TLIF) used exclusively in the treatment of degenerative spondylolisthesis.

To address this particular scientific problem, it was decided to conduct a randomized clinical trial to study the clinical efficacy and technical limitations of midline lumbar interbody fusion (MIDLIF) with CBT screws in order to develop an optimized algorithm for the assessment and treatment of patients with degenerative spondylolisthesis.

Materials and methods

A scientific and analytical study was carried out between 2017 and 2022 at the Department of Neurosurgery of the „Timofei Mosneaga" Republican Clinical Hospital, using the model of prospective and non-inferiority controlled clinical trials with randomized selection of group subjects. The study analysed the clinical and radiological effectiveness of the MIDLIF arthrodesis technique compared to the traditional lumbar interbody fusion techniques (TLIF or PLIF), used exclusively in the treatment of degenerative lumbar spondylolisthesis.

The scientific research project was favorably approved by the Research Ethics Committee of *Nicolae Testemitanu* SUMPh (minutes no.44 from December 12, 2016).

To achieve the planned objectives, after applying the inclusion and exclusion criteria, patients were assigned to two study groups:

- Research group L_1 included patients treated by the Midline Lumbar Interbody Fusion (MIDLIF) experimental technique;
- Control group L_0 was composed of patients treated by the traditional surgical techniques of PLIF or TLIF interbody fusion.

The sample size was estimated by applying the respective formula:

$$n = \frac{1}{(1-f)} \times \frac{2(Z_\alpha + Z_\beta)^2 P(1-P)}{(P_o - P_1)^2}$$

where:

- P_0 = proportion of patients in whom intervertebral fusion was achieved by the traditional method. According to the literature [12, 13], the success rate of achieving radiologic fusion in patients with spondylolisthesis using the traditional technique was 75.0% ($P_0 = 0.75$);
- P_1 = the proportion of patients in the research group who had successful intervertebral fusion. We assume that the success rate of treatment after application of the new modified surgical technique will increase to 95.0% ($P_1 = 0.95$);
- $P = (P_0 + P_1)/2 = 0.85$;
- Z_α – tabular value. When "α" – significance threshold is 5%, then the coefficient Zα = 1.96;
- Z_β – tabular value. When "β" – the statistical power of comparison is 80.0%, then the coefficient $Z_\beta = 0.84$;
- f = the expected study dropout rate for various reasons q = 1/(1-f), f = 10.0% (0.1).

Entering the data into the formula, we obtained:

$$n = \frac{1}{(1-0.1)} \times \frac{2(1.96+0.84)^2 x 0.85 x 0.15}{(0.75-0.95)^2} = 56$$

Therefore, two groups were created for the research: the L_1 research group, which included no less than 56 patients with degenerative spondylolisthesis to whom the experimental surgical technique was applied, and the L_0 control group, which included no less than 56 patients with degenerative spondylolisthesis to whom the traditional surgical technique was applied.

The randomization rate between the research groups was 1:1.

The study inclusion criteria were:

- the presence of indications for surgical treatment by arthrodesis in cases of degenerative spinal disorders such as spondylosis associated with spondylolisthesis, foraminal stenosis, severe disc degeneration, and spinal degenerative instability;
- low grade of spondylolisthesis (grade I-II);
- age: 18 years and over;
- the patient is competent to give informed consent;
- acceptance to participate in the research.

The study exclusion criteria were:

- spondylolisthesis with high degree of vertebral slippage (Meyerding grades III-V);
- need for arthrodesis at 3 or more vertebral levels;
- spinal canal stenosis of non-degenerative origin: tumor, trauma, etc.;
- previous lumbar interbody fusion surgery;
- active systemic or local infection;
- severe spinal osteoporosis (DEXA T-score of -2.5 or less);
- presence of contraindications for surgical treatment (such as severe medical co-morbidities, administration of immunosuppressive therapy, etc.);
- lack of a permanent residence address in the Republic of Moldova, emigrants;
- patient is unable to give voluntary consent;
- patient refusal to participate in research.

The primary outcome measurement for assessing treatment efficacy was the successful fusion rate at 1 year post-

operatively, as assessed by the presence of a continuous fusion mass either inside or outside the cage as seen on high-resolution, thin-slice three-dimensional computed tomography (CT) reconstructions.

To quantify the clinical effectiveness of the studied fusion techniques, data were collected prospectively from self-assessment questionnaires, which were completed by patients during the follow-up visits (preoperative, 1 month, 3 months, 6 months, and 1 year postoperatively).

The secondary outcome parameters included the intensity of low back pain and radiating pain, functional status, quality of life, and surgical morbidity. To assess the clinical effect on the improvement of the pain syndrome, the visual analogue pain scale (VAS) was used. Postoperative functional recovery was assessed using the Oswestry Disability Index (ODI) score questionnaire, which is a standard method of measuring the degree of disability associated with back pain. The influence of surgical treatment on health-related quality of life improvement was assessed by the SF-12 questionnaire. Surgical morbidity assessment included recording of operation time, incision length, estimated blood loss, need for blood transfusion, hospital stay, and encountered complications (dura mater lacerations, CSF leak, wound infections, pedicle and pars fractures, mechanical screw failures). Additionally, the degree of iatrogenic muscle injury was assessed by the increase in serum creatine kinase levels the first few days after surgery.

Results

The study enrolled 112 eligible patients with degenerative low-grade spondylolisthesis, randomly assigned into two groups. Patients were similar between groups considering demographic characteristics such as age, gender, body mass index, smoking status, comorbidities, and fused lumbar level ($p > 0.05$). The groups were also homogeneous preoperatively in terms of the VAS score for low back pain and radiating leg pain, the Oswestry Disability Index score, as well as the physical (PCS) and mental (MCS) components of the SF-12 score.

According to the CT scan, a solid interbody fusion (BSF grade 3) at one year after surgery was achieved in 47 patients (83.9%) in group L_0 and 50 patients (89.3%) in group L_1, with no significant difference between groups. A solid fusion through the vertebral plateaus associated with a horizontal area of radiolucency through the middle of the cage or intervertebral space (Fig. 2), described in the literature as "locked pseudoarthrosis", corresponding to a grade 2 fusion according to the Brantigan and Steffee (BSF) classification, was observed in 9 patients (16.1%) in the group L_0 and 6 patients (10.7%) in the group L_1. No cases of true pseudoarthrosis (BSF-1) were radiologically confirmed in either study group (Tab. 1). The difference in fusion rates between groups was not statistically significant, with the p-values for both the Pearson Chi-Square test ($p = 0.405$) and the Fisher exact test ($p = 0.580$) being much greater than 0.05.

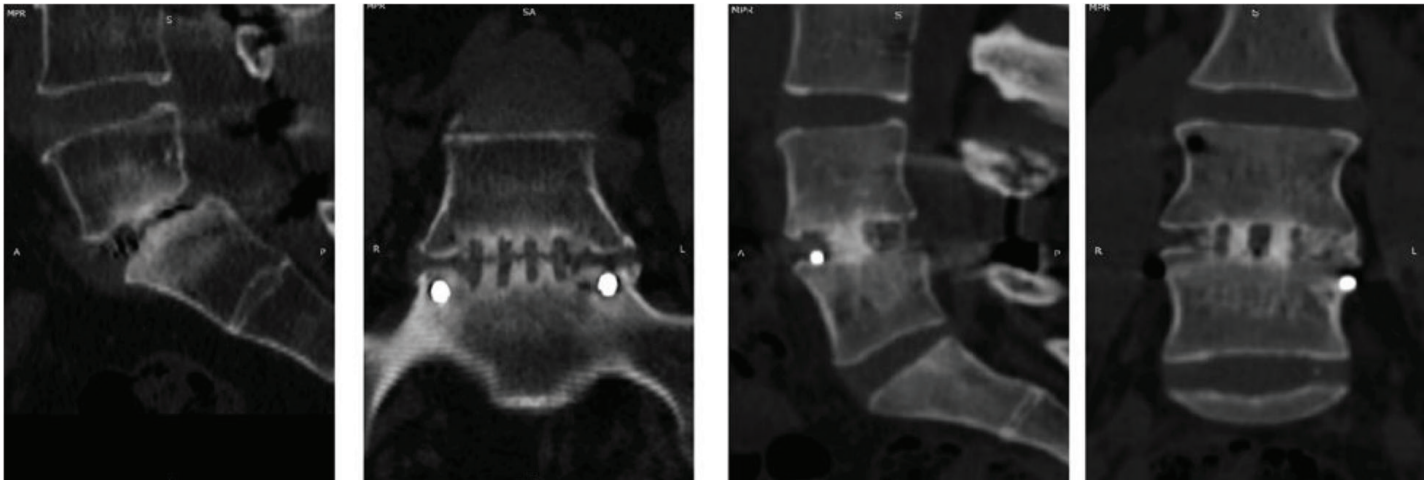

Fig. 2
Locked pseudoarthrosis BSF-2 (first two images from left) and Solid Fusion BSF-3 (last two images) on CT imaging.

Table 1. Success rate of interbody fusion.

Fusion type	Brantigan and Steffee	L_0 group	L_1 group
Radiographic pseudoarthrosis	BSF-1	0	0
Locked pseudoarthrosis	BSF-2	16.1%	10.7%
Radiographic fusion	BSF-3	83.9%	89.3%

Note: BSF – Brantigan and Steffee classification of pseudoarthrosis.

The VAS score for low back pain at 1 year after surgery was significantly lower than the preoperative level in both groups, with the mean score decreasing from 7.18±2.22 pre-op to 3.48±1.57 at 1-year post-op in the L_0 group and from 7.3±1.9 to 1.82±1.34 in the L_1 group. The VAS score for back pain at 1-year post-op was significantly lower in the L_1 group ($p < 0.001$). The VAS score for low back pain was also significantly lower in the L_1 group compared to the L_0 group

at 1 month and 6 months postoperatively ($p < 0.05$), but this difference was not identified at 3 months post-op.

Similarly, the VAS score for radiating pain in the lower limbs improved significantly in both groups after surgery, with the mean score decreasing from 7.34±2.08 preoperatively to 2.27±1.61 at 1 year postoperatively in the L_0 group and from 7.54±2.18 preoperatively to 0.73±1.29 at 1-year post-op. The difference between the groups at 1-year post-op was highly statistically significant ($p < 0.001$). Statistical analysis failed to identify a significant difference between the groups for the radiating pain VAS score at 1 month, 3 months, and 6 months post-op.

The Oswestry Disability Index score also improved significantly in both study groups after surgery, from 51.79±15.22 pre-op to 24.06±12.28 at 1-year post-op in the L_0 group and from 46.45±15.77 to 11.51±8.66 in the L_1 group, the difference being of strong statistical significance ($p < 0.001$). Also, a significant difference in ODI score between groups was found at 1 month ($p < 0.001$) and 6 months ($p < 0.001$), the functional improvement being more evident in the research group.

The improvement in health-related quality of life after treatment was assessed using the SF-12 score. The mental component summary (MCS) of the SF-12 score improved from 39.15±10.89 preoperatively to 51.05±9.2 at 1-year post-op in the L_0 group and from 42.01±12.19 to 54.84±7.15 at 1-year post-op in the L_1 group, the difference between groups being statistically significant ($p < 0.05$). There was also a significant difference between the study groups at 6 months postoperatively. However, no difference could be found between the groups at 1 month and 3 months post-op. At the same time, the physical component (PCS) of the SF-12 score improved from 27.15±7.33 pre-op to 37.41±8.09 in the L_0 group and from 27.58±7.43 pre-op to 46.34±7.39 at 1-year postoperatively in the L_1 group, the difference between groups being statistically significant ($p < 0.001$). The improvement of the physical component of the SF-12 score was significantly greater in the L_1 group at 1, 3, and 6 months postoperatively ($p < 0.05$).

Regarding surgical morbidity, the L_1 group was associated with better outcomes as compared to the L_0 group in terms of intraoperative bleeding volume ($p < 0.001$), need for blood transfusions ($p < 0.001$), operative time ($p < 0.05$), incision length ($p < 0.001$), and increase in creatine kinase serum levels the first few days after surgery ($p = 0.01$). The general complication rate did not differ significantly between groups. Due to the neuronavigation guidance, there were no screw misplacements in either group. Mechanical problems such as pars or pedicle fracture due to screw placement, screw fracture or migration were not encountered in any patients in either group.

However, in both groups there were cases of inadvertent injury to the dura mater (5 cases in the control group and 4 cases in the study group), with one patient in the L_1 group having postoperative CSF leakage through the wound and one patient in the L_0 group having superficial wound infection. Both complications (CSF leakage, wound infection) were resolved without repeated surgery. One patient from each group developed adjacent level disease (at 5 years post-op in the group L_1 and 2 years post-op in group $L_{0)}$. Both patients underwent minimally invasive decompression surgery without additional fusion.

Discussion

Lumbar fusion is widely used to treat lumbar spine disorders, including degenerative spondylolisthesis. Most of the spine fusions are performed with the aid of pedicle screw fixation because of the higher success rates. However, the use of pedicle screws has some important drawbacks. One is the need for a long skin incision and significant lateral muscle dissection due to the far lateral screw entry point. Pedicle screw fixation may lead to the risk of injuring the posterior medial branch of the spinal nerve, denervation of the paravertebral muscles, and superior facet joint violation, all of which lead to biomechanical failure and persistent lower back pain.

In 2009, Santoni et al. introduced a novel method of pedicle screw insertion known as the cortical bone trajectory (CBT), which follows a medial-to-lateral path in the transverse plane and a caudal-to-cephalad path in the sagittal plane through the pedicle. The purpose of the trajectory was to maximize the thread contact with the zone of higher bone density. Santoni found that CBT screws and traditional pedicle screws have equivalent pull-out strengths and toggle characteristics. CBT screws exhibit a 30% increase in uniaxial pull-out strength relative to pedicle screws [1].

Matsukawa et al. first highlighted in an in vivo study that the insertional torque of the CBT technique was higher than in the traditional pedicle technique.

The first comparison between CBT and traditional pedicle fixation for lumbar fusion was reported in 2015 by Lee et al. in a prospective randomized noninferiority trial [10]. Seventy-nine eligible patients were randomly assigned to either CBT or pedicle groups. The primary study endpoint was the fusion rate. Secondary end points included the intensity of lower back pain and pain radiating to the legs as measured by visual analogue scales, functional status improvement as measured by the ODI scale, surgical morbidity, and other outcomes such as mechanical screw failure and pedicle fractures. At the 12-month follow-up, similar fusion rates were observed in both groups. As for clinical outcome, CBT fixation provided similar improvements in pain and functional status. CBT fusion also resulted in a significantly shorter incision length, less blood loss, and a lower operative duration. Therefore, CBT screws in posterior lumbar interbody fusion provided similar clinical and radiologic outcomes compared with pedicle screws [14]. The study had some major limitations, like an insufficient sample size, a short follow-up period, and a broad spectrum of treated degenerative conditions.

Our study yielded several important findings. Similar fusion rates (>80%) were observed in both groups at the 12-month follow-up, with no significant statistical difference between the groups.

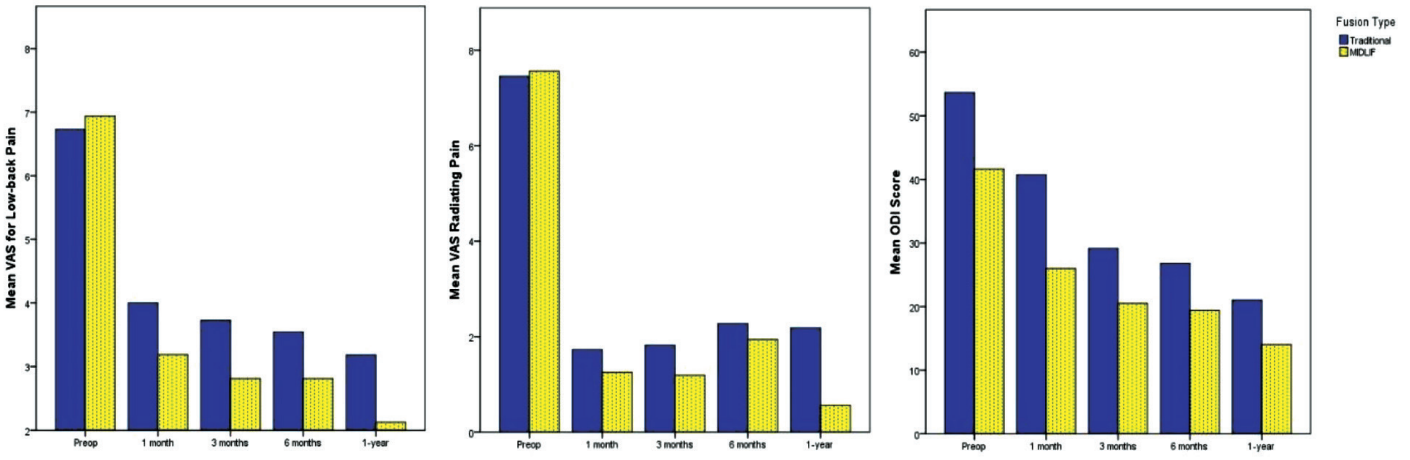

Fig. 3
Mean VAS for back pain (left), mean VAS for radiating pain to the lower extremity (center), and mean ODI (right) scores by time points. The VAS and ODI were collected at each follow-up.

All BSF-2 arthrodesis cases were assessed by CT 2 years after surgery. None of the cases showed negative progression to true pseudoarthrosis. If we accept the statement that "locked pseudoarthrosis" (BSF-2) is a biomechanically stable construct that can be accepted as a satisfactory interbody fusion, we can consider that in both groups the success rate of arthrodesis was 100%.

Clinically, both techniques have been very effective in relieving the back pain, the radiating pain, and the functional disability. However, MIDLIF provided a significantly better improvement in VAS of low back pain and radiating pain in the lower limb and a lower ODI score at 1 year post-operatively (Fig. 1), which is probably due to the minimally invasive nature of the experimental technique. In addition, MIDLIF resulted in lower surgical morbidity measured by incision length, operation time, blood loss and the need for blood transfusions, and the severity of muscle damage expressed by increased serum CK levels in the first postoperative days, compared to TLIF or PLIF. The reduction of surgery-related morbidity may be attributed to the medial insertion point and the medial-to-lateral trajectory of CBT screws, with shorter skin incisions and less lateral muscle dissection and retraction.

Our study partially confirms the results of the two other randomized controlled trials, which have demonstrated the efficacy of CBT screws in achieving a high fusion rate (>80%), lower surgical-related morbidity, and satisfactory clinical outcomes in the treatment of a broad spectrum of degenerative spinal pathologies [10, 15]. However, unlike our trial, the aforementioned studies found no difference between the groups in terms of back pain relief and functional recovery.

Conclusions

Based on the present study, we suggest that MIDLIF is a safe and effective technique for the treatment of degenerative spondylolisthesis. The MIDLIF technique provides the vertebral segment with adequate biomechanical stability, sufficient to achieve a solid interbody fusion. The success rate of intervertebral fusion in the MIDLIF technique is similar to that associated with traditional spondylolisthesis techniques. At the same time, MIDLIF offers all the specific benefits of a minimally invasive surgical technique, such as less postoperative pain, faster functional recovery, less bleeding, and fewer blood transfusions. Thus, MIDLIF might be a good alternative to the traditional fusion techniques in the treatment of degenerative low-grade spondylolisthesis.

Abbreviations

BSF – Brantigan and Steffee, CBT – cortical bone trajectory, MCS – mental component summary, MIDLIF – Midline Lumbar Interbody Fusion, PCS – physical component summary, PLIF – Posterior Lumbar Interbody Fusion, TLIF – Transforaminal Lumbar Interbody Fusion.

Declaration of conflict of interests

Nothing to declare.

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RESEARCH ARTICLE

Diagnostic and prognostic markers of seronegative rheumatoid arthritis

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ABSTRACT

Introduction. Rheumatoid arthritis (RA) is the most common inflammatory disease of the joints, the prevalence of which is increasing in the population, leading to the emergence of new cases of the disease in young and middle-aged people, which has enormous medical and social significance. The study objective was to optimize the diagnosis and prediction of seronegative early rheumatoid arthritis outcomes by identifying the most significant clinical, laboratory and instrumental predictors of joint destruction.

Material and methods. The study includes 82 patients (22 men and 60 women), aged 17 to 70 years (average of 45.02±12.4 years), with the presence of articular syndrome (arthritis). All subjects were classified in the following groups: group I – 41 patients - 11 men and 30 women, whose average age was 44.46±13.36 years (average duration of the disease 6.0±2.9 months) with early seronegative rheumatoid arthritis (eRA), and group II consisted of 41 patients aged 45.55±11.12 years - 11 men and 30 women, with a diagnosis of seropositive rheumatoid arthritis (RA) (average duration of the disease of 6.8±3.7 months).

Results. In the seronegative eRA group, the average Value of "prognostic index" (PrI) calculated from the data at the time of the initial survey was 5.67±1.72 points. PrI values in patients with transformation in RA within 1 year were significantly higher – 6.68±1.61, than without transformation in RA 4.52±0.96 points, $p < 0.0001$. At the same time, the values of PrI < 6 points were observed in 17 (20.7%) patients, PrI > 8 – in 25 (30.48%) patients, intermediate values (between 6 and 8 points) – in 40 (48.78%) patients, $p < 0.001$. Thus, in most patients with transformation in RA, the PrI values were more than 6 points.

Conclusions. In 53% of patients with seronegative RA, there is a transformation into seropositive rheumatoid arthritis during the first 18 months of the development of the disease. Features of early rheumatoid arthritis, in comparison with stabile RA are a polyarthritis presentation of the onset with damage to the joints of the hands, prolonged morning stiffness (more than 1 hour), moderate or high level of activity, the presence of productive synovitis and erosion during ultrasonography.

Keywords: seronegative, rheumatoid arthritis, prognostic index.

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Key messages

What is not yet known about the issue addressed in the submitted manuscript

A comprehensive assessment of the features regarding the course of seronegative rheumatoid arthritis is given and the predictive significance of the „prognostic index".

The research hypothesis

Additional clinical, serological, immunological and instrumental features of the debut, variants of the course and outcomes of early rheumatoid arthritis were revealed. It is demonstrated that the ul-

trasonography is a more sensitive method of studying peripheral joints than radiography in the initial stages of development of rheumatoid arthritis.

The novelty added by the manuscript to the already published scientific literature

The principles of determining diagnostic and prognostic risk factors for the development of erosive-destructive changes in the joints in patients with early seronegative rheumatoid arthritis are substantiated.

Introduction

Rheumatoid arthritis (RA) is the most common inflammatory disease of the joints, the prevalence of which is increasing in the general population [1-3], leading to the emergence of new cases in young and middle-aged people, which has enormous medical and social impact [4].

It has been established that it is the first years of the disease that are decisive in the development and progression of the pathological process. In the earliest period of RA, when the disease is in the primary, exudative phase, its reversibility is significantly higher, due to the not yet developed autoimmune mechanisms and the lack of a morphological basis for articular destruction – synovial pannus [2, 3, 5, 6].

However, to date, the specific signs of the early stage of the disease have not been fully determined [1, 7]. In recent years, an opinion has been expressed about the inconsistency of the criteria of the American College of Rheumatology (ACR, 1987) for early seronegative RA (eRA), while allowing with a sensitivity of 91-94% and a specificity of 89-90% to diagnose the disease with a detailed clinical picture [2-4].

Many researchers state that the main reason for the difficulty of diagnosing seronegative eRA is the lack of clear criterion signs [3, 7]. In this regard, with a lack of classification parameters of any rheumatic disease, but in the presence of persistent arthritis of one or more joints, a group of undifferentiated arthritis is currently distinguished, from which transformation into RA is possible [2-5]. However, the allocation of this medical category is still a controversial issue, and the main concern is about finding criteria to predict how undifferentiated arthritis progresses into rheumatoid arthritis [1-3].

Along with the above, the problem of early diagnosis of RA in the absence of rheumatoid factor (RF) in the blood remains poorly understood. Such questions as features of the immunological picture of seronegative RA in the debut, the rate of progression and functional disorders are not specified. At the same time, the diagnostic and prognostic values of the RF is questionable. An important addition to the verification of rheumatoid inflammation can currently be antibodies to cyclic citrullinated peptides (anti-CCP) [5, 8]. According to preliminary data, anti-CCP has 85-97% specificity and 60-85% sensitivity for RA [2, 6, 9], while detecting 34-69.3% seronegative in the RF. However, some authors note lower rates of occurrence of this marker in eRA - in 40% in the general group and only in 14% of patients negative RF [3, 5, 10], and therefore, new studies are needed to assess the value of anti-CCP as a diagnostic and prognostic marker of eRA, especially seronegative form.

Analysis of the data of recent years has proved that conventional radiography of the hands and feet for verification of seronegative eRA.is quite poor [1-3, 8]. In recent years, ultrasound (USG) and magnetic resonance imaging have been introduced into the diagnosis of seronegative eRA, but the diagnostic effectiveness of these innovative methods requires further study [7-9].

Thus, these prerequisites dictate the need to improve diagnostic approaches and develop new effective prognosis criteria, especially at an early stage of seronegative RA, which determined the goals and objectives of this study.

The purpose of the study was to optimize the diagnosis and prediction of early seronegative rheumatoid arthritis outcomes by identifying the most significant clinical, laboratory and instrumental predictors of joint destruction.

The objectives of the study were:

(1) Conduct a comparative analysis of clinical, laboratory and instrumental parameters in patients with undifferentiated and early seronegative rheumatoid arthritis.

(2) Identify predictors of transformation and assess the possibility of using the „Prognostic Index" to determine the risk of progression of undifferentiated arthritis to rheumatoid arthritis.

(3) Characterize the variants of the course of the disease in patients with early seronegative rheumatoid arthritis in the dynamics of observation.

(4) Assess the diagnostic and prognostic significance of clinical, serological, immunological, and instrumental indicators for the development of destructive arthritis.

Materials and methods

The study was carried out between 2019 and 2022. Subjects were selected from the arthrology and rheumatology departments of the Timofei Moşneaga Republican Clinical Hospital. In total 82 patients were selected (22 men and 60 women), aged between 17 to 70 years (average 45.02±12.4 years), with the presence of articular syndrome (arthritis). During observation, all subjects were identified in two groups.

In group I were included patients with early seronegative rheumatoid arthritis. A patient was included if at the time of the examination, he showed signs of inflammatory joint damage, but there were a sufficient number of criteria for the diagnosis of RA according to ACR, 1987, and there were no certain criteria for other nosology. This group consisted of 41 patients - 11 men and 30 women, whose aver-

age age was 44.46±13.36 years. The duration of the disease on average was 6.0±2.9 months.

Group II consisted of 41 patients aged 45.55±11.12 years - 11 men and 30 women, with a diagnosis of seropositive RA and an average duration of the disease of 6.8±3.7 months. The diagnosis of RA was made based on the criteria of the American College of Rheumatology/The European Alliance of Associations for Rheumatology (ACR/EULAR, 2010) for the early diagnosis of RA and ACR, 1987.

For the entire period of observation - from 12 to 18 months (on average 13.7±4.3 months) from the undifferentiated arthritis group, 7 (17%) patients had converted to seropositive RA, 6 (14.6%) - spontaneous remission, 5 (12.2%) - other rheumatic disease, the rest had an indefinite joint syndrome.

Special immunological studies included determination of rheumatoid factor (RF) in plasma by nephelometry; antibodies to anti-CCP by immunoelectrophoresis using the Set Anti-CCP ORG 220, OR-GENTEC (Germany) and other lab-tests according to the National Protocol for rheumatoid arthritis.

All patients underwent conventional radiography of the hands and distal parts of the feet from a direct (anteroposterior) projection using standard modes on the X-ray diagnostic complex "Bennett" (USA). The X-ray stage of RA was determined by the modified Steinbrocker method. All patients underwent USG of joints. USG diagnostics was carried out on the device "Toshiba 1200" linear sensor with a frequency of 7 to 17 MHz.

Statistical analysis of the results was carried out by using the standard packages of statistical programs SPSS for Windows (version 11.5) and STATISTICA (version 9.0). To create the database, the Microsoft Excel 7.0 spreadsheet editor was used. To describe the nature of the distribution of quantitative features, standard methods of variational statistics were used with the determination of the arithmetic mean value of the variable (M), the mean quadratic standard deviation (SD) and the standard error of the average value (t). The average values in the study were presented in the form M±SD. To assess the reliability of quantitative parameters, a paired Student's t-criterion was used, as well as the Mann-Whitney test. The Wilcoxon matched pairs test criterion was used to evaluate dynamic changes within groups. When assessing the differences in the distribution of rank variables, the X^2 criterion was used. The analysis of the relationship of variables was carried out using correlation analysis using Pearson and Spearmen methods, regression analysis, and binary logistic regression model. Differences were considered significant at the bilateral level of significance of $p < 0.05$.

Evaluation of the effectiveness of forecasting various parameters, indices, and tests was carried out by calculating the operational characteristics – building a characteristic curve, estimating the area under the curve with the determination of the predictive value of a positive result and the predictive value of a negative result, diagnostic efficacy, and diagnostic accuracy of the test.

Results

Of the 81 patients of the observation group, 41 patients were identified in the seronegative eRA group - 11 men and 30 women, the average age – 44.46±13.36 years. The duration of the disease on average was 6.7±2.9 months, while with the duration of the joint syndrome – from 3 to 6 months 14 (34.1%) patients were observed, from 6 to 12 months – 16 (39%), from 12 to 18 months – 11 (26.8%) patients.

A patient was included in the seronegative eRA group if at least one of the modified criteria for the alleged diagnosis of RA was present during the initial examination: (1) the presence of arthritis of at least one joint; (2) a positive symptom of compression of the hands and / or feet; (3) the presence of morning stiffness lasting 30 minutes or more.

Treatment of patients with seronegative eRA was carried out in accordance with the recommendations of EULAR, 2010 with the following scheme: the appointment of non-steroidal anti-inflammatory drugs (NSAIDs), if necessary, glucocorticoids (mainly intra-articular). With the persistence of arthritis during the 3-month observation period, the question of prescribing therapy with disease-modifying antirheumatic drugs (DMARDs) was decided. In most cases, sulfasalazine was prescribed as the first drug at a dose of 2-3 g/day – 20 (48.8%) patients and methotrexate - 21 (51.2%) patients.

In the seronegative eRA group, with an increased duration of the disease, the number of patients with transformation into seropositive RA increased. By the end of observation, 24 patients (58.5%) began to correspond to the diagnosis of seropositive RA.

During the observation of patients with seronegative eRA, to predict the development of RA in this category of patients, at the time of the initial examination, the prognostic index (PrI) was calculated using the method proposed by Van der Helm-van Mil *et al.* We used the same methods as the authors of this index.

In the seronegative eRA group, the average value of PrI calculated from the data at the time of the initial survey was 5.67±1.72 points. PrI values in patients with transformation in RA within 1 year were significantly higher 6.68±1.61, than without transformation in RA 4.52±0.96 points, $p < 0.0001$. At the same time, the values of PrI <6 points were observed in 17 (20.7%) patients, PrI > 8 - in 25 (30.48%) patients, intermediate values (between 6 and 8 points) – in 40 (48.78%) patients, $p < 0.001$. Thus, in most patients with transformation of RA, the PrI values were more than 6 points.

Application of prognosis criteria for patients with seronegative eRA, proposed by Van der Helm-van Mil *et al.* in 2007 it gave a satisfactory result in patients with high rates of diagnostic significance (Table 1). It is worth noting that in our study, RF and anti-CCP were used as immunological markers of rheumatoid inflammation.

In the group of patients with seronegative eRA, a positive result for the RF was recorded in 3 (7.3%), while in patients with transformation to seropositive RA, this immunological marker was detected in 5 (12.2%) examined. The result on

anti-CCP in this group was positive in 4 (9.75%) patients, and in patients with transformation to seropositive RA, positive anti-CCP were found in 7 (17.07) %) of patients, other remain seronegative.

Table 1. Diagnostic value of determining the „predictive index" of the transformation of seronegative eRA in seropositive RA.

Index, n = 82	PrI
Diagnostic sensitivity, %	82
Diagnostic specificity, %	94
Predictive value of a positive result, %	92
Predictive value of a negative result, %	74
Diagnostic effectiveness of the test, %	87
The plausibility relation of a positive result, %	5.1
The plausibility relation of a negative result, %	0.34

Note: PrI - predictive index, eRA – early seronegative rheumatoid arthritis, RA – rheumatoid arthritis.

The analysis showed a high diagnostic effectiveness of anti-CCP and not RF determination in patients with eRA (Table 2).

Table 2. Diagnostic value of anti-CCP and RF determination.

Index, n = 82	anti-CCP	RF
Diagnostic sensitivity, %	99*	65
Diagnostic specificity, %	87*	70
Predictive value of a positive result, %	91*	73
Predictive value of a negative result, %	99*	62
Diagnostic effectiveness of the test, %	94*	68
The plausibility relation of a positive result, %	7.8*	2.2
The plausibility relation of a negative result, %	0.01*	0.52
"Cut-off", IU/ml	20	

Note: * - reliability of differences in the analyzed indicators p<0,05, anti-CCP - antibodies to cyclic citrullinated peptides, RF – rheumatoid factor.

Thus, anti-CCP is a highly specific and sensitive method for diagnosing RA, which allowed us to use it as one of the estimated indicators of PrI.

The obtained results justify the expediency of allocating seronegative eRA as a separate nosological unit, since transformation into RA is possible from this group of patients, which requires more thorough diagnostic approaches. At the same time, the results of the study proved the high diagnostic effectiveness of the determination of anti-CCP for RA verification among patients with seronegative eRA.

The early RA group consisted of 41 patients, of which 28 (68.2%) were diagnosed within the first 4-8 months, 13 (31.7%) patients were identified in this group from the seronegative eRA. Persistent articular syndrome in 41 (100%) of the examined patients was preceded by the appearance of a typical RA picture of the prodromal period. Its most frequent manifestations were: arthralgia – in 37 (90.24%), increased fatigue – 33 (80.48%), a feeling of numbness of the hands/feet – 26 (63.41%), myalgia – 33 (80.48%), which appeared on average 10-12 weeks before the onset of arthritis.

The survey group was dominated by women (ratio M:F = 0.1:4.5). In the dynamics of observation, pre-erosion stages of RA were recorded in 17 (41.6%) patients; single erosions of articular surfaces were recorded in 23 (56.09%) cases. Patients with a moderate degree of RA activity prevailed – 23 (56.09%). Seropositive in anti-CCP arthritis was in 28 (68.29%) patients, in the RF – in 11 (26.82%) patients. The „classic" presentation of the RA debut (persistent symmetrical, gradually increasing polyarthritis with a predominant lesion of the joints of the hands) by the end of observation was recorded in 19 (46.34%) of the subjects. In 23 (56.09%) patients in the initial period of the disease, oligoarthritis was observed mainly in the knee, elbow and shoulder joints and in 11 (26.83) %) – monoarthritis. Prolonged (more than 1 hour) morning stiffness was recorded in 41 (100%) patients. The test of transverse compression of the hands/feet was positive in 41 (100%) patients with eRA. On average, extra-articular manifestations were observed after 6.7±3.1 months from the onset of the disease.

Analysis of the initial manifestations of the joint syndrome showed a certain dynamic of transformation of clinical and laboratory parameters. In the first 3-6 months from the debut of the disease, more than half of the patients recorded mono-, oligoarthritic nature of the lesion, which by the end of the year in 17 (40%) transformed into polyarthritis. Thus, after 18 months from the debut, the polyarthritis nature of RA was observed in 66% of the examined patients, which is significantly ($p < 0.05$) more than in the debut.

Taking into account the critical attitude of classification criteria ACR (1987) in patients with eRA, we conducted a comparative assessment of the diagnostic significance of these parameters and RA classification criteria ACR/EULAR (2010).

As a result of the study in patients with very early seronegative RA, the classification criteria ACR, 1987 corresponded to 30% and in patients with advanced RA – 70%; while the ACR/EULAR, 2010 – in 48% and 100%, respectively. Thus, the diagnostic efficacy of the ACR/EULAR criteria, 2010 was significantly higher than the ACR criteria, 1987 in patients with eRA.

As an immunological marker, along with the RF, we used the level of anti-CCP. It is noteworthy that in an earlier period, RA anti-CCP showed less sensitivity than in the advanced stage of the disease with a consistently high specificity. In patients with a duration of 3-6 months, 27 (65.85%) were positive for anti-CCP, for RF – 17 (41.46%), with a duration of 6-12 months and more than – 100% and 70.1%, respectively. Thus, in the group with eRA by the end of 12-18 months of observation, the diagnostic titer of anti-CCP was recorded in all (100%) patients. Thus, a high diagnostic value of the determination of anti-CCP in the verification of early RA was registered. At the same time, the data obtained indicate that there is no advantage in the diagnostic value of the combination of RF and anti-CCP, compared with the study of only the level of serum anti-CCP.

Currently, it is established that more than 50% of cases of erosive-destructive changes in the joints develop within the

first 3-6 months from the debut of RA, which determines an unfavorable prognosis for the course of the disease. At the same time, half of the most pronounced destructive changes in radiography are observed only after 2 to 6 years from the debut of the disease, which requires a revision of the importance of radiography of the joints in the early stages of the development of RA in favor of other methods of instrumental examination (Table 3).

Table 3. Diagnostic value of USG and radiography in patients with early seronegative RA.

Indicator, n = 82 (group I = 41; group II = 41)	USG	**PrI**
Diagnostic sensitivity, %	71*	29
Diagnostic specificity, %	63*	61
Predictive value of a positive result, %	92*	62
Predictive value of a negative result, %	88*	21
Diagnostic effectiveness of the test, %	97*	34
The plausibility relation of a positive result	5.7*	0.8
The plausibility relation of a negative result	0.04*	1.1

Note: * is the reliability of the differences between p < 0.01, RA – rheumatoid arthritis, USG – musculo-skeletal ultrasound, PrI - predictive index.

In our study, ultrasound signs of seronegative RA were: thickening of the synovial membrane, its hypervascularization in large (knee) joints; tenosynovitis, inflammation of the ligaments and fragmentation of articular surfaces in small joints. In most patients, signs of productive synovitis and destructive arthritis (bone erosion) were detected in the dynamics of observation in the absence of radiographic data.

Table 4. Frequency of occurrence of clinical and instrumental signs of structural changes in patients with different duration of eRA.

	Clinical signs of synovitis	X-ray-signs of structural changes	USG signs of synovitis / structural changes
RA > 3 < 6 months, n = 56 (from 82)	31 (59%)	1 (2%)	54 (96%) *
RA > 6th < 12 months, n = 67 (from 82)	45 (71%)	15 (24%)	65 (97%) *
RA > 12 < 18 months, n = 75 (from 82)	69 (92%)	20 (28%)	75 (100%) *

Note: * - reliability of differences between radiological and USG signs of structural changes in joints p < 0.001, RA – rheumatoid arthritis, USG – musculo-skeletal ultrasound.

In patients with very early stage of seronegative RA, clinical signs of synovitis of the joints were detected in 59%, radiological – in 2%, and ultrasound – in 96% of patients. With an increase in the length of the disease, significant differences between radiographic and ultrasound indicators of structural changes in the joints persisted (Table 4). In addition, in 39% of the examined patients with RA only with ultrasound diagnostics, signs of concomitant osteoarthritis were determined.

Thus, in patients in the early stage of rheumatoid inflammation, USG is a diagnostically more valuable method of studying the joints than radiography. As a result of the study, it was found that with the polyarthritis variant of RA, destructive changes are significantly more often detected by ultrasound of the joints at earlier stages – 64%, against 8% - with oligo-monoarthritis (p < 0.001); with radiography of the joints – 75% against 4%, respectively (p < 0.001).

Analysis of the distinctive features of seropositive and seronegative variants of RA showed the following: the seronegative variant of RA at an early stage was characterized by the absence of early symptoms in almost 70% of patients, while in the group of seropositive patients such signs are detected in more than 77% (p < 0.05); onset with mono-oligoarthritis (80% vs 57% with seropositive variant, p < 0.05), with primary lesions of the knee (80%), ankle (12%, p < 0.01), elbow (6%), joints of the feet (11%, p < 0.05), while for seropositive patients the debut of RA with damage to the small joints of the hands (54%, p < 0.01), knee (40% p < 0.05) of the shoulder joints (6%, p < 0,05). It is also characterized by a smaller number of swollen joints (p < 0.05) throughout the entire period of prospective observation; below the value of the DAS28 index (p < 0.05).

Conclusions

Seronegative RA is characterized mainly by an oligo-, monoarthritis presentation of the joint syndrome; low degree of activity of the inflammatory process; negative rheumatoid factor and anti-CCP antibodies; the absence of active synovitis in USG of joints and destructive changes on radiography. In 53% of patients with seronegative RA, there is a transformation into seropositive rheumatoid arthritis during the first 18 months of the development of the disease. Features of early rheumatoid arthritis, in comparison with stable RA are a polyarthritis version of the debut with damage to the joints of the hands; prolonged morning stiffness (more than 1 hour); moderate or high level of activity; the presence of productive synovitis and erosion during USG.

„Prognostic index" with a significant degree of reliability (diagnostic effectiveness of the test – 87%) allows predicting the development of rheumatoid arthritis in patients with seronegative arthritis. Early ultrasound signs of rheumatoid arthritis show an increase in the thickness of the synovial membrane and its hypervascularization in large joints; tenosynovitis, fragmentation and erosion of the articular surfaces of small joints. USG has a greater diagnostic efficacy – 91% than radiography – 32% (p < 0.01) for assessment of structural changes in peripheral joints in patients with early seronegative rheumatoid arthritis.

Predictors of the development of a destructive process in the joints in patients with early seronegative rheumatoid arthritis are a polyarthritis presentation of the debut with a high degree of activity; tenosynovitis and hypervascularization of the synovial membrane during USG.

Abbreviations

Anti-CCP - antibodies to cyclic citrullinated peptides; eRA – early seronegative rheumatoid arthritis; PrI - predictive index; RA - rheumatoid arthritis; RF – rheumatoid factor; USG – ultrasound.

Declaration of conflict of interest

Nothing to declare.

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RESEARCH ARTICLE

Metabolic syndrome in patients with gout

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ABSTRACT

Introduction. The definition of metabolic syndrome is not yet consistent. However, many studies have been conducted in the latest decades about the effect of increased uric acid on metabolic syndrome development. Large epidemiological studies on the association between hyperuricemia and MS showed that increased concentration of serum urea is often observed in subjects with metabolic syndrome. The aim of the study was to characterize specific dysmetabolic changes and features of extraarticular evolution in patients with gout.

Materials and methods. A descriptive study was conducted that included 501 patients with gout. The mean age of gout (423 men and 78 women) was 49.2 (36.9; 59.9). The diagnosis of gout was established according to the classification criteria for gout ACR/EULAR 2015. The raw data was processed in SPSS version 26.0.

Results. According to our data, the highest severity of obesity and LDL-cholesterol was detected in those with tophaceous gout, which also caused an increase in the frequency of high blood pressure and type 2 diabetes, with age differences in the frequency of detection of metabolic syndrome and insulin resistance. Our data have noted that serum levels of uric acid correlated with the risk of developing both metabolic syndrome as well as its components - obesity, hypertension, and dyslipidemia, but inversely correlated with hyperglycemia.

Conclusions. Gout is associated with a severe lipid metabolism dysfunction, significantly increasing the rate of metabolic syndrome especially among patients with chronic tophaceous gout, than in the group of patients with gout under the age of 59 years. On the other hand, in patients with the onset of gout who are ≤59 years lipid metabolism disorders occur significantly earlier than in patients with the onset of gout at the age of ≥60 years.

Keywords: gout, metabolic syndrome, dyslipidemia.

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Key messages

What is not yet known on the issue addressed in the submitted manuscript

Gout is classified in the group of metabolic diseases, but to this day, the interrelationships between blood lipid values and indicators of the evolution of gout remains unclear.

The research hypothesis

Studying the most important indicators of dyslipidemia and lipid metabolism disorder in gout will discern new possibilities in the correct management of these patients.

The novelty added by manuscript to the already published scientific literature

Knowledge of changes in blood lipids and disorders of carbohydrate metabolism in various forms of gout (tophus and non-tophus) will allow a correct management of patients that will help prevent complications and the development of comorbidities.

Introduction

While it's not clear why there has been a rise in complications related to gout, many experts believe that this may be due to a growing prevalence of factors that contribute to gout, such as higher rates of obesity and metabolic syndrome [1, 2]. Lifestyle factors have an important effect on the incidence of gout. This was confirmed by researchers, authors of a 12-year cohort study conducted on 47150 cases of gout. The risk of gout was more frequent among individuals who were either obese or had a greater consumption of alcohol overall, or in situations where both factors were present [3-5].

Tophus is a cardinal feature of gout; it is a complex mass consisting of monosodium urate crystals, a variety of immune and inflammatory cells, and a fibrous capsule [6, 7]. Tissue deposition of uric acid crystals initiates the formation of tophus with a local inflammatory response of fibrotic tissue. Clinically, it is difficult to separate the different components of a tophus, and these can vary between different anatomical sites in different patients. Stimulation of immune/inflammatory system cells through MUS crystals can lead to chronic inflammation, pain, and tissue remodeling, including bone erosions [8].

In 1998, the World Health Organization (WHO) described „metabolic syndrome" for the first time. The classification criteria for metabolic syndrome (MS) have been proposed by WHO, the National Adult Cholesterol Education Program Treatment Panel III (NCEP ATP III) and the International Diabetes Federation. However, the definition of MS is not yet consistent. Multiple studies have been conducted in recent decades about the effect of increased UA on MS development. Large epidemiological studies on the association between HU and MS showed that increased concentration of serum urea is often observed in subjects with MS. Scientists evaluated data from 8 669 participants in the third National Health and Nutrition Review Study (NHANES-III) (1988-1994) and demonstrated that MS prevalence increased with increasing serum UA levels [9-11].

In another study, out of a total of 2 374 subjects who received a health exam, subjects with HU had a 1.63 times higher risk of MS compared to those without HU based on MS criteria defined by the American Heart Association/National Institute of Heart, Lungs and Blood. In two other studies, the average level of serum UA in patients with MS was approximately 0.5-1.0 mg/dL higher than controls. In addition, another study showed that serum UA levels increased with the number of components of MS after adjusting for age, sex, creatinine clearance, and the use of alcohol/diuretic. Recently, HU is recognized as a distinct feature and/or major associated factor of MS [12-15].

The aim of this study was to characterize of specific dysmetabolic changes and features of extraarticular evolution in patients with gout.

Material and methods

A descriptive study was conducted, and 501 patients with gout were included. There were 423 males and 78 females in the study. The mean age of gout debut was 49.2 (36.9; 59.9). The study was carried out in accordance with the requirements of the Ministry of Health for „Clinical and financial-economic research" within the postdoctoral scientific program at the *Nicolae Testemițanu* State University of Medicine and Pharmacy of the Republic of Moldova, Department of Internal Medicine, Discipline of rheumatology and nephrology. The patients were separated into two groups, depending on their age when gout debuted: ≤59 years (group I, 233 subjects) and the age of onset ≥60 years (group II, 268 subjects).

From the electronic medical records of the Departments of Arthrology, Rheumatology and Nephrology of the Republican Clinical Hospital „Timofei Moșneaga" were extracted the clinical, laboratory and treatment data points on 693 patients with gout hospitalized between 2015-2022. Of the 693 patients, 501 met the including criteria and were selected for the statistical processing. The diagnosis of gout in the database was carried out in accordance with the classification criteria for gout according to ACR/EULAR 2015 [12]. The raw data was processed in SPSS version 26.0.

Results

Metabolic syndrome - a complex of metabolic, hormonal, and clinical disorders associated with atherosclerosis, is detected in most of our patients with gout, but in a higher frequency among those with chronic tophaceous gout. Thus, our study showed that the frequency of MS in patients with tophaceous gout is 1.6 – 2.8 times higher than in the population, reaching 65% in people over 60 years of age. The main components of MS currently include abdominal obesity, impaired lipid (Table 1, Figures 1-5) and carbohydrates metabolism (Figure 6), hypertension and insulin resistance. The frequency of detection of individual components of MS in patients with gout is also quite high and made it possible to diagnose MS in 68% of cases, insulin resistance in – 67%, diabetes mellitus type 2 – in 18% and hypertension – in almost 80%.

The analysis of our study included results from 268 patients who were >60 years, had a chronic course of gouty arthritis and poor drug control. Tophi, a classical characteristic of chronic gout, was present in 27.6% cases (74 patients), and absent in 72.4% cases (194 patients).

Thus, according to our data, in patients with tophaceous gout, the severity of obesity and LDL-cholesterol (including cholesterol) was higher, while HDL-cholesterol was lower, causing an increase in the frequency of high blood pressure and type 2 diabetes, with age differences in the frequency of detection of MS and insulin resistance. The independent association between insulin resistance and MS prevalence is confirmed by many studies [16]. Therefore, the data from the literature, have noted that serum levels of UA correlated with the risk of developing both MS and its components – obesity, hypertension, and dyslipidemia, but inversely correlated with hyperglycemia (Figure 1) [17, 18].

The prevalence of MS in patients with gouty chronic arthritis was 30%-42% according to NCEP ATP III guidelines and 50%-59% according to WHO obesity criteria, both of

which were significantly higher than normal control groups. These findings showed a concomitant increase of prevalence of MS and gout and suggested that the two diseases are related [19-22].

Table 1. Values of the basic components of metabolic syndrome

		Tophus	
		Not present	Present
Total cholesterol	*Minimum, Maximum*	2.10; 72.60	3.10; 8.00
	Mean	5.42	5.21
	Standard Deviation	3.71	0.89
	Median	5.20	5.20
	Percentile 25	4.60	4.70
	Percentile 75	5.80	5.80
HDL-cholesterol	*Minimum, Maximum*	0.26; 1.60	0.42; 1.45
	Mean	1.00	0.91
	Standard Deviation	0.25	0.20
	Median	0.98	0.85
	Percentile 25	0.81	0.79
	Percentile 75	1.20	1.02
LDL-cholesterol	*Minimum, Maximum*	1.99; 6.90	2.18; 6.90
	Mean	4.60	4.91
	Standard Deviation	0.87	.86
	Median	4.70	4.90
	Percentile 25	3.90	4.50
	Percentile 75	5.20	5.40
Triglycerides	*Minimum, Maximum*	0.47; 5.90	0.80; 12.40
	Mean	2.48	2.52
	Standard Deviation	0.99	1.22
	Median	2.10	2.20
	Percentile 25	1.80	1.90
	Percentile 75	3.30	2.70
Uric acid in serum	*Minimum, Maximum*	81.00; 747.00	160.00; 797.00
	Mean	432.70	480.02
	Standard Deviation	119.96	117.64
	Median	437.00	480.00
	Percentile 25	345.00	418.30
	Percentile 75	506.50	559.00
Hemoglobin A1C	*Minimum, Maximum*	0.90; 58.30	0.40; 10.50
	Mean	6.29	5.99
	Standard Deviation	3.83	1.18
	Median	5.90	6.00
	Percentile 25	5.20	5.30
	Percentile 75	6.80	6.40

Note: HDL-cholesterol – high-density lipoprotein; LDL-cholesterol – low-density lipoprotein.

The values of the main clinical and laboratory indicators of blood (glucose, UA, creatinine) exceeded the normal and showed significant differences in groups with predominance in those with chronic tophaceous gout. Indicators of lipid metabolism also had significant differences in groups: cholesterol, TG, LDL-C levels were higher, and HDL-cholesterol was below the optimal values in those with tophi. Hyperuricemia was detected in 2/3 of the patients in both groups (65% and 66%).

It was found that five metabolic risk factors have significant or moderately significant correlations with the UA levels in the general group, as demonstrated in Figures 1-6.

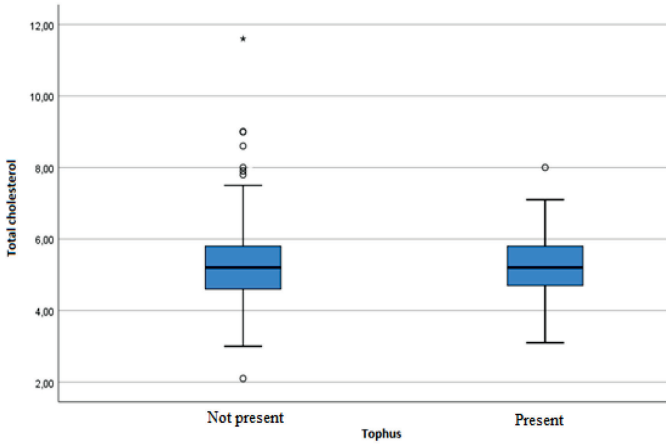

Fig. 1 Interdependence of the values of total cholesterol (mmol/L) and the presence of tophus

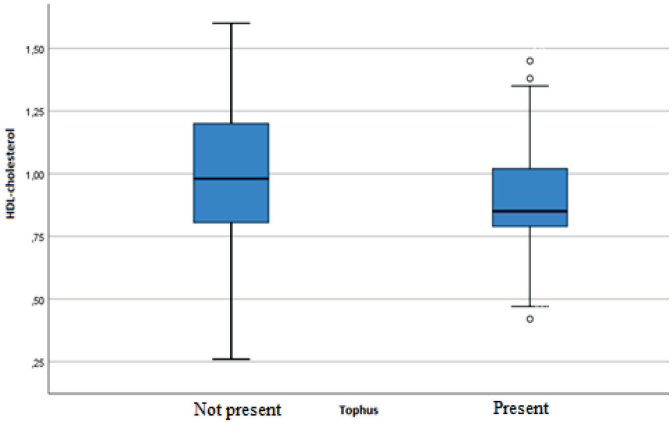

Fig. 2 Interdependence of HDL-cholesterol (mmol/L) values and the presence of tophus

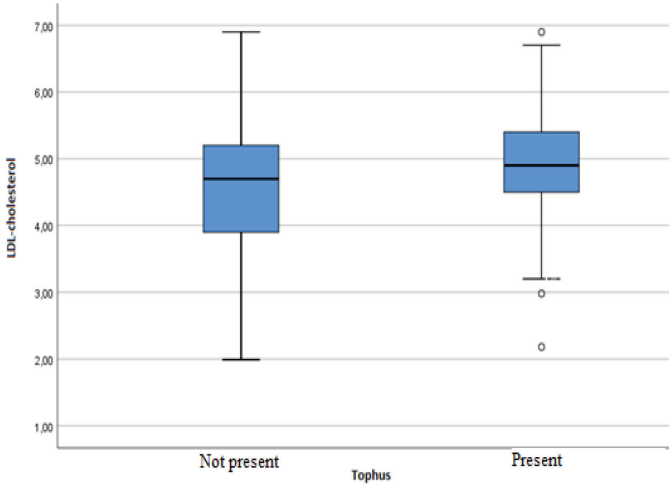

Fig. 3 Interdependence of LDL-cholesterol (mmol/L) values and the presence of tophus

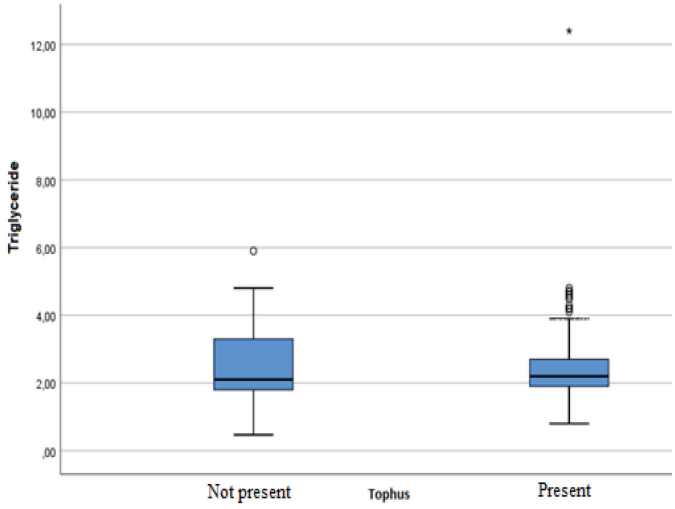

Fig. 4 Interdependence of triglyceride (mmol/L) values and the presence of tophus

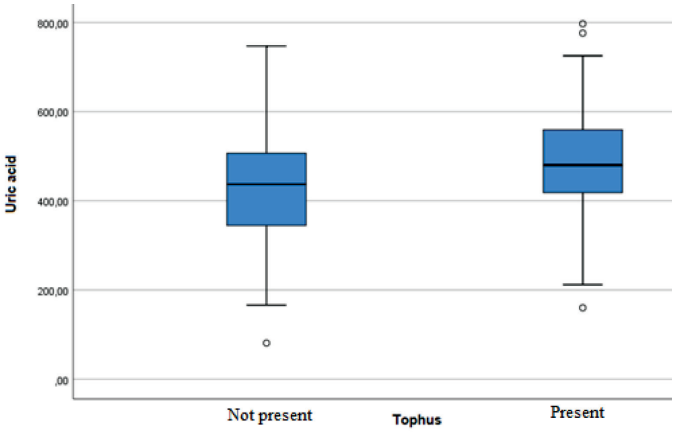

Fig. 5. Interdependence of uric acid values (mmol/L) and the presence of tophus

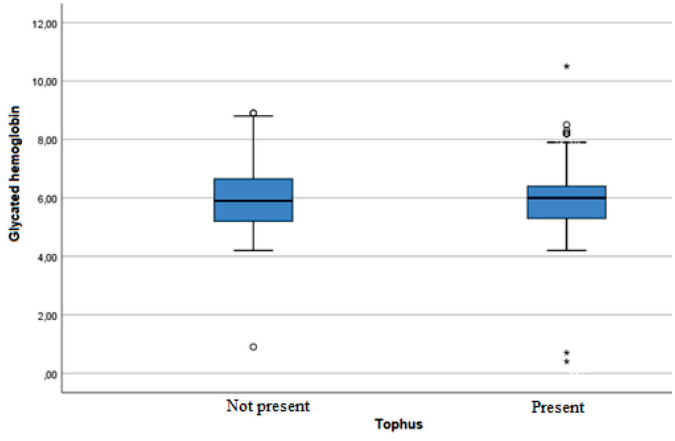

Fig. 6 Interdependence of glycated hemoglobin (%) values and the presence of tophus

In people without metabolic syndrome, uric acid levels did not exceed the upper reference value, except for fasting blood glucose (p = 0.146). In people with metabolic syndrome, it was found that the levels of glucose, triglycerides, HDL and waist circumference have a slight or moderate correlation, while blood pressure did not give such a correlation with uric acid [21]. The effectiveness of non-pharmacological measures to reduce the level of hyperlipidemia is confirmed by reducing the values of total cholesterol and LDL-cholesterol, but it does not have a significant rate.

Discussions

The prevalence of MS and hyperuricemia/gout has steadily increased. There are data on the epidemiology of gout and hyperuricemia in US adults in NHANES-III (1988-1994) and NHANES 2007-2008 that show that the prevalence of these conditions is substantial and has increased over the past two decades. In addition, the prevalence of MS has been shown to steadily increase according to the data from NHANES-III and NHANES 1999-2006 in the US population. Similarly, in the Korean population over the age of 20, scientists have demonstrated that MS's prevalence has gradually increased from 24.9% in 1998 to 31.3% in 2007. Several clinical studies showed a higher prevalence of MS in patients with gout compared to the general population [22].

In 2022, a few researchers studied the relationship of metabolic syndrome and gout, for an average of 7.4±1.2 years. 88 058 men with gout were examined. The incidence of gout was 3.36 per 1000 people. It has been found that gout in people with MS develops 2 times more often. Of all the components of MS, abdominal obesity came out on the first place; increased TG was in second place [23]. In a large cross-sectional study conducted in NHANES-III, the prevalence of MS in people with gout was 62.8% vs. 25.4% among people without gout according to age and gender [24]807 participants age >or=20 years in the Third National Health and Nutrition Examination Survey (1988-1994.

An analysis carried out in the ARIC study provides similar data for women. A total of 6263 women between the ages of 45 and 64, with no history of gout before inclusion in the study, were tracked for 9 years, identifying 106 cases of incident gout. Compared to women with a BMI <25 kg/m^2 at the baseline, the adjusted relative risk of gout was 1.63. In women with BMI 25–29.9 kg/m^2 and BMI ≥30 kg/m^2 at age 25 years, multivariate relative risks for gout were 3.36 and 2.84, respectively, compared to those with BMI <25 kg/m^2 at age 25 years. Women with the highest weight gain indicator (≥16.3 kg) from the age of 25 to the initial age had a 2 times higher risk of gout compared to people in the lowest indicator [25].

In the case study of the THIN database, persons with a BMI between 25-29 kg/m^2 had relative risk of 1.62 for gout and those with BMI of ≥30 kg/m^2, a relative risk of 2.34. With a BMI of 21-22.9 kg/m^2, the relative risk of gout in men was RR 0.85 in those with BMI <21 kg/m^2, increasing to a relative risk of 1.40 in those with BMI 23-24.9 kg/m^2, RR 1.95 with BMI 25-29.9 kg/m^2, RR 2.33 with BMI 30-34.9 kg/

m^2 and RR 2.97 with BMI ≥ 35 kg/m^2 [25]. In addition, the risk of the incidence of gout was increased in men who added 20-29 kg and ≥30 kg in weight from the age of 21, compared to those whose weight was stable. In contrast, weight loss of 10 kg or more reduced the risk of gout by 39% [26].

Although hyperglycemia and IR are recognized components of MS, the role of DM type 2 as a risk factor for the development of gout has received relatively little attention. Interestingly, in the case study THIN *database-control*, people with DM type 2 had a 33% lower risk of developing gout than those without DM type 2 (RR 0.67) [5, 19, 27]. This finding was more marked in men than in women. The risk of developing gout reduced with increased duration DM type 2: duration 0-3 years RR 0.81, 4-9 years RR 0.67, and 10 years or more RR 0.52. The risk was also lower for DM type 1 than for DM type2. Although these findings may seem counter-intuitive, the predisposition to HU and gout induced by hyperinsulinemia and IR in the pre-diabetic state is considered to be reversed by the uricosuric effects of glycosuria once DM type 2 complications develop [2, 17, 28].

Thus, the incidence of metabolic syndrome is 2 times higher in people with tophaceous gout compared without it (33.6% and 15.9%, respectively, after adjusting according to age, $p < 0.001$). This indicator increases with age, and after 60 years, MS occurs in 47.6% of those suffering from gout, which is 2 times more common than in people who do not suffer from gout of the same age (23.8%). Since MS increases the risk of atherosclerotic cardiovascular disease and DM type 2, its presence significantly aggravates the comorbid background, complicates treatment and worsens the prognosis for gout [23, 28].

Conclusions

Gout is associated with a severe lipid metabolism dysfunction, significantly increasing the rate of metabolic syndrome especially among patients with chronic tophaceous gout, than in the group of patients with gout under the age of 59 years. On the other hand, in patients with the onset of gout who are ≤59 years lipid metabolism disorders occur significantly earlier than in patients with the onset of gout at the age of ≥60 years ($p < 0.05$).

Abbreviations

BMI – body mass index, DM – diabetes mellitus, HU – hyperuricemia, IR – insulin resistance, RR – relative risk; MS – metabolic syndrome, MUS – mono-urate of sodium, NCEP ATP III – National Adult Cholesterol Education Program Treatment Panel III, NHANES – National Health and Nutrition Review Study, UA – uric acid.

Declaration of conflict of interest

Nothing to declare.

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RESEARCH ARTICLE

Clinical expression of parasitic arthritis – joint inflammatory process

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ABSTRACT

Introduction. Parasitic arthritis is associated with infestation of the patient's body of parasitic species of worms and protozoan microorganisms. Now it has been established that parasitic arthritis can be caused by almost any species of these creatures. The objective of the study was to research the clinical-evolutionary features of cases of parasitic infections associated with damage of the osteo-articular system in helminthic pathologies.

Materials and methods. 161 patients were included in the study, which were divided into 3 groups of differentiated observation by the pathogen of infestation and the clinical variant of parasitic arthritis. The first group (97 patients) consisted of patients with parasitic arthritis associated with echinococcosis infestation, the 2nd (31 patients) – patients with parasitic arthritis associated with *Toxocara canis* and the 3rd (33 patients) included subjects with parasitic arthritis associated with of *Giardia lamblia* infestation.

Results and discussion. Echinococcosis was manifested more often by the axial (65.67%) and peripheral (31.34%) clinical forms, while the mixed form was being extremely rare (2.98%) ($p < 0.001$). The power of connection with the type of arthritis has reached the degree of statistical significance ($p < 0.001$). Parasitic arthritis due to *Toxocara canis* showed an overwhelming predominance of peripheral forms of joint syndrome (70.96%), with an insignificant share of axial (12.9%) and mixed form (16.13%) ($p < 0.01$), with a connection to the peripheral form of parasitic arthritis ($p < 0.01$). The clinically developed form of parasitosis caused by *Giardia lamblia* manifested a predominantly mixed joint syndrome (57.89%), confirming a predominantly peripheral impairment (36.84%) of the axial (5%) with a statistically significant difference ($p < 0.05$), as well as a connection for the mixed-peripheral form of parasitic arthritis ($p < 0.05$).

Conclusions. Parasitic arthritis is characterized by the diversity of clinical joint manifestations, which fall into 3 clinical variants: induced by infestation with *Echinococcus*, *Toxocara canis* and *Giardia lamblia*, among which giardiasis correlates with a more severe clinical course, followed by echinococcosis and toxocariasis. Despite a large number of painful and inflamed joints that are also associated with an advanced radiological stage of joint damage, parasitic arthritis is characterized by a comparatively diminished articular painful syndrome.

Keywords: helminthiasis, parasitic arthritis, *Echinococcus*, *Toxocara canis*, *Giardia lamblia*.

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Key messages

What is not yet known about the issue addressed in the submitted manuscript

Despite the knowledge of the clinical picture of parasitic arthritis, to date there is no unanimously accepted set of diagnostic criteria for this nosology, which makes it difficult to issue the diagnosis.

The research hypothesis

All patients were divided into three batches, depending on the infestation agents detected. Parasitic arthritis is certain in the case

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when the appearance or aggravation of arthritis was preceded by phenomena of helminthic invasion of at least a month before.

The novelty added by the manuscript to the already published scientific literature

The presented study demonstrates that parasitic arthritis is characterized by the diversity of clinical joint manifestations, which fall into 3 clinical variants: induced by infestation with *Echinococcus*, *Toxocara Canis* and *Giardia lamblia*, of which giardiasis correlates with a more severe clinical evolution, followed by echinococcosis and toxocariasis.

Introduction

Parasitic arthritis is associated with infestation of the patient's body of parasitic species of worms and protozoan microorganisms. Now it has been established that parasitic arthritis can be caused by almost any species of these creatures. In most situations, they accompany such parasitic diseases as echinococcosis, taeniasis, schistosomiasis, filariasis, dracunculiasis, etc.

In recent years rheumatologists from all countries attest to a significant increase in the number of patients with parasitic arthritis [1]. According to specialized data, patients with parasitic arthritis constitute about 7% of the patients from rheumatology wards, 12% of patients develop a form of chronic peripheral arthritis and/or damage to the axial skeleton, which in 29% of cases cause disabilities [1, 2].

Parasitic arthritis can develop by two main mechanisms. The simplest of them is when the parasite itself or its eggs reach the joint cavity or surrounding tissues. The second mechanism is when lesions of the joint are caused by antibodies that are produced to fight the parasite located in other tissues or organs [3].

Echinococcosis is an anthropozoonosis that is usually asymptomatic in different mammals that are intermediate hosts for the parasite. Echinococcosis also affects humans, sometimes with a fatal outcome. Sensitization of the body by the products of parasitic metabolism leads to the development immediate hypersensitivity reaction and, after this, delayed type reaction of immunity. A vivid manifestation of an allergic reaction of an immediate type are eosinophilia and urticaria because of the outflow of echinococcal fluid, and in more severe cases (when opening the bladder) – anaphylactic shock. In the later stages of the disease, especially in multiple echinococcosis, immunopathological reactions play an important role. There are heterogenous clinical manifestations of echinococcosis and symptoms are determined by localization, size, multiplicity of invasion, rapid growth of the echinococcal cyst [4-8].

Another frequent form of helminthiasis widespread in our country is toxocariasis with a nematode causative agent of the *Anisakidae* family of the genus *Toxocara*. Several species of toxocariasis are known, but the most common are *Toxocara canis*, a helminth that mainly affects members of the canine family (dogs, wolves, foxes, arctic foxes, etc.) and *Toxocara mystas*, a helminth of the feline genus, sometimes called *Toxocara cati.* Clinical manifestations of toxocariasis are determined by the intensity of invasion, the distribution of larvae in organs and tissues, the frequency of re-inversion and the characteristics of the human immune response [5-7, 9]. There are several clinical forms of toxocariasis: (1) visceral; (2) cutaneous and (3) systemic.

Giardiasis is one of the most common protozoal diseases caused by *Lamblia intestinalis* (*Giardia lamblia*) [2, 4, 5, 9]. In the clinical manifestations, we can distinguish different lesions of the digestive tract (diarrhea, abdominal pain, vomiting, lack of appetite), hepatobiliary (dysfunction of the biliary tract), neurologic (irritability, fatigue, sleep disturbance, headache, dizziness), allergic manifestations, weight loss and anemia [3-5, 10, 11]. Hepatobiliary, cardiovascular, pulmonary, and musculoskeletal manifestations are common.

The purpose of the study was to research of the clinic and evolutional features of the cases of parasitic infections associated with damage of the osteo-articular system in helminthic pathologies.

Material and methods

In order to achieve the objectives, we have selected a group of 161 patients with the definite diagnosis of parasitic arthritis. The diagnosis was established in two stages – compliance according to the specific and serological criteria. The first stage included the diagnosis of inflammatory type of osteoarticular damage using the criteria. The second stage of diagnosis concerned the compliance with the positive results of the positive serological or parasitological diagnosis.

Patients were treated in the rheumatology and arthrology departments of the *Timofei Moșneaga* Republican Clinical Hospital and rheumatology department of the *Saint Trinity* Municipal Clinical Hospital, Chisinau, in the period 2017-2022 – according to the WMA Declaration of Helsinki with Favorable opinion of the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy (No. 88 from 19.06.2018).

Patients were divided into 3 groups depending on the pathogen and the clinical variant of parasitic arthritis. The first group (97 patients) consisted of patients with parasitic arthritis associated with echinococcosis infestation, the 2nd

(31 patients) – patients with parasitic arthritis associated with *Toxocara cannis* and the 3rd (33 patients) included patients with parasitic arthritis associated with *Giardia lamblia* infestation.

Criteria for inclusion in the study were age 18-70 years; the presence of parasitic infection; the appearance of symptoms of locomotor damage during a positive diagnosis of parasitic infection; damage to the musculoskeletal system for the first time (unsolved causes); patient's agreement to participate in the research. Exclusion criteria: history of rheumatic diseases (inflammatory, autoimmune); uncontrolled diseases (cardiovascular, hepatic, renal); neoplastic diseases; mental and cognitive disorders; <18 years or >70 years.

The index β-Tau was determined by applying the statistical method of trenchant analysis of homogeneous evolutionary data over time, with the calculation of the McNemar-Fisher index, which characterizes the rate of approach of the analyzed values to the ideal range (as an ideal interval it was considered the minimum and maximum value of the physiological norm). ANOVA's correlational factorial analysis established the degree of interaction between factors and the percentage share of different (independent) factors in the source of variation of another factor (dependent) [6-7]. This test has the advantage that it can compare the values of several batches at the same time, while the Student test cannot do so. The data were statistically processed in the software package STATISTICA 11.0.

Results

As can be seen from Table 1, the average age of the patients was 47.0±2.1 years, with the average debut of the disease 4.2±1.3 years, so only patients with chronic parasitic arthritis (the course approximately over 1 year) were included in the study. The joint function was classified according to the functional class (FC). FC stage I joint insufficiency was determined in 34.78% of patients, FC II – in 49.62% of patients, FC III – in 10.02% of persons and in 5.59% FC IV was determined. Sacroiliitis stage I (by Kellgren criteria (1963)), was diagnosed in 44 patients (27.32%), stage II – in 97 patients (59.46%), stage III – in 15 patients (10.1%) and stage IV in 5 (3.1%) patients. Recurrent bilateral conjunctivitis was detected in 97 (60.24%) patients. In 37 (22.98%) patients, oligoarthritis was detected, in 124 patients (77%) polyarthritis was present. Enthesopathy of the knee, hip, elbows, plants, spine was present in 141 (87.57%) patients, calcaneal spur 41 (25.46%) patients.

All patients were divided into three groups, depending on the infestation agents detected. Parasitic arthritis is certain in the event that the appearance or aggravation of arthritis was preceded by an episode of helminthic invasion at least a month before. Parasitic arthritis is certain if it has developed one month after a diarrheal stool or an unstable stool and requires coprocytogram to exclude the impact of pathogenic intestinal flora [6-10].

Table 1. General characteristic of the study batch.

Indices assessed		Total patients (n = 161)
Age, years (Mean±median)		47.0±2.1
Sex M/F		4/1
Age of the disease, years (M±m)		4.2±1.3
Joint functional insufficiency (%)	Functional class I	34.78±0.23*
	Functional class II	49.62±0.35**
	Functional class III	10.02±0.16
	Functional class IV	5.59±0.09
Activity by score DAREA (%)	Degree I	11.8±0.08
	Degree II	49.06±0.64**
	Degree III	39.13±0.71*
Unilateral sacroiliitis (%)	Stage I	27.32±0.21*
	Stage II	59.46±0.39**
	Stage III	10.1±0.07
	Stage IV	3.1±0.09

*Note: *p < 0.05; **p < 0.01; DAREA – disease activity reactive arthritis score; M/F – male/female.*

Due to not compliance to the study protocol, or due to adverse effects developed during the study, 15 patients were excluded, and their data were not included in the final analysis.

In order to differentiate the general status of the patients according to the clinical and evolutive variant of the parasitic arthritis, the separation was performed according to etiological category of infestation agent, as presented in Table 2.

Thus, reactive parasitic arthritis is characterized by a diversity of clinical joint manifestations, expressing itself through different clinical variants.

The spectrum of agents identified with clinical expression is presented as follows: Pulmonary echinococcosis was detected in 67 cases (41.61%), hepatic echinococcosis in 30 cases (18.63%), *T. canis* – in 31 cases (19.25%), *Giardia lamblia* – in 19 (11.8%), *Giardia lamblia* limited form (only joint syndrome predominates) in 14 cases (8.69%) (Table 3).

The pathogens of the infestation have been coprologically and serologically diagnosed according to the corresponding National Clinical Protocols and the diagnosis has been established based on the clinical picture, which is possible when the main clinical-paraclinical criteria of inflammatory arthritis and the confirmation of parasitosis are used, in the absence of other possible rheumatic diseases.

The results of the analysis of the frequency of detection of parasitic agents depending on the type of clinical expression e of the joint syndrome, is presented in Figure 1.

Pulmonary echinococcosis was more often manifested by the axial (65.67%) and peripheral (31.34%) clinical forms, the form of mixed clinical expression being extremely rare (2.98%) ($p < 0.001$). The power of connection with the type of arthritis has reached the degree of statistical significance (Kendall-Tau index = 0.92; $p < 0.001$).

Hepatic echinococcosis was similar to the as the pulmonary echinococcosis, with the predominance of the axial variant (63.33%), followed by the peripheral one (36.66%) in the absence of expressions of mixed variants (0%) ($p < 0.01$), with a major connection to the axial form of parasitic arthritis (Kendall-Tau index = 0.96; $p < 0.001$).

Table 2. Clinical-statutory parameters of the general study group, n = 161.

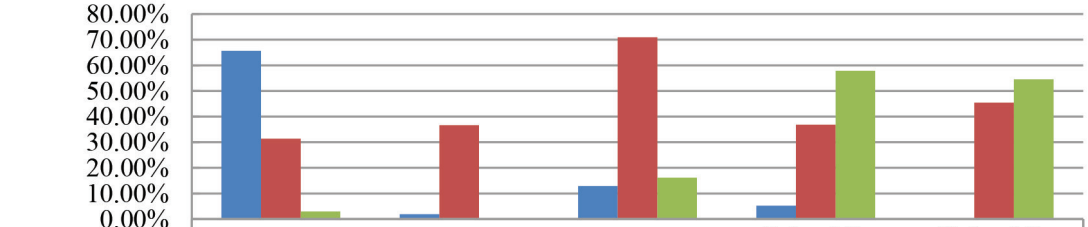
Clinical variant	Examined indexes		
Echinococcal form of parasitic arthritis (n = 97 patients)	Average age, years		29.0±1.1
	Sex M/F		6/1
	Age of the disease, years		3.3±0.98
	Joint functional incapacity (%)	FC I	21.6
		FC II	51.2
		FC III	17.5
		FC IV	9.7
	Activity, DAREA score (%)	Degree I	15.9
		Degree II	63.8
		Degree III	20.3
	Activity, ASDAS-CRP score (%)	<1.3	9.27
		>1.3 < 2.1	36.08
		>2.1 < 3.5	38.14
		> 3.5	16.49
	Unilateral sacroiliitis (%)	Stage I	23.2
		Stage II	65.4
		Stage III	7.9
		Stage IV	3.5
Toxocariasis form of parasitic arthritis (n = 31 patients)	Average age, years		33.0±3.4
	Sex M/F		1/2
	Age of the disease, years		2.4±0.56
	Joint functional incapacity (%)	FC I	27.9
		FC II	59.6
		FC III	10.2
		FC IV	2.3
	Activity, DAREA score (%)	Degree I	28.9
		Degree II	66.4
		Degree III	4.7
	Activity, ASDAS-CRP score (%)	< 1.3	22.58
		>1.3 < 2.1	61.29
		>2.1 < 3.5	16.12
		> 3.5	0
	Unilateral sacroiliitis (%)	Stage I	38.4
		Stage II	51.6
		Stage III	7.1
		Stage IV	1.93
Giardiasis form of parasitic arthritis (n = 33 patients)	Average age, years		48.5±2.7
	Sex M/F		3/1
	Age of the disease, years		5.3±0.41
	Joint functional incapacity (%)	FC I	10.5
		FC II	51.2
		FC III	28.7
		FC IV	9.6
	Activity, DAREA score (%)	Degree I	8.7
		Degree II	59.8
		Degree III	31.5
	Activity, ASDAS-CRP score (%)	< 1.3	0
		>1.3 < 2.1	54.54
		>2.1 < 3.5	36.36
		> 3.5	9.09
	Unilateral sacroiliitis (%)	Stage I	14.5
		Stage II	48.9
		Stage III	31.6
		Stage IV	5.0

Note: CRP – C-reactive protein; DAREA – disease activity reactive arthritis score; M/F – male/female; ASDAS - Ankylosing Spondylitis Disease Activity Score.

Table 3. Frequency of various parasitic forms in patients with parasitic arthritis.

Pathogen and expression	No of patients n = 161	%
Pulmonary echinococcosis	67	41.61
Hepatic echinococcosis	30	18.63
Toxocara canis unfolded form	31	19.25
Giardia lamblia unfolded form	19	11.8
Giardia lamblia limited form (only joint syndrome predominates)	14	8.69

Parasitic arthritis due to *Toxocara canis* showed an overwhelming predominance of peripheral forms of joint syndrome (70.96%), with an insignificant number of axial (12.9%) and mixed forms (16.13%) ($p < 0.01$), with a connection to the peripheral form of parasitic arthritis (Kendall-Tau index = 0.93; $p < 0.01$).



	PE	HE	T. canis UF	G. lamblia UF	G. lamblia J.syndrome
Axial form	65.67%	1.91%	12.90%	5%	0%
Periferic form	31.34%	36.66%	70.96%	36.84%	45.45%
Mixed form	2.98%	0.00%	16.13%	57.89%	54.54%

Fig. 1 The frequency of parasitic species presented depending on the clinical variant of the joint syndrome.
Note: PE – pulmonary echinococcosis; HE – hepatic echinococcosis; *T. canis* UF – *T. canis* unfolded form; *G. lamblia* UF – *Giardia lamblia* unfolded form; *G. lamblia* J.syndrome – *Giardia lamblia* limited form (only joint syndrome predominates).

The joint involvement associated with *Giardia lamblia* had a predominantly mixed joint syndrome (57.89%), a peripheral impairment – 36.84%, and axial – 5% with a statistically significant significance ($p < 0.05$), as well as a connection for the mixed-peripheral form of parasitic arthritis (Kendall-Tau index = 0.81; $p < 0.05$).

We have deduced from the results that the clinical expression of the joint syndrome correlates with the type of parasite, which dominates the disease, and this confirms the validity of etiology classification.

Discussions

The problem of parasitic infection in rheumatology has seen continuous increase of interest to various medical specialists – rheumatologists, infectious diseases, immunologists, biochemists, etc. Their interest is supported by the importance of parasitic agents in the development of rheumatic diseases, such as rheumatic syndromes associated with echinococcosis, toxoplasmosis, toxocariasis, etc. In all these cases, the parasite is starting a process of triggering of immunopathogenic processes in the „target" organs. The interest in this problem is also supported by the demonstrated ability of antiparasitic therapy to modulate the evolution of syndromes and rheumatic diseases. The effectiveness of antiparasitic therapy is well known in the invasions of parasites and other rheumatic diseases, in which the etiology of parasites is proven or suspected.

The severity of the inflammatory process is confirmed by significant values of the DAREA score (for giardiasis DAREA = 84.29±0.47, echinococcosis the score DAREA = 69.28±0.29 and toxocariasis – 59.55±0.51. The value $p < 0.01$ for parasitic arthritis by giardiasis *vs.* parasitic arthritis by toxocariasis, and $p < 0.05$ – for parasitic arthritis from echinococcosis, which is a sensitive indicator of disease activity . In addition, the VAS pain score presented significantly higher values in the group of patients with parasitic arthritis in *Giardia lamblia* (55.07±0.14) compared to *Echinococcus* (45.15±0.13) and *Toxocara canis* (37.04±0.19), with the deduced evidence showing statistical differences ($p < 0.05$).

The ASDAS-CRP score on aggregate confirmed the data of the DAREA score, but it drew them much more clearly and defined. Thus, according to the ASDAS-CRP score, the most severe form of parasitic arthritis by inflammatory syndrome was the parasitic arthritis of *Giardia lamblia*: manifested the highest values of the score, with the predominance of high activity of the disease (cumulatively 100% patients the score is >1.3), followed by parasitic arthritis in *Echinococcus* (cumulatively 90.73% patients the score is >1.3, $p < 0.05$) and the mildest variant of parasitic arthritis was presented the one with *Toxocara canis* (cumulatively 77.42% patients the score is >1.3, $p < 0.01$).

Given the existence of various forms of spinal damage in parasitic arthritis, we considered necessary to examine separately the values of the indices of vertebral damage BASDAI, BASFI and BASRI depending on the study group and the clinical form of parasitic arthritis. The study of these indices allowed us to confirm the heterogeneity of affecting the spine by chaotic migration of the values of the indices of statistical significance between groups.

When analyzing the BASDAI, BASFI and BASRI indices within the values of the group of patients with parasitic arthritis from echinococcosis, the giardiasis and toxocariasis groups, a statistically significant difference was determined only between the BASDAI index values (4.23±0.33; 5.07±0.28 vs 3.4±0.45; $p < 0.005$).

This confirms that damage to the spine is much more expressed in patients with parasitic arthritis of *Giardia lamblia* etiology and *Echinococcus*, than in those with parasitic arthritis associated with *Toxocara canis*. The data of the literature also confirms the direct link between the parasitic infestation and the spine involvement, which is considered a negative prognostic factor in the evolution of the systemic disorder [10, 11].

Conclusions

Parasitic arthritis is characterized by the diversity of clinical joint manifestations, which fall into three clinical variants: induced by infestation with *Echinococcus*, *Toxocara canis* and *Giardia lamblia*, among which giardiasis correlates with a more severe clinical course, followed by echinococcosis and toxocariasis. Despite a large number of tender and swollen joints that is also associated with a progression of radiological erosions, parasitic arthritis is characterized by a comparatively attenuated articular painful syndrome.

Abbreviations

ASDAS – Ankylosing Spondylitis Disease Activity Score; BASDAI – Bath Ankylosing Spondylitis Disease Activity Index; BASFI – Bath Ankylosing Spondylitis Functional Index; BASRI – Bath Ankylosing Spondylitis Radiology Index; CRP – C-reactive protein; DAREA – Disease Activity REactive Arthritis; FC – functional class; VAS – visual analogic scale.

Declaration of conflict of interest

Nothing to declare.

Authors' contribution

Study conception and design: MG, LG. Data acquisition: MG, ER. Analysis and interpretation of data: MG, ER. Drafting of the manuscript: MG, ER. Significant manuscript review with significant intellectual involvement: LG. Approval of the „ready for print" version of the manuscript: LG, MG, ER.

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RESEARCH ARTICLE

The impact of secondary pulmonary hypertension in elderly patients with chronic obstructive pulmonary disease

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ABSTRACT

Introduction. Chronic obstructive pulmonary disease (COPD) is considered an important disease in the structure of morbidity and mortality and a rising public health problem with increasing global age prevalence. Exacerbation of COPD in the elderly has a significant negative impact on the clinical and paraclinical picture, daily activity capacity, and quality of life. The need for correct diagnostic management is dictated by the severity of the pathology in the elderly and the high frequency of cardiac complications. Studying the clinical-paraclinical picture and the diagnostic capacity of natriuretic peptides in COPD patients with cardiovascular complications was the aim of the present research.

Material and methods. We conducted an analytical, observational, case-control study with the use of modern research methods. The study included 194 elderly patients (≥ 65 years old) divided into two study groups: those with only COPD (COPD group) and those with associated pulmonary hypertension (PHCOPD group). Patients underwent clinical, paraclinical, and functional investigation methods with subsequent mathematical-statistical data processing.

Results. The study groups were similar according to socio-demographic data. The clinical assessment revealed that PHCOPD patients had, in comparison with COPD patients, statistically significantly more frequent and severe complaints. The objective examination determined that in PHCOPD patients, the values of the objective examination indices were much more affected than in the COPD group. At the complex geriatric assessment, the COPD group of patients shows a lower degree of dependence and less cognitive impairment. The multidimensional assessment of COPD reflected more severe impairment according to the CAT, SGRQ, CCQ, mMRC, BODE questionnaires, and 6MWT test in the PHCOPD group compared to the COPD group. Spirographic, radiographic, ultrasonographic, and echocardiographic evaluations correlated with age, inflammatory syndrome, and natriuretic peptide level.

Conclusions. The PHCOPD group patients present a clinical picture, exercise capacity, objective data, complex geriatric assessment scores, and a multidimensional assessment of COPD that is statistically more frequent and severely affected compared to the COPD group. The impact of hemodynamic disturbances in COPD could be confirmed by the correlation of paraclinical data, such as the inflammatory response syndrome, increased natriuretic peptides, echocardiography data, and indices of internal organ ultrasound examination.

Keywords: chronic obstructive pulmonary disease, elderly, pulmonary hypertension, natriuretic peptides, NT-proBNP.

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Key messages

What is not yet known on the issue addressed in the submitted manuscript

The peculiarities of COPD patients in geriatrics who have hemodynamic complications according to the clinical picture, objective exams, complex geriatric evaluation, complex COPD assessment, and paraclinical findings. The significance of natriuretic peptides in COPD hemodynamic disorders was highlighted.

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The research hypothesis
Correlational analysis of paraclinical indices that may indicate the presence of subsequent hemodynamic changes.
The novelty added by manuscript to the already published scientific literature
The diagnostic capacity of natriuretic peptides in COPD-induced pulmonary hypertension in elderly patients.

Introduction

Chronic obstructive pulmonary disease (COPD), according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2020 report, is considered a rising public health problem with a significant socio-economic burden [1-4]. The magnitude of the condition is statistically suggested by its fourth place in the structure of death causes worldwide. The negative impact was revealed by the seventh place in the leading causes of disability in high-income countries and the tenth place in low- and middle-income countries, and it is an important cause of hospitalization worldwide. COPD mortality represents about 6% of all the deaths worldwide [4, 5].

The global prevalence of COPD varies by age: approximately 10% among adults aged 40-60 years, 18.8% among subjects aged 60-74 years, and 22.3% among men and 25% among women in the population aged 75 years and older [6-8].

Continuous exposure to COPD risk factors and demographic aging were the responsible predisposing factors for the condition level rising to third place in the structure of the death causes [9, 10]. COPD complications increase the risk of death due to other comorbidities; therefore, the health impact of COPD may be underestimated, considering that 50% of mortality in COPD patients is caused by non-respiratory diseases [5, 11, 12].

Specific to COPD in geriatrics are: (1) higher prevalence in the elderly; (2) higher severity of the disease; (3) a silent clinical picture in the early stages, with the disease being unrecognized and untreated until it progresses to a more severe degree; (4) the association with a large number of comorbidities (average of 9); (5) a higher mortality rate; (6) reduced therapeutic efficacy and (7) a significant quality of life impact [13-18]. Exacerbation of COPD in the elderly consisted of aggravation of the disease, the apparition of other symptoms and worsening of the pre-existing ones, a significant decline in functional and cognitive performance, a reduction in quality of life, and a poor prognosis. As a result, determining the evolution characteristics of COPD in elderly patients is regarded as critical for early COPD diagnosis, assessment of COPD secondary hemodynamic disorders, implementation of effective therapeutic interventions, and reduction of the economic impact on the public health system [11, 14, 19].

The high rate of cardiac comorbidities, installed early with a silent evolution, the existing interrelationship of cardiovascular complications with the severity of COPD, and the increase in exacerbations, hospitalizations, and fatal events due to cardiovascular implements dictate the need for correct diagnosis and management implementation of COPD in the elderly. Therefore, rational, generalizable, and cost-effective strategies were necessary to identify cardiovascular complications, especially pulmonary hypertension and heart failure, in elderly patients with stable or exacerbated COPD in order to reduce the impact of the disease on this group of patients [20-22].

Lately, the detection of hemodynamic impairment in COPD patients presented deficiencies since their non-invasive diagnostic methods did not always provide relevant data and invasive diagnostic methods were difficult to perform, requiring increased possibilities and costs. Highlighting the specific clinical and paraclinical aspects of hemodynamic impairment in COPD patients will allow the elucidation of relevant paraclinical methods for detecting cardiovascular involvement with the application of the adapted treatment strategy.

Timely, accurate diagnosis and subsequent management of PH are very important because, despite therapeutic progress, survival in this group remains suboptimal [12, 15].

Currently, the need to evaluate new biomarkers in COPD has been highlighted, but their implementation in clinical practice has been largely unsuccessful. BNP and NT-proBNP levels have not been adequately studied in patients with lung diseases and cardiac complications, specifically in COPD associated with PH or cor pulmonale, with the goal of estimating prognostic value and mortality risk in these cases [7, 23]. Moreover, data on natriuretic peptides in elderly patients are very rare, and examination of plasma NT-proBNP in elderly patients with COPD in the Republic of Moldova has not been performed.

The goal of the work presented is to perform a diagnostic evaluation of the clinical, objective, and paraclinical status of elderly COPD patients with or without hemodynamic disorders, with a focus on the relevance of NT-proBNP levels in those patients.

Material and methods

To accomplish our purpose, we performed an analytical, observational, case-control study with the use of modern research methods.

The study included 194 elderly patients (≥ 65 years old) who were admitted to the Geriatrics ward of the Ministry of

Health Clinical Hospital for inpatient treatment and met the inclusion criteria. The overall study group was divided into 2 subgroups according to the presence of PH: subgroup 1 – 97 patients with COPD without PH (COPD group); subgroup 2–97 patients with COPD and secondary PH (PHCOPD group). The data were collected during the inpatient stay, coded, and statistically processed.

Eligibility criteria

Eligible patients for the study included: (1) patients with chronic obstructive pulmonary disease with various severity degrees (obligatory presence of clinical and paraclinical features); (2) possible associations of hemodynamic disorders in COPD patients; (3) the age of patients older than 65 years; (4) agreeing to and signing the informed consent for inclusion in the study; and (5) integral mental health and intellectual capacities in the patients included in the study.

The study protocol was reviewed and approved by the Research Ethics Committee of *Nicolae Testemițanu* SUMPh nr. 1 on July 12, 2022. Each patient signed a consent form to participate in the study after being fully informed about the study's purpose and objectives, as well as the obligations, benefits, and risks of the investigations carried out in accordance with the study protocol.

Investigation methods

The patients were examined using biochemical methods, instrumental methods (spirometry, pulse oximetry, electrocardiogram, ultrasonography of internal organs, chest x-ray, and transthoracic echocardiography), a functional exam with the 6-minute walk test, a multidimensional assessment of COPD (estimation of dyspnea (modified dyspnea severity scale – mMRC), symptom assessment (COPD assessment test – CAT, COPD clinical questionnaire – CCQ), quantification of the quality of life (respiratory questionnaire of the „Saint George» hospital – *SGRQ*), and the prognoses determination (*BODE index*, 6-minute walk test – *6MWT*). All patients in the study were evaluated using the survey method.

The volume of the representative sample was calculated in Program F tests – ANOVA (fixed effects, omnibus, one-way analysis; A-priori sample size calculator) – and established the research requirement of 192 patients.

The primary materials of the study were entered into an electronic database and processed on the personal computer using the functions and modules of the programs "Statistical Package for the Social Science" (SPSS) version 16.0 for Windows (SPSS Inc., Belmont, CA, USA, 2008) and Microsoft Office Excel 2019 through descriptive and inferential statistical procedures.

For the comparative analysis of the indicator values, we applied mathematical-statistical techniques (dynamic series indicators, proportional indicators, average values, etc.).

Results

Socio-demographic data

The study groups included 97 (50.0%) patients with pulmonary hypertension secondary to COPD (PHCOPD group) aged 65–90 years and 97 (50.0%) patients with COPD without secondary pulmonary hypertension (COPD group) aged 65–86 years. The study groups were similar according to sex, living environment, social status, living conditions, risk factors (smoking and household pollution), number of comorbidities, general condition, duration of current hospitalization, and number of hospitalizations in the last year (Table 1). Exposure to occupationally harmful substances was more significant in the PHCOPD group (55–56.7%) vs. (38–39.2%) in the COPD group.

The mean age was statistically significantly higher in the PHCOPD patient group (73.35±0.7 years; $p < 0.01$) compared to patients in the COPD group (70.32±0.5 years; $p < 0.01$). In the COPD patients group, youngest-old were statistically significantly more frequently included (84–86.6% vs. 61–62.9%, respectively; $p < 0.001$), and in the group of patients with PHCOPD – middle-old (28–28.9 % vs. 12–12.4%, respectively; $p < 0.01$) and oldest-old (8–8.2% vs. 1–1.0%, respectively; $p < 0.05$) (Fig. 1).

Table 1. Socio-demographic parameters and risk factors in patients in the study groups.

Parameters	PHCOPD		COPD		p
	abs.	%	abs.	%	
Gender:					
- men	65	67,0	70	72,2	NS
- women	32	33,0	27	27,8	NS
Living environment:					
- rural	85	87,6	80	82,5	NS
- urban	12	12,4	17	17,5	NS
Social status:					
- intellectuals	27	27,8	28	28,9	NS
- workers	51	52,6	55	56,7	NS
- farmers	19	19,6	14	14,4	NS
Living conditions:					
- satisfactory	93	95,9	93	95,9	NS
- unsatisfactory	4	4,1	4	4,1	NS
Smoking:					
- never smoked	54	55,7	54	55,7	NS
- ex-smoker	30	30,9	28	28,9	NS
- currently smokes	13	13,4	15	15,5	NS

Note: PHCOPD – the COPD group with pulmonary hypertension; COPD – the COPD group without pulmonary hypertension; abs. – absolute values; p – probability rate; NS – insignificant.

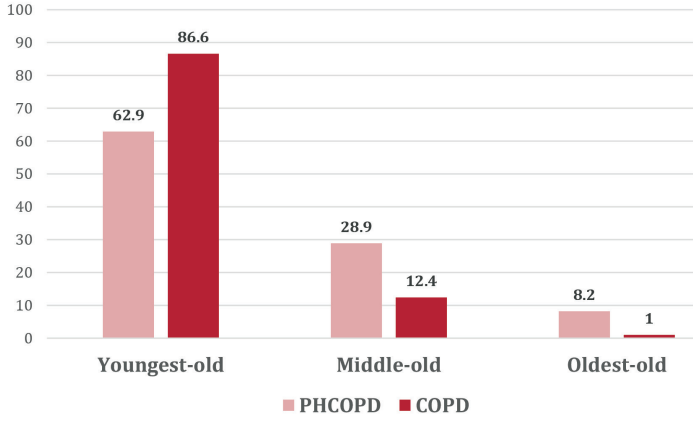

Fig. 1 Distribution of patients in the study groups according to age (%).
PHCOPD – the COPD group with pulmonary hypertension; COPD – the COPD group without pulmonary hypertension.

The evaluation of the patients autonomy according to the functional estate revealed that patients from the PHCOPD group had statistically more frequent pulmonary and cardiovascular causes of dependence and the IIIA degree of dependence (92.8% vs 73.2%, respectively; p < 0.001), but patients from the COPD group had somatic causes of dependence and the IIIB degree of dependence (24.7% vs 3.1%, respectively; p < 0.001).

Clinical picture

Cough and productive cough were comparable in both the PHCOPD and COPD patient groups. A small amount of morning or evening expectoration was statistically significantly more frequent in the COPD patients group (76–78.4% vs. 5–5.2%, respectively; p < 0.001), but an average amount (48–49.5% vs. 21–21.6%, respectively; p < 0.001) and a high amount (44–45.4% vs. 0%, respectively; p < 0.001) of expectoration throughout the entire day were statistically significantly more frequent in the PHCOPD patients group.

All patients from both study groups complained of dyspnea. The COPD group of patients had expiratory type dyspnea (62–63.9% vs. 36–37.1%, respectively; p < 0.001), dyspnea on mild exertion (64–66.0% vs. 10–10.3%, respectively; p < 0.001), the accentuation of respiratory signs at mild exertion (68–70.1% vs. 13–13.4%, respectively; p < 0.001) and at increased physical effort (13–13.4% vs. 3.1%, respectively; p < 0.01).

The PHCOPD group of patients had mixed dyspnea (60–61.9% vs. 34–35.1%, respectively; p < 0.001), which accentuates on minimal exertion (85–87.6% vs. 33–34.0%, respectively; p < 0.001), wheezing (77–79.4% vs. 16–16.5%, respectively; p < 0.001), accentuation of respiratory signs even during speech (8–8.2% vs. 1–1.0%, respectively; p < 0.05) and minimal exertion (73–75.3% vs. 15–15.5%, respectively; p < 0.001), low-grade fever (28–28.9% vs. 2–2.1%, respectively; p < 0.001) and dysphonia (62–63.9% vs. 12–12.4%, respectively; p < 0.001).

Although both study groups experienced chest pain (89–91.8% in the COPD group vs. 95–97.9% in the PHCOPD group), the COPD group had a more frequent mild degree of chest pain (55–61.8% vs. 9–9.5%, respectively; p < 0.001), while the PHCOPD group reported more frequently moderate (68–71.6% vs. 34–38.2%, respectively; p < 0.001) and severe pain (18–18.9% vs 0–0%, respectively; p < 0.001).

The mean number of comorbidities was also similar in both study groups: 8.4±0.3 (from 3 to 15 comorbidities) in patients from the PHCOPD group and 8.26±0.2 (from 4 up to 14 comorbidities) in patients from the COPD group (p > 0.05).

The objective examination

At the objective examination, the patients of the PHCOPD group had statistically significantly more frequent central cyanosis (19–19.6% vs. 3–3.1%, respectively; p < 0.001), forced body position (73–75.3% vs. 3–3.1%, respectively; p < 0.001), peripheral edema (90–92.8% vs. 70–72.2%, respectively; p < 0.001), emphysematous chest (43–44.3% vs. 9–9.3%, respectively; p < 0.001), hyperresonant percussion sound (97–100.0% vs. 51–52.6%, respectively; p < 0.001), diffuse dry rales, accentuation of the second sound over pulmonary artery (95–97.9% vs. 64–66.0%, respectively; p < 0.001).

The PHCOPD group presented higher mean values of systolic blood pressure (138.57±1.2 mmHg vs. 128.61±1.0 mmHg, respectively; p < 0.001), diastolic blood pressure (84.99±0.6 mmHg vs. 81.93±0.7 mmHg, respectively; p < 0.001), heart rate (75.71±1.2 beats per minute vs. 69.98±1.0 beats per minute, respectively; p < 0.01), respiratory rate (20.91±0.2 cycles per minute vs. 19.62±0.09 cycles per minute, respectively; p < 0.001). On the other hand, the COPD group more frequently presented higher mean values of oxygen saturation (96.79±0.07% vs. 95.36±0.1%, respectively; p < 0.001).

At the complex geriatric assessment, the COPD group of patients showed a higher degree of independence and more clarity of thought. Those facts were revealed by the higher mean values of the Katz score (11.41±0.09 points vs. 10.64±0.1 points, respectively; p < 0.001), of the Lawton score (14.26±0.2 points and 12.62±0.2 points, respectively; p < 0.001), and of the MMSE score (26.43±0.2 points vs. 25.14±0.2 points, respectively; p < 0.001) in the COPD group. On the other hand, the PHCOPD group of patients showed statistically significantly higher mean values of the Hamilton anxiety score (9.90±0.2 points and 8.4±0.3 points, respectively; p < 0.001), of the Hamilton depression score (7.03±0.3 points and 5.98±0.3 points, respectively; p < 0.05), and mild cognitive impairment according to the MMSE test (Fig. 2).

According to the Katz score, independent patients were statistically significantly more common in the COPD group (57–58.8% vs. 30–30.9%, respectively; p < 0.001), but patients with moderate dependence (19–19.6% vs. 2–2.1%, respectively; p < 0.001) were statistically significantly more frequent in the PHCOPD group.

According to the Lawton score, independent patients were statistically significantly more frequent in the COPD group (45–46.4% vs. 13–13.4%, respectively; p < 0.001), but there were statistically significantly more patients with moderate dependence (55–56.7% vs. 24–24.7%, respectively; p < 0.001) in the PHCOPD group.

Although COPD exacerbations were found in all study groups, patients in the COPD group mentioned rare COPD exacerbations (≤ 1) in the previous year statistically significantly more frequently (58–59.8% vs. 13–13.4%, respectively; p < 0.001), and PHCOPD patients mentioned frequent COPD exacerbations (≥ 2) statistically significantly more frequently (84–86.6% vs. 39–40.2%, respectively; p < 0.001).

According to the COPD GOLD classification (2007/2017), the moderate COPD in the COPD group of patients was found statistically more frequently (81–83.5% vs. 11–11.3%, respectively; p < 0.001), but the patients from the PHCOPD group had severe COPD (79–81.4% vs. 16–16.5%, respectively; p < 0.001), very severe COPD (7–7.2% vs. 0–0%, respectively; p < 0.01) and COPD of type D (96–99.0% vs. 89–91.8%, respectively; p < 0.05).

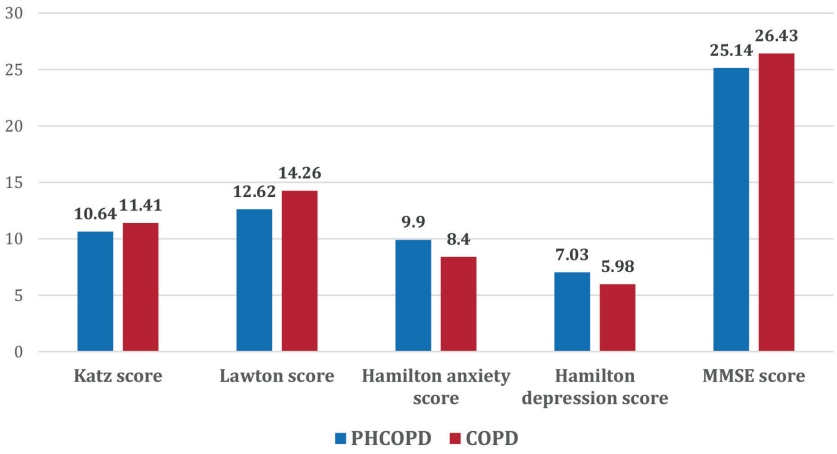

Fig. 2 Complex geriatric assessment of the patients from the study groups (points, mean values).
PHCOPD – the COPD group with pulmonary hypertension; COPD – the COPD group without pulmonary hypertension; MMSE – Mini Mental State Examination.

The multidimensional assessment of COPD found higher average values in the PHCOPD group of patients in comparison with the COPD group of patients. The impact of COPD on health and daily life activities is revealed by the higher value of the CAT scale in the PHCOPD group (29.81±0.5 points vs. 22.61±0.5 points, respectively; p < 0.001). The mean values of SGRQ questionnaire were higher in PHCOPD group, and namely total score (71.73±1.2 points vs. 52.73±1.0 points, respectively; p < 0.001), the SGRQ symptom score (74.63±1.3 points vs. 54.77±0.9 points, respectively; p < 0.001), the SGRQ activity score (75.40±1.1 points vs. 63.87±0.8 points, respectively; p < 0.001), and the SGRQ impact on daily activities score (68.69±1.5 points vs. 45.41±1.4 points, respectively; p < 0.001). The COPD influence on clinical picture and daily living activities was proved by CCQ score, that was higher in PHCOPD group: the CCQ total score (4.08±0.08 points vs. 2.97±0.07 points, respectively; p < 0.001), the CCQ symptom score (4.54±0.08 points vs. 3.17±0.07 points, respectively; p < 0.001), the CCQ functional status score (3.57±0.08 points vs. 2.43±0.08 points, respectively; p < 0.001), and the CCQ mental state score (4.16±0.1 points vs. 3.64±0.1 points, respectively; p < 0.01). The assessment of COPD dyspnea revealed higher mean values in the PHCOPD group according to the BODE index (5.04±0.1 points vs. 2.97±0.1 points, respectively; p < 0.001) and mMRC dyspnea scale (2.35±0.06 points vs. 1.27±0.06 points, respectively; p < 0.001) (Fig. 3).

In the COPD group of patients, the mean value of the Borg scale was statistically significantly higher compared to the PHCOPD group (3.02±0.05 points vs. 2.4±0.05 points, respectively; p < 0.001).

When comparing the mMRC scale, the second (59–60.8% and 28–28.9%, respectively; p < 0.001) and third degrees of dyspnea (33–34.0% and 2–2.1%, respectively; p < 0.001) were found statistically significantly more often in the PHCOPD group.

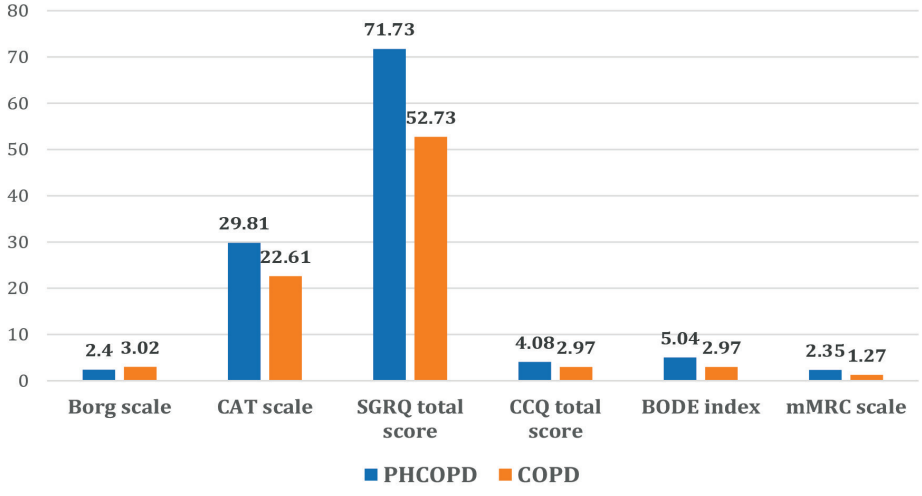

Fig. 3 Impact severity estimation of COPD according to the GOLD questionnaires in elderly patients from the study groups (points, average values).
PHCOPD – the COPD group with pulmonary hypertension; COPD – the COPD group without pulmonary hypertension; CAT – COPD assessment test; SGRQ – Saint George Respiratory Questionnaire; CCQ – Clinical COPD Questionnaire score; BODE – Index for COPD that incorporates body mass index, obstruction, dyspnea, exercise capacity; mMRC – modified Medical Research Council Dyspnea Scale.

Although both study groups had similar mean values of the Charlson comorbidity index (CCI) (6.56±0.2 points in the PHCOPD group vs. 6.33±0.2 points in the COPD group), according to the degree classification, patients in the COPD group had a statistically more frequently moderate CCI score (3-4 points) (18–18.6% vs. 8–8.2%, respectively; p < 0.05), whereas patients in the PHCOPD group had a more frequently high CCI score (≥ 5 points) (89–91.8% vs. 79–81.4%, respectively; p < 0.05).

Pre-test patient assessment when performing the 6MWT revealed statistically significantly higher mean values in the PHCOPD group compared to the COPD group according to the following indices: systolic blood pressure (137.32±1.1 mmHg vs. 128.11±0.9 mmHg, respectively; p < 0.001), diastolic blood pressure (84.32±0.6 mmHg vs. 81.82±0.6 mmHg, respectively; p < 0.01), heart rate (75.13±1.2 beats per minute vs. 69.77±0.9 beats per minute, respectively; p < 0.001), respiratory rate (20.84±0.2 cycles per minute vs. 19.60±0.09 cycles per minute, respectively; p < 0.001). On the other hand, the mean values of O2 saturation (96.82±0.07% vs. 95.45±0.1%, respectively; p < 0.001), Borg scale (3.01±0.05 points vs. 2.40±0.05 points, respectively; p < 0.001), and walking distance (321.57±4.7 meters vs. 255.19±5.4 meters, respectively; p < 0.001) were higher in patients from the COPD group (Table 2).

The analysis of the post-test data reveals the same tendency of statistically average values, which are higher in the PHCOPD group compared to the COPD group. The mean value of O2 saturation (94.09±0.1% vs. 91.78±0.2%, respectively; p < 0.001) was statistically significantly higher in patients from the COPD group.

Table 2. Parameters before and after the 6-minute walk test at patients from the study groups.

Parameters	PHCOPD group pre-test	COPD group pre-test	p	PHCOPD group post-test	COPD group post-test	p
SBP (mmHg)	137.32±1.1	128.11±0.9	< 0.001	151.09±1.2	139.07±0.8	< 0.001
DBP (mmHg)	84.32±0.6	81.82±0.6	< 0.01	92.86±0.6	90.05±0.4	< 0.001
HR (beats per minute)	75.13±1.2	69.77±0.9	< 0.001	89.43±1.6	81.55±1.1	< 0.01
Respiratory rate (cycles per minute)	20.84±0.2	19.60±0.09	< 0.001	28.13±0.8	26.76±0.8	< 0.001
SaO2 (%)	95.45±0.1	96.82±0.07	< 0.001	91.78±0.2	94.09±0.1	< 0.001
Borg scale (points)	2.40±0.05	3.02±0.05	< 0.001	2.58±0.2	2.45±0.07	< 0.05
Walked distance (m)	255.19±5.4	321.57±4.7	< 0.001			

Note: *SBP – systolic blood pressure; DBP – diastolic blood pressure; HR – heart rate; SaO2 – blood saturation with oxygen; PHCOPD – the COPD group with pulmonary hypertension; COPD – the COPD group without pulmonary hypertension; p – probability rate.*

As per the results of the 6MWT test according to the distance walked, the PHCOPD group had lower exercise tolerance (75–77.3% vs. 19–19.6%, respectively; p < 0.001), while moderate exercise tolerance (75–77.3% vs. 21–21.6%, respectively; p < 0.001) was statistically significantly more frequent in the COPD group.

Chronic respiratory failure of different degrees was detected in all patients included in the study. The first degree of respiratory failure was more frequent in patients from the COPD group (60–61.9% vs. 6–6.2%, respectively; p < 0.001), while the second degree of chronic respiratory failure (86–88.7% vs. 37–38.1%, respectively; p < 0.001) and the third degree of chronic respiratory failure (5–5.2% vs. 0–0%, respectively; p < 0.05) were detected statistically more often in the PHCOPD group.

Functional respiratory examination through spirometry revealed statistically lower mean values of the main respiratory indices in patients from the PHCOPD group compared to the COPD group: FEV1 (42.81±0.8% vs. 53.80±0.6%, respectively; p<0.001), FVC (59.31±1.2% vs. 65.05±0.9%, respectively; p<0.001), FEV1/FVC (56.17±0.8% vs. 63.53±0.5%, respectively; p < 0.001), and FEF (32.44±1.1% vs. 46.33±1.0%, respectively; p < 0.001) (Table 3).

Table 3. Spirometry parameters (%) before and after the bronchodilation test at patients from the study groups

Parameters	PHCOPD group pre-test	COPD group pre-test	p	PHCOPD group post-test	COPD group post-test	p
FEV1	42.81±0.8	53.80±0.6	< 0.001	45.64±0.8	55.90±0.6	< 0.001
FVC	59.31±1.2	65.05±0.9	< 0.001	62.03±1.3	67.02±0.9	< 0.01
FEV1/FVC	56.17±0.8	63.53±0.5	< 0.001	57.58±0.8	64.03±0.5	< 0.001
FEF	32.44±1.1	46.33±1.0	< 0.001	35.54±1.0	46.94±1.0	< 0.001

Note: FEV1 – forced expiratory volume in the first second; FVC – forced vital capacity; FEF – forced expiratory flow; PHCOPD – the COPD group with pulmonary hypertension; COPD – the COPD group without pulmonary hypertension; p – probability rate.

Spirometry parameters after the bronchodilator test had the same tendency, with lower mean values in the PHCOPD group, namely FEV1 (45.64±0.8% vs. 55.90±0.6%, respectively; p < 0.001), FVC (62.03±1.3% vs. 67.02±0.9%, respectively; p < 0.01), FEV1/FVC (57.58±0.8% vs. 64.03±0.5%, respectively; p < 0.001), and FEF (35.54 ±1.0% vs. 46.94±1.0%, respectively; p < 0.001).

Laboratory and instrumental examination (electrocardiographic, radiological, echocardiographic, and ultrasonographic examination of internal organs) revealed several parameters occurring statistically significantly more often in the PHCOPD group compared to the COPD group.

The radiological examination found that in the PHCOPD group, there were more frequent susceptible signs for

chronic bronchitis (96–99.0% and 81–83.5%, respectively; $p < 0.001$), susceptible signs for bronchial obstruction (50–51.5% and 9–9.3%, respectively; $p < 0.001$), and susceptible signs for pulmonary emphysema (34–35.1% and 6–6.2%, respectively; $p < 0.001$).

Electrocardiography revealed that the patients in the PHCOPD group presented more often with signs of right atrial hypertrophy, such as pulmonary P waves in II, III, aVF, and V1-V2 leads (33–34.0% and 2–2.1%, respectively; $p < 0.001$) and signs of right ventricle hypertrophy. The signs of right ventricle hypertrophy found more frequently in the PHCOPD group were right axis deviation (76–78.4% vs. 7–7.2%, respectively; $p < 0.001$), increase in wave amplitude in leads III, aVF, and V1-V2 (75–77.3% vs. 1–1.0%, respectively; $p < 0.001$) and ST segment depression in leads II, III, aVF, and V1-V2 (40–41.2% vs. 24–24.7%, respectively; $p < 0.05$).

Echocardiography revealed that the patients in the PHCOPD group presented more often with increased right ventricle wall thickness over 5 mm (93–95.9% vs. 4–4.1%, respectively; $p < 0.001$), right ventricle dilatation over 26 mm (94–96.9% vs. 3–3.1%, respectively; $p < 0.001$), and a higher mean pulmonary artery pressure (42.44±1.4 mmHg vs. 19.26±0.2 mmHg, respectively; $p < 0.001$).

The ultrasonography of internal organs found that dilation of the suprahepatic veins greater than 10 mm (58–59.8% vs. 0–0%, respectively; $p < 0.001$), dilation of the inferior vena cava greater than 20 mm (57–58.8% vs. 0–0%, respectively; $p < 0.001$), and venous congestion of the liver (56–57.7% vs. 2–2.1%, respectively; $p < 0.001$) occurred more frequently in the PHCOPD group.

Polycythemia was found to be more common in the PHCOPD group (20–20.6% vs. 8–8.2%, respectively; $p < 0.05$), as well as C-reactive protein (7.78±0.9 mg/dL vs. 5.77±0.8 mg/dL, respectively; $p < 0.001$), fibrinogen (4.23±0.4 mg/dL vs. 3.47±0.09 mg/dL, respectively; $p < 0.05$), and mean values of NT-proBNP (1807.16±91.5 pg/mL vs. 138.65±8.3 pg/mL, respectively; $p < 0.001$).

The microscopic examination of sputum in the PHCOPD group revealed increased number of epithelial cells (17.41±0.7 vs. 13.99±0.7 in the visual field, respectively; $p < 0.01$), macrophages (7.24±0.5 vs. 5.65±0.3 in the visual field, respectively; $p < 0.01$), and leukocytes (22.16±1.1 vs. 18.69±1.0 in the visual field, respectively; $p < 0.01$).

Discussions

Therefore, the complex geriatric assessment in the PHCOPD group, compared to the COPD group, revealed statistically significantly more frequent and severe complaints. The cough was more severe, and expectoration was moderate to severe throughout the day. Dyspnea was of mixed type and occurred with minimal physical exertion. The clinical picture in the PHCOPD group was completed by wheezing, accentuation of respiratory signs during speech, minimal physical exertion, low fever, moderate chest pain, and severe chest pain. PH in elderly COPD patients has a negative impact on the clinical picture and an unfavorable impact on daily activities.

The objective examination determined a statistically significant negative impact on the objective health status of elderly PHCOPD patients: lower mean values of SaO2 and statistically significantly higher mean values of blood pressure, cardiac contraction frequency, and respiratory frequency. Patients with PHCOPD had significantly more frequent signs of respiratory system impairment caused by disease severity, hemodynamic alterations, and advanced age, such as peripheral edema, central cyanosis, forced body position, emphysematous ribcage, participation of the rib cage in the act of breathing, widening of the intercostal spaces during inspiration, active participation of additional respiratory muscles in the process of breathing, hypersonic percussive sound, decrease in pulmonary respiratory excursion, diffuse dry rales, prolonged exhalation, and accentuation of the second sound over the pulmonary artery.

The complex geriatric evaluation outlined the repercussions of PH and COPD on quality of life and independence. The mean values of the Katz, Lawton, and Hamilton anxiety scores, the Hamilton depression score, and the MMSE score indicate that the COPD group has a statistically higher prevalence of mild dependence or independence, the absence of anxiety and depression, and the absence of cognitive disorders. Meanwhile, patients in the PHCOPD group had statistically higher rates of moderate dependence, mild cognitive impairment, moderate anxiety, and moderate depression, confirming the impact of the complex COPD on their daily lives.

Clinical functional examination of elderly COPD patients found a moderate degree of COPD (types B and C were statistically significantly more frequent in patients from the COPD group). The severe and very severe degrees of type D COPD are statistically significantly more frequent in patients from the PHCOPD group, thus presenting the correlation between the severity of COPD and hemodynamic disorders in elderly COPD patients.

In the PHCOPD group, the multimodal approach to COPD revealed statistically significantly higher mean values of the CAT scale, SGRQ score, CCQ score, BODE index, and mMRC dyspnea scale; this suggests the impact of COPD on the health status, quality of life, and outcome. The statistically significantly higher mean value of the Borg scale in the COPD group of patients suggests that a physical exertion reserve is available.

The COPD group of patients had statistically significantly more frequent values of 0 and the first dyspnea degree in the evaluation of patients according to the mMRC dyspnea scale, but the PHCOPD group of patients had statistically significantly more frequent second and third degrees of dyspnea.

The 6MWT test revealed that low exercise tolerance was statistically more common in the PHCOPD group and moderate exercise tolerance was statistically more common in the COPD patient group.

Although chronic respiratory failure was diagnosed in all patients in the study groups, the first degree of chronic respiratory failure was statistically significantly more com-

mon in the COPD group, but the second and third degrees of chronic respiratory failure were found to occur statistically significantly more often in the PHCOPD group of patients.

At the same time, the PHCOPD patients had more frequent electrocardiographic changes characteristic of right heart damage with right ventricular hypertrophy and right atrial hypertrophy that occur because of PH and the elderly body's reduced capacity to compensate for them.

Furthermore, the PHCOPD group of patients had statistically more frequent echocardiographic changes characteristic of right ventricular affection as a result of the PH secondary to the COPD.

Furthermore, the PHCOPD group had a statistically higher rate of inflammatory response syndrome in blood tests, as well as polycythemia, as a result of bronchial obstruction and its association with pulmonary hypertension. The presence of pulmonary hypertension and right atrial hypertrophy correlated with the increase in the value of natriuretic peptides (NT-proBNP), arguing the necessity of natriuretic peptides evaluation in geriatric patients as an informative and safe diagnostic method. Also, the level of natriuretic peptides correlated with the presence of ultrasonographic signs of right heart failure, such as dilation of the suprahepatic veins more than 10 mm, dilation of the inferior vena cava more than 20 mm, and venous congestion of the liver, a fact that once again recommends the application of the NT-proBNP marker in the diagnostic procedure of elderly patients with COPD complicated by PH.

Conclusions

1. The comparative complex geriatric evaluation of patients with COPD reveals the presence of the characteristic symptoms in all the patients, but they are much more severe in those from the PHCOPD group, with a more significant impact on the quality of life and a greater deterioration of the assessed paraclinical indices.
2. Arrhythmias, right ventricular hypertrophy, right atrial hypertrophy, and even right heart failure are more common in elderly patients in the PHCOPD group, which has a negative impact on quality of life, self-care capacity, physical effort capacity, and clinical and paraclinical indices.
3. Pulmonary hypertension has a significant impact on the clinical aspects of elderly patients with COPD, a fact reflected in the scores of the complex geriatric assessment, the multidimensional assessment scales of COPD, and the 6-minute walk test. Also, the impact can be confirmed by the data of the paraclinical laboratory examination, in which the inflammatory response syndrome and the increase in natriuretic peptides correspond to the data of the ultrasound examination of the heart and internal organs.
4. The significant negative impact of COPD on elderly patients argues for the need to assess each elderly patient's functional estate at the time of admission for an opportune diagnosis of COPD.
5. The important impact of PH secondary to COPD requires the mandatory implementation of new techniques of PH diagnosis, like natriuretic peptides.

Abbreviations

COPD – chronic obstructive pulmonary disease; PHCOPD – the group of patients with chronic obstructive pulmonary disease and pulmonary hypertension; COPD group – the group of patients with chronic obstructive pulmonary disease without pulmonary hypertension; GOLD – Global Initiative for Chronic Obstructive Lung Disease; CAT – COPD assessment test; SGRQ – the St George's Respiratory Questionnaire; CCQ – the clinical questionnaire for COPD; mMRC – Modified Medical Research Council Dyspnea Scale; BODE index – index of body mass, obstruction, dyspnea and exertion; NT-proBNP – B-type natriuretic peptide; 6MWT – the 6-minute walking test; PAP – pulmonary artery pressure; MMSE – mini mental examination test; FEV1 – forced expiratory volume in the first second; FVC – forced vital capacity; FEF – forced expiratory flow; PH – pulmonary hypertension.

Declaration of conflict of interest

Nothing to declare.

Authors' contributions

Both authors contributed equally to the manuscript's writing and have read and approved the final version of the manuscript.

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RESEARCH ARTICLE

The role of digital tomosynthesis in laryngeal cancer: comparison with radiography and computed tomography

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ABSTRACT

Introduction. Digital tomosynthesis is a relatively new imaging modality that is already used in the diagnosis of breast cancer and has shown promising results in evaluating patients with pulmonary, osteoarticular, and other pathologies. However, up to date, there are no published studies related to the usefulness of digital tomosynthesis in the evaluation of patients with laryngeal cancer. The purpose of this study was to evaluate the clinical role of digital tomosynthesis in the diagnosis of laryngeal cancer and compare the imaging results with those obtained by digital radiography and computed tomography.

Material and methods. The study was carried out between 2015 and 2019 at the Institute of Oncology in the Republic of Moldova and included 253 consecutive patients with laryngeal cancer referred to the Institute of Oncology during this period. All patients underwent digital radiography and digital tomosynthesis investigations. In 41 patients who provided written informed consent, computed tomography was performed. The results of all imaging investigations were compared.

Results. The statistical analysis revealed a high degree of agreement and a strong linear correlation between the data obtained with digital tomosynthesis and computed tomography, as well as concordance correlation coefficients for different parameters between 0.63 and 1.0 (mean value = 0.82±0.11). For comparison, the concordance correlation coefficients for the same parameters obtained for digital radiography versus computed tomography ranged between 0.08 and 0.93 (mean value = 0.43±0.25). An updated imaging algorithm that includes digital tomosynthesis has also been proposed for investigating patients with suspected laryngeal cancer.

Conclusions. The study demonstrated the usefulness of digital tomosynthesis for the evaluation of patients with laryngeal cancer. When compared to computed tomography, which is considered the gold standard, digital tomosynthesis revealed a much higher performance compared to digital radiography. Considering the availability of low-dose protocols for digital tomosynthesis, the modality might also be helpful for laryngeal cancer screening in a high-risk population. However, new studies are also required to confirm our findings and define the place of digital tomosynthesis in the imaging algorithm for patients with laryngeal cancer.

Keywords: laryngeal cancer, diagnosis, tomosynthesis, radiography, computed tomography.

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Key messages

What is not yet known on the issue addressed in the submitted manuscript

Up to date, there are no published studies related to the use of digital tomosynthesis for the evaluation of patients with laryngeal cancer.

The research hypothesis

Digital tomosynthesis is an emerging imaging modality with a much lower radiation dose compared to computed tomography

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and can provide valuable information for initial diagnosis as well as follow-up evaluation in patients with laryngeal cancer. It can also replace computed tomography in certain clinical situations.

The novelty added by the manuscript to the already published scientific literature

To our knowledge, this is the first study related to the use of digital tomosynthesis for the evaluation of patients with laryngeal cancer. The study showed the clinical role of digital tomosynthesis for diagnosing and staging laryngeal cancer. It has also demonstrated a high level of agreement and a strong linear correlation between the data obtained by digital tomosynthesis and computed tomography, which is considered the gold standard for imaging these patients.

Introduction

Laryngeal cancer represents about 3% of all malignancies and is associated with significant diagnostic challenges related to initial diagnosis, staging, and guiding treatment strategies [1-3].

In the Republic of Moldova, the incidence of laryngeal cancer has increased during the last few years, with the official records being as follows: 2.4% (109 cases) in 2000, 2.5% (138 cases) in 2010, 2.8% (143 cases) in 2016, and 2.9% (157 cases) in 2019. Furthermore, late referral cases predominate, with around 80–85% of patients being diagnosed in advanced stages of the disease (stages III-IV) [3, 4]. The 5-year survival rates in patients treated in the Republic of Moldova in this period varied from 83–92% in early disease stages (stages I–II) to about 35% in patients diagnosed with advanced disease (stages III-IV) [3, 4]. This underscores the importance of early diagnosis and optimal imaging investigations in these patients [3-6].

Radiographic imaging is associated with the lowest radiation dose and is commonly used as a first-line modality, being readily available in outpatient settings and primary care institutions. Laryngoscopy provides additional details and allows tumor biopsy; however, it is less accurate in determining tumor extension, especially since over 80% of laryngeal cancers are associated with infiltrative growth [3, 7].

Cross-sectional imaging with computed tomography (CT) and magnetic resonance imaging (MRI) allows excellent depiction of the intricate anatomy of the larynx and the characteristic patterns of submucosal tumor extension [8]. Additionally, certain imaging-based parameters like tumor volume and cartilaginous abnormalities have been used to predict the success of primary radiotherapy or surgery in these patients [8]. Due to the short acquisition time and the possibility to perform functional maneuvers, CT is commonly considered the tool of choice [9], but is also associated with a higher radiation dose. MRI allows better soft tissue differentiation but is more susceptible to movement artifacts and is complicated by disease-specific symptoms [9]. Higher costs, imaging time, and availability represent additional issues.

Digital tomosynthesis (DT), a new tomographic technique that is associated with a much lower radiation dose, may offer an alternative to CT. It uses a conventional radiograph tube, a flat-panel detector, a computer-controlled tube mover, and reconstruction algorithms to produce section images [10]. Digital tomosynthesis is already widely used in the diagnosis of breast cancer and has shown promising results in evaluating patients with other pathologies [10-15].

At the time of conducting the study, there were no data in the literature regarding the use of digital tomosynthesis in the imaging diagnosis of laryngeal pathology. The purpose of this study was to evaluate the usefulness of digital tomosynthesis in the diagnosis of laryngeal cancer and to compare the imaging results with those obtained by digital radiography and computed tomography.

Material and Methods

The study was carried out between 2015 and 2019 at the Institute of Oncology in the Republic of Moldova and included 253 consecutive patients with laryngeal cancer who provided written informed consent. All patients underwent histological investigations for confirmation of their diagnosis. The staging of tumors was done according to the existing TNM classification developed by the American Joint Committee on Cancer (AJCC) and the Union for International Cancer Control (UICC).

A total of 250 (98.81%) patients were male, and only 3 (1.19%) were female. The age limits varied between 48 and 76 years, with the mean value being 62.35±2.70 years. When divided into age groups, 15 (5.93%) patients were between 41 and 50 years old, 77 (30.43%) patients were between 51 and 60 years old, 132 (52.17%) patients were between 61 and 70 years old, and 29 (11.46%) patients were between 71 and 80 years old. According to the TNM classification, at the time of diagnosis, 15 (5.93%) patients had stage I disease, 68 (26.88%) patients had stage II disease, 136 (53.75%) patients had stage III disease, and 34 (13.44%) patients had stage IV disease.

All patients underwent digital radiography and digital tomosynthesis investigations. In 41 patients who provided separate informed consent for this purpose, computed tomography was performed. The results of all imaging investigations were

subsequently compared. A correlation was also made with intraoperative and histopathology findings, as well as with the final diagnosis indicated in the patient's chart on discharge.

The standard statistics kits were used for data analysis through Microsoft Excel, MedCalc (version 20.106), and IBM SPSS Statistics (version 26.0). The results obtained by the 3 imaging modalities were evaluated using comparison tests, linear regression, and agreement analysis.

The research was approved by the Research Ethics Committee of the *Nicolae Testemitanu* State University of Medicine and Pharmacy (No. 22 of November 7, 2016). All patients provided informed written consent.

Results

The imaging findings revealed by digital radiography, computed tomography, and digital tomosynthesis in the group of patients who underwent all 3 investigations (n = 41) are presented in Table 1.

When compared to computed tomography, which is considered the gold standard, digital tomosynthesis proved to have a much higher performance compared to digital radiography. For example, the tumor mass was detected in 100% of patients by computed tomography, in 95.12% of patients by tomosynthesis, and in only 63.41% of patients by digital radiography. Similarly, digital tomosynthesis was superior to digital radiography in evaluating local tumor spread and invasion of adjacent anatomical structures (Table 1). Thus, unilateral ligament thickening was noted in 85.37% of cases by computed tomography, in 78.05% of cases by tomosynthesis, and in only 48.48% of patients by digital radiography. The findings were confirmed by the results of statistical analysis that included calculation of concordance correlation coefficients, linear regression, and agreement analysis of the obtained data by all 3 imaging modalities.

Table 1. Radiological findings in patients with laryngeal cancer revealed by digital radiography, computed tomography and digital tomosynthesis.

Radiological signs	Digital Radiography n (%)	Computed Tomography n (%)	Digital Tomosynthesis n (%)
Tumor mass detection	26 (63.41%)	41 (100%)	39 (95.12%)
Unilateral ligament thickening	20 (48.78%)	35 (85.37%)	32 (78.05%)
Bilateral ligament thickening	3 (7.32%)	6 (14.63%)	5 (12.20%)
Asymmetrical arytenoid thickening	1 (2.44%)	6 (14.63%)	3 (7.32%)
Morgagni's sinus flattening	17 (41.46%)	33 (80.49%)	30 (73.17%)
Incomplete closure of laryngeal ligaments	13 (31.71%)	35 (85.37%)	32 (78.05%)
Subligamentous space flattening	14 (34.15%)	25 (60.98%)	21 (51.22%)
Enlarged prechondral space	8 (19.51%)	9 (21.95%)	9 (21.95%)
Cartilage invasion	8 (19.51%)	12 (29.27%)	13 (31.71%)
Unilateral piriform sinus invasion	13 (31.71%)	27 (65.85%)	22 (53.65%)
Bilateral piriform sinus invasion	2 (4.88%)	6 (14.63%)	5 (12.20%)

The concordance correlation coefficients for various radiological findings revealed by digital tomosynthesis and by digital radiography compared to the standard method (computed tomography) are provided in Table 2 and Table 3, respectively. As described in the literature, the concordance correlation coefficient reflects the degree of agreement between two methods or assessments and can take values between 0 and 1, being a non-directional coefficient. Values close to 0 indicate no agreement, while values close to 1 show perfect agreement [16]. Furthermore, the concordance correlation coefficient (ρ_c) contains a measurement of precision (ρ) and accuracy (Cb), i.e., $\rho_c = \rho C_b$, where ρ is the Pearson correlation coefficient, which measures how far each observation deviates from the best-fit line and is a *measure of precision,* and C_b is a bias correction factor that measures how far the best-fit line deviates from the 45° line through the origin and is a *measure of accuracy* [16]. The values of these coefficients obtained in our study are also provided in tables 2 and 3.

The statistical analysis of imaging parameters obtained by digital tomosynthesis and computed tomography (which is considered the gold standard) revealed a concordance coefficient between 0.63 and 1.0 for various parameters, with a mean value of 0.82±0.11 (Table 2). The Pearson correlation coefficient ranged from 0.68 to 1.0 for various parameters with a mean value of 0.83±0.10, while the C_b correction factor reflecting accuracy ranged from 0.93 to 1.0 for various parameters with an average value of 0.98±0.02 (Table 2). These data reflect a high concordance between the results obtained by digital tomosynthesis and computed tomography.

Table 2. Evaluation of radiological findings revealed by digital tomosynthesis in patients with laryngeal cancer as compared to the gold standard (computed tomography).

Radiological signs	Concordance correlation coefficient	Pearson ρ (precision)	Bias correction factor C_b (accuracy)
Tumor mass detection	0.66	0.70	0.94
Unilateral ligament thickening	0.85	0.86	0.99
Bilateral ligament thickening	0.90	0.90	0.99
Asymmetrical arytenoid thickening	0.63	0.68	0.93
Morgagni's sinus flattening	0.80	0.81	0.98
Incomplete closure of laryngeal ligaments	0.76	0.78	0.97
Subligamentous space flattening	0.80	0.82	0.98
Enlarged prechondral space	1.00	1.00	1.00
Cartilage invasion	0.94	0.94	1.00
Unilateral piriform sinus invasion	0.75	0.77	0.97
Bilateral piriform sinus invasion	0.90	0.90	0.99
Mean±standard deviation	0.82±0.11	0.83±0.10	0.98±0.02

Table 3. Evaluation of radiological findings revealed by digital radiography in patients with laryngeal cancer as compared to the gold standard (computed tomography).

Radiological signs	Concordance correlation coefficient	Pearson ρ (precision)	Bias correction factor C_b (accuracy)
Tumor mass detection	0.08	0.21	0.40
Unilateral ligament thickening	0.33	0.44	0.74
Bilateral ligament thickening	0.63	0.68	0.93
Asymmetrical arytenoid thickening	0.25	0.38	0.67
Morgagni's sinus flattening	0.29	0.41	0.71
Incomplete closure of laryngeal ligaments	0.15	0.28	0.52
Subligamentous space flattening	0.50	0.58	0.87
Enlarged prechondral space	0.93	0.93	0.99
Cartilage invasion	0.74	0.77	0.97
Unilateral piriform sinus invasion	0.39	0.49	0.79
Bilateral piriform sinus invasion	0.46	0.55	0.84
Mean±standard deviation	0.43±0.25	0.52±0.21	0.77±0.19

On the contrary, statistical analysis of the imaging parameters obtained by digital radiography versus computed tomography revealed a concordance coefficient between 0.08 and 0.93 for various parameters, with a mean value of 0.43±0.25 (Table 3). The Pearson correlation coefficient varied between 0.21 and 0.93 for various parameters with an average value of 0.52±0.21, while the C_b correction factor reflecting accuracy varied between 0.40 and 0.99 for various parameters with a mean value of 0.77±0.19 (Table 3). Overall, the statistical analysis reflects a much weaker concordance of the results obtained by digital radiography compared to those obtained by digital tomosynthesis (Tables 2-3).

The data obtained by the three imaging modalities were also compared using the mountain plot analysis for agreement evaluation (Fig. 1). A mountain plot (or "folded empirical cumulative distribution plot") shows the distribution of the differences between two methods and is commonly used to evaluate the difference between a new method and a reference method [17]. If two assays are unbiased with respect to each other, the mountain will be centered over zero [17]. Long tails in the plot reflect large differences between the methods [17]. The graphs shown in Figure 1 display the differences between the data obtained by digital tomosynthesis (square dots) and digital radiography (round dots) in relation to a gold standard (computed tomography). It is worth noting that the curve representing the data obtained by digital tomosynthesis has a relatively narrow, symmetrical shape that is centered much closer to 0 and has small deviations in its values between -1 and 5. On the contrary, the curve representing the data obtained by digital radiography is much wider, prominently skewed from 0 to the right, with significant variations in its values between 1 and 22. The diagrams demonstrate a very high degree of agreement between the data obtained by digital tomosynthesis and computed tomography (the standard method) and a much lower degree of agreement with the data obtained by digital radiography.

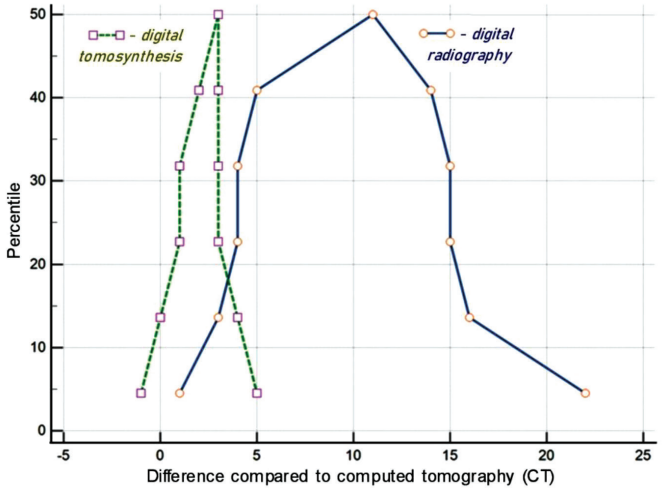

Fig. 1 Mountain plot diagram of the data obtained by digital tomosynthesis (square dots) and digital radiography (round dots) in comparison to computed tomography.
Of note is that the curve of digital tomosynthesis has a relatively narrow symmetrical shape centered much closer to 0 (variations between -1 and 5), while the curve of digital radiography is much wider and skewed to the right (variations between 1 and 22).

The results were also confirmed by the linear regression diagrams of the values obtained by digital tomosynthesis versus computerized tomography (Fig. 2A) and those obtained by digital radiography versus computerized tomography (Fig. 2B). As is well known, one of the most popular techniques for modeling the relationship between two pairs of numerical data, in this case obtained by different imaging modalities, is linear regression. In our analysis, the scatter plot of digital tomosynthesis versus computed tomography values (Fig. 1A) demonstrates a strong, positive relationship and much tighter clustering of data points compared to conventional radiography values (Fig. 1B), revealing also a stronger linear correlation of tomosynthesis data with a correlation coefficient $r = 0.993$ ($p < 0.0001$).

Discussions

Digital tomosynthesis has been recently introduced as an advanced clinical technique that removes overlying structures, enhances local tissue separation, and provides depth information about structures of interest by providing high-quality tomographic images [18]. One of its main advantages over standard computed tomography is the low radiation dose. For example, for imaging the chest region, previous studies have reported an effective dose for adults ranging between 0.12-0.21 mSv for thoracic tomosynthesis depending on selected parameters, patient constitution,

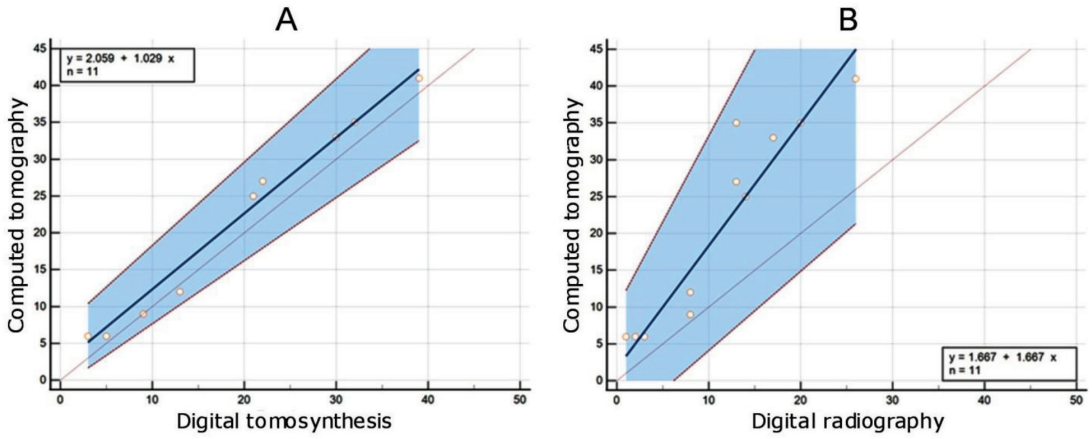

Fig. 2 Linear regression plots of the data obtained by digital tomosynthesis versus computed tomography (A) and by digital radiography versus computed tomography (B).

and the region of interest investigated [19, 20]. As comparative values reported within the same studies, the average radiation dose for chest radiography was 0.10 mSv, and for computed tomography of the chest region – 6.8 mSv [19]. Low-dose protocols with an effective dose of 98.87 +/- 0.08 microSv have also been created [21].

Although a relatively new imaging method, digital tomosynthesis is already widely used in the evaluation and screening of breast pathology, and there are an increasing number of reports related to its usefulness in the evaluation of other systems and pathologies [10-15, 18, 19, 21]. Due to its low radiation dose, digital tomosynthesis has also been used in pediatric patients as well as for screening purposes [20, 22-25].

In our study, we showed the usefulness of tomosynthesis for the evaluation of patients with laryngeal cancer. The statistical evaluation revealed a concordance coefficient with computed tomography between 0.63 and 1.0, as well as high agreement and a strong linear correlation of the obtained data on the mountain plot and linear regression analysis. To underline the practical value of digital tomosynthesis in patient care, a clinical case report from this study is also presented below.

A 57-year-old male presented to a primary care institution with voice changes and discomfort in his throat upon swallowing for two months. A computed tomography scan was performed and showed an invasive tumor mass arising from his left vocal cord with local extension to the anterior

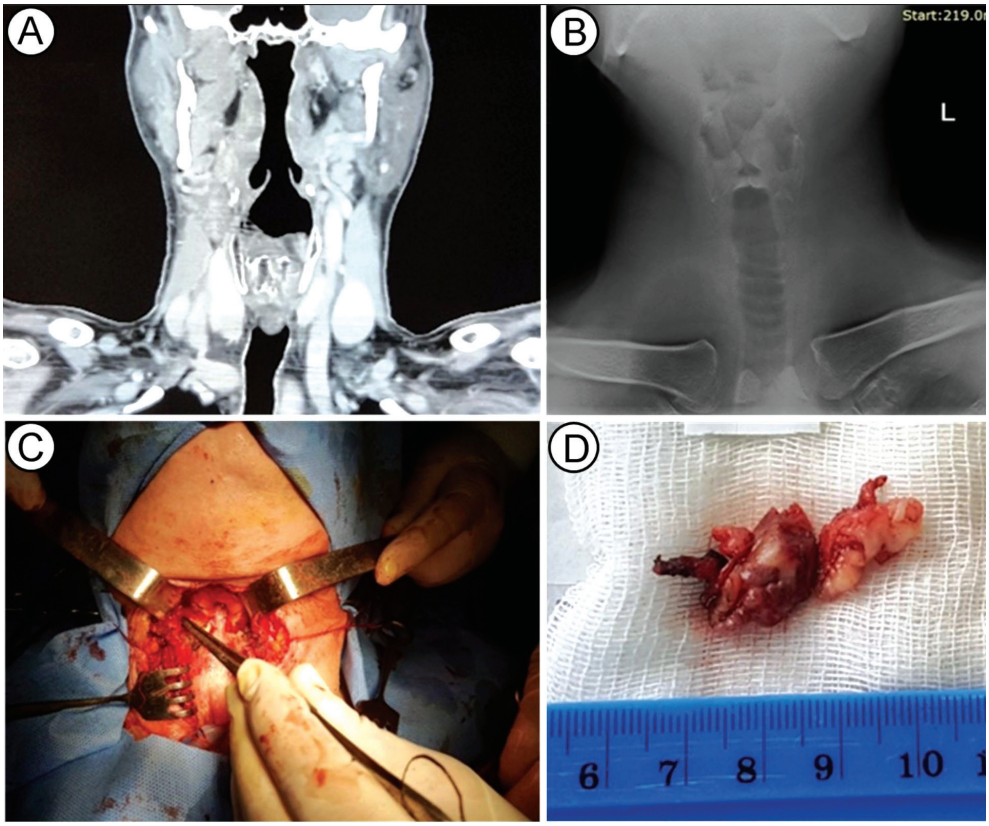

Fig. 3. A 57-year-old male with laryngeal cancer included in this study. (A) – CT showed a tumor mass arising from his left vocal cord with local extension to left supra-ligamentous space; (B) – digital tomosynthesis confirmed the findings and ruled out thyroid cartilage involvement; (C) – intraoperative picture; (D) – surgical sample following partial laryngectomy.

and posterior commissures of the larynx, the left supra-ligamentous space, as well as concern for potential involvement of his thyroid cartilage that required further investigation. A digital tomosynthesis performed at our institution ruled out tumor extension to the thyroid cartilage, and the patient underwent partial instead of total laryngectomy (Fig. 3). Histopathology results of the surgical sample showed negative margins for tumor infiltration.

The results of this study, as well as the acquired experience at the Institute of Oncology, allowed us to introduce digital tomosynthesis into the imaging algorithm for patients with laryngeal cancer (Fig. 4) [4]. Nevertheless, new studies are required to confirm our findings and to define the place of digital tomosynthesis in the imaging algorithm of patients with laryngeal cancer, especially since, to our knowledge; currently there are no published articles on this topic. Considering the availability of low-dose protocols for digital tomosynthesis, the modality might also be suitable for laryngeal cancer screening in high-risk populations; however, this also requires new studies on digital tomosynthesis.

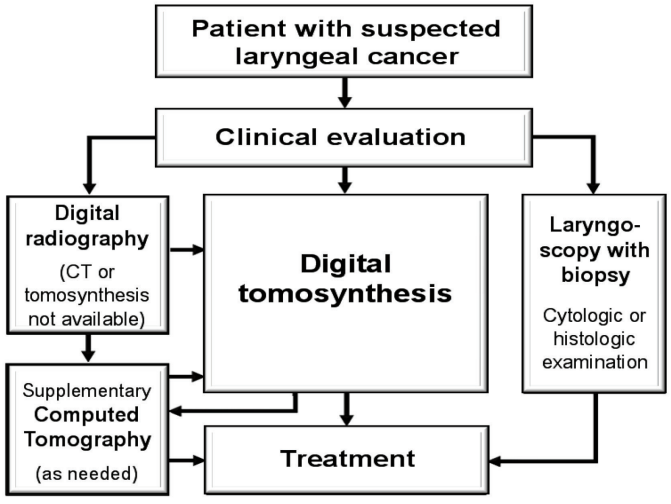

Fig. 4 Proposed diagnostic algorithm in patients with suspected laryngeal cancer.

Conclusions

1. The study demonstrated the usefulness of digital tomosynthesis for the evaluation of patients with laryngeal cancer. When compared to computed tomography, which is considered the gold standard, digital tomosynthesis revealed a much higher performance compared to digital radiography.
2. The statistical analysis revealed a high degree of agreement and a strong linear correlation between the data obtained with digital tomosynthesis and computed tomography, as well as concordance correlation coefficients for different parameters ranging between 0.63 and 1.0. The results show that digital tomosynthesis can replace computed tomography in certain clinical situations, significantly lowering the cost and the radiation dose for patients with laryngeal cancer who frequently require repeated imaging investigations.
3. The results of this study allowed us to introduce digital tomosynthesis into the imaging algorithm for patients with laryngeal cancer. However, new studies are also required to confirm our findings and define the place of digital tomosynthesis in the imaging algorithm for patients with laryngeal cancer.
4. Given the availability of low-dose protocols for digital tomosynthesis, the modality may be useful for laryngeal cancer screening in high-risk populations; however, additional research is needed to assess its suitability for this purpose.

Declaration of conflict of interest

Nothing to declare.

Authors' contribution

Study conception and design: DJP, IC, IG, MH. Data acquisition: DJP, IG, MH. Analysis and interpretation of data: DJP, IC, IG, MH. Drafting of the manuscript: DJP, IC, IG, MH. Significant manuscript review with significant intellectual involvement: DJP, IC, IG, MH. The final version has been read and approved by all authors.

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RESEARCH ARTICLE

Awareness of the culture of patient safety among medical staff in neurosurgery departments from Moldova

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ABSTRACT

Introduction. To improve outcomes for patients and prevent avoidable surgical errors, neurosurgeons must change the culture of patient safety. The purpose of the study was to explore the perception of Patient Safety Culture (PSC) and the factors influencing it among the staff in the neurosurgical departments from Republic of Moldova.

Material and methods. A cross sectional study was conducted in neurosurgical departments using the Hospital Survey on Patient Safety Culture (HSOPSC). Descriptive statistics were carried out, comprised the Cronbach "α" coefficient, frequency of positive answers (PPRs), level of minimum and maximum of 95% confidential interval, F. Galton correlation coefficient, Kendall rank coefficient, Harrington scale.

Results. Medical staff from neurosurgical departments from five hospitals voluntarily participated in the study n=345. Most of the respondents rated the patient's safety grade as "excellent" and "very good". The value of the frequency of positive responses to the dimensions of the survey varies between 37.3% (CI 95% [34.8-39.9]) (staffing) and 85.0% (CI.95% [83.1-86.9]) (teamwork within units). The dimensions with the highest score of the PPRs stand out: „teamwork within units", „organizational learning- continuous improvement" and „supervisor/manager expectations and actions promoting patient safety". Analyzing the effect of the influence of patient safety culture factors on the degree of patient safety appreciated by the staff, we notice that the dimensions with the greatest influence are "Feedback and Communication About Error", "Teamwork Across Units", "Management Support for Patient Safety", "Handoffs and transitions", "Communication openness". We found significant correlations among patient safety culture composites with the degree of patient safety with differences in the strength of the correlation.

Conclusions. The results reflected the positive attitude of the staff towards most composites of the patient safety culture. The study made it possible to highlight the strong and vulnerable points of the patient safety culture and the factors influencing the patient safety degree in neurosurgical departments from Moldova.

Keywords: patient safety culture, neurosurgery, patient safety.

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Key messages

What is not yet known on the issue addressed in the submitted manuscript It is a lack of research in the field of patient safety culture in neurosurgery and the factors that influence patient safety in neurosurgical departments from Republic of Moldova.

The research hypothesis

Patient safety culture as important factor that influence the patient safety and patient treatment outcomes during the hospital care.

The novelty added by manuscript to the already published scientific literature. For the first time in the Republic of Moldova, the perception of patient safety culture in neurosurgery departments was studied, using an international instrument. The study outlined the strongest and most vulnerable aspects of the patient safety culture that influence the patient safety degree.

Introduction

The Fifty-fifth World Health Assembly in May 2002 adopted resolution WHA55.18. This resolution recognized "the need to promote patient safety as a fundamental principle of all health systems and urged to pay the closest possible attention to the problem of patient safety and to establish and strengthen science-based systems, necessary for improving patients' safety and the quality of health care, including the monitoring of drugs, medical equipment, and technology. It was requested to support the efforts of Member States to promote a culture of safety within health care organizations and to develop mechanisms, for example through accreditation or other means, in accordance with national conditions and requirements, to recognize the characteristics of health care providers that offer a benchmark for excellence in patient safety internationally" [1].

The 72nd World Health Assembly (WHA) May 2019 recognized Patient Safety as a "global health priority". Global Patient Safety Action Plan 2021–2030 highlighted the strategic objectives: policies for zero patient harm, high-reliability systems, safety of clinical processes, patient and family engagement, health worker education, skills and safety, information, research and risk management, synergy, partnership and solidarity [2].

„Patient safety is a framework of organized activities that creates cultures, processes, procedures, behaviors, technologies and environments in health care that consistently and sustainably lower risks, reduce the occurrence of avoidable harm, make errors less likely and reduce impact of harm when it does occur. Patient safety is a strategic priority for modern health care and developing a culture of safety is cardinal to any sustainable efforts towards patient safety improvement" [3].

According to AHRQ "patient safety culture is the extent to which an organization's culture supports and promotes patient safety. It refers to the values, beliefs, and norms that are shared by healthcare practitioners and other staff throughout the organization that influence their actions and behaviors. Patient safety culture can be measured by determining the values, beliefs, norms, and behaviors related to patient safety that are rewarded, supported, expected, and accepted in an organization" [4].

„Changing our culture to advance patient safety" served as the theme of the 81st Annual Meeting of the American Association of Neurological Surgeons. "The neurosurgeon of the future has to embrace the ideals of individualism and innovation while never giving up the art of medicine, prioritizing the doctor-patient relationship, and changing our culture to practice the science of medicine within systems that help us to understand and prevent errors from occurring" [5]. Leaders should be educated in the importance of safety culture, and they need tools to help create this culture [6].

The HSOPSC is one of the most common tools being used to assess the culture of safety in hospitals. Studies that utilize this tool usually report the 12 composite scores and the scores on the patient safety grade and the number of events reported [7]. The areas of patient safety culture assessed by the AHRQ SOPS surveys include "Communication About Error, Communication Openness, Organizational Learning - Continuous Improvement, Overall Rating on Patient Safety, Response to Error, Staffing, Supervisor and Management Support for Patient Safety, Teamwork, Work Pressure and Pace" [4].

As of September 2022, there are 56 known translations for the AHRQ Surveys on Patient Safety Culture™ (SOPS®) [8] and 107 known countries where the AHRQ Surveys on Patient Safety Culture™ (SOPS®) have been administered [11]. The European Network for Patient Safety (EUNetPas) has been an important promoter of the „Culture of Patient Safety" and of this tool in Europe [10].

The original US Hospital Survey on Patient Safety Culture (HSOPS), designed by the American Agency for Healthcare Research and Quality (AHRQ) in 2004 was translated in Romanian and the psychometric properties was studied. The study found that Psychometric properties of the Romanian version of the HSOPS was acceptable for nine composites with 31 items [11]. Later a cross-sectional study was conducted in Moldovan healthcare settings, using the Romanian translation of the US Hospital Survey on Patient Safety Culture HSOPSC [12].

Assessing the status of safety culture in healthcare organizations and identifying the dimensions of safety culture that are the most important predictors of patient safety, are the first steps to improving that culture and enhancing patient safety [13]. Exploring the association between the patient safety composite scores and the hospital and respondent characteristics with the patient safety culture outcomes are not common in the literature [7].

Currently, the Republic of Moldova does not have in use any tool to assess patient safety culture in hospital settings [12]. Therefore, the topicality of the problem is related to the lack of research in the field of patient safety culture in neurosurgery and the necessity for the identification of patient safety culture factors that influence patient safety in neurosurgical departments from Republic of Moldova.

The purpose of the study was to explore the perception of organizational factors of patient safety culture among the staff in neurosurgical departments from Republic of Moldova and to determine their influence on the degree of patient safety rated by the staff.

Material and methods

A cross sectional study was conducted in neurosurgical departments from Moldova using the Hospital Survey on Patient Safety Culture (HSOPSC) Romanian version, developed by the US Agency for Healthcare Research and Quality, created by Sorra et al. [14]. The research project was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy, Republic of Moldova on 19.06.2018.

The paper form survey was distributed to 400 members of medical staff from January till September 2019 in neurosurgical departments from five hospitals from Republic of

Moldova. The questionnaire was anonymously completed. Overall, 345 completed questioners were returned, which constituted the 86% response rate. Completed surveys were collected and digitized using MS Excel and IBM SPSS Statistics 26. The survey contains forty-two questions and two output indicators: one question asks the respondents to appreciate the patient safety grade and another question asks about the number of events reported during the last 12 months. The survey questions used Likert scale of 5-point response options of degree of agreement: 1 point mean „strongly disagree", 5 points – „strongly agree", and the frequency 1 point mean „never", and 5 points mean „always". For negatively worded items, percentage of positive responses is the percentage of respondents who answered, „Strongly disagree" or „Disagree," or „Never" or „Rarely", because a negative answer on a negatively worded item indicates a positive response [14]. We recoded negatively worded items to calculate an item percent positive score. We averaged the percent of positive scores for each item included in the composite measure, to calculate score on a particular safety culture composite measure as described the AHRQ guide [14]. Descriptive statistics were carried out, comprised the Cronbach „α" coefficient, frequency of positive answers PPRs, variance, standard error, level of minimum and maximum of 95% confidential interval.

For us, it was interesting to study the organizational factors of the patient safety culture, which is why the nine dimensions of the HSOPSC survey that characterize this aspect of the patient safety culture were analyzed. We also studied the meaning and the relationship of dependence between the factors of the patient safety culture and the degree of patient safety appreciated by the medical staff, using the F. Galton correlation coefficient for this purpose. It gave us indications on the meaning and intensity of dependence between phenomena, without being able to specify, quantitatively, how much a phenomenon increases or decreases when the one with which it correlates increases or decreases by a certain amount [15].

In the study, the relationship between two types of variables was measured: independent variables- 32 questions of the questionnaire and the dependent variable which was the question: „Rate the degree of patient safety in your department from 1 to 10". The main task was to find out which of the 32 independent variables affect the dependent variable - the degree of patient safety. Since the experts' answers were expressed in the ordinal scale, the Kendall rank coefficient was used for non-parametric data, developed by the English statistician Maurice Kendall in 1938, being more precise than ρ Spearman's [16].

Results

We explored the staff perception about patient safety culture in neurosurgical departments from five hospitals providing in-patient hospital care. 345 persons from medical staff voluntarily participated in the study. From 345 respondents there were: doctors - 36.0%, nurses - 49.8%, residents - 14.2%. All of respondents were in direct interaction or contact with patients. Most of respondents 173 (50.1%; CI 95% [44.6-55.1]) were worked in neurosurgery units and 172 (49.9%; CI 95% [44.9-55.4]) were worked in anesthesiology and intensive care units where neurosurgical patients received medical care. The distribution of respondents by intervals of years of work experience in the hospital showed that a third of them have a work experience in the hospital 1-5 years-109 people (31.6%; CI 95% [27.0-36.5]), and another third had 21 and more years of work experience - 122 people (35.4%; CI 95% [30.1-40.6]). The distribution of respondents by intervals of years of work experience in the unit showed that: 1-5 years -38.6 % respondents, 6-10 years-18.6% respondents, 11-15 years- 10.7% respondents, 16-20 years- 9% respondents, > 20 years - 23.2 % respondents. The results showed that a half of respondents worked 40-49 hours per week- 49.9%, 20-39 hours- 20.9 %, 60-79 hours- 26.1%, less than 20 hours- 0.9% and more than 80 hours- 2.3%.

The frequency of adverse events reported in the last 12 months by respondents-output indicator, reflects that the most part of staff did not report any adverse events during the last 12 months- 90.7% (CI 95% [87.5-93.6]).

Most of the employees rated the patient's safety grade as "excellent"- 39.1 % and "very good" 43.8%. Table 1 reflects the staff perception of patient safety grade.

Table 1. Patient Safety Grade -output indicator

Patient Safety Grade -points	Frequency of responses			Patient Safety Grade
	Number of respondents	%	95% CI	
9-10	135	39.1	33.9-44.1	Excellent
7-8	151	43.8	38.6-48.0	Very good
5-6	44	12.8	9.3-16.5	Acceptable
3-4	11	3.2	1.4-5.2	Poor
1-2	4	1.2	0.3-2.3	Failing

Note: *CI* - level of minimum and maximum of 95% confidential interval

It was interesting for us to find out how the respondents assessed the safety of the patient depending on the position occupied. The results are reflected in the Table 2

Table 2. The comparisons of patient safety grade between different professionals

Nr.	Respondents Position	Number of respondents	Patient safety grade		Level of patient safety grade
			Score	CI 95%	
1	Residents	49	8.2	7.9-8.5	Very good
2	Doctors	124	7.8	7.6-8.0	Very good
3	Nurses	172	7.7	7.5-7.9	Very good
	Overall	345	7.8	7.6-8.0	Very good

Note: *CI* -level of minimum and maximum of 95% confidential interval.

The results obtained showed that the highest rating for patient safety was given by resident doctors. They gave 8.2 points out of 10 for patient safety, which corresponds to a "very good" level. The score given by doctors was lower (7.8 points out of 10), and the lowest score for patient safety was given by nurses (7.7 out of 10 points), which corresponds

to the "very good" level. The differences in the appreciation given by these three categories of respondents determined a significant statistical difference depending on the position of the participants (χ^2=20.056; gl=8; p=0.010)

Nine composites derived from the Agency for Healthcare Research and Quality's (AHRQ) Hospital Survey on Patient Safety Culture (HSOPSC) were used to investigate organizational aspect of patient safety culture. Table 2 express the item and composite positive scores for the patient safety culture with 95% confidence intervals.

Table 3. Item and Composite Percent Positive Scores for the Patient safety culture with 95 % confidence intervals

Code	Composites and items	Absolut number	%	95% CI
D I Teamwork within units		*1173*	*85.0*	*83.1- 86.9*
A1	People support one another in this unit	301	87.2	83.8- 90.7
A3	When a lot of work needs to be done quickly, we work together as a team to get the work done	310	89.9	86.7- 92.8
A4	In this unit, people treat each other with respect	292	84.6	80.9- 88.1
A11	When one area in this unit gets really busy, others help out	270	78.3	73.6- 82.9
D II Supervisor/manager Expectations and Actions Promoting Patient Safety		*1117*	*80.9*	*78.9- 83.0*
B1	My supervisor/manager says a good word when he/she sees a job done according to established patient safety procedures	311	90.1	87.0- 93.0
B2	My supervisor/manager seriously considers staff suggestions for improving patient safety	285	82.6	78.8- 86.7
B3r	Whenever pressure builds up, my supervisor/manager wants us to work faster, even if it means taking shortcuts	220	63.8	58.8- 68.7
B4r	My supervisor/manager overlooks patient safety problems that happen over and over	301	87.2	83.8- 90.7
D III Organizational Learning-Continuous Improvement		*839*	*81.1*	*78.7- 83.4*
A6	We are actively doing things to improve patient safety	276	80.0	75.7- 84.3
A9	Mistakes have led to positive changes here	288	83.5	79.4- 87.2
A13	After we make changes to improve patient safety, we evaluate their effectiveness	275	79.7	75.7- 83.8
D IV Management Support for Patient Safety		*613*	*59.2*	*56.2- 62.2*
F1	Hospital management provides a work climate that promotes patient safety	228	66.1	60.9- 71.3
F8	The actions of hospital management show that patient safety is a top priority	228	66.1	60.9- 71.3
F9r	Hospital management seems interested in patient safety only after an adverse event happens	157	45.5	40.3- 50.8
D VI Feedback and Communication About Error		*792*	*76.5*	*73.9- 79.1*
C1	We are given feedback about changes put into place based on event reports	300	87.0	83.2- 90.1
C3	We are informed about errors that happen in this unit	220	63.8	58.6- 68.7
C5	In this unit, we discuss ways to prevent errors from happening again	272	78.8	74.2- 82.9
D VII Communication openness		*491*	*47.4*	*44.4- 50.5*
C2	Staff will freely speak up if they see something that may negatively affect patient care	225	65.2	60.3- 70.4
C4	Staff feel free to question the decisions or actions of those with more authority	144	41.7	36.5- 47.0
C6r	Staff are afraid to ask questions when something does not seem right	122	35.4	30.7- 40.3
D IX Teamwork Across Units		*719*	*52.1*	*49.5- 54.7*
F2r	Hospital units do not coordinate well with each other	122	35.4	30.4- 40.9
F4	There is good cooperation among hospital units that need to work together	212	61.4	56.2- 66.9
F6r	It is often unpleasant to work with staff from other hospital units	140	40.6	35.1- 46.1
F10	Hospital units work well together to provide the best care for patients	245	71.0	66.1- 75.9
D X Staffing		*515*	*37.3*	*34.8- 39.9*
A2	We have enough staff to handle the workload	135	39.1	33.9- 44.6
A5r	Staff in this unit work longer hours than is best for patient care	103	29.9	25.5- 35.1
A7r	We use more agency/temporary staff than is best for patient care	147	42.6	37.1- 47.8
A14r	We work in «crisis mode» trying to do too much, too quickly	130	37.7	32.5- 42.9
D XI Handoffs and transitions		*854*	*61.9*	*59.3- 64.4*
F3r	Things "fall between the cracks" when transferring patients from one unit to another	202	58.6	53.3- 63.2
F5r	Important patient care information is often lost during shift changes	238	69.0	63.5- 73.9
F7r	Problems often occur in the exchange of information across hospital units	192	55.7	50.4- 60.9
F11r	Shift changes are problematic for patients in this hospital	222	64.3	59.4- 69.3

Note: CI -level of minimum and maximum of 95% confidential interval;A1…F11 – the item number in the survey; DI…D XI-the composite number in the survey. An "r" associated to the item number indicates items that are negatively worded and reverse-scored when calculating percentage positive scores.

Prin urmare actualitatea problemei a fost asociată cu lipsa cercetării privind Cultura Siguranței Pacienților de profil Neurochirurgical în serviciul spitalicesc, riscurile înalte ale asistenței medicale la Pacienții cu maladii neurochirurgical

Table 4. Classification of the results of patient safety culture dimensions according to the Harrington scale

Grade	Frequency of positive responses %	Level of Harrington scale	Dimension	The total value of dimension %
I.	80- 100%	Very good (excellent)	Teamwork within units	85.0
			Organizational Learning-Continuous Improvement	81.1
			Supervisor/manager Expectations and Actions Promoting Patient Safety	80.9
II.	63- 79%	Good	Feedback and Communication About Error	76.5
III.	37- 62%	Satisfactory	Handoffs and transitions	61.9
			Management support for patient safety	59.2
			Teamwork Across Units	52.1
			Communication openness	47.4
			Staffing	37.3

Table 5. Kendall rank correlation coefficient with patient safety degree

Code	Composites and items	Keywords	The Correlation Coefficient with the degree of Patient Safety	„p" Predicted probability
D I Teamwork within units				
A1	People support one another in this unit	support	0.207	p < 0.05
A3	When a lot of work needs to be done quickly, we work together as a team to get the work done	team	0.241	p < 0.05
A4	In this unit, people treat each other with respect	respect	0.277	p < 0.01
A11	When one area in this unit gets really busy, others help out	help	0.309	p < 0.01
D II Supervisor/manager Expectations and Actions Promoting Patient Safety				
B1	My supervisor/manager says a good word when he/she sees a job done according to established patient safety procedures	appreciation	0.212	p < 0.05
B2	My supervisor/manager seriously considers staff suggestions for improving patient safety	suggestions	0.245	p < 0.05
B3r	Whenever pressure builds up, my supervisor/manager wants us to work faster, even if it means taking shortcuts	faster	0.188	p>0.05
B4r	My supervisor/manager overlooks patient safety problems that happen over and over	overlook	0.234	p < 0.05
D III Organizational Learning-Continuous Improvement				
A6	We are actively doing things to improve patient safety	activities	0.253	p < 0.05
A9	Mistakes have led to positive changes here	changes	0.267	p < 0.01
A13	After we make changes to improve patient safety, we evaluate their effectiveness	evaluation	0.264	p < 0.01
D IV Management Support for Patient Safety				
F1	Hospital management provides a work climate that promotes patient safety	climate	0.268	p < 0.01
F8	The actions of hospital management show that patient safety is a top priority	actions	0.278	p < 0.01
F9r	Hospital management seems interested in patient safety only after an adverse event happens	adversity	0.312	p < 0.01
D VI Feedback and Communication About Error				
C1	We are given feedback about changes put into place based on event reports	feedback	0.262	p < 0.01
C3	We are informed about errors that happen in this unit	errors	0.335	p < 0.001
C5	In this unit, we discuss ways to prevent errors from happening again	discussions	0.343	p < 0.001
D VII Communication openness				
C2	Staff will freely speak up if they see something that may negatively affect patient care	free	0.302	p < 0.01
C4	Staff feel free to question the decisions or actions of those with more authority	question	0.254	p < 0.01
C6r	Staff are afraid to ask questions when something does not seem right	afraid	0.257	p < 0.01
D IX Teamwork Across Units				
F2r	Hospital units do not coordinate well with each other	uncoordinated	0.250	p < 0.05
F4	There is good cooperation among hospital units that need to work together	cooperation	0.317	p < 0.01
F6r	It is often unpleasant to work with staff from other hospital units	discomfort	0.179	p>0.05

F10	Hospital units work well together to provide the best care for patients	coordination	0.309	p < 0.01
D X Staffing				
A2	We have enough staff to handle the workload	workload	0.174	p>0.05
A5r	Staff in this unit work longer hours than is best for patient care	overtime	0.175	p>0.05
A7r	We use more agency/temporary staff than is best for patient care	temporary	0.201	p < 0.05
A14r	We work in «crisis mode» trying to do too much, too quickly	pressure	0.214	p < 0.05
D XI Handoffs and transitions				
F3r	Things "fall between the cracks" when transferring patients from one unit to another	things loss	0.280	p < 0.01
F5r	Important patient care information is often lost during shift changes	data loss	0.244	p < 0.05
F7r	Problems often occur in the exchange of information across hospital units	information exchange	0.314	p < 0.01
F11r	Shift changes are problematic for patients in this hospital	shift change	0.288	p < 0.01

Note: p - predicted probability*;* A1…F11 – the item number in the survey; DI…D XI-the composite number in the survey. An "r" associated to the item number indicates items that are negatively worded and reverse-scored when calculating percentage positive scores.

The above results made it possible to determine the composite rating by the effect on the degree of patient safety.

Table 6. Rating of dimensions according to the Effect on the Degree of Patient Safety

Composite number	Composites	The Correlation Coefficient with the degree of Patient Safety	Composite Rating
VI	Feedback and Communication About Error	0.313	1
IX	Teamwork Across Units	0.292	2
IV	Management Support for Patient Safety	0.286	3
XI	Handoffs and transitions	0.282	4
VII	Communication openness	0.271	5
III	Organizational Learning-Continuous Improvement	0.261	6
I	Teamwork within units	0.259	7
II	Supervisor/manager Expectations and Actions Promoting Patient Safety	0.230	8
X	Staffing	0.208	9

Note: VI -, IX – the composite number in the survey.

Discussions

The results express the attitude of the staff from the neurosurgery departments towards the organizational factors of patient safety culture. The value of the frequency of positive responses to the composites of the survey varies between 37.3% (staffing) and 85.0% (teamwork within units).

The mean value of patient safety grade was 7.8 points (CI 95% [7.6-8.0]) from 10 that correspond to „very good" level of patient safety grade. 39.1% of respondents appreciated as „excellent" the degree of patient safety, 43.8%- „very good", 12.8%- „acceptable", 3.2%- „poor" and 1.2%- „failing". The results reflected the high appreciation of the patient's safety degree by the medical staff in neurosurgical departments.

Our study highlighted the advantages of the dimensions „teamwork within units", „organizational learning and continuous improvement" and „supervisor/manager expectations and actions promoting patient safety" in neurosurgical departments where these dimensions were rated with the highest score of the frequency of positive answers. The composites with a lower score of the frequency of positive answers were „handoffs and transitions", „management support for patient safety", „teamwork across units", „communication openness" and „staffing".

Wang et al. (2017) described similar results in his study carried out in surgical departments where the PPRs for „teamwork within units" and „organizational learning and continuous improvement" were ≥75%, which denoted strengths, and the PPRs for „staffing" and „non-punitive response to errors" were ≤50%, which denoted weaknesses in surgical units and other units [17].

Like the data published by AHRQ in 2022, the composite with the highest score of positive answers in our study was the „teamwork within units"- 85% of PPRs. According to AHRQ the Highest Scoring Composite Measures „Teamwork" where 82% of respondents „strongly agreed" or „agreed" that "staff work together as an effective team, help each other during busy times, and are respectful" [18]. Nwosu et al. (2022) described in their study on patient safety culture in operating room that the „teamwork within units" had the highest average percentage positive score and was the only area of demonstrable strength (composite score >75%), with a score of 79.6% [19].

The second high rated composite was „Organizational Learning-Continuous Improvement". 81.1% of respondents „strongly agreed" or „agreed" that work processes are regularly reviewed, changes are made to keep mistakes from happening again, and changes are evaluated. Higher staff perceptions of the domain were associated with their positive perceptions of patient safety in one study (El-Jardali et al., 2011) [7].

Another high rated dimension in our study was "Supervisor/manager Expectations and Actions Promoting Patient Safety" where 80.9% of medical staff "strongly agreed" or "agreed" that managers promote patient safety. Similar results were reported by AHRQ in 2022 where 80% of respondents "strongly agreed" or "agreed" that supervisors, managers, or clinical leaders consider staff suggestions for improving patient safety, do not encourage shortcuts, and address patient

safety concerns [18]. Particularly positive assessments were found for the categories "nonpunitive response to errors", „teamwork within units", „supervisor/manager expectations and actions promoting patient safety" in hospital emergency departments from Switzerland [20].

The dimension „Staffing" was rated with the lowest score of positive responses overall. According to AHRQ database for 2022 the LOWEST scoring composite measures „Staffing and Work Pace" where 51% of respondents „strongly agreed" or „agreed" that there are enough staff to handle the workload, staff work appropriate hours and do not feel rushed, and there is appropriate reliance on temporary, float, or PRN staff [18]. The process of hiring, positioning, and overseeing employees in an organization, that is, staffing, is a well-known and important challenge for attaining a favorable patient safety culture [21]. Reis et al., (2018) explained that the staff felt overloaded by the unsuitability of personnel to their work activities, which can prejudice the quality of care provided [22]. Another reason of low score in Moldova could be the insufficient staff of both doctors and nurses, which is why they work more intensively and more hours per week.

„Communication Openness" is another low rated composite of patient safety culture. 47.4 % of respondents „strongly agreed" or "agreed" that staff speak up if they see something unsafe and feel comfortable asking questions. Communication is an essential part of the practice of medicine. It is also essential for patient safety. Communication is frequently a cause of, and a resource to prevent, threats to patient safety. The main areas for attention are communication with patients, within healthcare teams and across the various interfaces that occur within healthcare [24]. Han et al., (2015) said about the steep authority gradient that traditionally exists in many operating room settings [24].

Analyzing the effect of the influence of patient safety culture factors on the degree of patient safety appreciated by the staff, we notice that the dimensions with the greatest influence are „Feedback and Communication About Error", „Teamwork Across Units", „Management Support for Patient Safety", „Handoffs and transitions", „Communication openness". We found significant correlations among patient safety culture composites with patient safety degree with differences in the strength of the correlation. Evidence of relationships between patient safety culture and patient outcomes was related at the hospital and nursing unit level according to DiCuccio (2015) [25].

El-Jardali et al., (2011) identified that "patient safety culture predictors such as event reporting, proper communication, patient safety leadership and management, hospital size, and accreditation status are associated with the patient safety culture outcomes" [7].

He also observed significant correlations of the same variables against the frequency of events reported and the overall perception of safety [7]. Moreover, higher scores on hospital handoffs and transitions increased the likelihood of having a better perception of safety among respondents and the likelihood of respondents to report a higher patient safety grade [7]. Ito et al., (2018) has shown that the safety culture subdimensions show the relationship between Patient Safety Grade and one of the outcomes. However, not all safety culture subdimensions show a relationship with Number of Events Reported [26].

Wang et al. (2017) shown that six dimensions („teamwork within units", „organizational learning and continuous improvement", „staffing", „non-punitive response to errors", supervisor/manager expectations and actions promoting patient safety", and „hospital management support for patient safety") affected „overall perceptions of safety" with statistical significance. All these six dimensions had a positive correlation with the dimension „overall perceptions of safety" [17].

Conclusions

The study reflects the positive attitude of the staff from the neurosurgery departments towards most dimensions of the patient safety culture. The study made it possible to highlight the strong and vulnerable points of the patient safety culture in neurosurgical departments from Moldova and to determine which of them have the highest influence on patient safety. There is a room for improvement in patient safety in neurosurgical departments and a key to the quality puzzle is the continual need to assess the ever-changing landscape of patient safety in neurosurgery, as well as to track the impact that quality improvement interventions are having [24]. "At the root of this is the need to change the neurosurgical culture: to practice medicine with patient safety as a priority within systems that help clinicians understand, identify, and prevent errors in a systematic fashion, with a focus on solutions rooted in systems-based approaches" [24]. With regard to creating a culture of safety, considering everything that has been written, "it all really boils down to three main themes: teamwork training, better communication among surgical teams, not just during timeouts and debriefing; and getting rid of steep authority gradients that prevent people from speaking up when something is just not quite right" [5]. According to Han SJ et al., "all stakeholders and clinicians must change their culture to be more transparent and increase the reporting of outcomes, including adverse events and complications" [24].

As Berger MS et al., concluded in the presidential address at AANS 2013 "to improve outcomes for patients and prevent avoidable surgical errors, neurosurgeons must change the culture that currently exists in the operating room so that safety concerns are of the utmost importance and that each member of the care team has a personal sense of accountability. Doing this will involve implementing and consistently applying systems-based strategies to ensure an adequate level of safeguards; improving communication with all members of the care team and dismantling authority gradients; and maintaining a well-trained and well-rested workforce" [5].

Patient safety policies should ideally support a „learning health system" approach to safety, in which measurement on the front lines of care creates evidence for improvement.

Policy makers must promote knowledge sharing, such as through the creation of a national clearinghouse or coordinating center to promote rapid knowledge exchange among health systems [27].

Health system have to expand the patient safety capacity and infrastructure to meet the demands of safety issues [27].

Declaration of conflict of interest

Nothing to declare.

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RESEARCH ARTICLE

Qualitative and quantitative determination of proteins in extracts of some medicinal plants

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ABSTRACT

Introduction. Global consumption of plant protein is increasing and high-fiber plants have health benefits through valuable phytochemicals. Plant proteins serve as an alternative to animal proteins to meet consumer demand on the one hand and reduce the risk of disease on the other.

Material and Methods. We performed qualitative and quantitative determination of proteins in extracts by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and total content of proteins by Bradford assay.

Results. Applied methods for the qualitative and quantitative determination of proteins in dry extracts of medicinal plants: *Agrimonia eupatoria* L., *Cichorium intybus* L., *Galium verum* L., and *Solidago virgaurea* L. confirm that proteins were detected in all dry extracts and were determined the total content of them.

Conclusion. For all extracts obtained from aerial parts of: *Agrimonia eupatoria*, *Cichorium intybus*, *Galium verum* and *Solidago virgaurea* we find a low protein concentration, which implies minimizing allergic reactions and intolerance to the extracts studied.

Keywords: qualitative, quantitative determination, proteins, *Agrimonia eupatoria*, *Cichorium intybus*, *Galium verum, Solidago virgaurea.*

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Key messages

What is not yet known on the issue addressed in the submitted manuscript

Qualitative and quantitative determination of proteins in some medicinal plants from the collection of the Scientific Practical Center in the Field of Medicinal Plants of *Nicolae Testemițanu* State University of Medicine and Pharmacy.

The research hypothesis

The low concentration of protein in extracts and vegetal products implies the absence of allergic reactions and intolerance to the given plants.

The novelty added by manuscript to the already published scientific literature

The analysis of proteins from medicinal plant extracts that can be used in the pharmaceutical and food industry to assess probability of eliciting allergic reactions.

Introduction

Global consumption of plant protein has increased by 15% since the early 1960s, and positive health benefits of plant foods are linked to dietary fiber, vitamins, minerals, and phytochemicals. The food industry is developing plant-derived proteins as an alternative to meat and animal-derived proteins to meet consumer demand, which is largely based on cultural, dietary and religious choices [1].

The pharmaceutical industry uses vegetal proteins to treat various diseases such as rheumatoid arthritis, coronary thrombosis, multiple sclerosis and chronic lymphocytic leukaemia. Thus, pharmacological actions, such as: the potential to reduce the risk of developing metabolic syndrome or to manage diabetes and prevent cancer, are studied and discussed, as well as the different grading systems currently used to determine the quality of proteins from plant sources [1, 2]. Plant-derived proteins have increased bioavailability and may be more concentrated than animal protein sources [2, 3].

Despite its benefits, proteins, especially plant proteins, are also known as major sources of allergens that trigger an allergenic response via immunoglobulin E (Ig E)-mediated allergies [4, 5]. These allergens may diffuse into the body from the upper respiratory tract or enter the body through intake of vast range of plant food or may cause external skin irritations. Allergens mainly present in pollen, spores, and other plant associated products are responsible for symptoms such as rhinoconjunctivitis, asthma, edema, urticarial, and anaphylaxis [6].

The prevalence of allergic diseases has increased, especially in the last 20 years in most countries, with manifestations through gastrointestinal pathologies, atopic eczema, and asthma [6], pollen being one of the main allergens [7]. Allergic reactions to legumes by inhalation have rarely been described [8], however, there are studies describing the occurrence of allergic reactions manifested by rhinoconjunctivitis and bronchial asthma [9]. The most widespread groups of plant allergens that are reported belong to the seed storage proteins, structural proteins, and pathogenesis related proteins, induced as a defense response system under stressful conditions such as infections, insects, injuries, exposure to harsh chemicals and atmospheric conditions [10, 11]. In this context, we aimed to determine the molecular weight and the total protein concentration in some dried extracts obtained from plants: Agrimony, Chicory, Lady's bedstraw, and European goldenrod.

Agrimony (*Agrimonia eupatoria* L., Rosaceae). Among the major phytochemical constituents of this species are polyphenols: phenolic acids: p-coumaric acid, various caffeoyl-quinic acids [12]; flavonoids: luteolin, apigenin, quercetin, and kaempferol derivatives, including C-glycosides such as vitexin and iso-vitexin, which are derived of apigenin; procyanidins: catechin, procyanidins and ellagitannins-mainly agrimoniin [13]. Agrimony has been used as a remedy for a wide range of diseases and symptoms: antioxidant, inflammatory diseases [14], ailments of the gastrointestinal tract, including diarrhea and stomatitis, in liver disorders [15]. It is claimed to have antibacterial activity [16], particularly on Gram-positive species and, rather inappropriately, on probiotic species. Neuroprotective, hepatoprotective [17], diuretic [18], anti-inflammatory, through cytokine modulation [19], and anti-nociceptive, wound healing and cytotoxic [20] effects were also reported for various extracts, fractions or individual compounds isolated from *A. eupatoria*. Plants of the g. Agrimony, mainly used as pharmaceutical raw material, perfectly fit into the current trends in technology that are searching for organic raw materials with high contents of bioactive compounds, such as dietary polyphenols and fiber [21, 22].

Chicory (*Cichorium intybus* L.; Asteraceae) is also a medicinal plant with a long tradition of use across various geographic regions. The aerial parts of Chicory contain cichoriin, arginine, choline, chicoric acid, bitter principles, and microelements: Fe, P, Ca. The entire plant contains latex, whose major constituent is inulin- type fructans. Besides inulin, there are also sugars, tannins, essential and fixed oils, pectin, and resins. The plant also serves as a source of vitamins: A, C, E, K, PP, flavonoids make up about 3%. The roots are rich in bitter triterpenic substances, fructose, tannins, and essential oils [23, 24]. Its aerial parts have been used as salads, its roots have been employed as a coffee substitute, and all its parts have been attributed a variety of potential health benefits: anti-inflammatory, hypolipidemic, gastroprotective, analgesic, antidiabetic, reproductive enhancing, wound healing, anticancer, antimicrobial, and anthelmintic and others [24, 25]. Chicory is found in the food industry: as a salad, for teas, food supplements, coffee supplements or as a source for inulin production [26]. Some compounds present in chicory, such as polyphenols, hydroxycinnamic acids, inulin, protein, can be considered as potential carriers of food functionality, the main biological activities of the species being associated with the presence of bioactive compounds [27].

Lady's bedstraw (*Galium verum* L., Rubiaceae), a species that blooms on the summer solstice and has a special role in the traditions and spirituality of our people, but in the Republic of Moldova they are not studied until the present [28]. Based on investigations and phytochemical studies carried out, in the aerial parts of *G. verum* have been identified phenolic compounds, flavonoids (rutin, quercetin, isoquercitrin, apigenin, myricetin, luteolin, kaempferol); hydroxycinnamic acids (gentisic, caffeic, chlorogenic, p-coumaric, ferulic, sinapic, caftaric, rubiforic [29, 30]. The entire plant contains iridoid glycosides: asperulozide, asperulozidic acid, diacetyl-asperulozide, 3,4-dihydro-3-methoxi-asperuloside, monotropeine, acetyl-dafiloside and scandoside [31]. Anthracene derivatives, essential oils, tannins, saponosides and coumarins were also identified in smaller quantities [30]. The literature indicates that total polyphenols depend on both the nature and concentration of the solvent and the extraction technique applied, with large differences ranging from (2.44-5.16) mg/g in the dried plant product of *G. verum,* [32], up to 75.3 mg/g in the methanolic extract [33]. Regarding the application of different extraction tech-

niques: ultrasonic, maceration, reflux, it was demonstrated that the highest amount of phenolic compounds was obtained by applying the ultrasonic extraction method [28]. Previous pharmacological studies have shown that species of g. *Galium* possess antioxidant, diuretic, spasmolytic, cytotoxic, antimicrobial, endocrine and protective effects [30, 34].

European goldenrod (*Solidago virgaurea* L., Asteraceae) is widely used in traditional medicine and among the most researched species from g. *Solidago* [35]. The aerial parts of European goldenrod have long been used for urinary tract conditions as a diuretic and a disinfectant remedy and as an anti-inflammatory agent [36]. According to the latest research in the field and besides the fact of the positive effect on the urinary system, herb and extracts also manifest antioxidant, analgesic, antibacterial, antifungal, antispasmodic, immunostimulant, antiadipogenic and antidiabetic activities [35, 37-39]. The chemical profile of the plant is a various one, being mostly represented by flavonoids (mainly derived from quercetin and kaempferol), terpenes (mostly from the essential oils), and saponosides (mainly virgaureasaponins and solidagosaponins [35, 40-44]. Due to overexploitation of the plant in its natural habitats, this taxon has become a rare species in Europe. Currently, European goldenrod is cultivated in some European countries thanks to scientific agronomic research. Therefore, the plant micropropagation method and other *in vitro* growth systems allow the multiplication of plant biomass for phytochemical and biological research [36].

Materials and methods

The vegetal products: *Agrimoniae herba*, *Cichorii herba, Galii herba, Solidaginis virgaureae herba* were harvested from the collection of the SPCFMP, according to the nature of the herbal products, throughout the flowering period. The vegetal products have been processed in agreement to recommendations for the purposes of chemical studies. The powdered drying herbal products was passed through a sieve with the dimensions of 0,5 mm. Extracts were obtained by repeatedly extracting the plant products sprayed with a mixture of ethanol: water (60%, w/w) for half an hour at each extraction stage, until the plant products were exhausted, with extraction applied by magnetic stirring. The extractive solutions thus obtained were concentrated at 40 °C, using a rotary evaporator- Laborota 4011 [22].

Protein extraction was carried out on the dry extract of four vegetal products: *Agrimoniae herba, Cichorii herba, Galii veri herba* and *Solidaginis virgaureae herba*. The proteins were extracted using four extraction buffers: Tris-Glycine (pH 8.3), PBS (Phosphate-buffered saline with pH 7.4), Citrate buffer (pH 4.5) and Carbonate-bicarbonate buffer (pH 9.6). Each extract was weighed twice at 150 mg and added 750 ml of extraction solution. Samples were shaken and placed in a thermomixer: 600 rpm at 45 ºC, to exclude protein denaturation, for 3 hours. Every 30 minutes, the samples were vortexed. The supernatant was collected in a 2 ml Eppendorf tube and refrigerated overnight.

Qualitative determination of proteins was performed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and total content of proteins were determined using Bradford assay [45].

SDS-PAGE allows separating proteins according to their molecular weight. At the beginning were prepared 2 sandwiches (a sandwich is made of an alumina ceramic plate, two spacers and one glass plate) and put them into gel caster. After, according to the pipetting scheme was prepared separation gel solution and filled it into the sandwiches with a Pasteur pipette (~ 6 cm high). Each of sandwiches were overlaid with 100 µl of water saturated 1-butanol and left for 30 minutes to polymerize. The stacking gel solution, prepared according to the pipetting scheme, was pipetted into both sandwiches and were introduced the combs. After another 30 min of polymerization, the sandwiches were separated with a knife and marked the samples loading wells on the glass plate. The samples were prepared by taking 30 µl of standard or the fractions to be analyzed and mixed with the same amount of denaturation mix and heated it for 5 min at no more than 60°C [46].

The next step was electrophoresis preparation. Each sandwich was attached to the electrophoresis unit with two clamps, the cooling tubes were connected, and the water tap was turned on. The lower chamber was filled with buffer solution (sandwiches should be submerged about 1 cm), the comb was removed and the upper chamber was filled with buffer solution (behind the sandwiches). Loaded 5 µl of the standard proteins BSA (67 kDa), human γ-globulin (50 kDa), conalbumin (78 kDa), ovalbumin (45 kDa) and the samples, placed the safety lid onto the electrophoresis unit and plugged to a power supply. At the beginning, the current was 40 mA and the run took about 1 h. After the time elapsed, the sandwiches were lifted and the stacking gel was separated from the glass plate and placed in a tray filled with staining solution. Staining was carried out at 40°C for 30 min. The next step was destaining the gels using two solutions [47].

The Bradford assay is a spectrophotometric method, which is used to measure the concentrations of proteins according to the absorbance of Coomassie Brilliant Blue G-250 dye at 595 nm. The following dilutions were prepared from the BSA (bovine serum albumin) standard solution at a concentration of 1mg/ml to make the calibration curve: 500, 250, 200, 200, 150, 100, 50, 20, 10 µg/ml. Samples were diluted 1:100, 1 ml of Bradford working solution was added and incubated for 5 minutes. Thereafter, absorbance was measured at 595 nm [45, 48] by performing 5 technological repeats.

Results

Extracts obtained from the aerial parts of *A. eupatoria*, *C. intybus*, *G. verum* and *S. virgaurea*, in a mixture of ethanol: water (60%, w/w) until the vegetal products were exhausted and concentrated at 40°C using a rotary evaporator, were analyzed proteins according to the Protein Protocols Handbook [49].

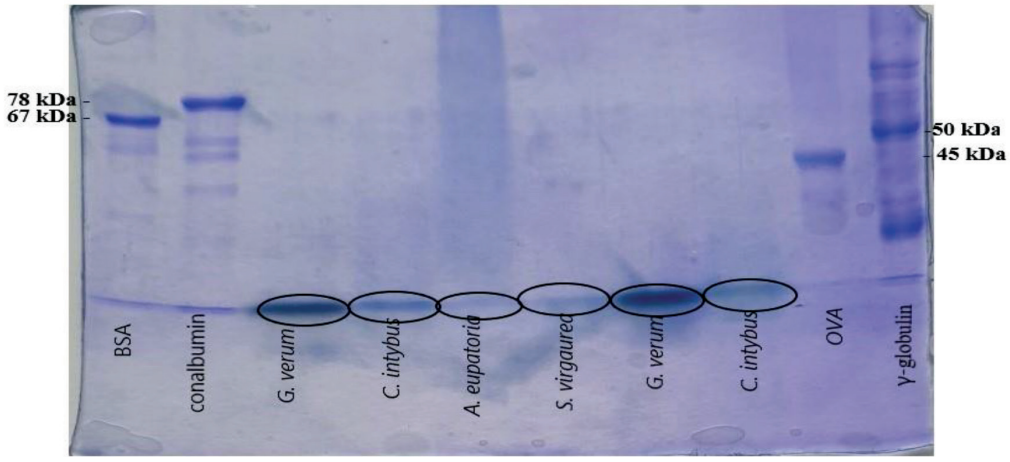


Fig. 1 Sodium dodecyl sulfate–polyacrylamide gel. *Standard proteins and samples were loaded in the following order (from left to right): bovine serum albumin, conalbumin, G. verum, C. intybus, A. eupatoria, S. virgaurea, G.verum. C. intybus, ovalbumin, γ-human globulin.*

Analyzing the obtained gel (*figure 1*), we confirm the presence of proteins in the extracts, but in all samples the proteins had a lower weight than used standards. The thickest bands belong to the extracts of *G. verum* and *C. intybus* and the thinnest to *A. eupatoria* and *S. virgaurea*.

Protein concentration was calculated through Bradford assay, from the calibration curve of concentration versus absorbance in µg/ml (*figure 2*).

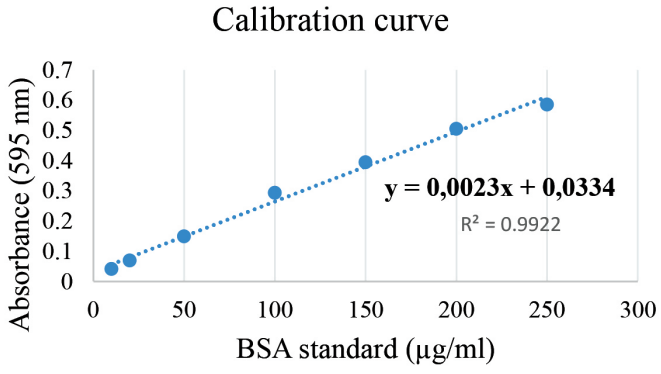


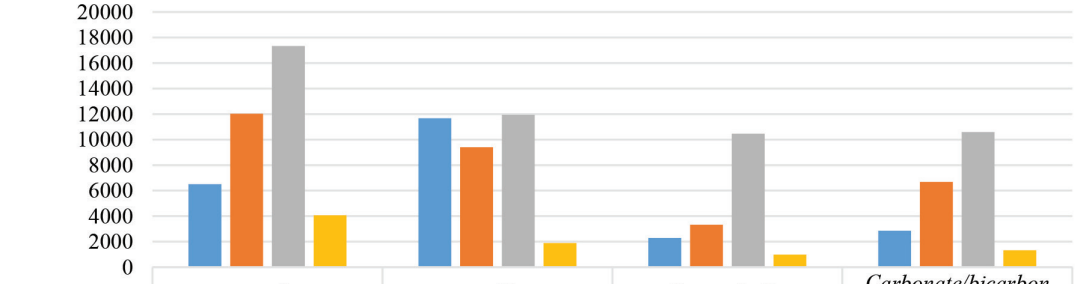
Fig. 2 Bradford assay standard curve of concentration versus absorbance

Protein extraction at different pH of buffer solutions showed that better protein extraction is achieved with neutral pH buffer, and with increasing buffer's pH in basic, the concentration decreases. Weaker protein extraction is observed with the acidic citrate buffer at pH 4.5.

As a result, a better protein extraction was obtained with Phosphate-buffered saline (PBS) extraction buffer (pH 7.4) which showed higher protein values in all extracts examined; followed by Tris-Glycin (pH 8.3), Carbonate-bicarbonate (pH 9.6) and Citrate (pH 4.5) extraction buffers. Analyzing the obtained results, we can conclude that a higher concentration of protein is found in the dry extract of *A. eupatoria*, followed by *C intybus*, *G. verum* and a lower concentration of protein we have in the dry extract of *S. virgaurea.*

Discussion

It is well known that the field of modern medicines has recently increasingly focused its interest on herbal medicines with minor side effects. The exploitation of natural resources used in particular in folk tradition is of particular interest because of their biological potential, and the pharmacotherapeutic value of plants is linked to their phytochemical components and secondary metabolites.



	PBS	Tris-Glycin	Citrate buffer	Carbonate/bicarbonate buffer
G. verum	6504.34	11668.18	2286.95	2852.17
C. intybus	12026.08	9395.45	3330.43	6678.26
A. eupatoria	17330.43	11940.9	10460.86	10591.3
S. virgaurea	4069.56	1895.45	982.6	1330.43

Fig. 3 Protein concentration obtained, with different buffer solutions, determined by Bradford assay (µg/ml)

The Bradford spectrophotometric test (absorbance 595 nm) used to measure protein concentrations as a function of absorbance of Coomassie Brilliant Blue G-25 dye show that, the richest in protein is the dry extract of Agrimony, with a protein maximum of 17330.43 μg/ml extracted with PBS buffer, followed by 11940.9 μg/ml with Tris-Glycin buffer, 10591.3 extracted by Carbonate-bicarbonate buffer and a concentration of 10460.86 with Citrate buffer.

The second plant, by protein concentration, is Chicory. The best protein extraction was obtained with PBS buffer 12 026.08 μg/ml, followed by Tris-Glycin buffer with 9395.45 μg/ml, 6678.26 μg/ml with Carbonate-bicarbonate buffer and last with Citrate buffer with a protein concentration of 3330.43 μg/ml.

The third plant, by protein concentration, is Lady's bedstraw. The dry extract of this plant showed a higher protein extraction with Tris-Glycin buffer, with a protein concentration of 11668.18 μg/ml, followed by 6504.34 μg/ml with PBS buffer, 2852.17 μg/ml with Carbonate-bicarbonate buffer and a lower protein value was received with Citrate buffer 2286.95 μg/ml.

A lower protein concentration had European goldenrod. All buffer solutions used, showed a low protein concentration. Extraction with PBS solution showed a higher concentration of protein 4069.56 μg/ml, followed by Tris-Glycin with 1895.45 μg/ml of proteins, 1330.43 μg/ml with Carbonate-bicarbonate buffer solution and a lower concentration was determining with Citrate buffer 982.6 μg/ml.

The low protein concentrations in dried plant extracts are due to the ethyl alcohol used as an extraction agent. Studies show that proteins have much lower solubility in polar solvents such as ethanol [50].

Conclusions

1. The development of the pharmaceutical and beneficial food industry branches for health and personalised treatment requires careful evaluation of phytocompounds in products to ensure the positive effect on human health and to minimise consequences and risks.
2. The results obtained show a low protein concentration, which suggests a potential lack of allergic reactions and intolerance to the given plant extracts for *A. eupatoria*, *C. intybus*, *G. verum* and *S. virgaurea*.

List of abbreviations used

BSA – bovine serum albumin; OVA – ovalbumin; PBS–Phosphate-buffered saline; SPCFMP – Scientific Practical Center in the Field of Medicinal Plants; SDS-PAGE – sodium dodecyl sulfate–polyacrylamide gel electrophoresis; SUMPh – State University of Medicine and Pharmacy

Declaration of conflict of interest

Nothing to declare

Authors' contributions

AO collected the data, performed the experimental part; MCM coordinated experimental activity and approved the manuscript; MCT drafted and revised the manuscript; TC interpreted the data and drafted the manuscript; NC revised the manuscript critically; CF collected the data and drafted the manuscript; CC analysis of data; AB interpreted the data; LU interpreted the data and drafted the manuscript. All authors revised and approved the final version of the manuscript.

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ORIGINAL ARTICLE

Morality, ethics, and professional deontology: non-traditional sources of medico-pharmaceutical law

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ABSTRACT

Introduction. Moral, ethical, and professional deontological rules have a substantial impact on the social relations of legal regulation regarding liability for abuses and violations of citizens' rights – people taking medication – patients as part of the health care system. The identification of the place and role of moral norms, ethics, and professional deontology, their quality as specific non-traditional primary sources in the development of the health care system, and the sub-branch of pharmaceutical law in the Republic of Moldova were the focus of the present study.

Material and methods. The secondary descriptive synthesis study of normative-legal acts as primary specific non-traditional sources, viewed through the lens of the protection of the rights of the consumer of medicines, the patient – the ultimate beneficiary of the social relations in the field of health care – spanned the years 1991 to 2021. The most relevant sources subject to analysis are the Constitution of the Republic of Moldova, the Code of Ethics for doctors and pharmacists in the Republic of Moldova, laws, and sub-legislative acts, etc. The study is based on the use of several recognized techniques and methods of analysis: systemic approach, synthesis, logical-legal deduction, content and comparative analysis, etc.

Results. Both the literature and recognized authors state that respect for moral, ethical, bioethical, and professional deontological norms in the field of health care has always been highly appreciated in society, which has led to the recognition of the nobility of medical and pharmaceutical activity. The results presented in this paper have made it possible to highlight aspects that recommend that the investigation of the role of legal regulation in medical and pharmaceutical activities also question the place and role of moral, ethical, and bioethical norms, as they generally have the same thematic orientation and influence on law, legislation, the practice of applying the law, and vice versa.

Conclusions. The accomplished study allowed the identification of the dialectical and organic unity between moral, ethical, bioethical, and deontological sources with the rules of law - the moral-legal foundation of medico-pharmaceutical law. The consolidation of legal and moral norms demonstrates the structuring of the sub-branch of medico-pharmaceutical law. The analysis of the normative-legal acts in the Republic of Moldova confirms both the functionality of the „moral-ethics-deontology-law" system and the importance given to the protection of the rights of the consumer of medicines, the patient, as the ultimate beneficiary of social relations in the field of health care.

Keywords: morality, ethics, bioethics, social relations, consumers of medicines, pharmaceutical activity, legal regulation of medical and pharmaceutical activities.

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Key messages

What is not yet known about the issue addressed in the submitted manuscript

Moral, ethical, and deontological rules as primary sources of legal regulation can substantially influence and change social relations regarding liability for misconduct and violations in health care and pharmaceuticals as integral parts of the health system. Currently, the system of „morality-ethics-deontology-law" (medical, pharma-

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ceutical, and medico-pharmaceutical) in terms of the protection of consumer rights – namely, the patient as the final beneficiary of social relations in the field of health care – remains a little-studied subject.

The research hypothesis

The Constitution of the Republic of Moldova, the Code of Ethics for doctors and pharmacists in the Republic of Moldova, the State Policy of the Republic of Moldova in the field of medicine, and the laws and normative acts issued all contribute to the consolidation of the role of moral, ethical, and deontological norms as primary sources in the development of pharmaceutical law in the Republic of Moldova.

The novelty added by the manuscript to the already published scientific literature

The evaluation of normative acts concerning the functionality of the system „morality-ethics-deontology-law" as specific non-traditional primary sources has revealed four stages that can be applied in the field of pharmaceutical law.

Introduction

Every human being, as a citizen of a certain state, lives and works in a certain legal area and in a certain ethical-moral comfort zone; therefore, the importance and role of legal and ethical regulations increase significantly. The state of his health and quality of life will also depend, in these cases, on how well coordinated, harmonious, effective, and promptly enforced moral, ethical, professional, and legal rules will work to defend the rights of citizens with this special status.

Health, medicine, and pharmacy are three interdependent components that can influence the quality of life of any individual. Medicine and pharmacy are also integrated parts of health care, their basic task being to ensure, maintain, and preserve human (and animal) health. Both simultaneously manifest, preserve, and guarantee freedom of expression, both in terms of professional ethics and deontology and in terms of the legal sciences. The quality of the medical and pharmaceutical acts provides a constructive and independent dialogue between all health professionals and the consumers of medicines, the patients, with a direct benefit for the latter. In this context, Professor Safta V. defines the ultimate goal of the pharmaceutical act as „ensuring the achievement of the ultimate goal of the health system through the active involvement of the pharmacist in providing effective, harmless, and accessible medicines and medical-pharmaceutical products for the treatment process of each individual and the entire society" [1]. Analyzing the sources of legal regulation of social relations concerning the protection of health and the protection of the rights of citizens with this special status, we note the positions of many authors who also emphasize the role of moral norms, ethics, and professional deontology, both in medicine and pharmacy [2-4]. And we propose that every time, when investigating abuses and violations in the field of health protection, the rights of the consumer of medicines, the patient, should also be approached through the prism of assessing the place and role of ethical norms, which generally have the same historical-legal origin, thematic orientation, and/or direct influence on the law and the practice of law enforcement.

The sources of law in the relations considered are a well-defined system of legal normative acts, which include, first and foremost, legal rules governing social relations regarding the protection of public health in general but also the protection of the health of individual citizens.

Material and methods

A secondary descriptive study of normative legal acts on the functionality of the „moral-ethics-deontology-law" system was carried out through the prism of the protection of the rights of the patient, the final beneficiary of social relations in the field of health care, as primary specific non-traditional sources.

The study was carried out from November 2016 to December 2021 and covered the period from 1991 to 2021. It involved data selection and systematization, a systematic approach to the literature and internet sources, and their analysis via Google search engine queries, Google Scholar using keywords appropriate to the topic, and manual evaluation of returned articles. The purpose was to identify the essence and functionality of the system „moral-ethics-deontology-law" in the protection of the rights of the consumer of medicines, the patient - the final beneficiary of social relations in the field of health care - and the description of the non-traditional sources of pharmaceutical law. The most relevant sources to analyze were the Constitution of the Republic of Moldova, the Code of Ethics for doctors and pharmacists in the Republic of Moldova, laws and sub-legislative acts, and various relevant bibliographic sources. The study is based on the use of several recognized techniques

and methods of analysis: systemic approach, analysis and synthesis, logical-legal deduction, comparative analysis, etc.

Results

What constitutes law in the health-care system is still an open question, and legal doctrine has yet to provide an answer. The conclusion that the protection of citizens' health is achieved by means of legal rules in various branches of law is nothing other than an acknowledgement of the current situation, which is binding on nothing and nobody. Many health-care laws are quite complex legal documents that include rules from civil, criminal, administrative, tax, and other areas of law.

Moral, ethical, and bioethical rules: the sources of medico-pharmaceutical law

If social relations in the field of health care are regulated by various branches of law and not by a single branch, there is a real risk of confusing the concepts of „law" and „legislation" applicable to this field. To reduce this risk, it is first necessary to determine the mutual interactions between morality, ethics, and law and, second, to determine the hierarchy by recognizing their importance in medical, pharmaceutical, and legal practice.

„Morality" is defined as „the attribute of what is moral; the nature, character, and value of an event or the conduct of a person or a community from a moral standpoint; behavior, conduct, and morals in accordance with moral principles; honesty, good conduct - from lat. moralitas, -atis, fr. moralité" by dexonline.ro. Similarly, morality also determines the spiritual qualities necessary for a person in society [5].

Ethics, according to the same dictionary, is „the theoretical study of human values and conduct in the light of moral principles and their role in social life; the totality of the corresponding rules of moral conduct; morality - from fr. éthique, lat. ethicus". Similarly, ethics is the science of morality, its meaning, principles, norms, and role in society, as well as the totality of norms of behavior, the morality of social groups, and professions.

Thus, medical and pharmaceutical ethics investigates and defines the value of the doctor and pharmacist's professional activities to society, as well as their personal characteristics. In this context, Professor Baciu Gh. mentions the position of the physician Albert Schweitzer (1875–1965), winner of the Nobel Peace Prize in 1952, who defined ethics as „respect for any life". By means of this universal respect, man encounters the world and is in harmony with its laws. Such a principle can lead to a profound and universal humanism, which must be the dominant element in the contemporary world [2].

In his book „Bioethics: Origins, Dilemmas, Trends," Professor Ţîrdea T. mentions that „the term ethics comes from the Greek word „ethos," which means „character," „conduct," and „custom." The Romans derived the adjective moralis (moralitas) from the word mos (mores), which means „habit" or „custom" in Latin. Therefore, these two words, „ethics" and „morality," etymologically coincide. Although originally the two nominalized terms, one Greek and the other Latin, had approximately the same meaning, their etymological evolution dissociated them, giving them different meanings [3].

In other words, if ethics is „character," „conduct," or „custom," then morality, according to the generally accepted conception, is how people understand the notion of right and/or wrong, „right" and „wrong" behavior. The norms and rules are formed based on these understandings, without which one cannot live in a society, community, etc.

The same dictionary, dexonline.ro [5], defines bioethics as the morality of science in general and medicine in particular, which prohibits the commercialization of the human body and organ trafficking (from fr. bioéthique, it. bioetica). Similarly, bioethics is a scientific discipline and a sphere of practical activity concerning the non-formal regulation of health care relationships. Bioethics is a much broader concept, its subject being the human attitude towards all living things. Bioethics „derives" from medical ethics, and through this „prism," it can be understood as part of professional medical and pharmaceutical ethics.

According to some scholars, which we also support, modern bioethics is defined by three representative levels: the theoretical level, the practical level, and the applied level. Theoretical bioethics represents the totality of knowledge about human attitudes towards all living things, expressed in the form of an axiological discourse. The institutionally formed regulation and value expertise of human attitudes toward all living things is known as „practical bioethics." These stipulations are duly formulated in the form of oaths, documents, and declarations, which, in essence, have no legal character. Applied bioethics is a description of specific cases and specific situations of human behavior in relation to all living things [3, 6].

Not only to promote a new science subfield in the Republic of Moldova - namely pharmaceutical law and medical law - but rather for the purpose of the present research, it is important to note that bioethics is a part of ethics (morality), being determined by some objective criteria such as profession, professional activity, and vocation. Bioethics, as a component part of ethics in the studied context, is intended to study and highlight the peculiarities of the moral component not only of the systems „patient-doctor", „patient-pharmacist", „doctor-doctor", „pharmacist-pharmacist", but also of the specific systems „drug consumer-pharmacist", „drug consumer-doctor-pharmacist" [7, 8]. The essential specific features observed in some cases recently require us to propose a new configuration of this system, namely: „consumer of medicines - doctor -pharmacist - lawyer."

Both medicine and pharmacy, from ancient times, have been most closely linked to the rules of morality. They began as public regulatory instruments that were very close to legal rules. At the same time, if law is a system of order in society endowed by the state with a constraining (coercive) force, then morality is a system of visions and reflections that possesses no such force. The systemic approach to contrasting law and morality enables a much deeper understanding of the content of these phenomena.

The organic link between morality and law–spheres of human life

Analyzing law and morality according to their scope, it can be seen that these notions are not similar: morality covers all sides of human behavior, while law covers only the most important social relations, those that require constraint measures by the state [9]. Consequently, the sphere of moral (ethical, bioethical) influence on an individual's behavior shows itself to be much wider than the sphere of legal regulation.

According to the influenced object, the concepts of law and morality in general are similar. Both law and morality involve the whole of society, all groups, and all categories of the population. On the other hand, as we know, different groups may simultaneously share different views of morality. The correlation of the legal norm with other categories of social norms is shown in Figure 1.

Legal and moral norms do not always, but frequently, coincide in content. Law is part of morality, but with the presence of a specific instrument - the force of coercion. At the same time, hypothetically, the adoption of unconstitutional rules cannot be ruled out. The practices of the Constitutional Court and the Parliament of the Republic of Moldova clearly prove this. Law and legal norms are only moral when they result from a social compromise; therefore, there may be contradictions between moral norms (ethics), which over time evolve either in favor of morality or in favor of law (legal norm), forming new values, provisions, regulations, and beliefs [10, 11].

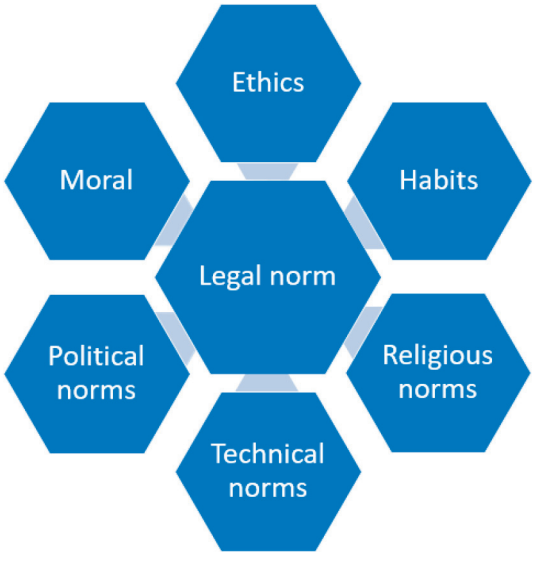

Fig.1 The correlation between the legal norm and other categories of social norms

Depending on the form of manifestation, moral and legal norms can be very different. Legal norms are promoted by means of decisions, acts of state power, administrative bodies, and courts. The special procedure of state registration is a feature of legal norms, while moral norms have no such characteristic. In terms of bioethical norms, it should be noted that some norms and regulations, particularly their common parts, do not require special registration, whereas others do. Various legislative acts can serve as examples, such as:

- The Code of Ethics for doctors and pharmacists, adopted by Government Decision No. 192 of 24.03.2017, based on the current legislation of the Republic of Moldova;
- Law no.261 of 01.11. 2013 about the College of Physicians of the Republic of Moldova, which in Article 4 provides that „in the field of training and professional development of physicians, the College of Physicians: 1) participates, through endorsement, in the development and approval of policies in the field of training and professional development of physicians, namely: a) submits to the Ministry of Health of the Republic of Moldova proposals on the amendment and completion of the Framework Code of Ethics (deontology) of the medical and pharmaceutical worker; 4) a) examines petitions and addresses of natural and legal persons exclusively on cases of deviations of physicians from the rules of professional ethics, medical deontology and the rules of good professional practice...", but does not specify who, which of the specialist associations participates in the drafting of the rules of medical ethics and the settlement of cases of their violation. We assume that it is a question of setting up specialized collegial bodies - the College of Physicians, the College of Pharmacists, the College of Dentists, etc. - that would, in our opinion, be the most competent bodies in drafting, promoting, and assessing compliance with the rules concerned.

Looking at how bioethical norms influence legal regulation in the health sphere, including pharmaceutical activity in general, not only in the Republic of Moldova but also in other countries, it can be seen that they go through a series of stages, the first being always the stage of regulating relations in the public sector. It includes both the drafting of legal rules and their adoption. Moral and ethical rules can be the subject of legal science studies in order to determine the interests of various communities and professional groups and to formulate legislative ideas and legal formulations on their basis. Bioethical rules, therefore, directly or indirectly, through a different degree of involvement, can influence the content of the actual law at any stage of the historical development of social relations in the system of „drug consumer-doctor" or „drug consumer-pharmacist." As an example, consider the National Health and Medicines Policies and Strategies In the Republic of Moldova, as in other countries, the National Health Policy 2007–2021 represented a set of priorities and development directions in the field of health, established for 15 years with the aim of strengthening the health of the population and reducing inequalities between different social groups and regions of the country [12]. The aim of the National Health Policy was „to create optimal conditions for the maximum realization of the health potential of each individual throughout life and to achieve adequate

standards of quality of life for the population." The general objectives of the National Health Policy are: (1) to increase life expectancy at birth and increase the duration of healthy life; (2) to ensure quality of life and reduce differences in terms of health for all social groups; (3) to strengthen intersectoral partnerships aimed at strengthening the health of the population; and (4) to make individuals responsible for their own health [13].

The State Policy of the Republic of Moldova in the field of medicine stated „the development of the health system in the Republic of Moldova requires health care for citizens on equal principles" [14]. Medicines are an important element in the prophylaxis, diagnosis, and treatment of diseases. The coordinated development of the pharmaceutical sector, especially in relation to its social importance, is one of the priority issues in health care. State policy in the field of medicines is an important component of the National Health Policy. The policy will serve as the foundation for the elaboration of programs for the development of the Republic of Moldova's pharmaceutical system (development, testing, authorization, manufacture, distribution, and rational use of medicines), as well as legislation governing medicines and pharmaceutical activity. We regret that this policy document has been ignored; furthermore, we believe that this guiding document for the development of legislation in the field of medicines and pharmaceutical activity has fallen into disuse.

In Romania, the principle on the basis of which the purpose of the National Health Strategies (2001) [15], as well as the purpose of the National Medicines Policy, was established, is that laid down in the Romanian Constitution [16]. According to this principle, the state is obliged to guarantee, through specific laws and regulations, the population's right to health services.

In other countries, the legislative bases on health protection state that: „the protection of citizens' health includes all political, economic, social, legal, cultural, scientific, medical, sanitary-hygienic and anti-epidemic measures aimed at protecting and strengthening the physical and mental health of each person, supporting the long life of active people, providing them with the necessary medical care in case of loss of health" [6].

Although there is direct interaction between medicine and law, medicine and bioethics in all of these acts, medicine remains both a component of its own system (health care) and a component of the larger system (law-medicine-bioethics).

One of the most eloquent examples of the legal assimilation of ethical norms is Article 9 of the Law concerning the Practice of the Medical Profession, No. 264 of 27.10.2005, according to which graduates of medical and pharmaceutical institutions of high education shall take the doctor's oath [7]. A simple analysis of the text reveals an analogy with the Hippocratic oath, known for hundreds of years to doctors throughout the world. In addition, in other countries, under the legislation in force, doctors bear legal responsibility for violating this oath [6].

It should also be noted that some bioethical documents are legally binding worldwide. For example, if a state accedes to the Convention on Biomedicine, the rules of the „Convention" become binding on that state [17].

Moral and legal principles - guarantees for the realization of the sources of medico-pharmaceutical law

Regarding the protection of the rights of the consumer of medicinal products, the principles of bioethics include the millenary experience of generations and express their unconditional recognition, used equally by both legal doctrine and the lawmaker, who as such can become specific non-traditional sources. Thus, on the one hand, the principles of bioethics represent, in relation to the content of the rules, legal customs, and on the other, the legal provisions outline the framework within which the principles of bioethics can operate.

The principle of patient autonomy (except in cases of self-medication) can be used as an example of compliance by the consumer of medicinal products with one of the basic principles of bioethics:

- In its most general form, it entails the consumer of medicinal products being asked for consent to treatment from the stage of prescribing the medicinal product, based on good information practice (and enshrined in law), with the doctor having the obligation to propose to the consumer of medicinal products all options for medicinal treatment, with justification and a forecast of the consequences. Thus, based on this bioethical principle, the doctor cannot prescribe a medicine in any case; instead, he should first propose to the drug user all possible options for drug treatment, including details of efficacy (bioavailability), contraindications, adverse effects, mode of administration, and so on, which will enable the drug consumer, together with the doctor, to identify, on the basis of their individual characteristics (age, sex, psychophysiological condition, social, professional, national, economic, religious status, etc.), their „medicine" (personalized medication);
- The principle of the autonomy of the will (consent) of the consumer of medicinal products simultaneously manifests itself as a legal principle. It has obtained legislative recognition in the Order of the Ministry of Health of the Republic of Moldova no. 303 of May 6, 2010 on ensuring access to information about medical data and the list of medical interventions requiring informed consent: p. II, „Parental interventions, including immunizations"; p. VI, „Therapeutic treatments with specific adverse effects or increased risk" (Annex 2);
- The principle of autonomy of will (agreement) of the consumer of medicinal products is a special one in relation to the principle of autonomy of will, characteristic of civil law, legislated in the Civil Code of the Republic of Moldova (CCRM), namely Art. 1, Book I, Title I, Chapter I of the CCRM, which states: „(1) Civil legislation is based on the recognition of the partici-

pants' equality in the relations regulated by it, the inviolability of property, freedom of contract, the inadmissibility of interference in private affairs, the need for the free realization of civil rights, the guarantee of the restoration of the person's rights (in case of violation), and the judicial defense. (2) Natural and legal persons shall be free to establish by contract their rights and obligations and other contractual conditions, provided that they do not contradict the law". Article 21 of the CCRM defines the notion of „consumer": (1) A consumer is any natural person who, in a civil legal relationship, acts predominantly for purposes that are not entrepreneurial or professional. A natural person is not a consumer if the other party to the civil legal relationship is not a professional. (2) Any natural or legal person governed by public or private law who, in a civil law relationship, is acting for purposes relating to his trade, business, or profession, even if that person is not acting for purposes relating to his trade, business, or profession for profit, shall be regarded as a trader. In general, civil law provides that „citizens (physical persons) and legal persons acquire and exercise their civil rights by their own will and in their own interest" [3].

Based on the postulates and principles set out above, the general outline of the systemic approach to the „doctor-drug user-pharmacist" correlations influenced by various legal rules is proposed (Figure 2).

Unquestionably, one of the main sources of ethical regulation of the behavior of the medical and pharmaceutical bodies in the Republic of Moldova is the Code of Ethics for doctors and pharmacists, adopted by Government Decision No. 192 of 24.03.2017 [18]. According to p.46, Section III of the Code, „Consent will be accepted only after fully informing the patient about the diagnosis, prognosis, therapeutic alternatives, their risks, and benefits (the primary objective being the life, health, and benefit of the patient)." Point 53, Section IV of the Code, also sets out patients' rights to medical confidentiality. Medical workers and pharmacists have a duty to protect the confidentiality of information about patients obtained during their professional activity through the processes of accumulating, storing, transmitting, receiving, or destroying personal data.

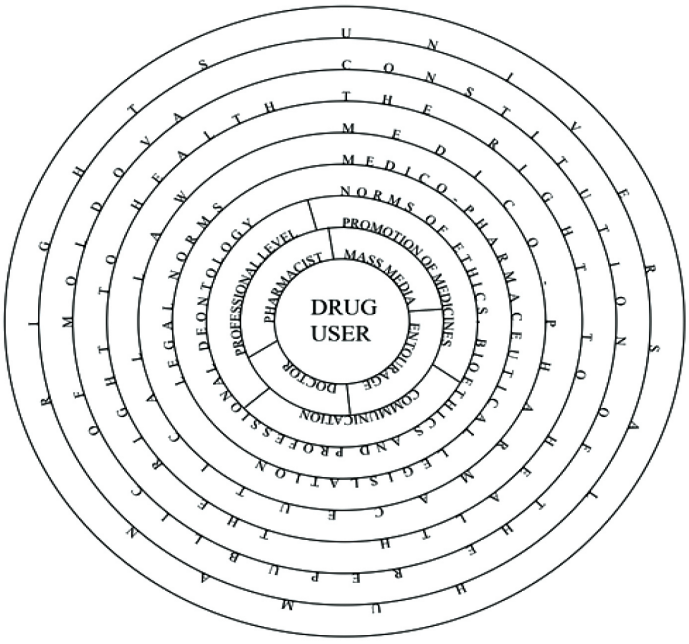

Fig. 2 General scheme of the systemic approach to the relationships in the „doctor-drug consumer-pharmacist" system, influenced by various legal norms

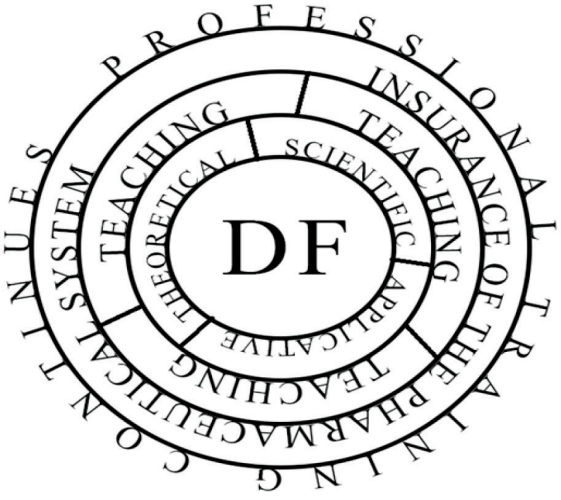

Fig. 3 Schematic diagram of the functionality of the pharmaceutical law system

Stages in the crystallization of medico-pharmaceutical law

As can be seen, ethical rules also have a visible impact on doctrine and case law. A number of such rules are also incorporated into legal norms, acquiring obligatory features and other features inherent in the legal norm. We understand and support the idea that their main function should be „to help those involved in the public health sphere avoid misunderstandings and violations, to point out the best ways to make decisions without inducing prejudice and accusations, to protect both the dignity of the consumer of medicines and the personal dignity of the doctors and pharmacists, who are daily burdened with the responsibility of making decisions and can become the target of severe and often unfounded accusations, and to overcome the conflicts between personalized, new, technological medicine and old ethics" [19].

Considering the results of the study and the opinions of other researchers in the field, we can see four stages of the functionality of the „morals-ethics-deontology-law" system, which can be applied to the field of pharmaceutical law: the theoretical stage, the applied (practical) stage, the scientific stage, and the teaching stage (Figure 3).

The first stage of the interaction between bioethics and pharmaceutical law is at *the theoretical level*, involving the use of existing ideas, concepts, and theories in the fields concerned.

The second stage is *the application level*, which involves:

- the emergence of legal relations through the formation of certain (guided) behaviors on the part of the consumer of medicines. At this stage, legal and ethical norms interact synchronously, forming an optimal social variant specific to relations and behavior in the health care system. Inevitably, they must ensure that the fundamental rights and obligations of both the consumer of medicines (the patient) and the healthcare workers are realized. In this particular case, the area of ethical evaluation is much wider than the legal area, since law regulates not all but only some of the relations within this functional system, which allows it to be a kind of law laboratory at the same time. Thus, with the emergence of partnership relations between doctor, pharmacist, and patient and the spread of the rules of the Civil Code on services and medical services, there is also the real possibility, with the agreement of the parties, of fixing the ethical rule in the provisions of the contract. Such a norm, obtaining the „residence visa" among the specific clauses of a contract for the provision of medical and/or pharmaceutical services, will acquire the status of an obligation for the parties and will no longer be accepted only as a purely ethical rule;
- the development of legal rules in the knowledge that selected individuals (based on legal and bioethical rules) can achieve a „model of behavior," often being totally „indifferent" to legal rules, only if there are no deviations from the chosen model;
- application of the law, which is not mandatory but characterizes the law as a specific regulator of social relations. The application of coercive measures by the state (of sanctions), expressed in individual prescriptions, is associated with law enforcement.

The third stage is *the scientific level*, which imposes the need for evidence-based argumentation of the correlations in the „moral-ethics-deontology-law" system applicable to social relations in the „doctor-drug consumer-pharmacist" system, which are strictly oriented towards obtaining health benefits.

The fourth stage is *the didactic level*, which involves continuous direct and/or distance training of all parties involved in the relationships under study (the „doctor-medicine consumer-pharmacist-lawyer" system).

Conclusions

There is no doubt that fundamental human rights and freedoms are supreme and constitutionally guaranteed values (see articles 1(3) and 16(1) of the Constitution of the Republic of Moldova). The life of the human being as a biological phenomenon acquires the legal status of a fundamental right (Article 24 of the Constitution), with the protection of health as a source of life protection [20].

These qualities are ensured by legal regulations, but as sources of law, they will not be sufficient if they are not supplemented and enriched by the moral, ethical, bioethical, and deontological sources of medical workers and pharmacists. And, while the literature extensively explains the significance of legal sources, much less is exposed and demonstrated about the aptitude of moral, ethical, bioethical, and deontological rules sources.

Through the research we conducted, we were able to identify the dialectical and organic unity between moral, ethical, bioethical, and deontological sources, and legal norms - the moral-legal foundation of medico-pharmaceutical law.

Declaration of conflict of interest

Nothing to declare.

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REVIEW ARTICLE

The current assessment and management of thyroid nodules

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ABSTRACT

Introduction. The widespread use of diagnostic imaging favored the increasing incidence of thyroid nodules. Although most of nodules are benign, their clinical importance lies in the need to exclude malignancy. In assessing and managing thyroid nodules may occur the phenomenon of overdiagnosis and overtreatment on one hand and the risk of missing an aggressive thyroid cancer on the other hand. The equilibrium that has to be reached by health care providers.

Materials and methods. We conducted a PubMed, MEDLINE, ISI Web of Science, Cochrane databases search for the relevant and recent guidelines, meta-analysis, randomized controlled trial, reviews articles related to „thyroid nodules assessment", „thyroid nodules management", „thyroid nodules guidelines", „thyroid nodules surgery".

Results. The initial assessment of thyroid nodules includes an evaluation of clinical, laboratory and sonographic risk factors. Due to the sonographic features and size, the nodules are selected for biopsy. Cytologically benign nodules are usually followed-up, minimally invasive techniques may be required in certain cases. In suspected or confirmed malignancy, the treatment options of thyroid nodules include surgery or active surveillance. The main controversies appear in management of nodules with inconclusive cytology, low-risk cancers, multinodular goiters, hyperfunctioning nodules, and thyroid incidentalomas.

Conclusions. Thyroid nodules due to the high incidence and heterogeneity of background diseases cannot be evaluated and managed in one standardized approach. In the existing literature, there are discussed multiple options for diagnosis and treatment of thyroid nodules. We have reviewed the guidelines recommendations, novel published data, and controversial questions for health care professionals, to understand and provide efficient, personalized, and cost-effective management of patients with thyroid nodules in order to avoid automatic intensive testing and intervention and balancing each case from the patient expectations and demands.

Keywords: thyroid nodules, cancer, assessment, management, surgery.

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Key messages

What is not yet known on the issue addressed in the submitted manuscript

Discussion and decision-making on the most appropriate management of the patients with thyroid nodules.

The research hypothesis

Thyroid nodules are very common medical findings in adults and represent a multidisciplinary matter in which specialists have not reached a unique algorithm and consensus of diagnosis and treatment.

The novelty added by the manuscript to the already published scientific literature

This article reviews the main available evidence in order to offer guidance in health care of patients with thyroid nodules and points up particular considerations in thyroid nodules assessment and management.

Introduction

Thyroid nodules, solitary or in multinodular goiter are prevalent findings in the adult general population, which means that every second, a person has at least one nodule [1]. According to American Thyroid Association (2015), a thyroid nodule is a radiologically distinct lesion from the surrounding thyroid parenchyma [2].

The clinical significance of thyroid nodules relates to interference with thyroid gland function, causing compressive symptoms because of mass effect, and harboring a thyroid cancer.

Despite the fact that cancer occurs in 7-15% of cases, differentiation of benign from malignant nodules is a major issue in managing thyroid nodules. There is the possibility of overdiagnosis and overtreatment of thyroid nodules or the opposite, of missing an important clinical thyroid malignancy [1-6].

Materials and methods

A literature search was performed to identify meta-analysis, randomized controlled trial and reviews articles related to „thyroid nodules assessment", „thyroid nodules management", „thyroid nodules guidelines", „thyroid nodules surgery" in the PubMed, MEDLINE, ISI Web of Science, Cochrane databases. There were selected the most relevant and recent articles. The key guidelines eloquent on the issue were considered: The 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer [2]; AACE/ACE/AME Task Force on Thyroid Nodules, American Association of Clinical Endocrinologists, American College of Endocrinology, and Associazione Medici Endocrinologi medical guidelines for clinical practice for the diagnosis and management of thyroid nodules – 2016 update [4]; ACR Thyroid Imaging, Reporting and Data System (TI-RADS): white paper of the ACR TI-RADS Committee [5] and the American Association of Endocrine Surgeons Guidelines for the Definitive Surgical Management of Thyroid Disease in Adults [6].

Results and discussions

Epidemiology and etiopathogenesis. The last 30 years has registered a substantial rise in the incidence of thyroid nodules. This phenomenon is due to the use of neck imaging studies. The prevalence rate in the detection of thyroid nodule by ultrasound imaging is 76%, by computed tomography (CT) or magnetic resonance (MR) imaging is 16%, by carotid duplex ultrasound is 9.4%, and around 2-3% by 18-fluorodeoxyglucose positron emission tomography (18-FDG PET) [7].

Other reasons, which contribute, to a high incidence of thyroid nodules and thyroid cancer are residency in iodine deficiency regions, obesity, cigarette smoking, exposure to ionizing radiation. These factors lead to low levels of thyroid hormones into the bloodstream and in response TSH is released and stimulates proliferation of follicular cells with enlargement of thyroid gland and formation of thyroid nodules [3, 8, 9].

Moreover, the thyroid nodules are 4 times more frequent in females compared to males and this gender disparity is associated with pregnancy and influence of human chorionic gonadotropin on follicular cells as a homolog of TSH [10]. The incidence of thyroid nodules is increasing linearly with age and declines after reaching the plateau in the sixth-seventh decade of life [11].

Most nodules are derived from thyroid follicular cells. Based on etiology, thyroid nodules can be divided into non-neoplastic and neoplastic. Non-neoplastic nodules might be colloid nodules, nodules within Hashimoto's thyroiditis or Graves' disease, simple or hemorrhagic cysts. Benign neoplastic nodules are represented by follicular or oncocytic (Hürthle cell) adenoma, while malignant nodules can be papillary carcinoma, follicular carcinoma, Hürthle cell (oncocytic) carcinoma, anaplastic carcinoma, medullary carcinoma, thyroid lymphoma and breast, renal or lung metastases [1, 11].

Medical history and physical examination. History and physical examination of patients with thyroid nodules should comprise assessment of risk factors for malignancy.

The most incriminated and associated historical factors to malignancy are a history of childhood head and neck radiation therapy, total body radiation for bone marrow transplantation, exposure to ionizing radiation in childhood or adolescence (e.g. Chernobyl accident), familial thyroid carcinoma, or thyroid cancer syndrome (Cowden's disease, Carney complex, Werner syndrome, or MEN 2, as a risk diseases for medullary thyroid cancer) in a first-degree relative, rapid nodule growth [2, 6, 12].

Physical examination including inspection and palpation of the thyroid gland must be focused on nodules' location, size, texture, and examination of cervical lymph nodes. A thyroid nodule even harboring a thyroid cancer may be asymptomatic, but most of them are detected by the patient himself or medical practitioners as a lump in the anterior cervical region [3, 11-14]. The physical findings suggestive for malignancy are hard consistency and fixed nodule to surrounding tissue, vocal cord paralysis, and regional cervical lymphadenopathy [2, 4]. Rapid enlargement of a thyroid nodule may be associated with hemorrhage, especially if pain persists [3, 15]. The AACE/ACE/AME guideline mentions about a higher risk of cancer in nodules bigger than 4 cm in diameter, but more recent papers noted the highest malignancy risk in nodules <2 cm, meaning that size are inversely proportional to malignancy rate [13-15]. Large nodules provoke compression of underlying structures of trachea, esophagus, and recurrent laryngeal nerves leading to dyspnea or wheeze, dysphagia, and hoarseness correspondingly [2-4, 13-15].

Just as importantly, signs of hyperthyroidism or hypothyroidism also need to be extracted during thyroid nodules evaluation. Patients with hyperthyroidism have complaints about palpitations, heat intolerance, weight loss as opposed to increased appetite, frequent bowel movements, and anxiety, while in patients with hypothyroidism due to a slow metabolism rate are noticed fatigue, cold intolerance, constipation [2, 4, 9, 10, 16].

Paraclinical assessment and management

The present guidelines concentrate on three elements in thyroid nodules management – measurement of serum thyroid stimulating hormone (TSH), thyroid gland ultrasound, and fine-needle aspiration (FNA) of nodules [2-6].

Measurement of serum TSH is the initial laboratory test for all patients with thyroid nodules. Normal or elevated levels of TSH express a nonfunctioning nodule, and in large studies are associated with a higher risk of thyroid cancer [16, 17]. Low or suppressed levels are associated with hyperthyroidism and a radioisotope scan with iodine-123 or technetium-99m pertechnetate must be performed. Scintigraphically, the thyroid nodule can appear „warm" which means an isofunctioning nodule, „cold" which attests hypofunctioning nodule, and „hot" as a hyperfunctioning nodule. As stated by the guidelines, „hot" nodules do not require FNA and cancer vigilance, due to minor risk of malignancy, meanwhile nonfunctioning or „cold" nodules which combine clinical or/and ultrasound criteria, should be subjected to FNA [2, 4, 6]. Classically, malignancy rate in „hot" nodules has been reported to be about 0.34%, contested by the latest studies that found an increased malignancy incidence in „hot" nodules ranging between 10% and 34%, meaning that „hot" nodules need to be assessed in the same volume as to exclude a carcinoma, especially if they meet other relevant criteria [18, 19].

Other laboratory tests are not recommended for routine use [2, 4, 6]. Apart from the recommendation, not all health care professionals share this opinion. Serum thyroid hormones T_3 and T_4 are valuable in hyperthyroidism and hypothyroidism, particularly in their subclinical forms. T_3 and T_4 serum concentrations must be obtained in residents of iodine-deficient areas, and Republic of Moldova is considered such an area [16, 23]. Low values of T_3 and T_4 in conjugation with increased levels of TSH are related to malignancy [24, 25]. Determination of serum thyroglobulin (Tg), the storage form of iodinated thyroid hormones, can be a useful predictive biomarker to differentiate thyroid cancer and high levels of Tg are important in the adoption of the decision for surgery. Furthermore, the postoperatively elevated Tg levels are linked to a recurrent, persistent, or metastatic disease with a poor prognosis in cases of differentiated thyroid cancers [16, 20-22]. Calcitonin, another hormone produced by thyroid parafollicular C cells it is the single serum biomarker for detection of medullary thyroid cancer, which can be applied as a screening tool [26]. Acknowledging that antithyroid antibodies denote an autoimmune process including Hashimoto's thyroiditis or Graves' disease, the measurement of peroxidase antibodies, anti-thyroglobulin antibodies, and TSH receptor antibodies has an influential role. In addition to this, in a cross-sectional study and meta-analysis it was establish a higher prevalence of positive antithyroid antibodies in patients with carcinoma compared to those with benign nodules [27, 28].

Thyroid gland ultrasound is an essential tool in the evaluation and the first-line imaging investigation to stratify the risk of malignancy in thyroid nodules. Each guideline recommends a sonographic classification of thyroid nodules in an attempt to distinguish the malignant nodules before cytological evaluation or before follow-up. The ACR-TIRADS guideline compared to ATA and AACE/ACE/AME guidelines is widely accepted, with decreasing the number of FNA and has the highest accuracy in recognition of suspicious nodules [5, 29]. In 2017 The American College of Radiology, on the basis of breast reporting system, has introduced the Thyroid Imaging, Reporting and Data System (TI-RADS) that describe the ultrasound features of thyroid nodules such as composition, echogenicity, shape, margin, presence of echogenic foci and aid in calculation for a predictive score from TR1 - benign to TR5 - highly suspicious (Table 1) [5]. Although, the dimensions of thyroid nodules are not important for categorization, the cutoff size of nodules are taken into consideration for next step management.

Table 1. TI-RADS - Thyroid Imaging Reporting and Data System.

Composition (choose one)	Echogenicity (choose one)	Shape (choose one)	Margin (choose one)	Echogenic foci (choose one or more)
Cystic or completely cystic *0 points*	Anechoic *0 points*	Wider than tall *0 points*	Smooth *0 points*	None *0 points*
Spongiform *0 points*	Hyper- or isoechoic *1 point*	Taller than wide *3 points*	Ill-defined *0 points*	Large comet-tail artifact *0 points*
Mixed cystic and solid *1 point*	Hypoechoic *2 points*		Lobulated or irregular *2 points*	Macrocalcifications *1 point*
Solid or almost completely solid *2 points*	Very hypoechoic *3 points*		Extra-thyroidal extension *3 points*	Peripheral or rim calcifications *2 points*
				Punctate echogenic foci *3 points*
Summation of points to obtain a TI-RADS level				
0 points	*2 points*	*3 points*	*4-6 points*	*≥7 points*
TR1	TR2	TR3	TR4	TR5
Benign	Not suspicious	Mildly suspicious	Moderately suspicious	Highly suspicious
no FNA required	no FNA required	≥ 1.5 cm follow up, ≥ 2.5 cm FNA	≥ 1.0 cm follow up, ≥ 1.5 cm FNA	≥ 0.5 cm follow up, ≥ 1.0 cm FNA

Note: TR – TI-RADS category; FNA – fine needle aspiration.

Mainly reported patterns associated with a malignant nodule are hypoechogenicity, a taller-than-wide shape, lobulated or irregular margin, and microcalcifications [4, 12, 16]. It is noteworthy that these sonographic findings are more common to papillary thyroid cancers as the predominant type, and less typical to follicular or Hürthle cell tumors, medullary carcinoma and follicular variant of papillary thyroid cancer [4, 6, 16, 30].

Ultrasound is a valuable test in the evaluation of thyroid nodules, but it is also informative in the evaluation of cervical lymph nodes. None of the guidelines clearly recommends the exploration of cervical lymph nodes compartments, despite that their assessment adds precision to diagnosis. Papillary thyroid cancer has been found to metastasize in 30-65% to central lymph nodes (compartment VI) and in 3-44.5% to lateral lymph nodes (compartments III, IV), while regional lymph nodes metastases occur in less than 10% of follicular and Hürthle cell carcinomas [16, 31, 32].

Ultrasound elastography and color Doppler sonography are novel modalities that improve the diagnostic confidence of malignancies in thyroid nodules. Strain elastography or shear-wave elastography assesses tissue elasticity. The nodule stiffness indicates a malignant process. Some authors brought scientific evidence that this indicator can be used as an independent predictor for thyroid cancer and the elastography informativity is comparable to FNA [16, 33-35]. Color Doppler studies provide details about vascular architecture of thyroid nodules. Nodules with greater intranodular vascularity are more likely to be malignant than those with peripheral patterns [4, 34]. The existing guidelines do not support elastography and color Doppler modules for extensive use, regardless of the possibility of advancement in risk stratification of thyroid nodules and besides the fact that these tests are performed as a continuation of conventional ultrasound, and not as a separate investigation.

The routine use of cross-sectional imaging studies (CT, MRI) and 18-FDG PET scanning in evaluation of thyroid nodules are not approved. The American Association of Endocrine Surgeons (AAES) and ATA guidelines recommend preoperatively CT or MRI with intravenous contrast as an addition to ultrasound in patients with clinical suspicion of advanced locoregional thyroid cancer. Both CT and MRI project the images from the skull base to the mediastinum and are preferable in patients with confirmed thyroid nodules accompanied by vocal cord paralysis, positional dyspnea, mass fixation to surrounding structures, rapid enlargement of the nodule, and retrosternal extension of the thyroid gland [3, 6]. 18-FDG PET has a significant role in detecting recurrence of differentiated thyroid carcinoma and was estimated as a strong predictor of poor outcome in patients with metastases [6, 16].

As claimed by ACR-TIRADS guideline, thyroid nodules of 1 cm or larger with suspicious sonographic features require aspiration. Fine-needle aspiration is the unique method that provides the cytologic results of thyroid nodules. With the aim to unify data reporting and to obtain an interobserver agreement, in 2007 The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) was developed. The system includes six general categories: TBSRTC I Nondiagnostic / Unsatisfactory ND/UNS; TBSRTC II Benign; TBSRTC III Atypia of undetermined significance / Follicular lesion of undetermined significance AUS/FLUS; TBSRTC IV Follicular neoplasm / Suspicious for a follicular neoplasm FN/SFN; TBSRTC V Suspicious for malignancy SUS, and TBSRTC VI Malignant. Cibas *et al.* in 2017 updated these categories due to the revised guidelines for the management of patients with thyroid nodules, the introduction of molecular testing as an adjunct to cytopathologic examination, and the reclassification of the noninvasive follicular variant of papillary thyroid carcinoma as noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) (Table 2). The revised version of TBSRTC reconsider as well the management options for thyroid nodules [36].

Nondiagnostic results (ND/UNS) occur in 5–15% of cases and nearby the updated TBSRTC recommendation, ATA, AACE/ACE/AME suggest resection for repeatedly nondiagnostic solid nodules and surgery or follow-up for cystic nodules [2, 4, 6, 36, 37]. Divergent opinions were published in several reports. Some researchers promote a conservative tactic because most often nondiagnostic nodules have benign outcomes and those with no suspicious features, mainly cystic, need only an ultrasound follow-up. Moon *et al.* advocated for resection of the ND/UNS nodules in repeated biopsies with at least one sonographic suspicious feature [37]. Raveh Gildin *et al.* divided the ND/UNS category in 3 subgroups – Blood, Thyrocytes and Cyst and showed that malignancy rates were different in each of them with the highest in the Blood subgroup succeeded by the Thyrocytes subgroup and the lowest in the Cyst subgroup, with the consideration of an individual subgroup approach [38].

Thyroid nodules are found to be benign on cytology in about 70% of all FNAs. The surveillance targets for these patients are to detect a missed malignancy, to observe nodule growth for the appearance of compressive symptoms or suspicious features [3, 6, 39]. The debatable point about benign nodules follow-up procedure, that none of the guidelines disclose is the exact length of this period, when one should stop the follow-up or to perform surgery to these nodules. Negro *et al.* in a 5-year monitoring study of benign nodules described the appearance of new nodule which require FNA, increase of nodule diameter > 50%, the appearance of compressive symptoms, and repetition of FNA on the existing nodule. These events occurred in 26.1% of patients, with more than one event occurring in the same patient in 27.7% of cases, summarizing that 71.9% of events were observed at 24- and 36-months of the monitoring process [40].

Table 2. The 2017 Bethesda System for Reporting Thyroid Cytopathology: Diagnostic Categories, Risk of Malignancy and Recommended Clinical Management.

Diagnostic categories	ROM if NIFTP is not cancer	ROM if NIFTP is cancer	Clinical management
I. Nondiagnostic or unsatisfactory Cyst fluid only; Virtually acellular specimen; Other (obscuring blood, clotting artifact, etc.).	5–10%	5–10%	Repeat FNA with ultrasound guidance
II. Benign Consistent with a benign follicular nodule (includes adenomatoid nodule, colloid nodule, etc.); Consistent with lymphocytic (Hashimoto) thyroiditis in the proper clinical context; Consistent with granulomatous (subacute) thyroiditis; Other.	0–3%	0–3%	Clinical and sonographic follow-up
III. Atypia of undetermined significance or follicular lesion of undetermined significance	6–18%	10–30%	Repeat FNA, molecular testing, or lobectomy
IV. Follicular neoplasm or suspicious for a follicular neoplasm Specify if Hürthle cell (oncocytic) type	10–40%	25–40%	Molecular testing, lobectomy
V. Suspicious for malignancy Suspicious for papillary carcinoma; Suspicious for medullary carcinoma; Suspicious for metastatic carcinoma; Suspicious for lymphoma; Other.	45–60%	50–75%	Near-total thyroidectomy or lobectomy
VI. Malignant Papillary thyroid carcinoma; Poorly differentiated carcinoma; Medullary thyroid carcinoma; Undifferentiated (anaplastic) carcinoma; Squamous-cell carcinoma; Carcinoma with mixed features (specify); Metastatic carcinoma; Non-Hodgkin lymphoma; Other.	94–96%	97–99%	Near-total thyroidectomy or lobectomy

Note: ROM - Risk of malignancy; NIFTP - Noninvasive follicular thyroid neoplasm with papillary-like nuclear features; FNA – fine needle aspiration.

Levothyroxine administration for TSH suppression is intended to decrease the volume of nodules and prevent the growth of existing or new formation of thyroid nodules. In clinical practice, this therapy registered a low efficacy with an increased risk of iatrogenic thyrotoxicosis, atrial fibrillation, and osteopenia. More beneficial to reduce nodule size is considered adequate dietary iodine intake (150 mg daily), especially in iodine deficiency areas [7, 9].

Some patients with benign nodules may complain of compressive symptoms, cosmetic concerns, and signs of hyperthyroidism due to an autonomously functioning thyroid nodule. Treatment options in these cases are surgery or minimally invasive techniques such as ethanol ablation (EA), image-guided thermal ablation (TA) procedures and radioactive iodine therapy (RAI). Among the currently available TA techniques are laser thermal ablation (LTA), radiofrequency ablation (RFA), microwave ablation (MWA), and high-intensity focused ultrasound (HIFU) that decrease nodule size by thermal coagulation necrosis induced by laser, radiofrequency, microwave, or high-intensity focused ultrasound respectively. TA is considered for nodules with a twice confirmed benignity on cytology, which does not exceed 3.0 cm in diameter. In case of cystic or predominantly cystic symptomatic nodules, initially is preferred EA with subsequent TA, if the nodule relapsed or the residual nodule has a solid component. TA should not substitute surgery in patients with compressive multinodular goiter, due to the need for multiple treatments and inadequate efficacy. In hyperfunctioning thyroid nodules, RAI may be considered, after decreasing hyperthyroidism with antithyroid drugs. Toxic multinodular goiter is not suitable for TA procedures [39, 41, 42].

Although, FNA differentiates benign from malignant thyroid nodules in an appropriate manner, in approximately 30% of patients persists diagnostic difficulties for nodules categorized with indeterminate cytology, comprising atypia of undetermined significance or follicular lesion of undetermined significance (TBSRTC III, AUS/FLUS) and follicular neoplasm (TBSRTC IV, FN/SFN) or Hürthle cell neoplasm (TBSRTC IV, HCN/SHCN) [42,43]. The AAES recommends for TBSRTC III nodules, in addition to the other guidelines, to reckon with clinical factors, radiologic features, and patient preference about repeat biopsy, molecular testing, diagnostic thyroidectomy, or observation [2, 4, 6]. Cytological assessment of a repeated FNA reclassifies this category in 60–65% and the nodules may be benign in up to 45% of cases. If the second cytological result is still indeterminate, diagnostic lobectomy provides the definitive pathological diagnosis [6, 39, 42, 43].

Molecular testing of the FNA samples is an innovative investigation introduced to refine the malignancy risk of cytologically indeterminate thyroid nodules. Genetic changes in thyroid carcinoma are developing directly in the thyroid tissue being nonhereditary – somatic mutations. Molecular

profiling includes point mutations in BRAF, RAS, RET, TERT, TP53, the fusion genes RET/PTC, PAX8/PPAR-γ, NTRK and/or micro-RNA (miRNA) expression [6, 43, 44]. The most studied genome for papillary thyroid cancer was performed by the Cancer Genome Atlas (TCGA) research network, which described the molecular portrayal of 496 papillary cancers in classical and follicular variants. Following this study, papillary thyroid cancers have a quite stable genome with genetic events recurring in a limited number of genes and a low mutation burden, hence one mutation rarely co-exists in the same tumor, so the most common mutations determined in 70% of papillary cancers with a mutual exclusion are RET rearrangements or point mutations of RAS or BRAF protooncogenes [44, 45].

The commercially available tests, mainly in the USA are: ThyroSeq.v3 – the third version of ThyroSeq molecular test uses the next-generation sequencing technique of DNA and RNA to evaluate 112 thyroid-related genes that include point mutations, gene fusions, copy number alterations, and gene expression alterations to diagnose or rule out malignancy; Afirma Gene Sequencing Classifier and Xpression Atlas – uses a classifier to diagnose or rule-out malignancy according to gene expression based on RNA sequencing and operate the same RNA sequencing data to determine mutations and gene fusions in common with Xpression Atlas Panel; ThyGenX and ThyraMIR - ThyraMIR uses an algorithm of 10 microRNAs and ThyGeNEXT panel identifies DNA mutations and the RNA panel identifies the number of gene fusions [6, 45, 46].

Several limitations of using the molecular testing must be mentioned. The genome of follicular-derived thyroid carcinomas is highly variable among the cancer histological types. While the molecular changes of papillary thyroid cancers are almost understood, the molecular panel of follicular thyroid cancers is still unclear; moreover poorly differentiated and anaplastic cancers are often characterized by multiple co-occurring mutations that cannot be covered by available tests. Molecular tests are useless in diagnosis of Hürthle cell neoplasms determined by distinct molecular changes such as mitochondrial DNA mutations, point mutations recurring in atypical genes for thyroid cancer, and karyotype alterations in chromosomes 7, 5, 12. Some mutations, including those in RAS genes (HRAS, KRAS, NRAS) are present in malignant and nonmalignant thyroid neoplasms, invasive and noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP), unhelpful in differentiation benign from malignant nodules. In the studied literature, there is uncertain data about the negative predictive value of the tests because in 93% of long-term follow-up, sonographic, cytologic, and hystologic data of the unresected thyroid nodules are absent. Also. It is important to mention that if no mutations are confirmed, the malignancy should not be excluded [16, 45, 46].

Accepted options for patients with nodules cytologically categorized as TBSRTC IV are lobectomy and/or molecular testing. Cytological examinations of specimens cannot distinguish carcinomas from benign adenomas, due to the low possibility of assessing for capsular or vascular invasion of a nodule, even when a core-needle aspiration is performed. Molecular testing are limited for the same reasons for this category and has no influence on improvement of patient outcome such as survival or quality of life, or to reduce the number of thyroid surgeries [6, 16, 46, 47]. Diagnostic lobectomy is considered the only option to obtain definitive histology. For a malignant nodule, complete thyroidectomy is often indicated. It has been estimated that almost 60% of patients undergoing lobectomy for indeterminate cytology may be over- or undertreated at initial surgery [12, 48, 49]. In this context, in TBSRTC IV and even in TBSRTC V cytology, many surgical teams use intraoperative frozen section that is helpful in diagnosing malignancy and guiding decisions about initial surgical extent [6, 49].

Still it is not yet clear which nodules should be subjected to histology rather than cytology when taking into consideration the dimensions, number and suspicious features, as long as current guidelines are based on evaluation of single or dominant nodule in goiter between 1.0 to 4.0 cm.

In the medical literature, reviews, guidelines and studies, we can find a shallow approach of multinodular goiter, although each nodule in a multinodular goiter harbor an independent risk of malignancy. An algorithm of multinodular goiter management with clearest terms was published in 2014 by Lam *et al.* in reliance on three directions – thyroid function, mass effect, and nodule pathology with meaning of presence or absence of malignancy. In goiter associated with hyperthyroidism, compressive symptoms, and suspicion or confirmed malignancy, surgery was indicated. In cases of non-toxic, asymptomatic, and benign nodules within the multinodular goiter, the approach was reduced to observation [50].

Remains controversial the volume of thyroidectomies in III-VI TBSRTC categories and multinodular goiter. Most recent studies suggest as initial surgery of thyroid nodules with a hemithyroidectomy implies removing of the entire ipsilateral thyroid lobe and isthmus, including low-risk thyroid cancers to avoid recurrent laryngeal nerve injury or hypoparathyroidism with the advantage of no levothyroxine replacement [6, 7, 9, 12, 16, 36, 39, 50-52]. Beyond this concern, specialized centers register a low complication rate, <1% risk of permanent recurrent laryngeal nerve injury or hypoparathyroidism. It should be underlined that reoperating in an explored surgical field increase the risk to 3–10 times of permanent recurrent laryngeal nerve lesion or hypoparathyroidism [50].

In clinical practice, the perceptions of „low-risk" nodules may be dubious as a result of insufficient information for risk stratification provided by ultrasound and FNA reports [12, 51, 52]. The recommendation of hemithyroidectomy instead of total thyroidectomy when counseling patients is not clear as suggested in guidelines on the volume of surgery and the patient preference, implications for a long-term sonographic or cytologic surveillance, the potential for a completion thyroidectomy, need for postoperative thyroid hormone replacement should be considered. In addition,

clinicians and patients must establish the advantages of less extensive surgery against higher recurrence rate [50-53]. Wang *et al.* estimated that the risk factors for contralateral recurrent malignancy are: contralateral nodules misdiagnosed as benign, multifocality of primary carcinomas, capsular invasion, and Hashimoto's thyroiditis and may be reduced by performing frozen section examination [52].

Not insignificant are thyroid incidentalomas (TI). These are some of the most common incidental found nodules on imaging studies that include the neck CT, MRI, nuclear medicine, ultrasound, performed for other indications than thyroid evaluation. In 2015, the ACR formed the Incidental Thyroid Findings Committee to deduct a practical approach of managing incidentalomas [54]. Emerging from the fact that small and indolent nodules are more likely to be micropapillary carcinomas, the Committee stated that malignancy rate in TI depends on the techniques used to diagnose malignancy. Malignancy rate of TI detected by ultrasound is 12%, by CT and MRI ranges from 0% to 11% and with focal uptake on FDG-PET scans is higher 33-35%. The Committee recommends evaluation of TI with ultrasound in all cases, except in PET-avid lesions where thyroid ultrasound and FNA are considered. Some authors share the opinion that each thyroid incidentaloma, of any size, must be subjected to a comprehensive clinical, laboratory, instrumental, and cytological examination [55].

Conclusions

Thyroid nodules are common findings in general adult population with increasing prevalence. The primary concern that requires a careful assessment of each symptomatic nodule is the exclusion of malignancy. The initial evaluation of patients with thyroid nodules follow a risk stratification strategy that includes an exhaustive history and physical examination to identify risk factors, measurement of serum thyroid hormones, TSH, relevant biomarkers and neck ultrasonography to appreciate the size and suspicious features to select nodules to biopsy. Patients with benign nodules are subjected to active surveillance and in specific cases to minimally invasive techniques. Surgery is recommended for nodules that are suspicious for malignancy or malignant and is indicated for indeterminate compressive or any other symptomatic nodules. The main controversies appear in management of nodules with indeterminate cytology, low-risk cancers, multinodular goiters, hyperfunctioning nodules, and thyroid incidentalomas. The objective for a better management of patients with thyroid nodules is to identify the best individual treatment options and include the patient in the discussion with the multidisciplinary team regarding appropriate tactics, in terms of disease outcomes and quality of life, avoiding the dangers of overdiagnosis and overtreatment.

We have reviewed the guidelines recommendations, novel published data, and controversial points for health care professionals, to understand and provide efficient, personalized, and cost-effective management of patients with thyroid nodules in order to avoid automatic intensive testing and intervention and balancing each case from the patient expectations and demands.

Declaration of conflict of interests

There is no conflict of interest to declare.

Authors' contribution

CC - the collecting of data, the conception and drafting of the manuscript; AB - the critical revising of the manuscript for important intellectual content and the approval of the final version of the manuscript. Both authors - the analysis and the interpretation of data, approval of the „ready for print" version of the manuscript.

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REVIEW ARTICLE

Loco-regional analgesia in oncology. Influence on cancer recurrence rate. Literature review.

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ABSTRACT

Introduction. A major role and, at the same time, a question mark, both for patients and doctors, is the possibility that drugs and anesthetic techniques influence cancer metastasis. Cancer is the leading cause of death worldwide. This trend will continue in the future. Most of the deaths of cancer patients are due to complications arising from metastases. The metastasis process of a tumor depends on its intrinsic properties and interaction with the host. The treatment of tumors by performing a surgical intervention, radical or palliative, has a significant impact. For these reasons, the rate of survival and migration of cancer cells in the perioperative period is studied quite insistently and complexly. Thus, surgical intervention and anesthetic support in cancer patients becomes of great importance, because it represents the vulnerable link, both from the point of view of the operation itself, as well as the possibility that drugs, anesthetic techniques may or may not influence tumor metastasis.

Material and methods. Primary scientific studies published from 1996 to 2021 dedicated to loco-regional anesthesia and its influence on the perioperative period and on cancer metastasis were studied. To achieve the proposed goal, scientific sources PubMed, Medscape, SCOPUS, MEDLINE were researched. Keywords used for searching: loco-regional anesthesia, fascia plane anesthesia, metastasis. More than 80 reference sources were identified, 67 were selected for analysis.

Results and discussions. The surgical procedure, itself, performed for curative purposes, also known as tumor resection – is a risk factor for metastasis by creating an environment with high potential for tumor cell survival. This stimulates tumor growth and angiogenesis, can remodel lymphatic pathways, allowing metastasis of tumor cells. Hemotransfusion is associated with increased risk of metastasis. Regional anesthesia could reduce cancer recurrence through several mechanisms.

Conclusions. Regional anesthesia could reduce cancer recurrence by reducing the need for opioids or inhaled anesthetics, or by reducing the stress response during surgery. There is scientific *in vitro* evidence of a protective effect of systemic lidocaine on recurrent cancer, although relevant clinical data are limited.

Keywords: cancer recurrence, general anesthesia, regional anesthesia, stress response, opioid analgesics, angiogenesis inducing agents, morphine, onco-anesthesia.

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Key messages

What is not yet known on the issue addressed in the submitted manuscript

The article is a literature review of recent medical publications describing loco-regional anesthesia in oncology and its role in cancer recurrence. Thus, specialists from the Republic of Moldova will have a synthesis of modern research in the field.

The research hypothesis

Local anesthesia/analgesia positively influences patients with can-

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cer and does not contribute to development of metastasis or lead to cancer recurrence.

The novelty added by manuscript to the already published scientific literature

Fascial plane anesthesia/analgesia in oncology is a link of multimodal analgesia and does not influence metastases occurrence. This is a proven, safe and effective method of relieving perioperative pain syndrome in oncology patients.

Introduction

A major role and at the same time a question mark, both for patients and doctors, is the possibility that drugs and anesthetic/analgesic techniques influence cancer metastasis. Cancer is the leading cause of death worldwide. This trend will continue in the future [1, 2]. Most of the deaths of cancer patients are due to complications arising from metastases. These may result from direct, lymphatic or hematogenous spread. The metastasis process of a tumor depends on its intrinsic properties and interaction with the host [3]. The treatment of tumors by performing a surgical intervention, radical or palliative, have a significant impact [4]. For these reasons, the rate of survival and migration of cancer cells in the perioperative period is studied quite insistently and complexly [5]. Thus, surgical intervention and anesthetic support in cancer patients becomes of great importance, because it represents the vulnerable link, both from the point of view of the operation itself, as well as the possibility that drugs, anesthetic techniques may or may not influence tumor metastasis [6, 7].

Material and methods

There were studied primary scientific studies published from 1996 to 2021 dedicated to loco-regional anesthesia/analgesia and its influence on the perioperative period and on cancer metastasis. To achieve the proposed goal, scientific sources PubMed, Google Scholar, Medscape, SCOPUS, MEDLINE were used. Keywords used for searching: loco-regional anesthesia, fascia plane anesthesia, metastasis. More than 80 reference sources were identified, and 67 were selected for analysis.

Results and discussions

After analyses of the sources, we can describe some of the principal mechanisms of metastasis that occur during the perioperative period. The surgical procedure, itself, performed for tumor resection – is a risk factor for metastasis by creating an environment with high potential for tumor cell survival [8]. Metastasis occurs when cancer cells succeed in suppressing the immune system (decreases the activity of natural kinase cells) [9]. Virtually, all perioperative antineoplastic treatment creates relative immunosuppression [10]:

- manipulations on the tumor during surgery favor the penetration of its cells into the systemic circulation;
- the presence of the primary tumor is an inhibitor of angiogenesis and its removal eliminates the defense mechanism;
- perioperative immunosuppression, which primarily influences cellular immunity. A negative role is played by neuroendocrine and inflammatory components in response to stress, but also by the action of preparations administered during anesthesia and postoperative analgesia;
- hypothermia can also be attributed to the suppression of immune function.

The physiological response to stress in surgery causes relative immunosuppression through the release of hormonal mediators: catecholamines, prostaglandins, and growth factors [11]. Prostaglandins and catecholamines can cause activation of receptors that increase the metastatic capacity of cancer cells (e.g., β2-adrenergic) [12] and cyclooxygenase-2 receptors [13]. Inflammation associated with tissue trauma results in the release of cytokines (interleukin-6 and prostaglandin E2) that can cause inhibition of natural killer (NK) cell activity [14]. The role of NK cells is essential in the perioperative phase as they are responsible for the detection and destruction of circulating tumor cells [15].

Another factor contributing to cancer recurrence is tissue hypoxia. This causes an increase in the expression of transcription factor 1-alpha (HIF1A), which plays an important role in promoting cellular pathways for angiogenesis, cell proliferation, and metastasis [16]. The mechanism of action of HIF1A is to determine the expression of vascular endothelial growth factor (VEGF) [17]. This, in turn, stimulates tumor growth and angiogenesis, which can remodel lymphatic pathways, allowing metastasis of tumor cells [18, 19].

Hemotransfusion is associated with increased risk of metastasis [20]. Transfused blood induces immunosuppression. There are scientific data that show decreased natural killer cells, T-helper cells, and likewise decreased cytokine production [21]. The term immunomodulation induced by hemotransfusion was introduced in 1973. It is also associated with increased risk of cancer recurrence in patients given blood components preoperatively [22]. Hemotransfusion is associated with increased risk of metastasis [20]. Transfused blood induces immunosuppression. There is scientific evidence of decreased natural killer cells, T-helper cells and decreased cytokine production [21]. The term

immunomodulation induced by hemotransfusion has been proposed in 1973. This is also associated with increased risk of cancer recurrence in patients who are transfused blood components perioperatively [22]. Perioperative hypothermia is a factor that increases the risk of wound infection. It is considered, that maintaining a normal temperature in a patient during the perioperative period is more effective, than antibiotic prophylaxis. Hypothermia during general anesthesia inhibits cellular immunity, especially activity of natural killer cells, thus increasing cancer recurrence [23]. For example, intraoperative decrease of body temperature down to 35.5 ºC, in patients who underwent abdominal surgery, has a the immunosuppressive effect[24].

Effects of anesthesia

Clinical trials on the effects of anesthetics on cancer are complicated to conduct because patients need a combination of anesthetic agents. It would be medically and ethically difficult to perform surgery without perioperative pain relief e.g., using only regional anesthesia. Usually, intraoperative analgesia includes both regional analgesic and general anesthetic components. In the following paragraphs, we describe some comparative studies between local and general anesthesia on cancer metastasis. These have been performed on patients with tumors form different location who underwent surgery.

The first large randomized trial in this field for breast cancer surgery and was published in 2019 on approximately 2100 patients who underwent mastectomy or local surgery with axillary dissection, and were randomly assigned to receive regional anesthesia/analgesia (paravertebral block combined with sedation) or general anesthesia (inhalation, opioid analgesia) [25]. Cancer recurrences were similar in both groups: general/combined anesthesia occurring in 10% of patients in each group during a 36-month follow-up. Another study that included more than 1,700 patients who underwent major abdominal or thoracic surgery and were similarly randomized: epidural + general anesthesia versus general anesthesia + postoperative opioids. Thus, overall survival and recurrence-free survival were similar in the two groups at more than five years of follow-up [26]. In a randomized trial including 400 patients with lung cancer who underwent video-assisted thoracoscopic surgery, we have the following results: relapse-free survival and overall survival were similar in both patients receiving general anesthesia + postoperative opioid analgesia and those receiving general anesthesia + postoperative epidural analgesia [27]. In the next paragraphs, we will show the results from other scientific publications in which research has been described regarding the role of anesthetics in the development of metastases according to their mode of action.

General anesthesia (inhalation)

Laboratory studies have suggested some possible mechanism by which inhalational anesthetics may promote metastasis [28-31]. Inhalational anesthetic agents (e.g., isoflurane, sevoflurane, desflurane, and halothane) also have pro-inflammatory effects [32].

General (intravenous) anesthesia

According to multiple researchers that studied the effects of anesthetics on natural killer (NK) cell activity and metastasis development in rats modelled with breast cancer, propofol did not suppress NK cell activity nor did metastases develop, while halothane, ketamine and thiopental both decreased NK cell activity and metastases developed [33].

Regional anesthesia/analgesia

Regional anestheisa/analgesia could reduce cancer recurrence through several mechanisms, for example:

- decreasing the stress response to surgery (by pain control or sympathetic blockade) [34, 35];
- reducing the need for opioids or inhalants;
- via direct effects related to absorption of local anesthetics.

This type of anesthesia/analgesia is the most intensely discussed by the medical community because of the prospects for their use in general surgery as well as in oncology. Thus, we will point to studies that have been performed on large groups of patients.

The first large international randomized trials were conducted comparing regional anesthesia + propofol and general anesthesia with sevoflurane + opioids. It found similar relapse rates after surgery for breast cancer [25]. The same results were obtainted in two other studies describing epidural versus intravenous general anesthesia + opioids [26, 27]. In 2014, more than 3,000 people were included in studies and underwent cancer surgery. According to these data, no difference in cancer recurrence or overall survival was observed in patients given general anesthesia + epidural and general anesthesia alone [36]. Subsequently in 2017 were conducted 28 studies (retrospective, observational and randomized) including more than 67,000 patients who underwent surgery for multiple cancers. Overall and relapse-free survival was the same in those who received regional anesthesia with or without general anesthesia [37]. Ten other retrospective studies including approximately 13,760 individuals, after radical prostatectomy, with a diagnosis of prostate cancer were also reviewed. According to the results of these studies, regional anesthesia with or without general anesthesia, was described with better overall survival, but similar cancer recurrences with general anesthesia alone [38]. In conjunction with follow-up studies, investigations of the immune status of breast cancer patients treated with surgery given propofol + paravertebral regional anesthesia and inhaled general anesthesia + opioids was performed. Thus, it has been shown that in patients receiving regional and intravenous anesthesia in breast cancer tissue there is both increased infiltration with immune cells, increased apoptosis of cancer cells and maintenance of NK cell cytotoxicity [29, 30, 39].

Amide local anesthetics, particularly lidocaine, have been used for a long time in pain management during general anesthesia, as well as systemic intravenous infusions, in neuraxial and peripheral nerve blocks. Lidocaine is a

short-acting substance with minimal toxicity. It blocks voltage-dependent sodium channels, which are responsible for the generation of impulses in sensory nerve endings and conduction of pain impulses through nerve fibers. [40-42] Along with its analgesic effect, lidocaine also exhibits antioncogenic and anti-inflammatory properties through various pathways [43-47]. In general, local anesthetics, according to some studies, have antitumor and antimetastatic properties. These effects are achieved through several mechanisms, such as:

(1) direct cytotoxicity, induction of apoptosis;
(2) inhibition of proliferation, migration and invasion;
(3) modulation of gene expression through methylation [48].

Local anesthetics (LA) in high concentrations are known to be cytotoxic to neuronal cells. This seems to be correlated with the lipid solubility of LA. As a result, this process includes cell death by necrosis or apoptosis. All local anesthetics cause necrosis, and lidocaine and bupivacaine cause apoptosis in neuroblastoma cells [49, 50] as well as in breast and thyroid cancer cells [51, 52]. Apoptosis is controlled by an intracellular cysteine group – caspases. Chang and colleagues demonstrated that treatment of breast cancer cells with clinically relevant concentrations of lidocaine and bupivacaine induced caspase formation [51]. As a result, cell viability decreased and the process of apoptosis was triggered [53, 54]. Another article studied the action of lidocaine and bupivacaine on thyroid cancer cells, the latter induced apoptosis. This effect was mediated via the protein kinase pathway [51]. Local anesthetics also inhibit proliferation, migration and invasion of cancer cells. Yoon and colleagues introduced tetracaine and lidocaine into the tumor region. This has been shown to inhibit microtubule expansion and the ability of tumor cells to promote aggregation and reattachment [55]. LAs may also influence proliferation and invasion through their effects on cell signaling pathways [56, 57].

Several studies have been carried out on this issue:

- Mammamoto demonstrated that lidocaine, in clinically relevant concentrations, decreased the invasiveness of HT1080 cells by inhibiting HB-EGF excretion [58];
- Sakaguchi showed that lidocaine inhibited EGF-induced proliferation of tongue cancer cells [59];
- Piegeler demonstrated that amide local anesthetics reduced TNF-α-induced Src activation and ICAM-1 phosphorylation in human lung cancer cells and inhibited cancer cells [60];
- Baptista-Hon and colleagues demonstrated that ropivacaine inhibiting sodium channels decreases cell invasion. This is due to direct effects on cancer cells [61], but also indirect effects by blocking noxious stimuli [62].

Local anesthetics also inhibit cancer cell proliferation by modulating gene expression through DNA methylation. Lidocaine has been shown to demethylate DNA in breast cancer cells [63].

Since, cancer treatment is mainly surgical, used anesthesia must also be appropriate, meeting certain criteria. In general, this needs to be effective, safe and with as lowest risk of early and late postoperative complications as possible. To achieve this goal, the anesthesiologist has several methods in his arsenal: strong general anesthetics, effective analgesics both opioid and non-opioid, different types of loco-regional anesthesia/analgesia. Thus, nowadays, the radical method of treatment in oncology is considered surgery. However, its late results are insufficient, because the percentage of tumor recurrence is considerable. Cancer metastases are the cause of death in 90% of cases [64]. It is necessary for anesthetists working in cancer centers to differentiate between anesthesia/analgesia methods and their effects on cancer recurrence. Thus, research conducted by Ovechkin A. M. in 2012 at the First Moscow State Medical University *I. M. Sechenov*, showed us in major lines the anesthetic/analgesic remedies that influence the immune status of the oncological patient (Table 1, 2) [65].

Table 1. Remedies with suppressive effect on immune system during the perioperative period [65].

Drug	Potential action on anti-cancer immunity
Ketamine	Decreases the amount and activity of natural killer cells.
Thiopental	Decreases the amount and activity of natural killer cells.
Midazolam	Decreases the plasma concentration of IL-8 cytokines. This favors immunosuppression, because IL-8 is a factor that activates neutrophil chemotaxis and adhesion (important components for the normal immune response to surgical aggression).
Inhalation anesthetics	In the experiment, it inhibits interferon stimulation of natural killer cells. Sevoflurane *in vitro* decreases the clearance of tumor necrosis factor by natural killer cells. Decreased long-term results in melanoma interventions under inhalation anesthesia compared to regional anesthesia are demonstrated.
Nitrous oxide	In experiments, it causes the appearance and accelerates the formation of metastases in the lungs and liver. It is the most powerful stimulator of the formation of metastases in the liver among all the anesthetic preparations studied
Morphine	In the experiment it inhibits cellular immunity and the activity of natural killer cells
Fentanyl	Decreases the amount and activity of natural killer cells in clinic
α2-adrenoreceptor agonists (clonidine)	It accelerates cell proliferation and inhibits apoptosis. In the experiment, it favors the progression of mammary gland tumor growth

Note: IL-8 - Interleukin 8.

Table 2. Remedies with positive effect on immune system during the perioperative period. [65]

Drug	Potential action on anti-cancer immunity
Propofol	It has an immunoprotective effect, decreases the metastatic potential of a line of cancer cells, induces the process of apoptosis, increases the synthesis of anti-inflammatory cytokines IL-10
Local Anesthetics	Lidocaine inhibits the activity of the receptors of the endothelial growth factor and the proliferation of tumor cells (*in vitro*). Ropivacain inhibits the growth of tumor cells (*in vitro*)
Tramadol	In the experiment and in the clinic it stimulates the activity of natural killer cells; it does not allow the metastasis of the tumor which is induced by the surgical intervention (experimental data)
Nonsteroidal anti-inflammatory drugs	In the experiment, the negative action on angiogenesis and tumor growth is demonstrated, induce apoptosis, balance the negative action of morphine on the immune status
Blockers of β-adrenoreceptors	In the experiment it inhibits tumor growth, which is determined by β-adrenergic stimulation

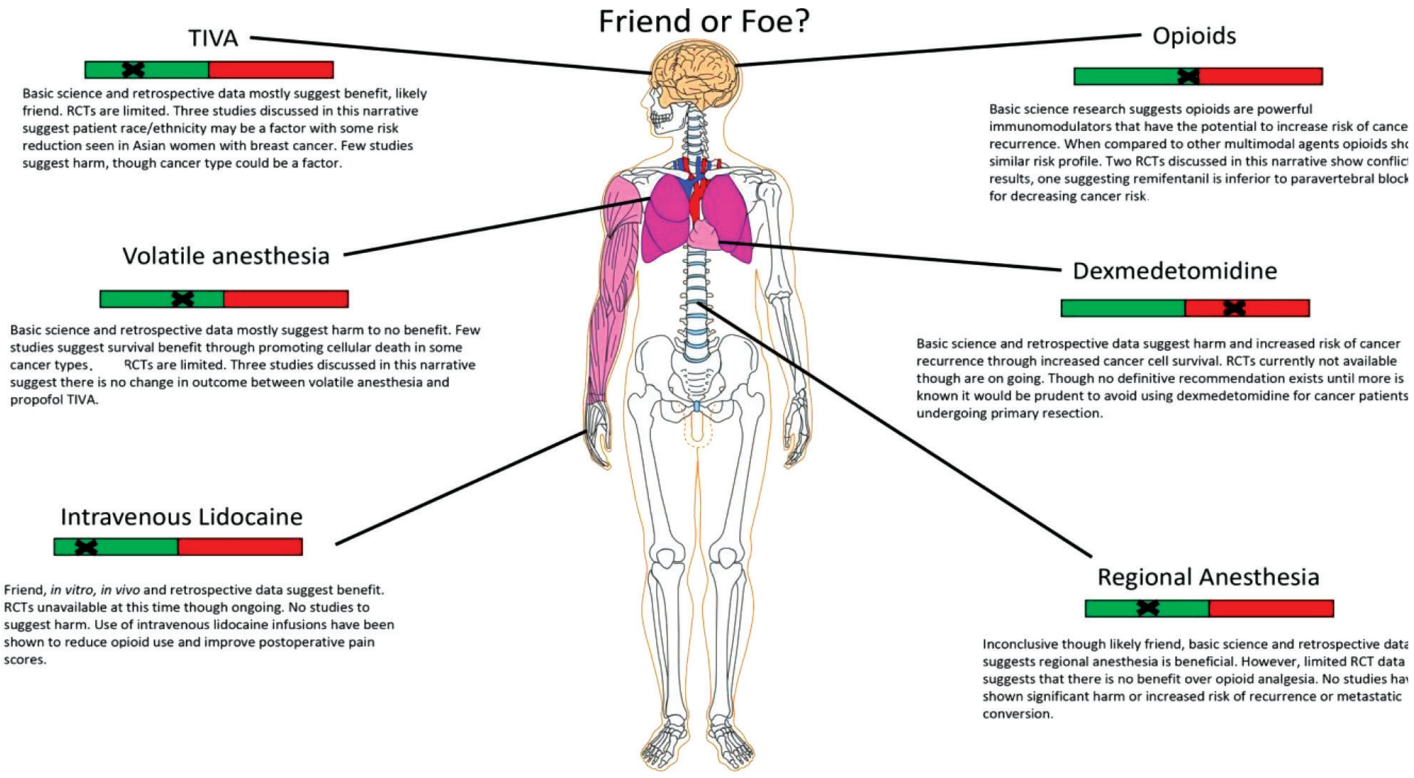

Fig. 1 Anesthesia and Cancer, Friend or Foe?

Julio Montejano and Vesna Jevtovic-Todorovic in 2021 wrote an article: *Anesthesia and cancer, Friend or foe? A narrative analysis.* In the article, they exemplified the types of anesthesia and their interaction with cancer (Fig. 1) [66].

Conclusions

Surgical treatment in cancer patients is associated with a multitude of factors that directly or indirectly may influence tumor cell survival, inflammatory and stress responses to surgical aggression. Inhalational anesthetics have pro-inflammatory effects and may influence cancer cell survival, including immune suppression and up-regulation of hypoxia-inducible factors (HIF1A). However, experiments on animal and human regarding cancer recurrence after the use of inhalational agents were conflicting. Propofol has anti-inflammatory and antioxidant effects that protect against immune suppression and may preserve natural killer (NK) cell activity. Clinical trials comparing intravenous and inhaled anesthetic agents have shown mixed results in terms of cancer recurrence. Regional anesthesia could decrease cancer recurrence by reducing the need for opioids or inhaled anesthetics, or by reducing the stress response during surgery. Other studies suggest that opioids might influence metastasis or tumor growth, however, the evidence is conflicting and inconclusive. There is *in vitro* scientific evidence of a protective effect of systemic lidocaine on recurrent cancer, although relevant clinical data are limited.

Conflict of interest

The authors declare no conflict of interest, financial and non-financial, associated with the subject of this paper.

Authors' contribution

All authors contributed equally to the drafting and writing of the manuscript. The authors read and approved the final version of the manuscript.

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MONOGRAPH REVIEW

"Gout: new advances in the diagnosis and management of an old disease"

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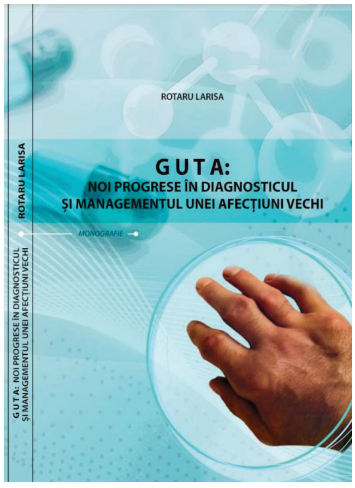

Gout is a metabolic disorder characterized by the deposition of uric acid in the form of excess sodium urate in the blood in the joints, leading to repeated episodes of arthritis.

Most commonly, the condition is monoarticular, with a preference for the joints of the lower limbs: metatarsophalangeal, tibiotarsal, and knee. Deposition also occurs in other systems and organs: renal, leading to uric lithiasis and uric nephropathy, and tegumentary, leading to the formation of gouty tophi.

Although it occurs more frequently in males (6:1 ratio), gout is also found in women, with a much-reduced incidence in the premenopausal period, but after the age of 65 it becomes more frequent and even causes the sex ratio to equalize after the age of 85. This distribution is largely due to estrogen hormones, the decrease in estrogen levels, as well as the development of degenerative joint damage explaining the higher frequency of gout in the post-menopausal period.

Genetic factors, alcohol consumption, diuretic treatment and kidney disease (especially chronic renal failure) are considered the main risk factors for gout, and the presence of metabolic syndrome (association of hyperglycemia or diabetes with obesity, dislipidemia and hypertension) is associated with a poor prognosis and a high mortality rate.

The topicality of the topic is conditioned by the increasing prevalence of gout everywhere. So far in many practical cases of gout treatment some signs and aspects have been detected, which imply that gout as a disease, is not characterized by a purely somatic nature, but also has a genetic aspect. Based on these considerations, early detection of patients with a genetic predisposition to gout is of considerable theoretical and practical interest.

Recently, the pathogenesis of gout has been described in more detail, new risk factors have been discovered, molecular mechanisms of renal urate transport, and mechanisms of crystal-induced joint inflammation have been studied. More and more often, gout has an atypical clinical picture, many cases of chronic evolution of the disease, oligo-, and polyarticular joint damage are observed, and therefore early diagnosis of the disease is difficult.

A major problem is comorbidity: hypertension, obesity, metabolic syndrome, type II diabetes, chronic kidney disease, osteoarthritis, psoriatic arthritis, which influence hyperuricemia. In NHANES research, the link between serum levels of AU and increased cardiovascular lethality was identified.

The monograph contains the introductory part and 12 chapters: definition, epidemiology, etiopathogenesis, genetic aspects, classification of gout, clinical picture, diagnosis of gout, management, prophylaxis, prognosis, conclusions, recommendations for diagnosis and treatment.

The materials and methods used will enable the study of changes in the general condition of patients with gout, the assessment of the degree of activity in patients with gout and the individual pharmacological correction of secondary changes detected in patients with gout.

In conclusion, I would like to emphasize that the monograph under the title ***"Gout: new advances in the diagnosis and management of an old disease"*** that the content of the monograph is argued in a complex way, which will allow to establish the main directions of conducting research and treatment of gout. The monograph meets all the conditions and requirements of a monograph, is current and useful for rheumatological practice, and is of interest primarily to students, residents, doctoral students, and medical practitioners.

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MONOGRAPH REVIEW

„Safety of anesthesia outside the operating room”

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Technological advances and the ageing population have increased the demand for complex diagnostic procedures and minimally invasive surgery. As a result, there is an increasing demand to anaesthetize patients undergoing procedures outside the operating room. Due to increased anesthetic safety, with the development of modern monitoring methods, new drugs, anesthesia protocols it has been possible to rapidly expand the areas requiring sedation, procedural analgesia. In a modern hospital, 10-15% of the work of an anesthesia department is carried out outside the operating room (not counting the field of intensive care). Almost every clinic/discipline needs anesthesiology support and anesthesiology work is required in various areas such as Radiology, Endoscopy, Gastroenterology, Cardiology, Psychiatry, Pediatrics, and Emergency Departments etc.

Some patients require procedural sedation, others anesthesia, sometimes with intubation, but the common factor is the obligatory need of monitoring the vital functions, the presence of equipment and staff trained to intervene in emergencies.

Performing anesthesia outside the operating room poses some specific difficulties. Most procedures are carried out in locations far from the operating room, which may make it necessary to organize extra conditions for patient safety, and in case of difficulties help may be late. In order to provide safe and skilled anesthetic care outside the operating room, it is important that anesthesiologists build a systemic and uniform structure that incorporates all provisions across all categories of anesthetic care. These criteria must be applied equally throughout the hospital, not only to ensure patient safety, but also to ensure the safety of healthcare providers. Since anesthesiologists are responsible for the safety of the patient as well as themselves, these standards should not be neglected. This review examines the potential problems and risks associated with sedation and anesthesia performed for various procedures outside the operating room.

The monograph is divided into an introductory part and 6 chapters. The introductory part covers general topics such as the challenges of anesthesia outside the operating room, preoperative assessment, monitoring, anesthetic techniques and recovery. Staff management and planning are discussed as well as issues related to outcomes, regulation and quality improvement.

The following chapters describe, in a detailed and informative manner, the anesthetic requirements in specific conditions, such as interventional radiology, magnetic resonance imaging, gastrointestinal endoscopy, electroconvulsive therapy, delivery room, in vitro fertilization, etc. Anesthesia in the cardiac catheterization laboratory is dealt with in a separate chapter together with anesthesiological care in pulmonology. This chapter deals with anesthesia and sedation for coronary angiography, cardiac catheterization, electrical cardioversion of the heart, transesophageal ultrasound - some of them are already known procedures, others are new both in the world and in the Republic of Moldova (such as transesophageal ultrasound).

This monograph provides useful recommendations on increasing patient safety as a result of collaboration and planning between anesthesiologists and team members depending on the procedure (cardiologists, pulmonologists, gastroenterologists, psychiatrists, obstetricians). A common knowledge base and mutual respect for each discipline contributing to the procedure are essential elements in the formula for success and patient safety.

The monograph is part of the efforts undertaken by the Department of Anesthesiology and Reanimatology No.1 „Valeriu Ghereg” to increase the safety of anaesthesia for any procedure both in and out of the operating room.

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Toate persoanele care au adus contribuţii importante la lucrul raportat în manuscris (de exemplu, ajutor tehnic, scris şi asistenţă la editare, suport general), dar care nu îndeplinesc criteriile de autor, sunt menţionate în secţiunea *„Mulţumiri",* iar acestea şi-au dat acordul în scris ca să fie menţionate. Dacă secţiunea *„Mulţumiri"* lipseşte din manuscris, atunci acest fapt semnifică că nu au existat contribuţii substanţiale din partea non-autorilor.

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Prezenta declaraţie este semnată de către toţi autorii:

(puteţi utiliza o fotocopie a formularului dat în cazul existenţei mai mult de 6 autori)

Numele autorului (tipărit)	Semnătura autorului	Data
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MJHS Moldovan Journal of Health Sciences
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AUTHORSHIP STATEMENT

Manuscript title: _____
_____
_____

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in the *Moldovan Journal of Health Sciences.*

Authorship contributions:

Please indicate the specific contributions made by each author (list the authors' initials followed by their surname(s), e.g., *A. Belîi, Gh. Rojnoveanu*). The name of each author must appear at least once in each of the three categories below.

Category 1

Conception and design of study: _____, _____, _____, _____;
Acquisition of data: _____, _____, _____, _____;
Analysis and/or interpretation of data:_____, _____, _____, _____.

Category 2

Drafting the manuscript: _____, _____, _____, _____;
Revising the manuscript critically for important intellectual content:_____, _____.

Category 3

Approval of the version of the manuscript to be published (the names of all authors must be listed):
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Acknowledgements:

All persons who have made substantial contributions to the work reported in the manuscript (e.g., technical help, writing and edit- ing assistance, general support), but who do not meet the criteria for authorship, are named in the „*Acknowledgements*" and have given us their written permission to be named. If we have not included an „*Acknowledgements*", then that indicates that we have not received substantial contributions from non-authors:

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Author's name (typed)	**Author's signature**	**Date**
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SCRISOARE DE ÎNSOŢIRE

Titlul manuscrisului: _____
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Relevanţa manuscrisului pentru scopul promovat de Revistă: _____
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Noi, autorii subsemnaţi ai manuscrisului, declarăm că (bifaţi):

- ☐ lucrarea menţionată este originală;
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- ☐ toţi autorii subsemnaţi au contribuit la elaborarea manuscrisului;
- ☐ de la subiecţii incluşi în studiu a fost obţinut consimţământul informat;
- ☐ toţi autorii subsemnaţi au aprobat versiunea finală a manuscrisului;
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Prin prezenta, autorii sunt de acord să transfere drepturile de proprietate (copyright) Revistei de Ştiinţe ale Sănătăţii din Moldova – Moldovan Journal of Health Sciences, în caz că manuscrisul va fi publicat.

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Datele de contact ale autorului corespondent:

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COVER LETTER

Manuscript title: _____
_____

Relevance of the manuscript for the Journal purposes: _____
_____
_____
_____

Contributions of the manuscript for to the research field: _____
_____
_____
_____

What is the added value of the manuscript to the already published scientific literature: _____
_____
_____
_____

We, the undersigned authors of the manuscript, declare that (please, tick):

- ☐ the paper contain original data;
- ☐ the paper has not been published before;
- ☐ the manuscript is not submitted for publication to another journal;
- ☐ all authors have contributed to the manuscript;
- ☐ the informed consent were obtained from all study subjects;
- ☐ all coauthors approved the final version of the manuscript;
- ☐ we agree for checking of the manuscript for plagiarism;
- ☐ any potential conflicts of interest were disclosed.

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Revista de Ştiinţe ale Sănătăţii din Moldova

Moldovan Journal of Health Sciences

Ghidul autorului

Criterii pentru publicare

Articolele originale trebuie să conţină cercetări noi (originale), rezultatele cărora contribuie la acumularea de noi cunoştinţe în domeniul publicat şi cu condiţia că rezultatele prezentate nu au mai fost publicate înainte sau nu sunt depuse, în paralel, la o altă revistă, în vederea publicării.

Manuscrisele prezentate trebuie să corespundă standardelor STROBE (http://www.strobe-statement.org).

Prezentarea manuscrisului

Manuscrisele trebuie să fie prezentate doar în formă electronică, în limba română sau engleză. Dacă manuscrisul a fost depus doar în limba română, odată ce a trecut procedura de recenzare internă, acesta va fi tradus integral de către autori în limba engleză, pentru a putea trece procedura de recenzare externă. În acelaşi manuscris se permite utilizarea *US English* sau *British English*, dar nu mixt. Varianta tipărită (hârtie) nu va fi acceptată. Doar autorul corespondent va putea depune manuscrisul la redacţie; tot el va deţine responsabilitatea completă de procesul de depunere, de corespondenţa cu redacţia pe durata procesului de publicare.

Procesul de publicare poate fi amânat, întrerupt sau anulat, la discreţia autorului corespondent. Odată manuscrisul depus, autorul corespondent va primi un cod electronic de identificare a manuscrisului, pe care îl va folosi în corespondenţa ulterioară cu redacţia.

În scopul menţinerii integrităţii editoriale şi a standardelor internaţionale de calitate, Redacţia *Moldovan Journal of Health Sciences – Revista de Ştiinţe ale Sănătăţii din Moldova* utilizează un sistem de detectare a plagiatului şi va supune manuscrisul unei verificări antiplagiat. Depunerea manuscrisului pentru publicare înseamnă, implicit, acordul tuturor autorilor cu verificarea lui antiplagiat. În cazul suspectării că manuscrisul depus a încălcat politicile de publicare, acesta poate fi suspendat sau respins, indiferent de etapa procesului de publicare.

Scrisoarea de însoţire

La depunere, autorul corespondent va anexa la manuscris o scrisoare de însoţire. Formularul tipizat al Scrisorii de însoţire este oferit de către Redacţie. Scrisoarea de însoţire include: (1) titlul manuscrisului; (2) o scurtă descriere despre relevanţa manuscrisului pentru scopul promovat de Revistă; (3) contribuţiile aduse de manuscris pentru domeniul său; (4) modul în care manuscrisul adaugă valoare la literatura ştiinţifică de specialitate; (5) numele şi semnăturile tuturor coautorilor; (5) datele complete de contact ale Autorului corespondent, cu menţionarea instituţiei şi adresei instituţionale, nr. de telefon, nr. de fax şi adresa e-mail.

Instructions for Authors

Criteria for publication

Original articles should contain new (original) results, which bring new knowledge in the field. The submitted manuscripts should contain data unpublished before and not submitted in parallel for publication to another journal.

Manuscripts submitted must meet STROBE standards (http://www.strobe-statement.org).

Manuscript submission

Manuscripts must be submitted only in electronic form in Romanian or English. Once past the internal reviewing procedure, the manuscript was submitted only in Romanian will be fully translated by the authors in English to pass the external reviewing procedure. In the manuscript are allowed to use U.S. English or British English, but not mixed. Printed version (paper) will not be accepted. Only the corresponding author may submit the manuscript. The corresponding author holds full responsibility of the submission and correspondence with the editor during reviewing and publication process.

The publication of the manuscript may be postponed, stopped or canceled at the request of the corresponding author. Once the manuscript is submitted, the corresponding author will receive an electronic identification code of the manuscript, which should be used for subsequent correspondence with the editor.

In order to maintain editorial integrity and international quality standards, editor of the Moldovan Journal of Health Sciences reserves the right to use a plagiarism detection system. Thus the submitted manuscript will be checked for plagiarism. Manuscript submission involves agreement of all coauthors for checking for plagiarism. If the submitted manuscript violates copyright policies; it can be suspended or dismissed, regardless of the stage of the publishing process.

Cover letter

A submitted manuscript should be accompanied by a Cover letter. A template of Cover letter is provided by editor. Cover letter should include: (1) the title of the manuscript; (2) a short statement regarding the relevance of the manuscript for the journal proposes; (3) contributions of the manuscript for to field; (4) what is the added value of the manuscript to the already published scientific literature; (5) the names and signatures of all coauthors; (5) the full contact details of corresponding author, indicating the institution and institutional address, no. telephone, no. fax and e-mail.

In the Cover letter, the corresponding author should clearly indicate that: (1) the paper contain original data; (2) the paper has

În scrisoarea de intenție, Autorul corespondent trebuie să indice în mod clar că: (1) lucrarea menționată este originală; (2) lucrarea menționată nu a fost publicată anterior; (3) lucrarea menționată nu este depusă pentru publicație în altă revistă; (4) toți autorii subsemnați au contribuit la elaborarea manuscrisului; (5) de la subiecții incluși în studiu a fost obținut consimțământul informat; (6) toți autorii subsemnați au aprobat versiunea finală a manuscrisului; (7) acordul implicit de verificare antiplagiat al manuscrisului; (8) au fost declarate orice potențiale conflicte de interes. De asemenea, Autorul corespondent poate include orice informație suplimentară în Scrisoarea de intenție, dacă consideră că aceasta poate fi utilă pentru Redacție.

Consimțământul informat

Orice manuscris care comunică rezultate experimentale, obținute de la subiecți umani, trebuie să fie bazat pe studii, în care a fost obținut consimțământul informat de la subiect (ți) și/sau tutore (i). În scrisoarea de intenție, autorul corespondent trebuie să indice în mod clar obținerea consimțământului informat. În caz de necesitate, Redacția este în drept să solicite probe suplimentare, care atestă obținerea consimțământului informat.

Comitetul de Etică

Pentru orice studiu experimental, efectuat pe oameni sau animale, este necesar de a menționa evaluarea etică a proiectului de cercetare. În acest sens, în articol vor fi menționate numărul procesului verbal și data ședinței Comitetului de Etică, când a fost aprobat proiectul de cercetare.

Permisiuni

În conformitate cu ghidurile Comitetului Internațional al Editorilor Revistelor Medicale (*ICMJE Guidelines*), în cazul când în manuscrisul prezentat este folosită sau reprodusă o informație publicată anterior, sau un material cu drepturi de autor, este de responsabilitatea Autorului corespondent să obțină permisiunea în scris a deținătorului de drepturi (*Copyright*) și să citeze corect sursa originală. Cu scopul de a menține transparența, se recomandă ca această permisiune, sub formă de copie, să fie depusă împreună cu manuscrisul.

Fotografii cu pacienți identificabili

În conformitate cu ghidurile internaționale ale Comitetului de Etică a Publicațiilor (*COPE Guidelines*), în cazul când în imaginile prezente în manuscris (fotografii, radiograme, rezultate de laborator, rezultatele investigațiilor paraclinice, înregistrări video sau sonore ș. a.) o persoană este identificabilă fizic, de la aceasta trebuie obținută o permisiune în scris de utilizare a imaginii date. Se recomandă ca permisiunea dată să fie depusă împreună cu manuscrisul, iar în manuscris să fie stipulat în mod clar, că această permisiune a fost obținută.

Specificarea medicamentelor și dispozitivelor

În manuscris se vor utiliza nume generice de medicamente, urmate, dacă este cazul, de denumirea lor comercială între paranteze. Pentru medicamente și dispozitive, includeți numele producătorului și localizarea acestuia (țara de origine).

Formatul fișierelor

Se acceptă următoarele formate de text pentru manuscrisul principal: Microsoft Word (97, 2003, 2007, 2010) și formatele „.rtf", „.doc", „.docx". Se acceptă următoarele formate pentru imag-

not been published before; (3) the manuscript is not submitted for publication to another journal; (4) all authors have contributed to the manuscript; (5) the informed consent were obtained from all study subjects (6) all coauthors approved the final version of the manuscript; (7) agreement for checking of the manuscript for plagiarism; (8) any potential conflicts of interest were disclosed. Corresponding author may include in the Cover letter any other additional information which could be useful for the editor.

Informed consent

Manuscripts that report experimental results obtained on human subjects must be based on studies in which informed consent was obtained from study subjects and/or their legal representative. The corresponding author should clearly indicate in his letter of intention about the obtaining of the informed. Editor reserved the right to request additional evidence attesting the obtaining of the informed consent.

Ethic Committee

For any experimental study conducted on humans or animals, it is necessary to mention in the article the ethical evaluation of the research project (such as date of evaluation and reference number of approval).

Permissions

In accordance with the guidelines of the International Committee of Medical Journals Editors (ICMJE Guidelines) if the submitted manuscript used or reproduced information/material previously published or copyrighted is the responsibility of the corresponding author to obtain a written permission from the owner of the copyright and properly cite the original source. In order to maintain transparency, it is recommended to submit the permission, as a copy, along with the manuscript.

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In accordance with international guidelines of the Publications Committee of Ethics (COPE Guidelines), if the manuscript contains pictures (photographs, radiograms, laboratory results, results of laboratory investigations, videos or sound etc.) which allows physical identification of the person, it must be obtained a written permission for the use of the image data. It is recommended to submit the permission along with the manuscript. Also in the manuscript text should be clearly stated that permission was obtained.

Drugs and devices specifications

In manuscript generic names of drugs, followed by their trade name in parentheses (if appropriate) should be used. For drugs and devices, manufacturer's name and location (country of origin) should be mentioned.

Files format

The following file formats for manuscript text are accepted: Microsoft Word (97, 2003, 2007, 2010) ".rtf", ".doc", ".docx". Pictures should be submitted in one of the following formats: ".jpeg", ". tiff", ".eps ", ".ppt", , ".pptx". The images could be transmitted also, in a format item ".ppt" or ".pptx" (one image one slide). Scanning

ini: „.jpeg", „.tiff", „.eps", „.ppt". „.pptx". Este posibil ca imaginile articolului să fie transmise în format „.ppt" sau „.pptx" (o imagine – un slide). Calitatea imaginilor, indiferent de format, trebuie să fie, minim: pentru desene – 800 dpi, pentru imagini cu detalii fine – 1000 dpi, pentru imagini alb-negru – de 300 dpi.

Structura manuscrisului

Publicaţia Periodică *Moldovan Journal of Health Sciences – Revista de Ştiinţe ale Sănătăţii din Moldova* respectă recomandările STROBE de raportare a cercetărilor observaţionale biomedicale. Pentru a vă uşura procesul de elaborare şi structurare a manuscrisului, vă recomandăm să consultaţi informaţia respectivă, disponibilă online, pe site-ul www. strobe-statement.org.

Volumul textului unui manuscris nu trebuie să depăşească 6000 de cuvinte. Cu toate că numărul figurilor şi tabelelor în manuscris rămâne la discreţia autorilor, se recomandă ca numărul lor să fie limitate la 5, pentru a nu reduce din lizibilitatea articolului pe paginile Revistei.

Structura unui articol original trebuie să respecte următoarea consecutivitate:

- Titlul lung (formulat în conformitate cu ghidurile STROBE)
- Numele şi prenumele complete ale autorului (autorilor)
- Afilierile autorului (autorilor)
- Datele de contact ale autorului corespondent
- Titlul scurt (va fi utilizat în calitate de colontitlu pe paginile Revistei)
- Elementele scoase în evidenţă din articol:
  - Ce nu este, deocamdată, cunoscut la subiectul abordat (descris în 1-3 fraze)
  - Ipoteza de cercetare (formulată în 1-2 fraze)
  - Noutatea adusă de articol literaturii ştiinţifice din domeniu (limitată la 1-3 fraze).
- Rezumatul articolului (compus din: introducere, materiale şi metode, rezultate, concluzii), limitat la maximum 350 de cuvinte.
- Cuvinte cheie
- Introducere
- Materiale şi metode
- Rezultate
- Discuţii
- Concluzii
- Lista abrevierilor utilizate (dacă este cazul)
- Declaraţia de conflict de interese
- Contribuţiile autorilor
- Mulţumiri şi finanţare (dacă este cazul)
- Referinţe bibliografice
- Tabele şi legende la tabele (dacă este cazul)
- Ilustraţii şi figuri (dacă este cazul)
- Legendele figurilor (dacă este cazul)
- Descrierea datelor suplimentare, anexe (dacă este cazul)

Pe pagina de titlu a manuscrisului trebuie să fie prezente următoarele elemente:

- **Titlul manuscrisului:** formulat în conformitate cu ghidurile STROBE, trebuie să fie laconic, relevant pentru conţinutul manuscrisului, să reflecte tipul (*design*-ul) studiului şi să nu depăşească 25 de cuvinte. Nu se admit prezenţa abrevierilor în titlu.
- **Titlul scurt** (ce va fi utilizat drept colontitlu pe paginile Revistei) reprezintă o versiune scurtă, de esenţă, a titlului complet.

resolution should be as follows: drawings – at least 800 dpi, fine line images – 1000 dpi and greyscale images – at least 300 dpi.

Structure of the manuscript

Moldovan Journal of Health Sciences follows STROBE recommendations for reporting observational biomedical research studies. To facilitate the development of the manuscript, please consult this information available online at www.strobe-statement.org.

The volume of the manuscript text should not exceed 6000 words.

Although, the number of figures and tables in the manuscript is at the discretion of the authors, in order to not reduce article legibility it is recommended to limit their number to five.

Structure of original article must comply with the following sequence:

- Full title (according to the STROBE guidelines)
- Full authors' name
- Authors' affiliations
- Contact details of corresponding author
- Short title (to be used as a running head on the journal)
- Article highlights:
  - What is not yet known on the issue addressed in the submitted manuscript (described in 1-3 sentences)
  - The research hypothesis (described in 1-2 sentences)
  - The novelty added by manuscript to the already publishedscientific literature (limited to 1-3 sentences).
- Abstract (consisting of background, materials and methods, results and conclusions), to not exceed 350 words.
- Keywords
- Introduction
- Materials and methods
- Results
- Discussions
- Conclusions
- List of abbreviations used (if applicable)
- Declaration of conflict of interests
- Authors' contributions
- Acknowledgements and funding (if applicable)
- References
- Tables and tables' captions (if applicable)
- Pictures and figures (if applicable)
- Figures' legends (if applicable)
- Description of additional data, appendices (if applicable)

The cover page of the manuscript should include:

- **Title of the manuscript:** written according to the STROBE guidelines, should be concise, relevant to the content of the manuscript, and reflect the study design. The title length should not exceed 25 words. It is not allowed the presence of abbreviations in the title.

- **Short title:** (to be used as a running title) is a short version of the essential of the full title. Short title will be limited to 40 characters, including spaces.

- **Author(s) name:** Authors list must include only those persons who had a substantial contribution to the work. Exam-

Va fi limitat la 40 de caractere, inclusiv spaţiile.

• **Numele autorului (autorilor).** Autori sunt numiţi doar acele persoane, care au avut o contribuţie substanţială la lucrare. Exemple de contribuţie esenţială la lucrare sunt: elaborarea *design*-ului studiului, recrutarea pacienţilor, participarea în colectarea datelor, analiza datelor, interpretarea rezultatelor, scrierea propriu-zisă a articolului, realizarea tehnică a testelor, investigaţiilor, realizarea imaginilor, formularea concluziilor. Pot fi citaţi până la 10 autori individuali. În cazul când grupul de lucru depăşeşte 10 autori individuali, vor fi citaţi în secţiunea „Numele şi prenumele autorilor" doar primii doi, iar restul vor fi menţionaţi la sfârşitul articolului, la secţiunea „Mulţumiri şi finanţare".

Membrii grupului de lucru, care nu îndeplinesc criteriile formale de autor enumerate, dar au avut o oarecare contribuţie la lucrare, pot fi menţionaţi în secţiunea „Mulţumiri şi finanţare".

Notă: Pentru a diferenţia autorul corespondent şi autorii care au contribuit în aceeaşi măsură la lucrare, folosiţi caractere speciale, ca exponenţi, la sfârşitul numelor lor:

(*) – pentru Autorul corespondent;

(†) – pentru Autorii care au avut o contribuţie egală. (De exemplu: Adrian Belîi*, Adrian Belîi†)

Nu se vor menţiona gradele şi titlurile ştiinţifice şi cele ştiinţifico-didactice.

• **Afilieri.** Afilierea autorilor se va scrie după secţiunea „Numele autorului (autorilor)". În acest sens, se va menţiona numele complet al instituţiei de afiliere a autorului (autorilor), localitatea şi ţara.

Afilierea se marchează cu cifre arabe, în superscript (de exemplu: Adrian Belîi[1])

- Elementele scoase în evidenţă din articol:
  - Ce nu este, deocamdată, cunoscut la subiectul abordat (descris în 1-3 fraze)
  - Ipoteza de cercetare (formulată în 1-2 fraze)
  - Noutatea adusă de articol literaturii ştiinţifice din domeniu (limitată la 1-3 fraze).

Din pagină nouă:

Rezumatul

Rezumatul trebuie să fie scris la timpul trecut, persoana a treia. Acesta trebuie să ofere un sumar concis al scopului, obiectivelor, rezultatelor semnificative şi concluziilor studiului, în limitele la 350 de cuvinte, organizate în următoarele secţiuni:

- **Introducere** – unde se va reflecta, pe scurt, contextul şi scopul principal al studiului;
- **Materiale şi metode –** cum a fost realizat studiul şi ce teste statistice au fost aplicate;
- **Rezultate** – prezintă rezultatele principale ale studiului;
- **Concluzii** – o scurtă trecere în revistă a constatărilor făcute, cu posibile implicări pentru studii ulterioare.

Nu utilizaţi abrevieri şi citaţii în rezumatul articolului.

Cuvintele cheie

Enumeraţi 4-10 cuvinte cheie, care sunt reprezentative pentru conţinutul articolului. Pentru a uşura găsirea articolului Dvs. de către motoarele de căutare ale bazelor de date, folosiţi termeni recomandaţi din lista de titluri cu subiect medical de pe http://nlm.nih.gov/ mesh.

Înregistrarea trialului clinic

În caz dacă articolul Dvs. comunică rezultatele unui trial clinic,

ples of essential contribution to the work are: developing of the study design, patients recruitment, participation in data collection, data analysis, interpretation of results, writing of the manuscript, performing of the tests, pictures taking, drawing conclusions. The authors list should not exceed 10 persons. If the research group exceed 10 individual authors, in the "Authors name" section first two will be cited, all others should be mentioned at the end of the article, in the "Acknowledgements and funding" section.

Members of the research group who do not meet the formal criteria of the authorship, but have had some contribution to the paper, may be mentioned in the "Acknowledgements and funding" section.

Note: To differentiate the corresponding author, as well as authors who have an equal contribution to the work, using special characters as a superscript index at the end of their names is recommended:

(*) – Corresponding author;

(†) – Authors with equal contribution. (e.g. Adrian Belii*, Adrian Belii†)

• **Affiliation:** Please state the full name of institution, city and country to which the author(s) is affiliated. Affiliation should be marked with Arabic numerals in superscript after the author(s) name (e.g. Adrian Belii[1])

- **Article highlights:**
  - What is not yet known on the issue addressed in the submitted manuscript (described in 1-3 sentences)
  - The research hypothesis (described in 1-2 sentences)
  - The novelty added by manuscript to the already published scientific literature (limited to 1-3 sentences).

From new page:

Abstract

The abstract should be written using the past tense, third person. It should provide a concise summary of the purpose, objectives, significant results and conclusions of the study. The summary text should not exceed 350 words organized into the following sections:

- **Introduction** – reflect in short the context and purpose of the study;
- **Materials and methods** – describe how the study was conducted and specify the applied statistics;
- **Results** – present the key results of the study;
- **Conclusions** – a brief overview of the findings, with possible implications for further studies.

Do not use abbreviations or citations in the abstract of the article.

Key words

List 4-10 keywords that are representative for the contents of the article. To facilitate finding of your article by search engines of electronic databases, use MESH keywords list (available on http://nlm. nih.gov/mesh).

Registered clinical trial

In case if your article reported the results of a clinical trial, please indicate Trial Register and the unique registration number of the trial.

vă rugăm să indicaţi Registrul trialului şi numărul unic de înregistrare a trialului.

Exemplu: *„Current Controlled Trials ISRCTN61362816”*. Atenţie! Nu trebuie să existe niciun spaţiu între literele şi cifrele numărului unic de înregistrare a trialului. Pentru mai multe informaţii, va rugam să accesaţi http://www.isrctn.org (*International Standard Randomised Controlled Trial Number*) şi http://www.icmje.org (*International Committee of Medical Journal Editors*).

Din pagină nouă:

Introducerea

Introducerea, scrisă la timpul trecut, persoana a treia, trebuie:

- să ofere informaţii care ar permite cititorilor din afara domeniului să intre în contextul studiului, să-i înţeleagă semnificaţia;
- să definească problema abordată şi să explice de ce aceasta este importantă;
- să includă o scurtă trecere în revistă a literaturii recente din domeniu;
- să menţioneze orice controverse sau dezacorduri relevante în domeniu;
- să formuleze ipoteza de cercetare şi să prezinte parametrul principal şi cei secundari de rezultat;
- să concludă cu scopul lucrării şi cu un comentariu care să ateste dacă scopul propus a fost atins.

Materiale şi metode

În secţiunea „Materiale şi metode” trebuie să fie descrise cu detalii suficiente procedurile efectuate. Aici se vor menţiona protocoalele detaliate privind metodele utilizate precum şi informaţii justificative. Se vor include: *design*-ul studiului, descrierea participanţilor şi materialelor implicate, descrierea clară a tuturor intervenţiilor şi comparaţiilor efectuate, precum şi testele statistice aplicate. Se vor specifica denumirile generice de medicamente. Atunci când în cercetare sunt folosite branduri, se indică în paranteze denumirea lor comercială. În cazul studiilor pe subiecţi umani sau pe animale, trebuie să fie menţionată aprobarea etică (data şi nr. procesului verbal al şedinţei Comitetului de Etică, preşedintele CE şi denumirea instituţiei, în cadrul căreia activează CE), precum şi consimţământul informat al persoanelor.

Rezultate

Rezultate şi discuţiile vor fi prezentate în secţiuni separate.

Autorii trebuie să prezinte rezultate clare şi exacte. Rezultatele prezentate trebuie explicate (nu justificate sau comparate, în această secţiune) cu constatări fundamentale, evident, referitoare la ipoteza care a stat la baza studiului. Rezultatele trebuie redate concis şi logic, cu accentuarea celor noi.

Discuţii

Se va descrie impactul, relevanţa şi semnificaţia rezultatelor obţinute în domeniul respectiv. Rezultatele obţinute se vor compara cu cele provenite din studiile anterioare din domeniu şi se vor trasa potenţiale direcţii viitoare de cercetare. Discuţiile trebuie să conţină interpretări importante ale constatărilor şi rezultatelor, în comparaţie cu studiile anterioare. De asemenea, se vor menţiona limitele studiului şi factorii potenţiali de *bias*.

Concluzii

Această secţiune trebuie să concludă laconic întregul studiu şi

E.g.: “Current Controlled Trials ISRCTN61362816”

Attention! There should be no space between letters and numbers of the unique record number of the trial. For more information, please visit http://www.isrctn.org (International Standard Randomized Controlled Trial Number) and http://www.icmje.org (International Committee of Medical Journal Editors).

From new page: Introduction

The Introduction section should be written using past tense, third person, and should:

- provide information that would allow readers outside of the field to enter the context of the study, to understand its meaning;
- define the problem addressed and explain why it is important;
- include a brief review of recent literature in the field;
- mention any controversy or disagreement existing in the field;
- formulate research hypothesis and present the main and secondary assessed outcomes;
- conclude with the research’ propose and a short comment whether the purpose has been achieved.

Materials and methods

“Materials and methods” section should present in sufficient details all carried out procedures. Here should be described protocols and supporting information on the used methods. It will include study design, subjects’ recruitment procedure, clear description of all interventions and comparisons and applied statistics. In the manuscript text the generic names of drugs should be used. When drug brands are used their trade name will be shown in parentheses. For studies on humans or animals a statement about ethical approval and informed consent of study subjects should be include. Please specify date and number of Ethics Committee (EC) decision, chair of the EC as well as institution within EC is organized.

Results

Results and discussion should be presented in separate sections. Authors must present results in a clear and accurate manner. Results should be explained (not justified or compared in this section) and include fundamental statements related to hypothesis behind the study. The results should be presented concisely and logically, emphasizing on new original data.

Discussions

Describe the impact, relevance and significance of the obtained results for the field. The results are compared with those from previous publications and draw potential future research directions. Discussions should include important interpretations of the findings and results compared with previous studies. Also, study limitations and potential bias should be mentioned.

Conclusions

This section should conclude laconically entire study, and highlight the added-value brought on the studied issue. The conclusions should not provide new information or double (repeat) those presented in the “Results” section.

să specifice, care este plus-valoarea adusă la informaţiile disponibile despre subiectul abordat. În concluzii nu se vor oferi informaţii noi şi nu se vor dubla (repeta) cele prezentate în secţiunea „Rezultate".

Abrevieri

Folosiţi numai abrevieri standard. De asemenea, pot fi formulate şi alte abrevieri, cu condiţia că acestea vor fi descifrate în text atunci când sunt utilizate pentru prima dată. Abrevierile din figuri şi tabele vor fi descifrate în legendă. Abrevierile trebuie folosite cât mai rar posibil.

Declaraţia de conflict de interese

După publicare, persoanele sau organizaţiile implicate în studiu vor deveni publice şi astfel poate fi influenţată reputaţia lor. Prin urmare, autorii trebuie să dezvăluie relaţia financiară sau non-financiară cu persoane sau organizaţii şi să declare conflictele de interese pentru datele şi informaţiile prezentate în manuscris. În conformitate cu ghidurile ICMJE, Autorul (autorii) trebuie să completeze o declaraţie privind Conflictele de interese, care va fi prezentată la sfârşitul articolului publicat.

Completând declaraţia referitoare la Conflictele de interes, se vor lua în consideraţie:

Pentru Conflicte de interese financiare

- specificaţi dacă vreo organizaţie are relaţie financiară cu lucrarea ştiinţifică reflectată în manuscris, inclusiv de finanţare, salariu, rambursări;
- menţionaţi, dacă articolul are un impact asupra organizaţiei date, ce ar genera pierderi sau profituri după publicare, în prezent sau în viitor;
- autorul (autorii) trebuie să precizeze dacă deţin cote de proprietate în orice organizaţie care ar putea să suporte pierderi sau să aibă profituri după publicare, în prezent sau în viitor. De asemenea, se recomandă să se specifice dacă autorul (autorii) deţin(e) sau aplică pentru orice drepturi de proprietate (brevet) în legătură cu conţinutul utilizat în manuscris;
- precizaţi dacă există oricare alte conflicte de interese.

Pentru Conflicte de interese non-financiare

- Vă rugăm să specificaţi oricare conflicte de interese non-financiare legate de politică, individuale, religioase, ideologice, educaţionale, raţionale, comerciale etc., care au legătură cu manuscrisul.

Contribuţia autorilor

Această secţiune a manuscrisului are rolul de a specifica contribuţia şi gradul de implicare a fiecărui autor. În acest sens, vă rugăm să respectaţi formatul exemplului propus: *„HW a conceput studiul, a participat la design-ul studiului şi a ajutat la redactarea manuscrisului. MG a efectuat procesarea exemplarelor, a metodelor de cultură ale ţesutului şi a elaborat manuscrisul. TK a efectuat testele de imunofluorescenţă. PN a participat la colorarea probelor şi la analiza citometrică prin flux. AR a participat la elaborarea design-ului studiului şi a efectuat analiza statistică. Manuscrisul final a fost citit şi aprobat de către toţi autorii".*

Fiecare Autor trebuie să aibă o contribuţie individuală în desfăşurarea cercetării, pregătirii manuscrisului şi publicării lucrării. Un Autor trebuie să contribuie semnificativ la conceptul şi *design*-ul lucrării, la efectuarea procedurilor experimentale, la colectarea datelor, la compilarea, analiza, interpretarea şi validarea rezultatelor.

Conform recomandărilor Comitetului Internaţional al Editorilor Revistelor Medicale, ICMJE, (www.icmje.org), drept autor poate fi considerată persoana care se încadrează în toate cele 4 criterii:

Abbreviations

Use only standard abbreviations. Other abbreviations may be defined and provided when are used for the first time in the manuscript. Abbreviations in the figures and tables will be explained in legend. Abbreviations should be used as rare as possible.

Declaration of conflict of interests

Following publication, persons or organizations involved in the study become public and thus their reputation may be influenced. Therefore, authors must disclose financial and non-financial relationship with people or organizations and to declare conflicts of interest related to the data presented in the manuscript. In accordance with the ICMJE guidelines, authors must fulfill a statement of conflicts of interest, which will be published at the end of the article.

Complementing the declaration of conflicts of interest the following will be taken into consideration

For financial conflicts of interest

- specify whether any organization has financial relationship with research presented in the manuscript, including funding, salary, reimbursements;
- mentioned, if the article has any impact on the eventually involved organization and could generate losses or profits after publication, now or in the future;
- authors must indicate if they have shares ownership in any organization that may incur losses or take profits after publication, now or in the future. Also, you should specify whether the author (s) own (s) or apply to any property rights (patent) on the content used in the manuscript;
- indicate if there are any other conflicts of interest.

For non-financial conflicts of interest

- Please specify any non-financial conflicts of interest: political individual, religious, ideological, educational, rational, commercial etc. related to manuscript.

Authors' contributions

This section of the manuscript is to specify the input and involvement of each author. In this regard, please follow the suggested format: *"HW conceived the study and participated in study design and helped drafting the manuscript. MG performed the processing of specimens and tissue culture methods and drafted the manuscript. TK performed immunofluorescence tests. PN participated in staining and flow-cytometry. AR participated in the study design and performed the statistical analysis. Final manuscript was read and approved by all authors".*

Each author must have an individual contribution to the research, manuscript preparation and work publication. An author should contribute substantially to one of the following: the concept and design of the work, performing of the experimental procedures, data collection, compilation, analysis, interpretation and validation of results.

According to the International Committee of Medical Journals Editors, ICMJE (www.icmje.org), as author may be a person who fit all four of following criteria:

o has made a substantial personal contribution in desi-gning,

- o a adus o contribuţie individuală substanţială conceperii, elaborării design-ului cercetării, sau a colectat, analizat sau interpretat datele;
- o a elaborat manuscrisul sau l-a revăzut în mod critic, aducând o contribuţie intelectuală importantă;
- o a aprobat versiunea finală a manuscrisului, gata pentru publicare;
- o este de acord să fie responsabilă pentru toate aspectele legate de cercetarea efectuată şi de manuscrisul depus pentru publicare şi să dea asigurare, că toate întrebările referitoare la acurateţea sau integritatea lucrării vor investigate şi rezolvate în mod corespunzător.

Notă: Persoanele, care au contribuit la realizarea lucrării, însă nu se încadrează în toate cele 4 criterii enunţate mai sus, nu pot fi considerate drept autori; contribuţia acestora va fi menţionată în secţiunea „mulţumiri şi finanţare" a manuscrisului. De asemenea, persoanele care au fost implicate doar în colectarea datelor, supraveghere, asistenţă tehnică şi finanţare, nu deţin drept de Autor, dar ei pot fi menţionaţi în secţiunea „mulţumiri şi finanţare". Simpla deţinere a funcţiei de şef de unitate, departament sau instituţie, în cadrul căreia s-a efectuat cercetarea, fără îndeplinirea tuturor celor 4 recomandări ale ICMJE, nu oferă dreptul de a fi (co)autor al lucrării.

Mulţumiri şi finanţare

Persoanele care au contribuit la elaborarea design-ul studiului, colectarea datelor, analiza şi interpretarea acestora, la pregătirea manuscrisului şi la redactarea lui critică, au oferit suport general sau tehnic, au contribuit cu materiale esenţiale pentru studiu, dar care nu îndeplinesc criteriile ICMJE de Autor, nu vor fi considerate drept Autori, dar contribuţia lor va fi menţionată în secţiunea „mulţumiri şi finanţare". Tot în această secţiune se vor menţiona sursele de finanţare ale lucrării. Menţionarea persoanelor fizice sau juridice, care au contribuit la realizarea lucrării şi manuscrisului, poate fi făcută doar după obţinerea unei permisiuni de la fiecare dintre ele.

Tabelele

Fiecare tabel va fi creat cu dublu-spaţiere şi amplasat pe o pagină separată, după textul manuscrisului. Enumerarea tabelelor va fi consecutivă, cu cifre arabe, în ordinea primei lor citări în text, scris cu caractere grase **(bold)**, alinierea – pe stânga, deasupra tabelului. Fiecare tabel va avea un titlu laconic, care va fi scris cu caractere normale (regular) sub numărul tabelului. Nu utilizaţi caractere bold în interiorul tabelului. Urmaţi exemplul prezentat:

Tabelul 1
Evenimente adverse intra-anestezice şi imediat post-extubare

	Lot experimental (n=100)	Lot control (n=100)	p
Disritmii	6,0%	3,0%	0,49
Instabilitate hemodinamică	7,0%	1,0%	0,034
Trezire prelungită*	11,0%	4,0%	0,19
GVPO† post-extubare	8,0%	27,0%	0,007
Durere intensă la trezire	17,0%	19,0%	1,0

Notă: * – trezire neobişnuit de lentă, după ce concentraţia cerebrală a reziduurilor de anestezice a trecut sub pragul de inducere a hipnozei; † – greaţă şi vomă postoperatorie. Analiza statistică utilizată: testul Fisher.

developing research protocol, or collected, analyzed and interpreted data;

- o developed or reviewed critically the manuscript bringing a significant intellectual contribution;
- o approved the final version of the manuscript ready for publication;
- o agrees to be responsible for all aspects of the conducted research and submitted manuscript and to assure that all questions relating to accuracy or completeness of the work was adequately assessed and resolved.

Note: Persons who have contributed to the work, but not fit the four criteria mentioned above cannot be considered as authors. Their contribution will be mentioned in the "Acknowledgment and funding section" of the manuscript. Also, people who have only been involved in data collection, monitoring, technical assistance and funding, are not eligible as coauthors, but they may be mentioned in the "Acknowledgements and funding" section. Mere position of head of unit, department or institution, on which the research was conducted, without fulfilling all four ICMJE criteria, doesn't provide the right to be a coauthor of the work.

Acknowledgements and funding

People who contributed to the study design, data collection, analysis and interpretation, manuscript preparation and editing, offered general or technical support, contributed with essential materials to the study, but do not meet ICMJE authorship criteria will not be considered as authors, but their contribution will be mentioned in section "Acknowledgements and funding". Also in this section must be specified the sources of work funding. Mention of persons or institutions who have contributed to the work and manuscript can be made only after obtaining permission from each of them.

Tables

Content of each table should be double-spaced and placed on a separate page after the text of the manuscript. Tables numbering will be done using consecutive Arabic numerals in the order of their first citation in the text; it is should be written in **bold**, align to left and place above the table. Each table should have a concise title that will be written in bold (regular) under table number. Do not use bold within the table. Please follow the example:

Tabelul 1
Intra-anesthetic and immediately post-extubation adverse events

	Experimental Cohort (n=100)	Control Cohort (n=100)	p
Dysrhythmia	6,0%	3,0%	0,49
Hemodynamic instability	7,0%	1,0%	0,034
Prolonged awakening*	11,0%	4,0%	0,19
PONV† post-intubation	8,0%	27,0%	0,007
Strong pain on awakening	17,0%	19,0%	1,0

Note: * – Unusually slow awaking, after that cerebral concentration of the anesthetic reach the under hypnotic level; † – postoperative nausea and vomiting. Used statistical analysis: Fisher's exact test.

Legendele şi notele explicative vor fi făcute sub tabel. Toate abrevierile non-standard se vor explica în notele de subsol, folosind următoarele simboluri, în următoarea ordine: * , † , ‡ , § , | | , , ** , † † , ‡ ‡ , § § , | | | | , ¶ ¶ etc.

Menţionaţi, de asemenea, testele statistice aplicate şi tipul de date prezentate. Asiguraţi-vă că fiecare tabel este citat în text. Dacă utilizaţi date din altă sursă publicată sau nepublicată, trebuie să obţineţi permisiunea şi să declaraţi pe deplin sursa sub tabel.

Figurile

Figurile vor fi prezentate atât în manuscris, cât şi pe fişiere separate. În manuscris, figurile vor fi prezentate după textul lucrării, fiecare pe pagină separată şi vor fi numerotate consecutiv, cu cifre arabe, în ordinea citării lor în text. Numerotarea va fi scrisă abreviat (**Fig. 1**), cu caractere grase (**bold**), alinierea – pe stânga, sub figură. Fiecare figură va avea un titlu laconic, care va fi scris cu caractere normale (regular) în dreptul numerotării.

Figurile trebuie să fie calitative, vizibile în detaliu. Fotografiile cu persoane potenţial identificabile trebuie să fie însoţite de permisiunea scrisă de a utiliza fotografia. În caz contrar, faţa persoanelor trebuie acoperită cu o bandă neagră. În cazul în care o figură a fost publicată anterior, faceţi referinţă la sursa originală şi prezentaţi permisiunea scrisă de la deţinătorul drepturilor de autor pentru a reproduce figura. Permisiunea poate fi luată atât de la autorul figurii, cât şi de la editor, cu excepţia documentelor din domeniul public.

Pentru figuri, sunt acceptate următoarele formate de fişiere:

- o TIFF
- o JPEG
- o EPS (format preferat pentru diagrame)
- o PowerPoint (figurile trebuie să fie de mărimea unui singur diapozitiv)

Titlul fişierului va consta din numărul figurii şi un titlu scurt, identificabil.

Legendele figurilor

Legenda figurii va fi scrisă în continuare, imediat după titlul figurii. Descrierea figurii nu trebuie să repete descrierea din textul manuscrisului. Când sunt folosite simboluri, săgeţi, numere sau litere pentru a identifica, descrie părţi ale ilustraţiilor, identificaţi-le şi explicaţi-le pe fiecare în mod clar în legendă. Explicaţi scala internă şi identificaţi metoda de colorare în microfotografii.

Vă rugăm să reţineţi că este de responsabilitatea autorului (autorilor) de a obţine permisiunea de la deţinătorul drepturilor de autor pentru a reproduce figuri sau tabele care au fost publicate anterior în altă parte. Imaginile color vor fi tipărite din contul autorilor.

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Toate referinţele bibliografice trebuie să fie numerotate consecutiv, între paranteze pătrate [ ], în ordinea în care sunt citate în text. Citatele de referinţă nu trebuie să apară în titluri sau subtitluri. Fiecare referinţă trebuie să aibă un număr individual. Citările multiple din cadrul unui singur set de paranteze trebuie să fie separate prin virgulă şi spaţiu. În cazul în care există trei sau mai multe citări secvenţiale, acestea ar trebui să fie indicate sub formă de serie. Exemplu: [1, 5-7, 28].

Vă rugăm să evitaţi folosirea excesivă a referinţelor. În cazul în care se folosesc sisteme automate de numerotare, numerele de

Legends and notes will be place under the table. All non-standard abbreviations should be explained in footnotes, using the following symbols, in the following order: *, †, ‡, §, | |, ¶, **, † †, ‡ ‡, § §, | | | |, ¶ ¶ etc.

Applied statistical tests and the type of presented data should be also mentioned. Make sure that each table is cited in the text. If you use data from another published or unpublished source, you must obtain permission and cited the source below the table.

Figures

Figures will be included in the main manuscript, and also submitted as separate files. The manuscript figures should be presented, each one on a separate page and should be numbered consecutively with Arabic numerals in the order of their citation in the text. Figure numbering will be written abbreviated (Fig. 1), using bold fonts, left alignment, and placed under the figure. Each figure should have a laconic title that will be written using regular font and place in the right of the figure's number. Figures' quality should assure the visibility of details. Pictures of persons potentially identified must be accompanied by written permission to use it. If a figure has been previously published, please cite the original source and submit the written permission to reproduce the figure from the copyright owner. Permission can be taken from both the author and the publisher, except the documents of public domain.

For figures, the following file formats are accepted:

- o TIFF
- o JPEG
- o EPS (preferred format for diagrams)
- o PowerPoint (figures should be of the size of a single slide)

The file title should include the figure number and an identifiable short title.

Figures' legends

Figure's legend should be written immediately after the figure's title. Figure's description should not repeat the description in the text of the manuscript. When used symbols, arrows, numbers or letters to describe parts of the figure, explain clearly each one of them in the legend. Explain the internal scale and identify the staining method of the photomicrographs.

Please note that it is the responsibility of the author(s) to obtain permission from the copyright holder to reproduce figures or tables that have been published previously elsewhere. Color images will be printed at the expense of the manuscript authors.

References

All references must be numbered consecutively, in square brackets [ ], in the order they are cited in the text. Reference citations should not appear in titles or subtitles. Each reference should have an individual number. Multiple citations within a single set of brackets must be separated by commas and spaces. If there is a sequence of three or more citations, they have to be given as a range (e.g. [1, 5-7, 28]).

Please avoid excessive use of references. If an automatic system of citation is used, reference numbers must be finalized and the bibliography must be fully formatted before submission. Reference list should include all authors. Journals' abbreviation must be in accordance with Index Medicus/MEDLINE. It may be cited only

referinţă trebuie să fie finalizate, iar bibliografia trebuie formatată complet înainte de depunere. Lista de referinţă trebuie să conţină toţi autorii. Abrevierea revistelor trebuie să fie în conformitate cu Index Medicus / MEDLINE. Pot fi citate doar articolele sau rezumatele care au fost publicate şi care sunt disponibile, accesibile prin intermediul serverelor publice. Orice rezumate sau articole nepublicate sau cu caracter personal nu trebuie să fie incluse în lista de referinţă, dar pot fi incluse în text şi citate în mod corespunzător, indicând cercetătorii implicaţi. Obţinerea permisiunii printr-o scrisoare de la autori pentru a le cita comunicările sau datele nepublicate sunt în responsabilitatea autorului corespondent al articolului.

Formatul referinţelor

Autorii sunt rugaţi să furnizeze cel puţin un link pentru fiecare referinţă bibliografică (preferabil PubMed).

o *Referinţă la revistă*

Numele şi iniţialele autorului sau al autorilor, separate prin virgulă (regular). Titlul articolului (regular). Forma abreviată a denumirii revistei (italice), urmat de anul, numărul volumului: numărul paginilor (regular). Articolele în curs de publicare citate vor fi menţionate cu „***In press***" (italic, bold), după numărul paginilor. Se vor menţiona toţi autorii articolului.

Ex: „1. Belîi A., Cobâleţchi S., Casian V., Belîi N., Severin G., Chesov I., Bubulici E. Les aspects pharmaco-economiques dans la gestion de la douleur perioperatoire. Mise au point. *Ann Fr Anesth Réanim*, 2012; 31: 60-66."

o *Referinţă la carte*

Numele şi iniţialele autorului sau al autorilor, separate prin virgulă (regular). Titlul capitolului (regular) (numărul paginii sau paginilor citate). În: Titlul cărţii. Detalii privind Editorul. Editura, locul, anul editării.

Ex: „1. Belîi A. Gestiunea riscului şi siguranţa pacientului în anestezie şi terapie intensivă (p. 115-134). În: Recomandări şi protocoale în anestezie, terapie intensivă şi medicină de urgenţă. Editori: Săndesc D., Bedreag O., Păpurică M. Ed. Mirton, Timişoara, România, 2010."

o *Referinţa la Web*

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Exemplu: „Agency For Healthcare Research and Quality (AHRQ). Production pressures. WebM&M. Disponibil la adresa: [http://webmm.ahrq.gov/case.aspx? caseID=150]. Accesat pe: 18.06.2010."

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Serghei Popa, dr. şt. med., conf. univ.,
Redactor-şef
tel: +373 60907799
e-mail: editor.mjhs@usmf.md

articles or abstracts that have been published and are available through public servers. Any abstracts or unpublished data or personal items should not be included in the reference list, but may be included in the text and cited accordingly, indicating the involved researchers. It is of manuscript authors' responsibility to obtain the permission to refer to unpublished data.

References format

Authors are asked to provide at least one link for each citation (preferably PubMed).

o *Journal article reference*

Surname and initials of the author(s), separated by commas (regular). Title of article (regular). Abbreviated name of the journal (in italics), followed by the year, volume number: pages number (regular). Articles in press should be specified as "***In press***" (italic, bold), after the pages number. All the authors should be listed.

e.g.: "1. Belîi A., Cobâletchi S., Casian V., Belîi N., Severin G., Chesov I., Bubulici E. Les aspects pharmaco-economiques dans la gestion de la douleur perioperatoire. Mise au point. *Ann Fr Anesth Réanim*, 2012; 31: 60-66. "

o *Book reference*

Surname and initials of the author (s), separated by commas (regular). Title of chapter (regular) (cited page(s) number). In: Title of book. Details of the editor, publisher, place, year of publication.

e.g. "Belii A. Risk management and patient safety version anesthesia and intensive care unit (p. 115-134). In: Recommendations and Protocols in Anesthesia, Intensive care and Emergency medicine. Editors: Sandesc D., Bedreag O., Papurica M. Ed. Mirton, Timisoara, Romania, 2010".

o *Web reference*

Name and initials of the author(s), separated by commas, or Copyright holder (regular). Title. Site Name. Available at: [URL]. Accessed: date.

E.g.: "Agency for Healthcare Research and Quality (AHRQ). Production Pressures. WebM & M. Available at: [http://webmm.ahrq.gov/case.aspx? caseID = 150]. Accessed on: 18.06.2010".

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