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CUPRINS

CONTENTS

NEXT GENERATION SEQUENCING (NGS) AS IMPORTANT TOOL FOR THERAPEUTIC APPROACH IN MELANOMA

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ABSTRACT

Sequencing technology proposes new insights into neoantigens and mutation-targeted therapies derived from transcriptional patterns. Combined with classic diagnostic methods, immune profile and microenvironmental assessment, can provide in-depth information that can reframe our understanding of human cancer biology. In order to know tumor biology, it is necessary to obtain information about the genetic changes in the DNA of the tumor cells. We performed an extensive genomic characterization by next-generation sequencing (NGS) of three melanoma cell lines with the aim of analyzing the genetic changes occurring in melanoma and their clinical and therapeutic relevance. We identified known melanoma driver mutations, such as BRAF V600E, NRAS Q61R, and additionally found other mutated genes that appear in melanoma pathogenesis and some of them targeted by novel therapies. These cell lines are used to improve the genetic understanding of melanoma and to study the functional significance of individual mutational changes, but turning cancer genomic data into knowledge remains a challenge. For establishing the appropriate therapeutic approach in melanoma, the personalized treatment should be envisioned, based on both next generation sequencing, surface markers expression, as well as microenvironment assessment.

Key words: NGS, melanoma, BRAF, targeted therapy

INTRODUCTION

The application of next-generation sequencing (NGS) has increased over the past ten years, facilitating the identification of cancer driver genes and opening new opportunities for cancer genomic research, including melanoma. The skin cancer associated with the highest mortality is cutaneous melanoma. It has a somatic (sporadic) or germline (familial) origin. Almost 90% of melanoma cases are represented by the somatic form [1]. The main germline mutations found in the familial form are the *CDKN2A* and *CDK4* genes. The most frequent genetic alterations occurring in melanoma are related to the *BRAF*, *NRAS*, *KIT* and *NF1* genes. According to the presence of these mutations, melanoma is classified into four genomic subtypes: *BRAF*-mutant, *NRAS*-mutant, *NF1*-loss and triple wild-type [1].

Cutaneous melanocytes are located in the basal layer of the epidermis and they represent the cells from which cutaneous melanoma develops. UV radiation (UVA and also UVB) is one of the main causes of DNA damage having harmful effects on skin cells. Intermittent and intense exposure to sun, as well as exposure to UV rays from artificial sources, has been associated to an increased risk of melanoma development [2].

The different clinical subtypes of melanoma have specific genetic alterations and are classified as follows based on sun-induced damage: non-CSD (non-chronic sun-induced damage): melanomas on skin without chronic sun-induced damage; CSD (chronic sun-induced damage): melanomas on skin with chronic sun-induced damage; and acral: melanomas on the palms, soles, or sub-ungual sites. Outside of the skin, melanocytes exist as well and can give rise to non-cutaneous melanomas on mucosal membranes, the uveal tract of the eye, or leptomeninges. Different genetic profiles important for therapeutic decisions have been identified in different

melanoma subtypes [3]. Non-CSD subtype was described to have the highest amount *BRAF* mutations (56%) compared to CSD, acral, and mucosal subtypes (6%, 21%, and 3%, respectively). In contrast, the incidence of *KIT* aberrations was 28%, 36%, and 39% in CSD, acral, and mucosal subtypes, respectively. *NRAS* mutations were described in 5% to 20% of the subtypes [3].

Considering the different subtypes of melanoma and their different genetic profiles, sequencing techniques used have included: targeted sequencing (focusing on specific regions of the genome), whole-exome sequencing: WES (the entire coding region of the genome), and whole-genome sequencing: WGS (the entire coding and non-coding regions of the genome) [1,4].

Identifying the presence of targetable mutations is an important step in melanoma management because effective targeted therapies are available. With the decreasing cost of next-generation sequencing (NGS), precision medicine can be clinically practiced based on the genetic profile of the patient's melanoma that is used for diagnosis and personalized treatment [4,5].

MATERIALS AND METHODS

We used 3 melanoma cell lines for morphological, immunophenotypic characterization and NGS analysis of single nucleotide variation (SNV).

The cells lines were purchased from ATCC, cultured and expanded according to producer specified conditions, at 37°C, in 5% CO_2 in air atmosphere: A375 (CRL-1619; in Dulbecco's Modified Eagle's Medium, 10% FBS), A2058 (CRL-11147; in Dulbecco's Modified Eagle's Medium, 10% FBS) and SK-MEL-2 (HTB-68, in Eagle's Minimum Essential Medium, 10% FBS).

Cells were submitted to immunophenotypic analysis using FACSVerse 3 lasers capable flowcytometer and a 12 markers panel including: CD90, CD105, HLA-DR, PD1, PD-L1, MICA/B, CD146, CD29, Fas, FasL, CD56 and EGFR.

The typical NGS workflow comprises different steps, from nucleic acids extraction to variant annotation. The process generally begins with converting nucleic acids (RNA or DNA) from biological samples to a biomaterial compatible with the sequencing system intended for the study. NGS experiment can be divided into the following four main steps: (1) DNA purification from tumor cells (2) preparation of libraries, (3) gene sequencing, and (4) gene analysis.

NGS - ThermoFisher Scientific kits and reagents / IonTorrent platform: DNA purification-GeneJET Genomic DNA Purification Kit; Library Preparation-Ion Ampliseq Library Preparation on the Ion Chef System + Ampliseq Cancer Hotspot Panel v2 Chef-Ready Kit (49 genes, 207 hotspots); Automated template preparation, chip loading, and sequencing-Ion 510 and Ion 520 and Ion 530 Kit Chef + Ion GeneStudio S5 System; Analysis - Ion Reporter and Oncomine Reporter software.

RESULTS

Currently, cultured melanoma cell models are widely used to study the mechanisms involved in tumor cell progression and to develop personalized therapies. In the present study, we performed an extensive genomic characterization by next-generation sequencing (NGS) of three melanoma cell lines. We also performed a morphological and immunophenotypic analysis. We used the following melanoma cell lines: A375, A2058 and SK-MEL-2. A375 is derived from a skin primary melanoma of a 54-year-old female. A2058 has been isolated from a lymph node metastasis of a 43-year-old male patient. SK-MEL-2 has been isolated from a metastatic site on the thigh of a 60-year-old male.

The A375 cell line expressed increased levels of the following markers on the cell surface: CD105, HLA-DR, PD-L1, CD146, CD29, Fas, CD56, EGFR (Figure 1). High expression of CD105, CD29 and CD146 are associated with a high risk of metastasis. CD146 is correlated with melanoma progression being highly expressed on metastatic melanoma cells.

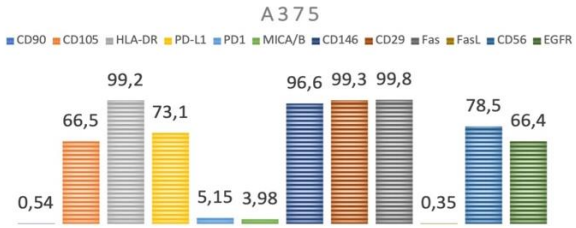


Figure 1. Expression of surface markers of the A375 cell

Also, the A2058 cell line expressed high amounts of PD-L1, CD146, CD29, Fas, CD56, EGFR as well as PD-1. This cell line has not expressed CD105 and HLA-DR (Figure 2).

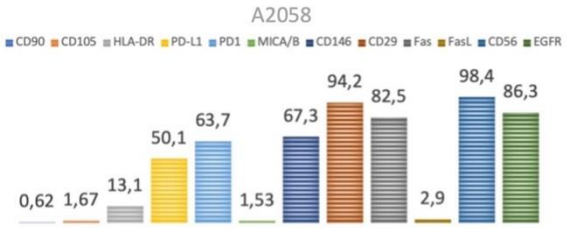


Figure 2. Expression of surface markers of the A2058 cell

The SK-MEL-2 had a completely different expression of the markers on the cell surface with a good level of expression of PD-L1, CD146, CD29, CD56, EGFR (Figure 3).

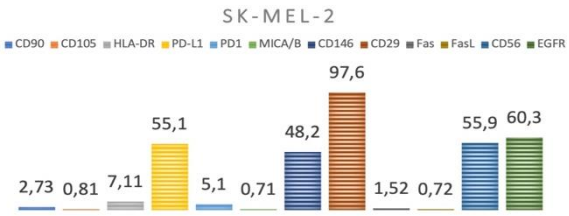


Figure 3. The expression of surface markers for SK-MEL-2 cell

All three cell lines have good expression of PD-L1, used in clinical practice to evaluate melanoma before checkpoint inhibitors therapy [3].

The E-cadherin is found on normal melanocytes and is important for attachment to keratinocytes. Down-regulation of E-cadherin is involved in melanoma progression, reflecting the ability of melanocytes to invade the dermis. All three cell lines did not express E-cadherin.

In order to obtain the genetic profile of these melanoma cell lines, we performed next-generation sequencing. We analyzed the data using Oncomine Reporter software, with which we identified relevant biomarkers currently targeted by approved therapies by: FDA, NCCN, EMA and ESMO. The report also contains information about the clinical trials targeting the mutations identified in the sample.

The NGS analysis revealed for A375 cell line the presence of *BRAF V600E* mutation and *CDKN2A E61* and *E69* mutations (Figure 4).

Variant Details

DNA Sequence Variants

Gene	Amino Acid Change	Coding	Variant ID	Locus	Allele Frequency	Transcript	Variant Effect
BRAF	p.(V600E)	c.1799T>A	COSM476	chr7:140453136	99.60%	NM_004333.6	missense
CDKN2A	p.(E69*)	c.205G>T	COSM13281	chr9:21971153	99.10%	NM_001195132.2	nonsense
CDKN2A	p.(E61*)	c.181G>T	COSM13486	chr9:21971177	100.00%	NM_001195132.2	nonsense

Relevant Biomarkers

Genomic Alteration	Relevant Therapies (In this cancer type)	Relevant Therapies (In other cancer type)	Clinical Trials
BRAF V600E B-Raf proto-oncogene, serine/threonine kinase	**atezolizumab + cobimetinib + vemurafenib** [1] **binimetinib + encorafenib** [1,2] **cetuximab + encorafenib** [1,2] **cobimetinib + vemurafenib** [1,2] **dabrafenib** [1,2] **dabrafenib + trametinib** [1,2] **trametinib** [1,2] **vemurafenib** [1,2] BRAF inhibitor + MEK inhibitor encorafenib ipilimumab + nivolumab	**binimetinib + encorafenib** [1,2] **cetuximab + encorafenib** [1,2] **dabrafenib** [1,2] **dabrafenib + trametinib** [1,2] **trametinib** [1,2] cobimetinib + vemurafenib dabrafenib + MEK inhibitor encorafenib + panitumumab selumetinib vemurafenib	56
CDKN2A E61, CDKN2A E69** cyclin dependent kinase inhibitor 2A	None	None	6

Public data sources included in relevant therapies: FDA[1], NCCN, **EMA**[2], ESMO

Figure 4. NGS analysis for A375 cell line.

For A2058 we identified the following mutations: *BRAF V600E, PTEN L112Q, TP53 V274F* and *SMARCB1*.

The identification of certain mutations may contraindicate the choice of treatment, as for example in this cell line with a *PTEN* mutation, treatment with dabrafenib leads to a shorter median progression-free survival (PFS) [2], outlining the importance of NGS analysis in guiding the therapeutic decision (Figures 5, 6).

Variant Details

DNA Sequence Variants

Gene	Amino Acid Change	Coding	Variant ID	Locus	Allele Frequency	Transcript	Variant Effect
BRAF	p.(V600E)	c.1799T>A	COSM476	chr7:140453136	36.09%	NM_004333.6	missense
PTEN	p.(L112Q)	c.335T>A	.	chr10:89692851	49.47%	NM_000314.8	missense
TP53	p.(V274F)	c.820G>T	COSM165075	chr17:7577118	99.00%	NM_000546.6	missense
SMARCB1	p.(?)	c.1119-41G>A	COSM1090	chr22:24176287	51.60%	NM_003073.5	unknown

Figure 5. Mutations identified by NGS analysis for A2058

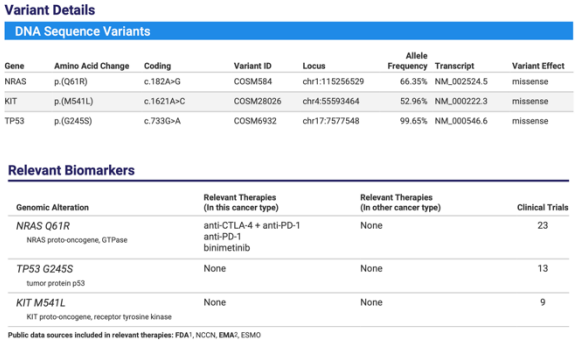
Relevant Biomarkers

Genomic Alteration	Relevant Therapies (In this cancer type)	Relevant Therapies (In other cancer type)	Clinical Trials
BRAF V600E B-Raf proto-oncogene, serine/threonine kinase	**atezolizumab + cobimetinib + vemurafenib** [1] **binimetinib + encorafenib** [1,2] **cetuximab + encorafenib** [1,2] **cobimetinib + vemurafenib** [1,2] **dabrafenib** [1,2] **dabrafenib + trametinib** [1,2] **trametinib** [1,2] **vemurafenib** [1,2] BRAF inhibitor + MEK inhibitor encorafenib ipilimumab + nivolumab	**binimetinib + encorafenib** [1,2] **cetuximab + encorafenib** [1,2] **dabrafenib** [1,2] **dabrafenib + trametinib** [1,2] **trametinib** [1,2] cobimetinib + vemurafenib dabrafenib + MEK inhibitor encorafenib + panitumumab selumetinib vemurafenib	55
PTEN L112Q phosphatase and tensin homolog	None	**niraparib** [1] bevacizumab + olaparib	28
TP53 V274F tumor protein p53	None	None	13
SMARCB1 c.1119-41G>A SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1	None	None	1

Public data sources included in relevant therapies: FDA[1], NCCN, **EMA**[2], ESMO

Figure 6. Relevant biomarkers identified by NGS analysis for A2058 cell line.

The SK-MEL-2 cell line presented mutations in *NRAS Q61R, TP53 G245S*, and *KIT M541L* (Figure 7).



Variant Details

DNA Sequence Variants

Gene	Amino Acid Change	Coding	Variant ID	Locus	Allele Frequency	Transcript	Variant Effect
NRAS	p.(Q61R)	c.182A>G	COSM584	chr1:115256529	66.35%	NM_002524.5	missense
KIT	p.(M541L)	c.1621A>C	COSM28026	chr4:55593464	52.96%	NM_000222.3	missense
TP53	p.(G245S)	c.733G>A	COSM6932	chr17:7577548	99.65%	NM_000546.6	missense

Relevant Biomarkers

Genomic Alteration	Relevant Therapies (In this cancer type)	Relevant Therapies (In other cancer type)	Clinical Trials
NRAS Q61R NRAS proto-oncogene, GTPase	anti-CTLA-4 + anti-PD-1 anti-PD-1 binimetinib	None	23
TP53 G245S tumor protein p53	None	None	13
KIT M541L KIT proto-oncogene, receptor tyrosine kinase	None	None	9

Public data sources included in relevant therapies: FDA[1], NCCN, EMA[2], ESMO

Figure 7. NGS analysis for SK-MEL-2 cell line.

DISCUSSIONS

The most common mutation was *BRAF V600E* which appeared in two samples (A375 and A2058), in a mutually exclusive manner with the *NRAS Q61R* mutation, which we found in one cell line (SK-MEL-2). For melanoma cell lines A375 and A2058, apart of *BRAF V600E* we identified other mutations which are not targeted yet by current approved therapies: *CDKN2A E61* and *E69*, *PTEN L112Q*, TP53 V274F, and SMARCB1. In SK-MEL-2 cell line we found the association between *KIT* mutation, frequently found in mucosal melanoma and *NRAS* mutation, found in acral melanoma.

Our study has several limitations. Unfortunately, we were unable to find out from the manufacturer of the cell lines the location of the primary tumors in the case of A2058 and SK-MEL-2 cell lines. We also lacked clinical data about tumors, such as exposure to the sun and the existence of other cases of melanoma in the family. It would be interesting to know the location of the primary tumor, its NGS analysis, the comparison with the one from the metastasis, but also if the patient underwent therapy because over the course of the disease, the genome of a cancer changes, especially in response to the selection pressure of treatment.

Tumors contain a multitude of mutations, few of which are essential for maintaining a malignant state. Advances in DNA sequencing technologies, including next-generation sequencing (NGS) facilitate the identification of potential targetable changes. In cutaneous melanoma, mutations in the signaling components *BRAF*, *NRAS* and *NF1* commonly lead to activation of mitogen-associated protein kinase (MAPK) pathway. The MAPK pathway can be targeted with FDA approved small molecule inhibitors: BRAF inhibitor + MEK inhibitor (dabrafenib + trametinib, binimetinib + encorafenib).

BRAF is the most common genetic alteration in cutaneous melanoma, being present in 40–60% of cases and representing one of the main therapeutic targets. *BRAF* mutations mainly affect the 600th codon. Replacement of valine with glutamic acid in the 600th codon (*V600E*) is the most common mutation, occurring in over 80% of cases. *V600K* is found in 15% of melanomas and *V600R/M/D/G* are described in less than 5% [3]. Currently, there is an association of clinical features of the patient with the *BRAFV600E* mutation, such as: younger age, skin sites with non-chronic sun-induced damage and superficial melanoma subtype. *BRAFV600K* mutation has been correlated with chronic sun-induced damage and older age [2,3].

In patients with *BRAF V600*-mutated melanoma, selective *BRAF* inhibitors have shown remarkable therapeutic activity. Unfortunately, within a few months of starting *BRAFi +/ MEKi* treatment, nearly all patients with *BRAF*-mutated metastatic melanoma will develop resistance to therapy and experience tumor recurrence, outlining the need for more clinical research to discover the processes that lead to the development of treatment resistance. As the disease progresses, the genome of a tumor evolves, especially in response to the selection pressure of treatment. In order to find presumably de novo mutations and the mechanisms that led to resistance to therapy, a pre- and post-treatment NGS analysis may facilitate their identification [6,7].

The presence of *NRAS* mutations has been described in 10-25% of cutaneous melanomas, in nodular subtypes and melanomas developing in skin sites with chronic sun induced damage, raising the question that they are a result of UV-related mutagenesis. In contrast to *BRAF, NRAS* is not a therapeutic target. *BRAF* and *NRAS* mutations are usually mutually exclusive, nevertheless there have also been descriptions of their coexistence [2].

Neurofibromin 1 (*NF1*) is a negative regulator of Ras and has frequently undergone loss of function in malignancy and thus considered as a tumor suppressor. Loss of *NF1* has been found to be significantly increased in cutaneous melanoma without either *BRAF* or *NRAS* mutations and has been

associated with increased activation of the MAPK pathway [8].

The melanoma subtypes with the most *C-KIT* mutations are mucosal and acral melanomas. *KIT* is a transmembrane receptor tyrosine kinase that is widely expressed in a range of normal cell types and is also essential for the regulation of normal melanocyte differentiation, growth and migration. Mucosal melanoma is a rare and aggressive subtype of melanoma with a less favorable outcome, and *KIT* mutations are more prevalent in this tumor. In mucosal melanoma there is a lack of understanding and identification of oncogenic drivers, and NGS analysis could help to reveal them. Tumors such as gastrointestinal stromal tumors (GISTs) and cutaneous melanoma with *KIT* exon 11 or exon 13 mutations have a better response to *KIT* targeted therapy suggesting that specific changes may be more sensitive to *KIT* inhibition, outlining the importance of NGS analysis. Imatinib, nilotinib, and dasatinib are *KIT* inhibitors that have demonstrated variable clinical efficacy in the treatment of *KIT* mutated mucosal melanoma, but further research is needed [8,9].

Neoantigens can develop from somatic mutations of tumor DNA, representing antigens derived from mutations that can be recognized and attacked by cells of the immune system. The probability of neoantigen formation increases with the number of somatic mutations a tumor contains and tumor mutational burden (TMB) might serve as a helpful indicator of tumor neoantigen load. The highest amounts of TMB have been found in melanoma, according to numerous studies that have been able to map and describe TMB variants in different types of cancer [9,10].

CONCLUSIONS

The molecular pathways of melanoma are crucial to be revealed, because understanding the mechanisms of operation and the significance of genetic changes will lead to new targeted therapies for personalized treatment with better chances of cure. The management of metastatic melanoma has been greatly improved by targeting the MAPK signaling pathway, with *BRAF* mutations being the most common and important alteration that can be targeted. For patients with metastatic melanoma who have a *BRAF* mutation, the combination of *BRAF/MEK* inhibition is the first line of treatment. However, drug resistance is highly common, so further research is required to find new targeted treatments and additional mutations causing resistance to therapy will need to be identified.

Cultured cell models of melanoma are often used to study response to treatment and mechanisms underlying therapy resistance. Due to the genetic diversity of melanoma cell lines, it would be useful to have access to broad panels of well-characterized cell lines, and with the help of NGS analysis we can define this database.

Given that significant advances have already led to the development of a variety of analysis tools for NGS results, it seems likely that NGS will soon be available for large-scale uses, for diagnosis and personalized treatment.

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SECVENȚIEREA DE GENERAȚIE URMĂTOARE (NGS) CA INSTRUMENT IMPORTANT PENTRU ABORDAREA TERAPEUTICĂ ÎN MELANOM

REZUMAT

Tehnologia de secvențiere propune noi perspective asupra neoantigenelor și a terapiilor țintite. Metodele clasice de diagnostic împreună cu evaluarea profilul imun și micromediului tumoral, pot oferi informații noi pentru o mai bună înțelegere a biologiei tumorilor maligne. Pentru o cunoaștere aprofundată a biologiei tumorale, este necesară obținerea de informații referitoare la modificările genetice din ADN-ul celulelor tumorale. În studiul de față, am efectuat o caracterizare genomică extinsă prin secvențierea de generație următoare (NGS) a trei linii celulare de melanom, cu scopul de a analiza modificările genetice prezente în aceste linii celulare și relevanța clinică și terapeutică a acestora. Am identificat mutații cunoscute implicate în patogeneza melanomului, precum BRAF V600E, NRAS Q61R dar și mutații noi pentru care terapii țintite se află în dezvoltare. Aceste linii celulare sunt folosite pentru a studia semnificația funcțională a modificărilor genetice individuale. Pentru stabilirea abordării terapeutice adecvate în melanom, trebuie avut în vedere tratamentul personalizat, bazat atât pe secvențierea de generație următoare, expresia markerilor de suprafață, cât și pe evaluarea micromediului tumoral.

Cuvinte cheie: NGS, melanom, mutația BRAF, terapii țintite

DECIPHERING THE INTRICATE NETWORK: IMMUNOLOGIC FACTORS IN INFERTILITY AND THEIR IMPACT ON REPRODUCTIVE HEALTH

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ABSTRACT

This paper investigates the intricate interplay of fetal human leukocyte antigen (HLA) and maternal killer cell immunoglobulin-like receptor (KIR) interactions, focusing on their implications for pregnancy outcomes. The study explores the impact of HLA mismatches, particularly in the context of the HY immunity system, on the maternal immune response. The presence of HLA antibodies is assessed, with consideration given to the correlation with total immunoglobulin levels, aiming to discern associations with potential pregnancy complications. Complement activity is examined, adding a layer of understanding to the dynamics of fetal HLA and maternal KIR interactions.

Furthermore, the paper delves into the delicate balance between Th1 and Th2 helper T cells, crucial components of the immune system, and their potential influence on maternal-fetal immune tolerance. Natural Killer Cell Cytotoxic Activity (NKa) is investigated, providing insights into the cytotoxicity of these immune cells and their role in the context of maternal-fetal interactions. The findings presented contribute to a nuanced comprehension of the immunological factors shaping pregnancy outcomes and may inform future research and clinical approaches aimed at optimizing maternal and fetal health.

Key words: immunological factors, infertility, reproductive health

INTRODUCTION

Recurrent implantation failure (RIF) is characterized by the inability to achieve pregnancy after three or more embryo transfer cycles involving high-quality blastocysts. RIF can stem from various factors, including altered uterine, male, or embryo-related factors. Disruptions in endometrial receptivity, gene expression in multiple pathways, immunologic disturbances in peripheral blood and/or the endometrium, and epigenetic changes are linked to RIF. Immunologically, women with RIF often exhibit an altered Th1/Th2 ratio and changes in NK cell and macrophage numbers. However, the diversity of immune dysfunction among women with RIF suggests a heterogeneous condition with varied immune responses, emphasizing the need for personalized therapies based on individual immune statuses. Tacrolimus is commonly recommended for those with a high Th1/Th2 ratio, while intravenous IgG is suggested for those with elevated NK cell numbers or HLA mismatch. Women with a hyperactivated immune status may receive progesterone support, prednisolone, vitamin E, and intralipid treatment to mitigate inflammation and oxidative stress. Conversely, those with a hypo-active immune status might benefit from endometrial scratching and intrauterine hCG administration. Standardized tests for immune status evaluation and well-powered randomized controlled trials are essential to determine the efficacy of personalized therapies for women experiencing RIF.

In this paper we present a set of tests that should be performed in case of RIF, related to the immune status of both partners.

1. Parental histocompatibility

Fetal HLA and maternal KIR interactions

The mother's uterine natural killer cells recognize the unique HLAs of a developing embryo through receptors known as killer immunoglobulin-like receptors (KIRs).

Some maternal KIRs exhibit better responsiveness to embryos expressing HLA-C2, influencing the flow of oxygen and nutrition through the placenta via spiral artery remodeling. Furthermore, if the embryo possesses a greater (or in some cases, an equal) number of HLA-C2 alleles compared to the mother, it may pose a potential risk. This examination assesses the patient's HLA-C2 content, the anticipated embryo's HLA-C2 content, and whether the maternal KIRs form a compatible match with HLA-C2.

Significant compatibility issues arise between the patient's KIR genes and the fetal HLA-C alleles, potentially leading to a compromised immune tolerance toward the embryo's HLAs and hindered spiral artery remodeling. The patient and their healthcare provider may consider investigating the advantages of immune-modulating treatments to enhance immune tolerance [1-6].

Clinical studies have revealed that the combination of maternal KIR-AA and fetal HLA-C2, as opposed to fetal HLA-C1, is associated with an elevated risk of recurrent losses [4], preeclampsia [3], and low birth weight [5]. Notably, the frequency of maternal KIR AA is higher in pregnancies that result in loss or are affected by preeclampsia, especially when the fetus has more C2 genes than the mother or when fetal C2 is inherited paternally [4]. Additionally, a study comparing HLA-C and KIR haplotypes in couples with three or more spontaneous miscarriages (RPL) versus those with no fertility issues demonstrated a significant association. The absence of activating KIR in the affected woman (KIR AA genotype) and an increased HLA-C2 group frequency were observed in couples experiencing recurrent pregnancy loss [6].

HLA mismatches

For a healthy pregnancy, a mother's immune system must establish tolerance to the embryo, acknowledging its genetic uniqueness. Yet, if the embryo inherits paternal HLAs closely resembling the maternal ones, a robust tolerance may not develop. This test assesses the extent of mismatches between maternal and paternal HLA Class II alleles to gauge the potential for immune tolerance development.

If there are fewer than 4 mismatches, there is a moderate risk that the similarity of HLA Class II alleles could hinder the maternal immune system from developing tolerance toward the embryo. The patient and their healthcare provider may consider investigating immune-modulating treatments to enhance immune tolerance.

Human Leukocyte Antigen (HLA) genes, commonly known as the major histocompatibility complex (MHC) genes, constitute a group of genes located on chromosome 6 and play a crucial role in presenting antigens to T cells to initiate immune responses. Typically, this immune reaction results in the elimination of cells displaying "non-self" peptides. There are two classes of HLA molecules: class I, which includes HLA-A, -B, -C, and class II, which includes HLA-DR, -DQ, and -DP. HLA molecules are pivotal in organ transplantation and are implicated in various diseases, including autoimmune disorders [7-8].

In organ transplantation, a mismatch in HLA between a donor and recipient may lead to graft rejection, often due to antibody formation [9]. Conversely, a certain degree of difference in HLA alleles between the mother and father [10], inherited by the embryo and defined as a mismatch, is essential for actively establishing immune tolerance of the embryo [11]. Consequently, couples with significant matching of HLA alleles may be more susceptible to infertility, repeated implantation failure, and recurrent pregnancy loss [12].

HY immunity

Embryos possessing a Y chromosome express proteins known as HY antigens (male-specific minor histocompatibility antigens) on their cells. On occasion, when a mother gives birth to a baby with a Y chromosome, her immune system may generate an immune response against these HY antigens, potentially affecting subsequent pregnancies. The mother's HLAs play a crucial role in triggering this immune response, with certain HLA alleles increasing the likelihood of such a reaction. This test examines whether the patient carries these higher-risk HY-restricting alleles, which could elevate the risk, especially if they have previously given birth to a boy. (An allele refers to an alternative version of a gene at a specific chromosome location.

In the initial pregnancy carrying a male fetus, the maternal immune system may be triggered by allogenic fetal cells containing male-specific minor histocompatibility inherited antigens (HYrHLA allele) encoded by genes situated on the Y chromosome [13]. In certain women, this activation can result in an immediate immune response, prompting the production of HY antibodies by B cells. These antibodies may persist in the maternal serum for an extended period, potentially contributing to subsequent recurrent miscarriages (regardless of the embryo's gender) and the delivery of male infants with lower birth weights [14-15].

Although found in 30% of women, anti-HY antibodies have been associated with secondary recurrent miscarriage in subsequent pregnancies, along with other complications like stillbirth, placental abruption, or fetal growth retardation. These occurrences are outcomes of an inflammatory environment. In a comprehensive cohort study involving women with unexplained secondary recurrent pregnancy loss (RPL), findings indicated that these patients faced a higher likelihood of miscarriage in their subsequent pregnancies when their firstborn was a boy compared to a girl (46% versus 24%, respectively) [16].

For those patients with a firstborn son, the presence of

H-Y restricting HLA class II alleles (HLADRB115, HLA-DQB105:01/02, and HLA–DRB3*03:01) has been correlated with a diminished chance of live birth and a lower male/female ratio among subsequent births (indicating an increased loss rate of male embryos) [17]. Patients with no copies of HY-restricting alleles have a subsequent live birth rate of 73%, which decreases to 58% with one allele copy and further to 49% with two allele copies.

HLA antibodies

Occasionally, an individual's immune system generates antibodies against HLAs. These antibodies may either be directed against "self" HLAs (autoantibodies), target "non-self" HLAs from prior full-term pregnancies or blood transfusions, or specifically focus on the HLAs of a partner that the embryo will inherit. While HLA antibodies are common and typically not problematic, partner-specific HLA-C antibodies (a subset of Class I antibodies) can present a significant risk and are associated with early miscarriages and secondary infertility. This test evaluates the presence, quantity, and type of HLA antibodies in a patient, if any.

Pregnancy is characterized by profound alterations in the maternal immune system, facilitating the implantation and growth of the semi-allogeneic fetus within the uterus. B lymphocytes (B cells) play a crucial role in the maternal immune adaptation to fetal implantation among immune cells.

Certain subsets of B cells can generate antibodies targeting components of the embryo/fetus determined by paternal genetics, notably paternally derived HLA molecules. Anti-HLA antibodies are present in approximately one third of healthy and successful pregnancies [18]. However, the presence of partner-specific anti-HLA antibodies, particularly those capable of fixing complement, can be detrimental to the maintenance of pregnancy, potentially leading to miscarriage or subsequent complications such as preeclampsia, intrauterine growth restriction, or stillbirth.

2. Inflammation

Total immunoglobulin level

Whether stemming from an autoimmune condition, infection, allergies, or another origin, various inflammatory markers have been linked to difficulties in reproduction. In the course of a healthy pregnancy, the body needs to transition to a relatively anti-inflammatory state to sustain the pregnancy. The following tests represent some well-established markers of inflammation in reproductive immunology. The outcomes of these tests can be utilized to assess whether interventions to alleviate inflammation might enhance a patient's likelihood of reproductive success.

Complement activity

The complement system, an integral part of the innate immune system, acts as a potent initiator of inflammation upon activation. Within this system, the proteins C3 and C4 play pivotal roles, and their activation has been linked to pregnancy complications, particularly pre-eclampsia.

Complement is a system of serum proteins that forms a crucial effector arm of the innate immune system, associated with macrophage activation and inflammation. There are three distinct pathways through which the complement system is activated: the classical pathway activated by antigen-antibody complexes, the alternative pathway activated by microbial surfaces, and the lectin pathways activated by microbial surfaces. These pathways converge, leading to the generation of anaphylatoxins (C3a, C4a, and C5a) and the membrane attack complex MAC (C5b,6,7,8, and 9), which are potent inflammatory molecules. The complement and coagulation pathways closely interact, with C5a inducing procoagulant activity and reducing fibrinolysis. Additionally, C3a and C5a activate endothelial cells and platelets, resulting in increased levels of adhesion factors and procoagulant activity.

Numerous studies have delved into complement activation in the circulation of women with preeclampsia, revealing heightened complement activity compared to pregnancies in healthy control subjects [19-21]. Similarly, clinical investigations support the significance of complement activity, evident in lower levels of C3 and C4 complement levels associated with obstetrical complications in patients with antiphospholipid syndrome [22-25] or Systemic Lupus Erythematosus [26]. Notably, a correlation has been established between elevated levels of complement activation fragments (Bb and C3a) and preterm birth [27-28]. Intriguingly, certain proteins involved in complement activation, such as factor B and H, have been identified as useful biomarkers for predicting preterm birth as early as 15 weeks into pregnancy [29].

The profound effects of complement activation on the placenta, leading to inflammation and placental injury, stand out as major contributors to fetal loss in APS, in addition to triggering pre-eclampsia.

Th1/Th2 helper T cells ratio

Certain immune cells, such as T helper cells, generate cytokines that instruct the immune system to intensify its activity (pro-inflammatory) or to ease off (anti-inflammatory). Th1 and Th2 cells, specific types of T helper cells, generate pro-inflammatory compounds, and the balance between Th1 and Th2 can play a role in gauging the extent of inflammation in a patient.

CD4+ T cells, CD8+ T cells, NKT cells, and NK cells constitute four major effector cell types within the immune

system. These cells can react to foreign antigens, including those derived from the paternal side present in conceptuses, and can initiate either immunogenic or tolerogenic responses. Upon antigen priming by antigen-presenting cells (APCs), naïve CD4+ T cells can differentiate into various lineages, such as Th1, Th2, Th17, and Treg cells (regulatory T cells), depending on the involved APCs and the soluble molecules secreted during priming. These lineages are distinguished by unique cytokine expression profiles, with flow cytometry identifying cells expressing specific cytokines (IFNγ for Th1, IL-4 for Th2, IL-17 for Th17, and IL-10 for Treg). Analogous lineages exist for CD8+ T cells, NKT cells, and NK cells, and their profiles can be similarly characterized. The presence of TNFα-positive cells serves as a general marker of cellular activation.

The relative balances of these lineages within each cell type can help characterize underlying immune conditions. For instance, certain autoimmune conditions, like rheumatoid arthritis, exhibit Th1 dominance, while others, such as systemic lupus erythematosus, show Th2 dominance. Conditions like endometriosis, PCOS, and atopy are also characterized by distinct intracellular cytokine profiles. These profiles can manifest many years before the full clinical manifestation and diagnosis of an underlying autoimmune or inflammatory condition. Combined with other genetic and cellular data, they can be employed to characterize preclinical or asymptomatic conditions influencing the immune response to foreign antigens. Changes in intracellular cytokine profiles can also be used to monitor the maternal immune response during pregnancy, evaluate the effectiveness of immune treatments, and identify whether any pregnancy failures have an immunological basis.

Women with a history of recurrent implantation failure or recurrent miscarriage exhibit altered intracellular cytokine profiles in peripheral blood. These alterations encompass an increased ratio of Th1 to Th2 cells, elevated levels of TNFα-positive cells, reduced levels of IL-10-positive cells, and heightened levels of Th17 cells [30-31]. The typical (non-pathological) response to pregnancy is characterized by a tolerogenic reaction to paternal antigens within the conceptus. This tolerogenic response involves specific modifications to the intracellular cytokine profile of various immune cell types. Such tolerogenic responses include a shift towards Th2 dominance, indicated by a decrease in the Th1/Th2 ratio, and an elevation in levels of IL-10-positive cells [32-33].

Pathological immune responses to a conceptus indicate a failure to establish proper immunological tolerance to paternally derived antigens. Instead, an immunogenic response is triggered, potentially leading to cellular and/or humoral (antibody-mediated) reactions. Such pathological immune responses to a conceptus can manifest in various clinical outcomes, including implantation failure, spontaneous abortion, preeclampsia, intrauterine growth restriction (IUGR), and stillbirth. The defective development of immunological tolerance during pregnancy is characterized in peripheral blood, marked by a failure to shift towards Th2 dominance, a failure to increase IL-10-positive cells, and an elevation in levels of IL-17-positive cells [34-35].

Natural Killer Cell Cytotoxic Activity (NKa)

Natural killer (NK) cells, often labeled as "killers" due to their cytotoxic capabilities, possess a broader range of functions beyond cell elimination. These cells contribute to various activities, such as promoting the healthy development of the placenta in the uterus. This test assesses the extent to which the patient's NK cells are inclined toward cytotoxic activity. Elevated NK cytotoxic activity has been linked to recurrent pregnancy loss.

Natural Killer (NK) cells represent a subset of cytotoxic innate lymphocytes [36]. Elevated natural killer cell activity has been observed in individuals with autoimmune conditions like Graves' disease and Hashimoto's thyroiditis when compared to a healthy control group [37]. NK cells may play a direct role in these diseases, either through their potential autoreactivity or by interacting with dendritic cells, macrophages, or T lymphocytes, thus contributing to excessive inflammation or promoting adaptive autoimmune responses [38]. During early pregnancy, NK cells dominate the lymphocyte population in the decidua [39]. While endometrial NK cells exhibit distinct phenotypic features from circulating NK cells—possessing weak cytotoxicity and immunoregulatory activities [40] - it is speculated that peripheral blood NK cells maintain a strong correlation with NK cell populations found in the decidua [41].

Natural Killer (NK) cell activity has been linked to the pathogenesis of recurrent pregnancy loss [42-43], even in cases where the embryo exhibits normal chromosomal characteristics [44]. A clinical investigation has demonstrated that women with elevated NK cell activity before conception face a substantially higher risk of pregnancy loss (3.5-fold higher risk) compared to those with normal levels [42]. Recent studies further emphasize a robust association between heightened NK cell activity and recurrent spontaneous abortion [45-47], suggesting that NK cell activity could serve as a predictive biomarker. Intravenous immunoglobulin (IVIG) has been shown to reduce NK cell activity in women experiencing recurrent pregnancy losses [48], potentially enhancing the overall pregnancy outcome. However, the significance of pre-conceptional NK activity as a predictive indicator remains debated, as one study found no correlation between NK activity and the risk of subsequent loss [49]. It's worth noting that this study may be biased as it exclusively considers clinical losses as miscarriages, excluding all chemical losses.

Regulatory T cells

Specialized immune cells known as regulatory T cells (Treg cells) play a crucial role in suppressing inflammation and preventing the rejection of the embryo by the uterus. Insufficient Treg levels in the uterus have been associated with infertility, recurrent miscarriage, and pregnancy complications. This test evaluates the patient's circulating Treg cell levels, offering insights into the potential recruitment of Treg cells to the uterus during pregnancy.

3. Autoimmunity

Antinuclear antibodies (ANAs)

The likelihood of experiencing adverse pregnancy outcomes is elevated in individuals with autoimmune diseases, making expectant mothers with conditions such as antiphospholipid syndrome, lupus, rheumatoid arthritis, and other autoimmune disorders classified as high-risk pregnancies. Nevertheless, a significant number of individuals go undiagnosed for autoimmune conditions. These tests aid in assessing a patient's susceptibility to different autoimmune conditions, enabling tailored treatment approaches for each specific condition.

Within a cell, the nucleus houses essential DNA and various proteins crucial for maintaining cellular function. Occasionally, an individual's immune system may initiate an attack on nucleus-associated proteins, producing antinuclear antibodies (ANAs). Elevated concentrations of ANAs could suggest the presence of an autoimmune disease, such as lupus. These tests evaluate the existence of various ANAs: ANA Ds-DNA, Anti-Jo1 Ab, ANA RNP, ANA SM, ANA SCL, Antichromatin, ANA CENT.

Antiphospholipid antibodies (APAs)

Phospholipids play a vital role in the composition of human cell membranes. On occasion, an individual's immune system may initiate an attack on its own phospholipids, producing antiphospholipid antibodies (APAs). As phospholipids are present in blood cells, the presence of APAs can contribute to issues such as blood clots, miscarriages, or complications during pregnancy. These tests are designed to evaluate the existence of various APAs: anticardiolipin (IgG, IgM, IgA), anti-beta2 glycoprotein I (IgG, IgM, IgA), anti-phosphatidylserine (IgG, IgM, IgA), lupus anticoagulant dPT, PTT-LA, Thrombin time.

Antiphospholipid antibody syndrome (APS) is an autoimmune disorder characterized by the production of antiphospholipid antibodies by the immune system, targeting its own components. The existence of these antibodies can instigate a thrombophilic disorder, resulting in excessive clotting and contributing to conditions such as venous thromboembolism, stroke, multiple miscarriages, and other complications during pregnancy. APS is categorized as primary when it occurs without an underlying disease and secondary when associated with an underlying pathology, such as systemic lupus erythematosus (SLE).

Among various antiphospholipid antibodies, anti-beta 2 glycoprotein I (anti-β2-GP1) antibodies are particularly instrumental in supporting the diagnosis of APS [50]. Numerous studies indicate that anti-β2-GP1 antibodies are more specific for APS compared to anti-cardiolipin antibodies [50-52]. All three isotypes of anti-β2-GP1 (IgG, IgM, and IgA) have been linked to thrombosis [53-55]. The presence of either or both β2-GP1 IgG and IgM antibodies serves as an independent risk factor for thrombosis and pregnancy-related complications [52].

APS stands out as the most prevalent acquired risk factor for recurrent pregnancy loss, exerting its influence by disrupting placental function and compromising maternal-fetal blood exchange. This heightened risk extends to various pregnancy complications, including stillbirth, intrauterine death, pre-eclampsia (PE), premature birth, and fetal growth restriction.

Among women experiencing recurrent pregnancy loss (RPL), 26.4% of cases were associated with the presence of antiphospholipid antibodies [55], while pregnancy complications were identified in up to 20% of pregnancies affected by APS [56]. A comprehensive meta-analysis involving over 200,000 participants revealed a 2.5-fold increase in the risk of spontaneous abortion in women with APS [57]. Additionally, another meta-analysis found that moderate to high levels of anti-cardiolipin antibodies (aCL) were linked to an elevated risk of PE [58]. In instances where anti-phospholipid antibodies are both high and persistent, the risk escalates for preterm birth and fetal growth restriction [59-60]. Notably, heightened titers of aCL and anti-β2GPI antibodies were associated with a 3- to 5-fold increased likelihood of stillbirth [61].

Collectively, these studies underscore the importance of detecting, closely monitoring, and providing appropriate care to pregnant women with APS to facilitate successful pregnancies.

CCP antibodies and rheumatoid factor

Antibodies such as cyclic citrullinated protein (CCP) and rheumatoid factor serve as indicators for rheumatoid arthritis and certain other autoimmune conditions. These tests evaluate the presence of specific antibodies, including anti-CCP antibodies IgG/IgA, and levels of rheumatoid factor.

CONCLUSION

Progress in reproductive medicine has markedly improved the efficacy of fertility treatments. Despite these advancements, certain women encounter recurrent

implantation failure (RIF) following in-vitro fertilization (IVF) or recurrent pregnancy loss (RPL). Imbalances in the immune system and the inability to establish immune tolerance to the fetus have been identified as potentially modifiable factors contributing to idiopathic RIF and RPL. Consequently, there is a growing trend in the use of immunomodulatory agents as a therapeutic approach to enhance the likelihood of a successful pregnancy in these cases. Advancements in immunological diagnostics are crucial to align with the evolving clinical requirements in this emerging field, providing clinicians with the necessary guidance to make optimal and safe therapeutic decisions.

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DESCIFRAREA UNEI REȚELE COMPLICATE: FACTORII IMUNOLOGICI ÎN INFERTILITATE ȘI IMPACTUL LOR ASUPRA SĂNĂTĂȚII REPRODUCTIVE

REZUMAT

Această lucrare cercetează intricata interacțiune dintre antigenul leucocitar uman fetal (HLA) și receptorii asemănători imunoglobulinei (KIR) de pe celulele NK materne, concentrându-se asupra implicațiilor lor asupra rezultatelor sarcinii. Studiul investighează impactul nepotrivirilor HLA, în special în contextul sistemului imunitar HY, asupra răspunsului imun matern. Se analizează prezența anticorpilor HLA, având în vedere corelarea cu nivelurile totale de imunoglobuline, în încercarea de a identifica eventualele asocieri cu complicațiile posibile ale sarcinii. Activitatea complementului este examinată pentru a adăuga o înțelegere mai profundă a dinamicii interacțiunilor dintre HLA-ul fetal și KIR-ul matern.

De asemenea, lucrarea investighează echilibrul subtil dintre celulele T helper Th1 și Th2, componente esențiale ale sistemului imunitar, și influența lor potențială asupra toleranței imune materno-fetale. Se analizează activitatea citotoxică a celulelor ucigașe naturale (NKa), oferind perspective asupra citotoxicității acestor celule imune și a rolului lor în contextul interacțiunilor materno-fetale. Descoperirile prezentate contribuie la o înțelegere complexă a factorilor imunologici care influențează rezultatele sarcinii și pot ghida cercetările și abordările clinice viitoare menite să optimizeze sănătatea atât a mamei, cât și a fătului.

Cuvinte cheie: factori imunologici, infertilitate, sănătate reproductivă

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ENDOMETRIOSIS - A CAUSE OF INFERTILITY?

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ABSTRACT

Endometriosis, marked by endometrial tissue outside the uterus, is a an important factor in infertility due to tubal and ovarian dysfunction. The lack of consensus on detection methods leads to delayed diagnoses, impacting therapeutic strategies. Around half a million patients in Romania suffer from this condition. This study aimed to correlate endometriosis with infertility. The study included 45 diagnosed patients endometriosi were aged 30 to 40 years.

The study explored correlations between endometriosis and infertility in 45 diagnosed patients, noting a peak occurrence at age 40. Early menarche (11-14 years) and high nulliparity percentages aligned with previous findings linking younger menarche and infertility in endometriosis. Various correlations between pregnancies, lesion size, and serum Ca-125 were found, indicating a potential link between larger lesions and higher Ca-125 values, suggesting contributions of these factors to infertility. The study's emphasis on the multifactorial nature of the disease and its small sample size underscore the need for further investigation.

The thesis choice stems from the lack of consensus on endometriosis etiopathogenesis, diagnosis, and management, compounded by the normalization of symptoms due to societal stigmatization of menstrual discussions. Clinical and paraclinical links between endometriosis and infertility were identified, highlights the multifactorial nature of infertility and calling for a comprehensive approach to diagnosis and management. Further research is crucial to unravel the complex interplay between genetic and environmental factors in endometriosis.

In summary, endometriosis poses challenges to fertility through debated mechanisms, delayed diagnoses, and societal stigmatization. The study highlights correlations and emphasizes the multifactorial nature of infertility, stressing the need for tailored management and further research.

Key words: endometriosis, early menarche, lesion size, Ca-125, nulliparity, infertility

INTRODUCTION

Endometriosis, an incompletely elucidated inflammatory disease, is the presence of endometrial tissue outside the uterus. It is associated with subfertility and chronic pelvic pain. The links between this disease and infertility are a much-debated topic in today's literature. With this study, we aimed both to establish an answer to the question "To what extent can endometriosis be considered a cause of infertility?" and to establish some predictors of infertility in endometriosis patients. This could prove useful in early diagnosis and more efficient and personalised management of the disease.

There are several ways in which endometriosis affects fertility. These include tubal dysfunction, ovarian dysfunction, adverse influences of peritoneal fluid on fertility-related phenomena, mechanical obstructions in the tubal lumen, changes in tubal-ovarian anatomical relationships.

In addition, there is still no consensus on effective minimally invasive methods for detecting endometriosis, leading to delayed diagnosis.

Both the early diagnosis of endometriosis and the predictors of infertility associated with it are necessary for the development of the most effective therapeutic strategy tailored to the patient's needs. A set of well-defined investigations would contribute significantly to this approach.

It occurs in about 10-15% of women in the reproductive period and in about 20-50 to 70% of women with infertility, according to some studies (1,2,3).

The highest prevalence was recorded between 25 and 35 years (4). Reproductive women younger than 35 years

of age have twice the risk of infertility if they have endometriosis than those without the condition (5). In addition, many cases are asymptomatic for a long period, with prevalence ranging from 6% to 43% (6), so the true prevalence becomes difficult to estimate. Unfortunately, for many women there is also a delay in the diagnosis of endometriosis, leading to unnecessary suffering and reduced quality of life (7).

In Romania it is estimated that there are half a million patients with endometriosis. 70% of patients with chronic pelvic pain and 50% of infertile patients have the dsease, but an accurate diagnosis is difficult to make. Numerous studies show a 7–10-year delay in diagnosing women in developing countries. 47% of patients see more than 5 doctors before the diagnosis of endometriosis is established (8).

MATERIAL AND METHOD

The aim of the study was to look for a correlation between endometriosis and infertility and to detect the possible existence of predictive factors for these two entities.

We included in the study all patients who were admitted between 01.01.2019 - 31.12.2019 in the Obstetrics and Gynecology I and II wards of the Emergency Clinical County Hospital "Pius Brînzeu" Timisoara. The main inclusion criterion was the diagnosis of endometriosis. Patient data were collected from observation sheets provided by the clinic archive. Data related to anamnestic, clinical and paraclinical examination, drug treatment, surgical procedures, discharge diagnoses were reviewed. Microsoft Excel and SPSS were used for the database and statistical analysis.

A total of 45 patients diagnosed with endometriosis were registered. In the first stage, we described the database using tables and graphical representations, as well as descriptive statistics specific to the variables studied.

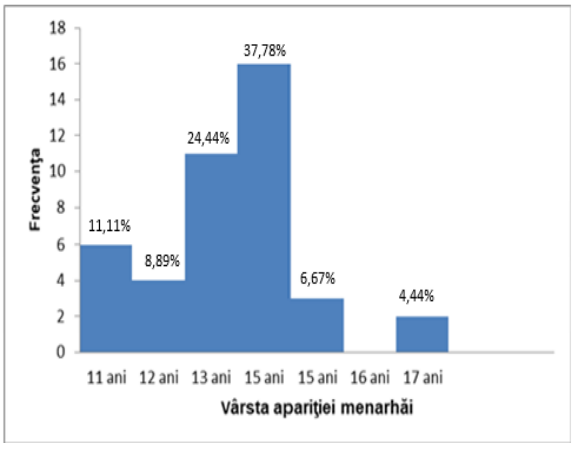

Fig 1. Histogram of patient distribution by age group

Most of them are aged between 30 and 40 years (48.89%). We also found that the number of patients diagnosed with endometriosis increases with age up to 40 years and then decreases again. The mean age of patients with endometriosis included in our study was 33 years. The youngest patient was 17 years old and the oldest 49 years old. According to Medscape, this trend is similar to other studies, and it is generally accepted that endometriosis is a disease of women of reproductive age, with most diagnoses being made between the ages of 25 and 35 (9).

RESULTS AND DISCUSSIONS

Many women with endometriosis have an increased risk of infertility, i.e. their ability to conceive spontaneously is affected. The percentage of women able to do so varies according to the severity of the disease, i.e. the more advanced the disease, the lower the chance of spontaneous conception. There are numerous mechanisms potentially involved in the development of infertility in women with endometriosis, e.g. mechanical changes in the reproductive organs, chronic inflammation, pathophysiological mechanisms affecting the ovum, especially in ovarian endometriosis (10).

Regarding the age of menarche onset, in our study most of the patients had menarche at 13 (24.44%) - 14 years (37.78%), but it can be seen that there is a tendency of menarche onset at younger ages (11-14 years), because after the age of 14 years, the percentages decrease significantly. The lowest age of menarche onset was 10 years and the highest 17 years. (Figure 2). We observed also that the number of patients diagnosed with endometriosis increases with age until 40 years and then decreases again. The mean age of endometriosis patients included in our study was 33 years. The youngest patient was 17 years old and the oldest 49 years old. According to Medscape, this trend is similar to other studies, and it is generally accepted that endometriosis is a disease of women of reproductive age, with most diagnoses being made between the ages of 25 and 35 (11).

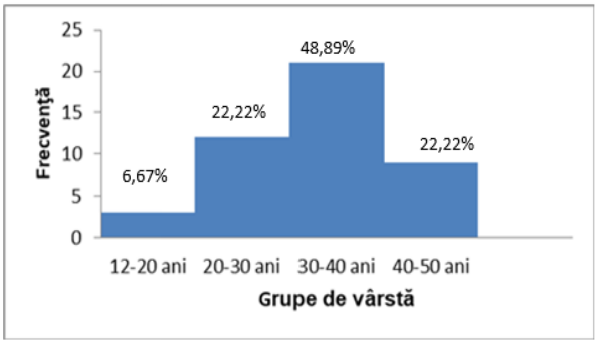

Fig. 2. Histogram of the distribution of patients according to the time of menarche onset

Multiple studies mention a trend similar to our study, i.e., the occurrence of menarche at a younger age in people suffering from endometriosis, some even considering this as a risk factor for the development of endometriosis, being explained by the theory of retrograde menstruation (12,13).

Table I. Distribution of patients by number of pregnancies and births

	No. of patients	**Percentage of total patients**
No. of patients with no pregnancies	26	57,78%
No. of patients with at least one pregnancy	19	42,22%
No. of patients with at least one birth	18	40,00%

Approximately 60% of the patients had no previous pregnancies, which may be partly explained by their younger age (Table 1). However, endometriosis may be a cause of infertility, which could explain this high percentage of women who had not had a spontaneous conception at the time of the study. The most pregnancies recorded in one patient were 3. Again, the literature shows an increased infertility rate in women with diagnosed endometriosis. In a large cohort study, the risk of infertility was doubled in women under 35 diagnosed with endometriosis compared to those without endometriosis (14).

The endometriotic lesion locations were diverse in our group of patients: ovary, uterus, pelvis, parietal - at the level of post-caesarean section scar and other locations perecum omentum, rectum, intestinal loops, vagina. However, by far the most common location was the ovary (73.33%). In terms of which ovary is more commonly affected, our study found a relatively similar incidence between the right and left ovary, with one third of cases having bilateral involvement. According to Medscape, the most common locations of endometriosis are the ovaries, with some studies showing a frequency of up to 96.4% of endometriosis located in the ovary (15).

Table II. Distribution of patients according to the location of the endometriotic lesion

Lesion site	**No. of patients**	**Percentage of total patients**
Ovary	33	73,33%
Uterus	5	11,11%
Pelvis	4	8,89%
Post-Cesarean section scar	9	20,00%
Other sites	2	4,44%

Quite a large percentage of patients had no Ca-125 value mentioned in the observation sheet (46.67%). Of the remainder, 35.56% had a serum Ca-125 value within normal limits, almost double those with Ca-125 greater than or equal to 46 U/mL. The mean Ca-125 value was 39.37 U/mL, the highest value being 102.8 U/mL and the lowest 8.6 U/mL (Table 3). Similar to our study, another study detected a link between increased serum Ca-125 level and endometriosis (16). However, there are no relevant data in the literature at the acute time point recommending the use of Ca-125 as a screening method for endometriosis, nor as a disease severity parameter (17,18).

Table III. Distribution of patients according to serum Ca125 value

Serum Ca125 level	**No. of patients**	**Percentage of total patients**
<46 U/mL	16	35,56%
≥46 U/mL	8	17,78%
Unknown	21	46,67%

The neutrophil/lymphocyte ratio was within normal parameters for the majority of patients (86.67%), suggesting a low degree of body stress. (Table 3), However, the results are not statistically conclusive in our study, and a number of studies in the literature prove the opposite situation, in which increased neutrophil/lymphocyte ratio is strongly correlated with endometriosis, especially in combination with increased Ca-125 value (19,20).

Table IV. Distribution of patients according to neutrophil/lymphocyte ratio

Neutrophile/lymphocyte ratio	**No. of patients**	**Percentage of total patients**
Between 1 and 6	39	86,67%
Between 6 and 9	2	4,44%
Between 9 and 18	0	0,00%
≥18	1	2,22%

Unknown	3	

Next, we calculated descriptive statistics corresponding to the numerical variables of the study. These are: central tendency parameters (mean, median, mode), dispersion parameters (standard deviation, variance, standard error, minimum, maximum, amplitude), degree of skewness and degree of flattening of the distribution. All these calculations are reported in the tables below (see Table 5-6).

Table V. Descriptive statistics corresponding to the numerical variables of the study

	Age (years)	**Highest lesion measurement (cm)**	**Volume of biggest lesion (ml)**	**Menarche (age)**
Average	33,3777778	5,382571	35,6177	13,32558
Standard error	1,2161742	0,384963	13,85112	0,222542
Median	33	5	11,375	14
Mode	24	4	6,24	14
Standard deviation	8,15834455	2,277472	80,76519	1,459304
Variant	66,5585859	5,186878	6523,016	2,129568
Symmetry index	-0,4685499	0,862616	19,40859	0,847647
Flattening index	0,158539	0,892024	4,202307	0,073556
Amplitude	32	10,07	435,2545	7
Minimum	17	1,93	1,545544	10
Maximum	49	12	436,8	17

Table VI. Descriptive statistics corresponding to the numerical variables of the study (continued)

	No. of pregnancies	**No. of births**	**Ca-125 level (UI/mL)**	**Neutrophile /lymphocyte ratio**
Average	1,02272727	0,613636	39,37417	3,479481
Standard error	0,18244474	0,113636	5,217265	0,489377
Median	0,5	0	32,95	2,727454
Mode	0	0		4,923077
Standard deviation	1,21020153	0,753778	25,55927	3,171524
Variant	1,46458774	0,568182	653,2765	10,05857
Symmetry index	-1,1494377	0,988515	0,299851	23,77434
Flattening index	0,69697561	1,129548	0,967657	4,420711
Amplitude	3	3	94,2	20,16963
Minimum	0	0	8,6	0,905637
Maximum	3	3	102,8	21,07527

We also wanted to examine the extent to which certain variables in the study are influenced by each other. To achieve this, we performed correlation and regression analysis. We tested whether there is a possible association between the number of loads, lesion size, lesion volume, Ca-125, and neutrophil/lymphocyte ratio, respectively. We also examined the degree of association between lesion size, i.e. lesion volume and age, serum Ca-125 and neutrophil/lymphocyte ratio. We used Pearson's coefficient for the degree of association of the variables, and to demonstrate statistical significance we set as level of significance. The results obtained are illustrated in the following tables and figures (see Table 7-16, Figure 3-5).

Table VII. Correlation between number of tasks and highest lesion measure

		Pregnancies no.	**Lesion size**
Pregnancies no.	Pearson correlation	1	-.068
	Sig. (2-tailed)		.697
	N	35	35
Lesion size	Pearson correlation	-.068	1
	Sig. (2-tailed)	.697	
	N	35	35

In this case we obtained a weak but statistically insignificant negative association (r = -0.68, p = 0.697).

Table VIII. Correlation between number of loads and lesion volume

		Pregnancies no.	**Lesion volume**
Pregnancies no.	Pearson correlation	1	.223
	Sig. (2-tailed)		.205
	N	34	34
Lesion volume	Pearson correlation	.223	1
	Sig. (2-tailed)	.205	
	N	35	34

In the given situation, we obtained a positive, statistically insignificant association (r = 0.223, p = 0.205).

Table IX. Correlation between number of loads and Ca-125 value

		Pregnancies no.	Lesion volume
Pregnancies no.	Pearson correlation	1	.249
	Sig. (2-tailed)		.252
	N	23	23
Ca-125 value	Pearson correlation	-.249	1
	Sig. (2-tailed)	.252	
	N	23	23

Here we obtained a statistically insignificant negative association (r = -0.249, p = 0.252).

Table X. Correlation between number of loads and neutrophil/lymphocyte ratio value

		Pregnancies no.	Neutrophils/ lymphocytes ratio
Pregnancies no.	Pearson correlation	1	-.030
	Sig. (2-tailed)		.853
	N	41	41
Neutrophils/ lymphocytes ratio	Pearson correlation	-.030	1
	Sig. (2-tailed)	.853	
	N	41	41

In this case, we obtained a very weak negative association, not statistically significant (r = -0.030, p = 0.853).

Table XI. Correlation between highest lesion measurement and Ca-125

		Lesion size	Ca-125 value
Lesion size	Pearson correlation	1	.365
	Sig. (2-tailed)		.104
	N	21	21
Ca-125 value	Pearson correlation	-.365	1
	Sig. (2-tailed)	.104	
	N	21	21

Here we obtained a positive, statistically insignificant correlation (r = 0.365, p = 0.104).

Table XII. Correlation between highest lesion measurement and neutrophil/lymphocyte ratio

		Lesion size	Lesion volume
Lesion size	Pearson correlation	1	.003
	Sig. (2-tailed)		.986
	N	32	32
Ca-125 value	Pearson correlation	-.003	1
	Sig. (2-tailed)	.986	
	N	2332	40

In this situation we obtained an extremely weak, positive, statistically insignificant association (r = 0.003, p = 0.986).

Table XIII. Correlation between highest lesion measurement and age

		Age	Lesion size
Age	Pearson correlation	1	.003
	Sig. (2-tailed)		.988
	N	35	35
Lesion size	Pearson correlation	.003	1
	Sig. (2-tailed)	.988	
	N	35	35

In this case, we obtained an extremely weak, positive, statistically insignificant association (r = 0.003, p = 0.988).

Table XIV. Correlation between lesion volume and Ca-125

		Lesion volume	Ca-125 value
Lesion volume	Pearson correlation	1	.156
	Sig. (2-tailed)		.511
	N	20	20
Ca-125 value	Pearson correlation	-.156	1
	Sig. (2-tailed)	.511	
	N	20	20

Here we obtained a weak, positive, statistically insignificant association (r = 0.156, p = 0.511).

Table XV. Correlation between lesion volume and neutrophil/lymphocyte ratio

		Lesion volume	Neutrophils/ lymphocytes ratio
Lesion volume	Pearson correlation	1	-.118
	Sig. (2-tailed)		.527
	N	31	31
Neutrophils/	Pearson correlation	-.118	1

lymphocytes ratio	Sig. (2-tailed)	.527	
	N	31	31

In this case we obtained a weak, negative, statistically insignificant correlation (r = -0.118, p = 0.527).

Table XVI. Correlation between lesion volume and age

		Lesion volume	Age
Lesion volume	Pearson correlation	1	.034
	Sig. (2-tailed)		.849
	N	34	34
Age	Pearson correlation	-.034	1
	Sig. (2-tailed)	.849	
	N	34	34

In this situation we obtained a very weak, positive, statistically insignificant correlation (r = 0.034, p = 0.8849). The highest association was present between number of loads and lesion volume, number of loads and serum Ca-125 and the highest lesion measurement and serum Ca-125.

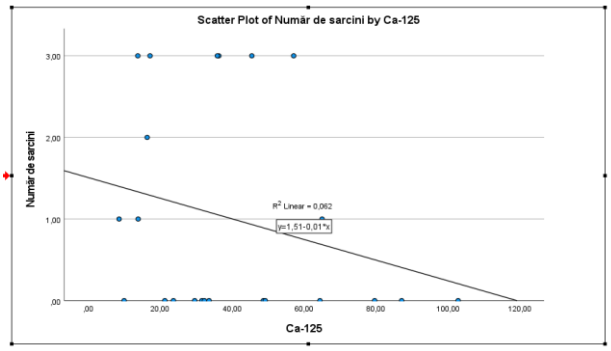

Fig 3. Degree of association between number of loads and lesion volume
serum Ca-125

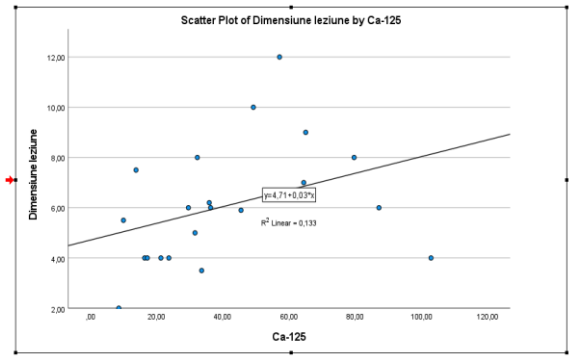

Fig. 4. Degree of association between number of loads and serum Ca-125

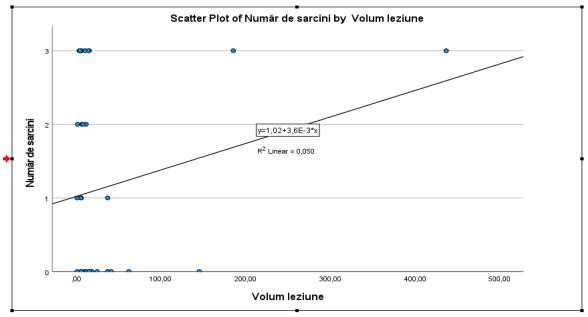

Figure 5. Degree of association between lesion size and serum Ca-125 value

In the correlation and regression analysis we observed a low degree of association between the variables studied, which may be attributed to the small number of samples in the study group, especially given the incompletely elucidated multifactorial nature of the disease. There are a few associations worth mentioning. The positive association between the number of pregnancies and lesion volume (r = 0.22, p = 0.2) can be explained by the association of these two variables independently with patient age. The negative association between number of pregnancies and Ca-125 value (r = 0.24, p = 0.2) is interpreted as an increase in Ca-125 is correlated with a low number of pregnancies in a patient (Figure 6).

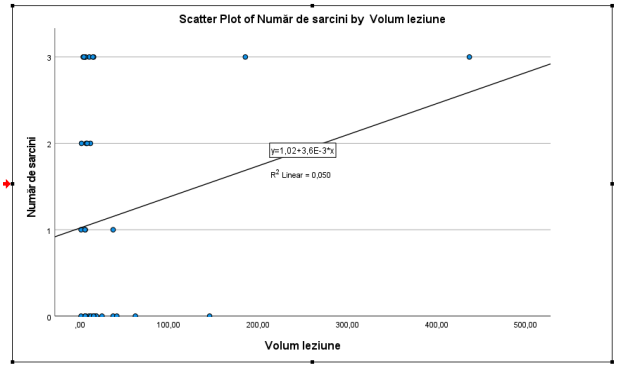

Fig. 6. Degree of association between number of loads and serum Ca-125

Last but not least, the strongest positive association in our study was between lesion size and Ca-125 value (r = 0.36, p = 0.1) - the larger the lesion size, the higher the Ca-125 value.

The strongest association was present between number of loads and lesion volume, number of loads and serum Ca-125 value and largest lesion size and serum Ca-125. These three measurements will be represented by scatterplots to show their distribution (see Figure 7).

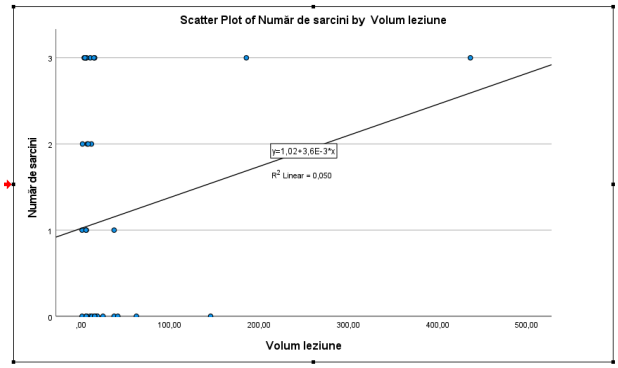

Fig.7. Degree of association between number of loads and lesion volume

The lesion size was generally small, but larger in patients without previous pregnancies compared to those with previous pregnancies. This result is consistent with the hypothesis that ovarian reserve shrinkage, being replaced by endometriotic cyst, is a possible cause of infertility in women with this diagnosis (21), but there are a number of factors that could contribute to infertility in this disease (22).

CONCLUSIONS

The lack of consensus regarding the etiopathogenesis, diagnosis and management of endometriosis, the impact that this disease has on a significant number of patients, as well as the stigmatization of menstrual cycle-related discussions that have led to the normalization of symptoms such as pelvic pain are the main reasons why I chose this topic for my thesis. I believe that endometriosis is a complex disease with diverse implications in women's lives especially in terms of fertility, so the existence of predictive factors in terms of correlation between the two pathologies would be very beneficial in clinical practice.

We believe that many of the hypotheses studied were relevant to clinical practice, especially as more information is needed regarding predictive, diagnostic and prognostic factors for this disease, and this work can be a starting point for possible future more conclusive studies.

In terms of the questions we have tried to answer since the beginning of the paper, our patient cohort showed that there are clinical and paraclinical links between endometriosis and infertility, but the exact pathophysiology of this link remains unelucidated and a comprehensive approach to the diagnosis and management of infertility in endometriosis is needed. So we can say that although the link between infertility and endometriosis exists, the view today is that infertility in endometriosis is multifactorial.

In conclusion, in recent years, endometriosis is seen as a multifactorial disease, with both genetic and environmental risk factors, and its contribution to subfertility status is not yet fully elucidated, but it can no longer be seen as the sole causative factor. A comprehensive approach to the diagnosis and management of this disease is therefore needed.

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INFECȚIA ȘI COLONIZAREA BACTERIANĂ LA FEMEILE INSARCINATE ȘI LA NOU-NASCUȚI

REZUMAT

Diferite tipuri de microorganisme, bacterii, paraziți, virusuri și fungi, pot coloniza și pot determina infecții la nivelul tractului genito-urinar feminin. Mai mult decât atât, nu puține femei expuse la infecțiile genitale bacteriene rămân însărcinate, crescând astfel semnificativ riscul de transmitere al infecțiilor la nou-nascuți. Infecțiile neonatale au o importanță deosebită datorită riscului crescut de morbiditate și mortalitate. Scopul studiului de față a fost identificarea tulpinilor bacteriene din culturi de probe cervicale provenite de la paciente însărcinate pe perioada unui an de zile, determinarea prevalenței și a rezistenței microbiene pentru fiecare patogen și corelarea rezultatelor cu etapele sarcinii, respectiv cu diferite patologii de sarcină. Datele studiului prezent au evidențiat următoarele: cel mai frecvent patogen Gram-negativ detectat a fost *E. coli,* în timp ce streptococul de grup B a prezentat cea mai mare prevalență din grupul bacteriilor Gram-positive. Important de menționat este faptul că rezultatele au arătat un număr substanțial de microorganisme depistate în culturile pacientelor cu sarcină în evoluție/sarcină la termen. Cu toate acestea, nu puțini patogeni au fost identificați în culturile pacientelor care au prezentat avort sau alte patologii de sarcină, unele tulpini având fenotipuri de rezistență care necesită o atenție sporită în ceea ce privește îngrijirea medicală și tratamentul infecțiilor din timpul sarcinii sau din timpul travaliului la pacientele însărcinate. În mod expectativ, achiziționarea și implementarea unei noi linii automate de prelucrare microbiologică a probelor ar putea ajuta la identificarea mult mai rapidă a microorganismelor și ar putea crește vigilența în supravegherea infecțiilor la pacientele însărcinate și la nou-născuți.

Cuvinte cheie: infecții genitale, sarcină, patologii neonatale, fenotip de rezistență

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INTRACRANIAL ANEURYSM UNVEILING AS NEUROLOGICAL DEFICITS: A LITERATURE REVIEW AND DIAGNOSTIC CHALLENGE

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ABSTRACT:

Intracranial saccular or berry aneurysms are abnormal blood vessel formations within the brain, responsible for the majority of nontraumatic subarachnoid hemorrhages (SAH). Advances in medical imaging have improved detection rates, though the prevalence of these aneurysms increases with age, particularly in women. Moreover, studies suggest a higher risk of de novo aneurysm formation in females and highlight the role of unstable blood pressure as a risk factor. Aneurysms are also associated with variations in estrogen levels and cardiometabolic risk factors, including hypertension, body mass index, and diabetes. Smoking is identified as a significant risk factor, with smoking-related vascular endothelial dysfunction contributing to aneurysm rupture. To illustrate the diagnostic and management challenges of intracranial aneurysms, a case report is presented. The case involves a 29-year-old female patient who initially presented with symptoms mimicking ischemic stroke. Subsequent evaluation revealed a right internal carotid artery aneurysm, cervical-lumbar discopathy, and thyroid pathology. The case highlights the importance of thorough assessment and consideration of atypical etiologies in patients with stroke-like symptoms. The study supports the proactive management of symptomatic aneurysms, as well as the role of embolization and direct arterial reconstruction in addressing aneurysms at risk of neurological complications and underscores the need for a comprehensive approach to their diagnosis and management, particularly in cases with atypical clinical presentations.

Keywords: intracranial aneurysm; risk factors; estrogen; cardioembolic; smoking; coffee; physical activity.

1.Epidemiology

Intracranial saccular or berry aneurysms are abnormal, bulging blood vessel formations in the brain that are typically acquired and are responsible for approximately 80% of nontraumatic subarachnoid hemorrhages (SAH) [1]. The detection rate of these aneurysms on arteriograms and magnetic resonance imaging (MRI) studies ranges from 1 to 2% [2], whereas autopsy studies indicate a slightly higher detection rate of 1 to 9% [3]. It's worth nothing that advances in medical imaging techniques, such as cross-sectional computed tomography (CT) and high-quality MRI, have improved the chances of identifying these aneurysms in recent years, contributing to the increase in their diagnosis [4,5].

Furthermore, the prevalence of unruptured intracranial aneurysms seems to rise with age, which is partly due to the aging of the population [6]. Surprisingly, women may have a higher likelihood of developing aneurysms compared to men, with a study on unruptured aneurysms revealing a 3:1 ratio of women to men [7,8]. When it comes to the development of new aneurysms, extensive long-term follow-up studies and meta-analyses have revealed a significantly greater risk of de novo aneurysm formation in females when compared to males [9,10]. Numerous studies have indicated that it's not necessarily high blood pressure but rather fluctuations in blood pressure that pose a risk factor for aneurysm rupture [11]. Irregular use of antihypertensive medication has been associated with increased variability in blood pressure [11].Additionally, research has shown that the incidence of aneurysm rupture is lower in white patients compared to african -american patients [12].

2.Risk factors

2.1. Estrogen levels

The activation of different estrogen subtypes and receptors has been demonstrated to have varying effects on the formation and rupture of intracranial aneurysms in mice. In female mice that underwent ovariectomy, the administration of an estrogen receptor-β agonist notably decreased the incidence of aneurysms. However, the administration of an estrogen receptor-α agonist or 17β-estradiol did not lead to a reduction in the occurrence of intracranial aneurysms [13].

In estrogen-deficient mice, there is an elevation in the serum levels of IL-6, and this cytokine seems to be involved in promoting the rupture of cerebral aneurysms associated with estrogen deficiency in mice. This promotion occurs through an increased infiltration of macrophages at the circle of Willis [14].

The connection between estrogen deficiency and the heightened risk of aneurysm development has also been established in humans [15,16]. The difference in the incidence of unruptured intracranial aneurysms (UIA) based on gender became increasingly noticeable in individuals aged 50 and older, implying a potential contribution of estrogen deficiency to this discrepancy [15]. Furthermore, a case-control study that investigated the relationship between the age at menopause and the presence of intracranial aneurysms found a trend suggesting that an earlier age at menopause was associated with the presence of cerebral aneurysms [17].

2.2. Cardiometabolic risk factors

When it comes to cardiometabolic risk factors, prior research has consistently shown that hypertension is linked to an increased risk of aneurysmal subarachnoid hemorrhage (aSAH) [18,19], supported by magnetic resonance (MR) results for both systolic and diastolic blood pressure [20].

However, findings have been conflicting for factors like body mass index and diabetes. For instance, the association between body mass index and aSAH varies between genders [21]. On the contrary, a borderline significant positive association between body mass index and aSAH was noted in previous MR studies conducted in both women and men [20,22]. Notably, high body mass index is a well-established risk factor for type 2 diabetes, which has shown an inverse association with aSAH in a meta-analysis of case-control studies and a non-significant inverse association in a pooled analysis of cohort studies [21]. The relationship between GHB (glycated hemoglobin, a marker of long-term blood sugar control) and the risk of intracranial aneurysm (IA) rupture is not straightforward and exhibits a nonlinear pattern. In cases where individuals with diabetes mellitus (DM) who have a single IA achieve good glycemic control, there is a reduction in the risk of IA rupture. Conversely, if glycemic control is poor, the risk of IA rupture increases [23].

Regarding lipid levels, the available observational data, primarily from case-control studies, suggest that hypercholesterolemia and high levels of high-density lipoprotein are associated with a reduced risk of aSAH [24]. Genetically determined levels of HDL-C (high-density lipoprotein cholesterol) and LDL-C (low-density lipoprotein cholesterol) have been associated with a reduced risk of intracranial aneurysms and recurrent intracranial aneurysms (rIA) [25].

2.3. Modifiable risk factors

Findings of earlier research have consistently highlighted smoking as a significant risk factor for aSAH [21,22]. A multivariate analysis comparing individuals who are current smokers to those who have never smoked, demonstrates that smoking is significantly correlated with multiple aneurysms, and an increased prevalence of aneurysms located at the basilar apex [26]. Tobacco smoke contains nicotine as well as a multitude of other compounds that have the potential to promote dysfunction in the inner lining of blood vessels, known as vascular endothelial dysfunction, and ultimately contribute to the rupture of intracranial aneurysms [27]. Mechanistic studies further reveal that nicotine exposure amplifies the risk of IA rupture by acting on vascular cell nicotinic acetylcholine receptors that contain α7 subunits. This action leads to elevated levels of vascular endothelial growth factor, platelet-derived growth factor-B, and inflammatory cytokines, all of which are processes that can compromise blood vessel integrity and increase the likelihood of an IA rupturing [27,28].

Research on the relationship between coffee consumption and the risk of aneurysmal subarachnoid hemorrhage is limited, and the findings have been inconclusive. In some studies, an inverse association between coffee consumption and aSAH risk was observed, such as in cohorts of Swedish women [29]. However, in other studies, a neutral association was reported, as seen in Finnish male smokers [30]. The latest research provides evidence suggesting that the consumption of coffee may be linked to an increased risk of IA and the subsequent occurrence of hemorrhage [31].

Regular physical activity has been linked to a lowered risk of aSAH in various population groups, including men of Japanese ancestry [32], Finnish adults [33], and over 1 million UK women [34]. Physical activity's protective effect against aSAH may be attributed to its ability to enhance endothelial function, lower blood pressure, and reduce systemic inflammation [20].

3.Case Presentation:

In the realm of neurology and vascular pathology, patients often present with a diverse array of symptoms that challenge the diagnostic acumen of healthcare providers. While ischemic strokes are typically attributed to a well-defined set of cardiovascular risk factors, such as hypertension, diabetes, and atherosclerosis, there exist uncommon scenarios that confound conventional diagnostic expectations [35-38].

This article delves into a unique case that uncovers the intricate relationship between intracranial aneurysms and neurological symptoms. Unlike the more conventional association of aneurysms with hemorrhagic strokes, in this instance, the aneurysm itself is the root cause of debilitating symptoms, as described in the literature [39]. The patient's clinical presentation is not characterized by the usual manifestations of an ischemic stroke but rather by neurological deficits directly resulting from the presence of the aneurysm.

Patient Information:

A 29-year-old female with a known history of endocrine pathology and a rural background presented with sudden-onset right limb weakness.

Clinical Examination:

The patient was in good general condition, conscious, and cooperative. Neurological examination revealed: absence of neck stiffness, preserved ocular motility in both axes, symmetric facial appearance with no nystagmus, right-sided hemiparesis, mild hypotonia, bilaterally present osteotendinous reflexes, bilateral plantar flexion. Blood cell count data and biochemical findings are stated in Table I and II.

Imaging:

Cranial CT scan revealed the absence of recent vascular lesions. However, it did indicate the presence of an aneurysmal dilation in the right infra-cavernous internal carotid artery. Additionally, there was observed thickening of the mucous membrane in the right sphenoidal sinus, along with a pre-communicating artery on the left side displaying a hypoplastic appearance. Furthermore, the ventricular system was found to be aligned along the midline.

Cranial MRI demonstrated a lack of signal abnormalities or diffusion in favor of hemorrhagic lesions and showed no current demyelination. However, it did reveal a saccular aneurysmal dilation in the internal carotid artery, specifically in the C5-C6 clival segment, measuring 8/9 mm. Additionally, the left pre-communicating artery displayed a hypoplastic appearance, while the venous sinuses remained patent, and no signs of pathological intraneural intra-axial tumor-like contrast enhancements were identified (Fig. 1).

The MRI investigation reveals a lack of signal abnormalities or diffusion in favor of hemorrhagic lesions and showed no current demyelination, with a saccular aneurysmal dilatation in the internal carotid artery, specifically in the C5-C6 clival segment, measuring 8/9 mm (Fig. 2).

Spine X-ray: The X-ray of the cervical spine revealed a slight anterior bending or compression in the C5-C6 intervertebral space in the lower half, along with a straightening of the cervical spine. The X-ray of the lumbar spine indicated an accentuated lumbar lordosis, suggesting narrowing or compression of the intervertebral spaces in the L4-S1 region.

ANGIO MRI revealed the presence of a right ICA aneurysm, encouraging the patient's transfer to the Department of Vascular Neurosurgery, where a cerebral angiography of the 4 major vessels reveals the presence of the aforementioned aneurysm (Fig. 3).

By combining the patient's medical history, general clinical examination, neurological assessment, laboratory results, and clinical explorations, we have directed our diagnosis towards: Right hemiparesis. Aneurysm in the territory of the right internal carotid artery, supported by the sudden symptomatology, manifested by a motor deficit of 4 out of 5 in the right upper limb, 4 out of 5 in the right lower limb, and mild hypotonia.

In the acute phase, the patient was prescribed Aspenter 100 mg thrice daily, later reduced to a once-daily regimen. To maintain stability, the patient received 500 ml of NaCl twice daily, and proton pump inhibitor therapy was initiated with Pantoprazole FI once daily. In addition, Famotidine at a dose of 40 mg was administered once daily.

Subsequent interventions took place, with an interventional procedure targeting the aneurysmal formation in the ophthalmic segment of the right internal carotid artery. This procedure involved a right femoral approach, using a 6F sheath and a Chaperon 6F catheter. The guiding catheter was placed within the right internal carotid artery, enabling access to the aneurysm sac via a microcatheter PX Slim. While coil embolization was attempted, it was temporarily postponed to facilitate the placement of a flow diverter. A follow-up intervention occurred to address the same aneurysmal formation in the ophthalmic segment of the right internal carotid artery. During the hospitalization period the patient received Algocalmin 500mg/2 ml, NaCl 0.9% 500 ml, Dexamethasone 8 mg/2ml, Esomeprazole 40 mg (powder for IV administration), Heparin 25,000 IU/5 ml, Iodine 10% 1000 ml, Iomeron 350 mg/100 ml, Omniopaque 350 mg/ml, Pantoprazole 40 mg (powder for injection), Paracetamol solution 100 ml, Protecardin

75 mg tablets, and Ringer's solution 500 ml.

Upon discharge, the patient was advised to follow a hygienic and dietary regimen, including low sodium, low lipid, and low carbohydrate intake. Additionally, the patient was instructed to limit sun exposure, especially avoiding extreme temperatures, and engage in physical activities within their tolerance level. For chronic home treatment, the patient was prescribed Aspenter 75 mg once daily, Controloc 20 mg twice daily for 20 days per month, and Brilique 90 mg once daily for six months, with a planned angiographic follow-up.

In conclusion, we presented the case of a 29-year-old female patient, without significant risk factors for arterial hypertension, admitted for clinical and imaging evaluation to exclude endovascular treatment of a saccular aneurysm located in the right internal carotid artery (ICA) ophthalmic segment. The decision was made to perform embolization with stent-assisted coiling, for which dual antiplatelet therapy with Aspirin and Ticagrelor was initiated.

4.Discussion

Intracranial aneurysms are typically asymptomatic but can present with neurological deficits in rare cases, as discussed by Wiebers et al. [40] note that small, unruptured aneurysms rarely cause symptoms unless they press on adjacent neural structures or cause microembolic events. The motor deficit observed in our patient aligns with findings from a study, which suggests that aneurysms in the carotid artery can present with focal neurological symptoms due to compression or microembolization [41]. For instance, bilateral extracranial aneurysms of the internal carotid artery, emphasize the rarity and potential severity of such presentations, underscoring the importance of surgical intervention in preventing complications [42].

Furthermore, the association of carotid artery aneurysms with systemic diseases such as Takayasu's arteritis, as described by Funiu et al. (2004), highlights the need for a comprehensive evaluation of the vasculature in patients presenting with atypical symptoms [43]. This is particularly relevant given the rapid enlargement and high risk of rupture associated with such aneurysms.

The therapeutic approach in this case, involving Aspenter and proton pump inhibitors followed by interventional coiling, is supported by the ISUIA study (International Study of Unruptured Intracranial Aneurysms), which found the intervention to be beneficial in patients with symptomatic aneurysms, particularly when the risk of rupture is considered high. The ISUIA study highlights the importance of intervention in preventing the potential complications associated with unruptured aneurysms [44]. In parallel, the management of extracranial internal carotid artery aneurysms often involves surgical treatment to mitigate the risk of cerebral ischemia due to thrombus formation or rupture, as discussed by Yamaguchi et al. (1997). This approach is consistent with the interventional measures taken in our patient's case, where embolization was deemed necessary to address the symptomatic aneurysm [45]. Both sources advocate for a proactive approach to aneurysms that present with symptoms or carry a significant risk of neurological complications.

Additionally, the treatment of high cervical carotid aneurysms, with bypass and carotid ligation, provides insight into the complex surgical strategies that may be required when direct arterial reconstruction is not feasible [46].

This case adds to the literature by highlighting the need for a high index of suspicion for aneurysms in atypical presentations of neurological deficits and supports a multidisciplinary approach to management, as suggested by the success seen in similar cases [47].

This case reinforces the importance of considering intracranial aneurysms in the differential diagnosis of acute neurological deficits and suggests that tailored therapeutic strategies are essential for optimal outcomes.

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Index	Value	Reference range
WBC (White Blood Cell Count)	7.96 x 10^9/L	4.0 - 11.0 x 10^9/L
BAS# (Basophil Count)	0.02 x 10^9/L	less than 0.1 x 10^9/L
BAS% (Percentage of Basophil)	0.2%	less than 1%
NEU# (Neutrophil Count)	5.88 x 10^9/L	2.0 - 7.5 x 10^9/L
NEU% (Percentage of Neutrophils)	73.9%	40 - 75%
EOS# (Eosinophil Count)	0.06 x 10^9/L	0.04 - 0.4 x 10^9/L
EOS% (Percentage of Eosinophils)	0.7%	0.5 - 5%
LYM% (Percentage of Lymphocytes)	1.58%	20 - 40%
MON# (Monocyte Count)	0.42 x 10^9/L	0.2 - 0.8 x 10^9/L
MON% (Percentage of Monocytes)	5.3%	2 - 8%
RBC (Red Blood Cell Count)	4.33 x 10^12/L	4.2 - 5.4 x 10^12/L
HGB (Hemoglobin)	13.2 g/dl	12.0 - 16.0 g/dl
MCV (Mean Corpuscular Volume)	93.3 fL	80 - 100 fL
MCH (Mean Corpuscular Hemoglobin)	30.6 pg	27 - 33 pg
MCHC (Mean Corpuscular Hemoglobin Concentration)	32.8 g/dl	32 - 36 g/dl
RDW-CV (Red Cell Distribution Width Coefficient of Variation)	12.4%	11.5 - 14.5%
RDW-SD (Red Cell Distribution Width Standard Deviation)	45.5 fL	39.0 - 46.0 fL
HCT (Hematocrit)	40.4%	36 - 46%
PLT (Platelet Count)	269 x 10^9/L	150 - 450 x 10^9/L
MPV (Mean Platelet Volume)	10.5 fL	7.4 - 11.0 fL
PDW (Platelet Distribution Width)	15.8%	9.0 - 17.0 fL
PCT (Plateletcrit)	0.282%	0.15 - 0.35%
ESR (Erythrocyte Sedimentation Rate)	5 mm/30 min	0-20 mm/30 min

Table I: Blood cell count

Index	Value	Reference range
Glucose	86 mg/dl	70 - 100 mg/dl
Serum Urea	18.7 mg/dl	7 - 20 mg/dl
Serum Creatinine	0.83 mg/dl	0.6 - 1.1 mg/dl

Total Serum Cholesterol	139.2 mg/dl	Less than 200 mg/dl
AST (Aspartate Aminotransferase)	11.7 U/L	10 - 40 U/L
ALT (Alanine Aminotransferase)	19 U/L	7 - 56 U/L
Cl (Chlorine)	102.7 mmol/L	96 - 106 mmol/L
K (Potassium)	3.75 mmol/L	3.5 - 5.0 mmol/L

Table II: Biochemical findings

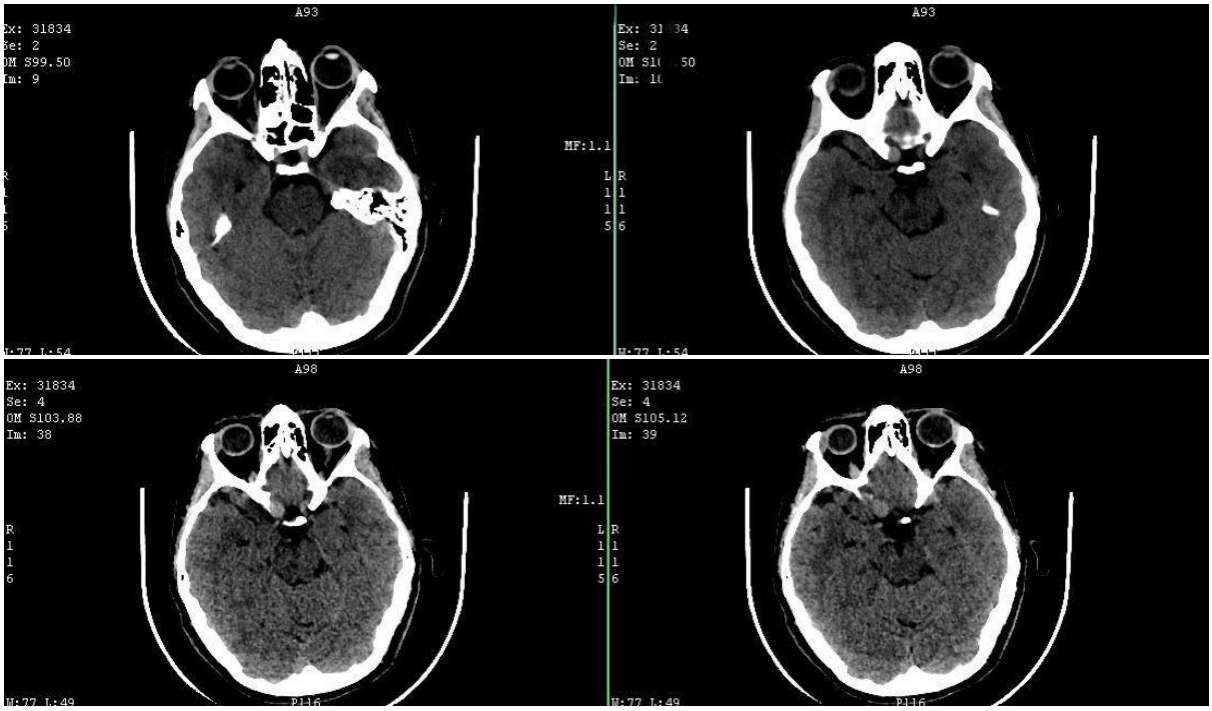

Fig. 1: CT scan evaluation reveals an aneurysmal dilation in the right infra-cavernous internal carotid artery.

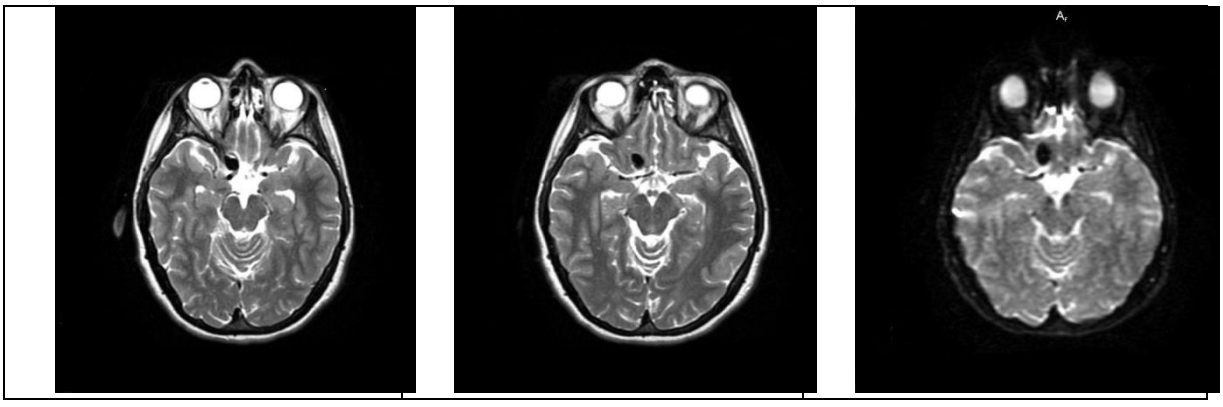

Fig. 2 MRI investigation

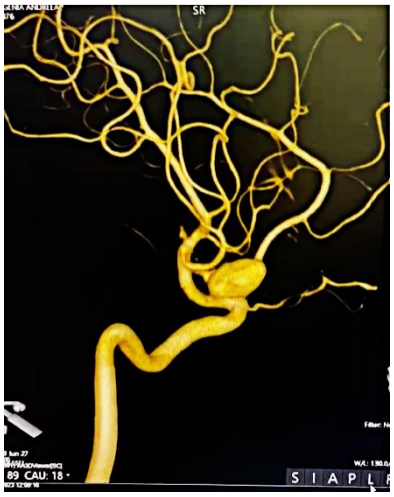

Fig. 3 Cerebral angiography of the 4 major vessels reveals the presence of the aforementioned aneurysm.

ANEVRISM INTRACRANIAN DEZVALUIT PRIN DEFICIT NEUROLOGIC - O EXPLORARE A LITERATURII ȘI O PROVOCARE DIAGNOSTICĂ

Rezumat:

Anevrismele intracraniene de tip sacular sau de tip boabă reprezintă formări anormale ale vaselor de sânge în interiorul creierului, responsabile pentru majoritatea hemoragiilor subarahnoidiene nontraumatice (HSA). Progresele în domeniul imagisticii medicale au îmbunătățit ratele de detectare, cu toate că prevalența acestor anevrisme crește odată cu înaintarea în vârstă, în special la femei. Mai mult, studiile sugerează un risc mai mare de formare a anevrismelor de novo la femei și evidențiază rolul tensiunii arteriale instabile ca factor de risc. Anevrismele sunt, de asemenea, asociate cu variații ale nivelurilor de estrogen și factori de risc cardio-metabolici, inclusiv hipertensiunea arterială, indicele de masă corporală și diabetul. Fumatul este identificat ca un factor de risc semnificativ, contribuind la disfuncția vasculară endotelială asociată fumatului și la ruptura anevrismelor. Pentru a ilustra provocările diagnostice și de gestionare ale anevrismelor intracraniene, este prezentat un studiu de caz. Cazul implică o pacientă de 29 de ani care a prezentat inițial simptome asemănătoare unui accident vascular cerebral ischemic. Evaluarea ulterioară a relevat un anevrism de arteră carotidă internă dreaptă, o discopatie cervicală-lombară și o patologie tiroidiană. Cazul evidențiază importanța evaluării amănunțite și a luării în considerare a etiologiilor atipice la pacienții cu simptome asemănătoare accidentului vascular cerebral. Studiul susține managementul proactiv al anevrismelor simptomatice, precum și rolul embolizării și reconstrucției arteriale directe în abordarea anevrismelor cu risc de complicații neurologice și subliniază necesitatea unei abordări cuprinzătoare în diagnosticul și managementul acestora, în special în cazurile cu prezentări clinice atipice.

Cuvinte cheie: anevrism intracranian; factori de risc; estrogen; cardioembolic; fumat; cafea; activitate fizică.

EXPLORING PREDICTORS AND OUTCOMES IN ISCHEMIC STROKE

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ABSTRACT

Stroke, a critical neurological event, often leaves an indelible mark on a patient's life, demanding a comprehensive understanding of its multifaceted facets for effective management and prognosis. This study focuses on ischemic strokes, which account for a significant proportion of stroke cases. With the prevalence of strokes escalating globally, identifying robust prognostic markers becomes imperative for tailoring interventions and improving patient outcomes. Key risk factors, including advanced age, hypertension, and comorbid vascular conditions, play pivotal roles in shaping the trajectory of ischemic stroke outcomes. Recognizing the intricate interplay between these factors is vital for clinicians to devise targeted therapeutic strategies. Prognosis post-ischemic stroke remains a complex puzzle, with variables such as the severity of the infarct, associated complications, and the efficacy of medical interventions influencing the clinical course. This study conducted retrospectively on a cohort of 200 patients, delves into these nuances, shedding light on correlations and patterns that can refine our understanding of ischemic stroke prognosis. In unravelling the intricacies of these factors, this research aims to contribute valuable insights that can inform evidence-based clinical practices and enhance the overall management of ischemic stroke patients.

Keywords: stroke, risk factors, prognosis;

1. Introduction

Stroke is currently defined according to the World Health Organization (WHO) definition, introduced in 1970 and still in use, as: "the rapid development of clinical signs of focal (or global) disturbance of brain function, lasting more than 24 hours or leading to death, with no apparent cause other than vascular origin" [1].

However, this broad definition encompasses strokes, cerebral and subarachnoid hemorrhages, without keeping pace with scientific progress that allows their separation based on imaging and histopathological criteria. In 2013, the American Heart Association proposed redefining these terms: stroke represents secondary cell death due to focal ischemia in the cerebral, spinal, or retinal tissue, based on pathological, imaging, or other objective evidence of ischemic injury to the cerebral, spinal, or retinal tissue in a defined vascular territory or clinical evidence of focal cerebral, spinal, or retinal ischemic injury, based on symptoms persisting for ≥24 hours or leading to death, excluding other etiologies [2].

Ischemic stroke is defined as an episode of neurological dysfunction caused by focal cerebral, spinal, or retinal infarction. Limiting the definition of ischemic stroke to a focal injury takes into account several considerations.

There are significant differences between the pathological mechanisms of focal and global ischemia, with focal ischemia resulting from the loss of perfusion in an arterial territory due to stenosis or obstruction, leading to cell death in the tributary tissue. In focal ischemia, the lesions are limited, with an ischemic core and a penumbral zone where neuronal and supporting cells are affected. Global ischemia results from a decrease in

cerebral perfusion due to decreased blood pressure (cardiogenic shock, hypovolemia) or a sudden increase in intracranial pressure (trauma), resulting in selective neuronal loss in sensitive areas (hippocampus, basal ganglia, thalamus, neocortex, cerebellum) [3].

Risk factors are any characteristics of an individual that increase the risk of a stroke compared to individuals who do not have that characteristic [4]. Some risk factors are non-modifiable, such as family history of cardiovascular diseases, age, male gender, and race, while others can be modified, leading to a reduction in the risk of the disease. These risk factors, which often coexist, account for approximately 60-80% of the risk of stroke in the general population [4]. Although complex genomic studies attempt to identify genes susceptible to stroke, with the identification of certain loci, the clinical relevance is still modest [5].

Risk factors have a cumulative effect on the structure and function of blood vessels, both locally and systemically. Many of these risk factors alter vascular structure with a pro-atherosclerotic effect, leading to arterial stiffening, narrowing, and twisting of arterioles and capillaries [6]. At the cerebral level, these morphological changes are often associated with reduced cerebral blood flow due to alterations in cerebrovascular regulation mechanisms.

Advanced age, hypertension, diabetes, and hypercholesterolemia disrupt vital adaptive mechanisms responsible for adequate perfusion [7]. The endothelial ability for microvascular regulation is compromised, while the increase in blood flow secondary to neural activity is suppressed, resulting in a desynchronization between energy demand and supply [8].

Hypertension and diabetes alter protective vascular mechanisms that maintain stable cerebrovascular flow during periods of blood pressure decline, facilitating the occurrence or increased intensity of ischemic events during decreased perfusion [9]. These vascular changes increase cerebral vulnerability to ischemia following vascular occlusion by compromising the development of collaterals from adjacent unobstructed vessels, a crucial factor in limiting the infarcted area [10].

In addition to vascular risk, advanced age and diabetes increase the susceptibility of brain cells to injury, amplifying the effects of ischemia, though the underlying mechanisms for this are not yet fully understood [11].

The assessment of stroke severity is conducted through neurological examination and is likely the most crucial factor affecting short or long-term symptoms [12]. Increasingly, in both research studies and clinical practice, neurological deficit is measured using the NIHSS (National Institutes of Health Stroke Scale), which employs a 15-point score. Several conducted studies have demonstrated that the NIHSS score effectively assesses the prognosis of an ischemic stroke [13,14]. In one report, over 1200 patients were analyzed within the first 24 hours of acute stroke symptoms onset, utilizing the NIHSS score. Each added point reduced the likelihood of a favorable outcome at 3 months by 17% [13]. An NIHSS score <6 signifies good recovery - the ability to live independently, while a score >16 is associated with an increased risk of death or severe disability [14].

The relationship between the NIHSS score and the outcome depends on the time elapsed since the onset of the first stroke symptom, partly because early deficits tend to be unstable, and many patients experience gradual recovery [14,15].

The Canadian Neurological Score (CNS) is also useful in predicting the outcome after an acute ischemic stroke. A CNS score <6.5 is associated with increased mortality at 30 days and a poor outcome at 6 months. However, compared to the CNS score, the NIHSS score more precisely relates to the prognosis at 3 months [16,17].

Following thrombolysis or thrombectomy according to guideline recommendations, patients with ischemic stroke have shown a dramatic reduction in neurological deficits.

This study aims to correlate and analyze the clinical, biological, and imaging parameters of a cohort of 200 patients who have experienced an ischemic stroke, to identify prognostic factors that influence the favorable or unfavorable progression of ischemic stroke. The specific objectives include highlighting prognostic factors associated with ischemic stroke, analyzing common and distinct aspects of the correlation between prognostic factors and the evolution of ischemic stroke, examining the influence of factors such as origin, gender, and age, identifying vascular comorbidities, and conducting an in-depth analysis and correlation of the results obtained from the studied patient cohort.

2. Materials and Methods

2.1. Study design

This investigation adopts a retrospective approach by integrating observational records obtained from the Neurology Clinic at the Clinical Hospital of Neuropsychiatry in Craiova. The study focuses on patients diagnosed primarily with Ischemic Stroke during the timeframe spanning from January 1, 2018, to June 30, 2018. To compile comprehensive insights, pertinent diagnostic information was extracted from diverse sources, encompassing patient histories, clinical examinations, neurological assessments, biological and imaging features, as well as details concerning

administered treatments and the subsequent progression of patients. This method allows for a thorough examination of the multifaceted factors influencing the course of Ischemic Stroke within the specified period.

2.2 Methodology

The extracted data encompassed various parameters across different assessment domains. From the patient history (anamnesis), information included age, gender, origin, primary diagnosis, secondary diagnoses, admission reasons, pathological personal history, and associated risk factors. The clinical examination involved evaluating language impairments such as dysarthria and aphasia, assessing expressive and spontaneous speech, repetitive and automatic speech, color and object naming, as well as expressive lexia through reading aloud, recognition of names, and explanation of read words. Writing abilities were also evaluated by the patient's ability to write their name or desired content. In the paraclinical domain, recorded parameters consisted of serum creatinine, blood glucose, and cholesterol levels. The CT examination included noting the presence or absence of recent or residual vascular lesions, cranio-cerebral traumas, intracranial hemorrhages, aneurysms, hematomas, intracranial tumors, affected vascular territories, and sequelae. Neurological examination parameters involved observing unique attitudes, and assessing involuntary movements in terms of rhythm, recurrence, frequency, speed, amplitude, coordination disorders, and balance examination results. This comprehensive dataset provides a foundation for the correlation and analysis of clinical, biological, and imaging parameters in understanding the prognosis of ischemic stroke.

3. Results

3.1. Cohort characteristics

In the initial phase of our study, our focus was on examining the distribution of ischemic stroke patients based on age, investigating the prevalence of ischemic stroke within our patient cohort according to gender, and analyzing the distribution of patients across rural and urban environments with a gender-based breakdown. Additionally, we delved into the distribution of patients based on the number of hospitalization days, aiming to gain insights into the duration of care received. Furthermore, we explored the distribution of patients concerning associated prognostic factors and pathological personal histories. These preliminary analyses form the foundation for our comprehensive examination of the clinical, demographic, and prognostic landscape of ischemic stroke in our study population. We present in Table I the cohort characteristics.

Regarding the analysis of the studied patient cohort based on age, Table I illustrates that the highest frequency of ischemic strokes occurs in the age group between 70 and 95 years (119 patients, representing 59.5% of the total studied patients), followed by the age group of 60-69 years (59 patients, representing 29.5% of the total 200 patients). In the age groups of 50-59 years, there were 16 patients (8%), and in the 40-49 age group, 7 patients were identified, accounting for 3.5%.

Secondly, the distribution of patients by gender in the studied group shows an approximately equal percentage of males at 49.5% (99 patients) and females at 50.5% (101 patients), indicating a balanced prevalence of ischemic stroke between both genders. Examining the origin environment, it highlights a higher incidence of ischemic stroke in rural areas (63%) compared to urban areas, where the percentage is 37%. These percentages suggest potential differences in medical accessibility and health education, possibly associated with an aging population and lower living standards in rural areas.

The graphic representation of hospitalization days for the 200 studied patients reveals that 61% of patients required a hospitalization period of 1-9 days, 34% needed a more extended period of 10-15 days, and only 5% required hospitalization for approximately 16-28 days. This breakdown provides insights into the duration of care received by the patients.

Hypertension stands out as a major risk and prognostic factor for ischemic stroke. Out of these, 170 patients were diagnosed with hypertension before admission, constituting 85%, while the remaining 15% were normotensive. Within the hypertensive group, the majority had grade II hypertension (69.5%), followed by grade I (11%) and grade III (4.5%).

Focusing on atrial fibrillation (AF), a potent risk and prognostic factor in ischemic stroke, 29.5% of the studied patients (59 individuals) were identified with AF, while the remaining 70.5% (141 patients) did not exhibit atrial fibrillation. The presence of AF underscores the need for correct treatment and frequent monitoring to prevent complications or unfavorable outcomes associated with ischemic stroke.

Among the total of 200 ischemic stroke patients, 62.5% (125 patients) had a history of chronic ischemic heart disease, while the remaining 37.5% (75 patients) had no known chronic ischemic heart disease. Additionally, 18% (35 patients) had a history of type II diabetes, and 34% (68 patients) had blood glucose levels exceeding 126 mg/dl. Patients with diabetes have a higher susceptibility to atherosclerosis, leading to an increased risk of ischemic stroke. While 25.5% (51 patients) of the total studied patients had dyslipidemia, the majority (74.5%) had elevated cholesterol, LDL-cholesterol, or triglyceride

levels but did not meet the criteria for dyslipidemia.

The presence of chronic kidney disease (CKD) among the studied patients is 9% (18 patients). CKD serves as both a risk factor for ischemic stroke and a negative prognostic factor for the post-stroke evolution. The impairment of renal function contributes to complications such as hypertension, anemia, sodium and water retention, nitrogen retention, hyperlipoproteinemia, and acidosis, negatively impacting the quality of life and survival of patients post-ischemic stroke.

3.2 Complications

3.2.1 Urinary tract infections

Among the myriad complications associated with stroke, we specifically directed our attention toward two prevalent issues within the studied patient group: urinary tract infections (UTIs) and pneumonia. These complications are of significant concern due to their frequency and potential impact on the overall health and recovery of stroke patients.

Urinary tract infections are a common complication observed in stroke survivors. The compromised mobility and altered neurological function often lead to challenges in maintaining proper hygiene and bladder control, contributing to an increased susceptibility to UTIs. Furthermore, issues such as urinary retention and the use of catheters can elevate the risk of bacterial infections in the urinary tract. UTIs, if not promptly addressed, can lead to systemic complications, exacerbating the recovery process for stroke patients.

Within the cohort of studied patients, 9.5%, equivalent to 19 individuals, presented with urinary tract infections (UTIs). Among these cases, 7.5% (15 patients) exhibited UTIs caused by Escherichia coli (E. coli), 1.5% (3 patients) were diagnosed with Klebsiella infection, and 0.5% (1 patient) had an infection attributed to Citrobacter. The occurrence of urinary tract infections represents a noteworthy prognostic factor that can unfavorably influence the trajectory of ischemic stroke patients, particularly among the elderly, those immobilized for extended periods, or those experiencing cognitive impairments. The identification of specific pathogens causing UTIs underscores the importance of tailored interventions and vigilant management to mitigate the impact of these infections on the overall well-being and recovery of stroke patients (Fig. 1).

3.2.2 Pneumonia

Pneumonia is another noteworthy complication frequently encountered in the aftermath of a stroke. Reduced mobility, impaired cough reflex, and compromised swallowing function contribute to an increased risk of aspirating oral or gastric contents into the lungs, leading to pneumonia. Additionally, the immobility associated with stroke may result in a weakened respiratory system, making stroke survivors more vulnerable to respiratory infections. Pneumonia, when coupled with the existing challenges posed by stroke, can significantly impede the rehabilitation and recovery of affected individuals.

Within the studied patient cohort, 8.5% (17 patients) manifested bacterial pneumonia. Bacterial pneumonia stands out as a critical complication in individuals with strokes, primarily attributed to aspiration. Aspiration is most commonly observed in patients with swallowing disorders and those experiencing altered consciousness. Therefore, oral feeding should be postponed until the patient can adequately ingest small quantities of water or even cough effectively. This highlights the significance of proactive measures in managing swallowing difficulties to mitigate the risk of bacterial pneumonia, a prominent concern in the post-stroke scenario (Fig. 2).

3.2.3 Other associated pathology

Moreover, our investigation delved into additional comorbidities, as outlined in Table II.

3.3 Stroke distribution

The distribution of the patient cohort based on the size of ischemic cerebral infarction visualized through computer tomography is depicted.

Specifically, acute ischemia at the brainstem was observed in 2 patients (1%), while acute cortical ischemia was present in 34 patients (17%). Additionally, 6 patients (3%) exhibited acute lacunar ischemia, 12 patients (6%) had acute subcortical ischemia, and 28 patients (14%) demonstrated acute cortico-subcortical ischemia. Acute cerebellar ischemia was identified in 3 patients, constituting 1.5%. Furthermore, 95 patients (47.5%) showed no recently constituted lesions. This comprehensive analysis provides insights into the varied manifestations of ischemic cerebral infarction in the studied population.

The increased size and location of the infarct are associated with an unfavorable prognosis in the evolution of patients with ischemic stroke. Most patients experienced cortical infarcts (17%), followed by 14% with cortico-subcortical infarcts and 6% with subcortical infarcts. Large-sized infarcts were identified in a total of 74 patients, and the involvement of a large area of cerebral tissue leads to various motor, sensory, mixed, language, cognitive, behavioral, memory, and consciousness disorders. In contrast, only 3% had lacunar infarcts, 1% in the brainstem, and 1.5% in the cerebellum—these being particularly severe infarcts with a severe evolution due to their impact on a multitude of vital structures in the body. This detailed analysis

highlights the variety of manifestations and consequences of infarcts among patients with ischemic stroke (Fig. 3).

The distribution of patients based on the affected cerebral territory is particularly crucial, as the evolution and prognosis of ischemic stroke depend on the specific area affected. Within the studied patient cohort, the distribution is as follows: the majority of patients suffered ischemic strokes in the territory of the left carotid artery, accounting for 28% (56 patients); 21% experienced ischemic strokes in the territory of the right carotid artery (42 patients); 14.5% in the vertebro-basilar territory (29 patients); 17% in the territory of the right middle cerebral artery (34 patients); 18.5% in the territory of the left middle cerebral artery (37 patients); 2% in the territory of the right posterior cerebral artery (4 patients); 1% in the territory of the left posterior cerebral artery (2 patients); 1% in the territory of the anterior cerebral artery (2 patients); and 3% in the total carotid territory (6 patients). This detailed breakdown underscores the diverse patterns of cerebral involvement and their significance in understanding the impact of ischemic stroke on patients (Table III).

4. Discussions

Prognostic factors for patients with ischemic stroke include the patient's age, the severity of the infarct, the mechanism of infarct production, associated comorbidities, and complications of ischemic stroke, all of which contribute to the current morbidity and mortality rates for these patients. The clinical study aimed to analyze a cohort of 200 patients diagnosed with ischemic stroke at the Neurology Clinic II over 6 months. Examining the patient group reveals that the majority of ischemic stroke patients were found in the 70-95 age group, with a relatively equal distribution between males and females, and a higher representation from rural areas. These findings align with existing literature in the field [18-20].

The study identified several associated comorbidities, out of which hypertension emerged as a major aggravating factor, affecting 85% of the studied patients, with a predominance of grade II hypertension. The next aggravating factor was diabetes mellitus, present in 18% of patients, followed by atrial fibrillation encountered in 29.5%, and chronic ischemic heart disease observed in over half of the patients.

Another important parameter aiding prognosis evaluation is neuroimaging, which provides information about infarct volume and location [21,22]. In the studied patient group, the volume of the infarct correlated as follows: cortical infarcts accounted for 17%, subcortical infarcts for 6%, cortico-subcortical infarcts for 14%, lacunar infarcts for 3%, brainstem infarcts for 1%, and cerebellar infarcts for 1%.

Infarct location is another prognostic factor determining the evolution of ischemic stroke [23,24]. Obstruction of the basilar, vertebral arteries, or any of the major intracranial vessels is associated with a very high risk of unfavorable outcomes. In this study, obstruction of the right carotid artery occurred in 21%, obstruction of the left carotid artery in 28%, and vertebro-basilar territory involvement in 14.5%.

Additionally, the literature suggests that obstruction of the middle cerebral artery is associated with a very high mortality risk due to vital structures at this level [25,26]. In the studied patient cohort, obstruction of the right middle cerebral artery occurred in 17%, while obstruction of the left middle cerebral artery occurred in 18.5%.

Complications of ischemic stroke represent prognostic factors for post-stroke outcomes [27-30]. In the studied patient group, bacterial pneumonia and urinary tract infections were encountered at percentages of 8.5% and 9.5%, respectively. These findings contribute to a comprehensive understanding of the multifaceted factors influencing the prognosis of patients with ischemic stroke.

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Age – interval	**40-49 yo**	**50-59 yo**	**60-69 yo**	**70-95 yo**
(%)	3,5%	8%	29,5%	59,5%
Sex				
M (%)	49,5%			
F (%)	50,5%			
Days of hospitalisation	**1-9 days**	**10-15 days**	**16-28 days**	
(%)	61%	34%	5%	
Comorbidities	**YES**	**NO**		
High blood pressure, N (%)	170 (85%)	30 (15%)		
Diabetes mellitus, N (%)	35 (18%)	165 (82%)		
Dyslipidaemia, N (%)	9 (25,5%)	161 (74,5%)		
Atrial fibrillation, N (%)	59 (29,5%)	141 (70,5%)		
Chronic ischemic cardiopathy, N (%)	125 (62,5%)	75 (37,5%)		
Chronic kidney disease, N (%)	18 (9%)	152 (91%)		

Table I – Cohort characteristics

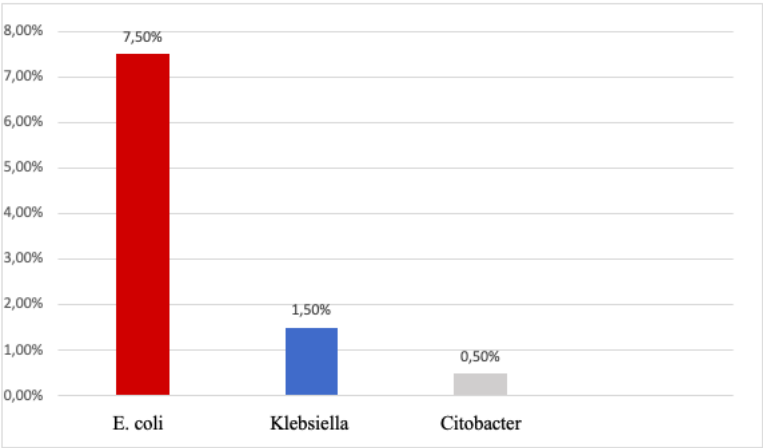

Fig. 1 – Main pathologic agents involved in UITs

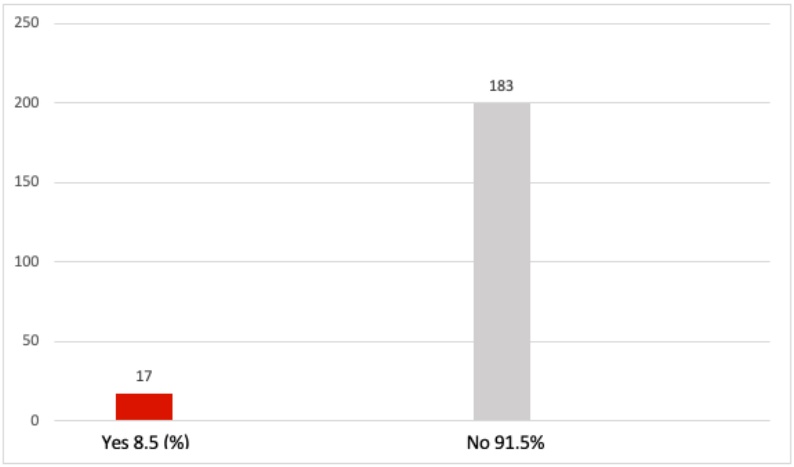

Fig. 2 – Pneumonia distribution among patients

Associated pathology	No. of patients
Previous Myocardial Infarction	5
Vascular Dementia	7
Chronic Obstructive Pulmonary Disease (COPD)	3
Asthma	2
Acute Exacerbation of Chronic Bronchitis	5
Acute Respiratory Failure	2
Chronic Kidney Disease	2
Right Lateral Basal Pleuritis	6
Congestive Heart Failure	2
Decubitus Ulcer	5
Seizures	2
Gangrene in Lower Limbs	2
Chronic Obliterative Arteriopathy	1
Colon Neoplasm	2
Prostate Neoplasm	1
Cervical Cancer	1

Table II – Associated pathology

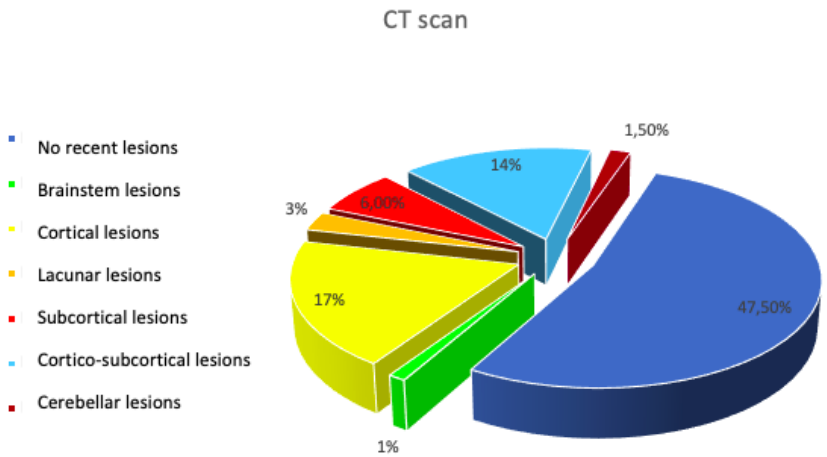

Fig. 3 - Representation of the CT examination results in the studied patient cohort.

Affected cerebral territory	No. (%)
Left carotid artery	56 (28%)
Right carotid artery	42 (21%)
Vertebro-basilar territory	29 (14.5%)
Right middle cerebral artery	34 (17%)
Left middle cerebral artery	37 (18.5%)
Right posterior cerebral artery	4 (2%)
Left posterior cerebral artery	2 (1%)
Corotid territory	6 (3%)

Table III – Affected cerebral territory

EXPLORAREA FACTORILOR DE RISC ȘI PROGNOSTIC ÎN ACCIDENTUL VASCULAR CEREBRAL ISCHEMIC

Rezumat

Accidentul vascular cerebral, un eveniment neurologic critic, lasă adesea o amprentă indelebilă asupra vieții unui pacient, solicitând o înțelegere cuprinzătoare a aspectelor sale multiple pentru managementul și prognosticul eficient. Acest studiu se concentrează asupra accidentelor vasculare ischemice, care reprezintă o proporție semnificativă din cazurile de accident vascular cerebral. Cu prevalența accidentelor vasculare în creștere la nivel global, identificarea unor markeri prognostici robusti devine imperativă pentru adaptarea intervențiilor și îmbunătățirea rezultatelor pacienților. Factorii de risc cheie, inclusiv vârsta înaintată, hipertensiunea și afecțiunile vasculare comorbide, joacă roluri pivotale în modelarea traiectoriei prognosticului accidentului vascular cerebral ischemic. Recunoașterea interacțiunii intricate dintre acești factori este vitală pentru ca medicii să elaboreze strategii terapeutice direcționate. Prognoza post-accident vascular cerebral ischemic rămâne un puzzle complex, cu variabile precum severitatea infarctului, complicațiile asociate și eficacitatea intervențiilor medicale influențând cursul clinic. Acest studiu, realizat retrospectiv pe o cohortă de 200 de pacienți, explorează aceste nuanțe, aducând în prim-plan corelații și modele care pot rafina înțelegerea asupra prognosticului accidentului vascular cerebral ischemic. Dezvăluind complexitățile acestor factori, acestă cercetare își propune să contribuie cu perspective valoroase care pot infuența practicile clinice bazate pe dovezi și pot îmbunătăți în mod general managementul pacienților cu accident vascular cerebral ischemic.

Cuvinte cheie: accident vascular cerebral, factori de risc, prognostic.

EXTRACELLULAR VESICLES IN ISCHEMIC STROKE: POTENTIAL BIOMARKERS AND THERAPEUTIC AVENUES

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ABSTRACT

Ischemic stroke, a global health concern, demands comprehensive exploration due to its substantial impact. This review delves into critical aspects, notably excitotoxicity and calcium dysregulation, which play pivotal roles in irreversible neuronal damage. Excitotoxicity, initiated by glutamate pathways, underscores the urgency of understanding these intricate mechanisms. The focus shifts to calcium dysregulation, a key contributor to ischemic cell death. Various ion channels, pumps, and receptors are intricately involved in this process, emphasizing the need for a nuanced understanding. Amidst these complexities, extracellular vesicles (EVs) emerge as crucial mediators in intercellular communication. Once considered cellular debris, EVs now present promising potential in stroke therapy. However, challenges in EV isolation and analysis are acknowledged, emphasizing the importance of reproducible biomarkers. As EVs exhibit multifaceted impacts on neuroinflammation, neurogenesis, and angiogenesis, their role in stroke pathophysiology becomes increasingly complex. With ongoing studies that provide a comprehensive perspective, EVs are positioned as promising candidates for both diagnostic biomarkers and therapeutic interventions in ischemic stroke.

Keywords: stroke, EVs, biomarkers.

1. Introduction

Stroke stands as the primary contributor to long-term disability and ranks as the fifth most prevalent cause of death in the United States [1]. This debilitating condition, characterized by the sudden disruption of blood flow to the brain, not only poses a significant threat to life but also has enduring consequences for those who survive [2]. The impact of stroke extends beyond its immediate life-threatening nature, often resulting in long-term impairments that affect individuals' quality of life and independence [3]. The European Union is projected to experience a 27% rise in the number of individuals affected by stroke from 2017 to 2047. This increase is primarily attributed to population aging and enhanced survival rates [4].

The findings from the BASIC study (Brain Attack Surveillance in Corpus Christi) spanning the years 2000-2010 revealed a noteworthy decline in the occurrence of ischemic strokes among individuals aged 60 and above. In contrast, rates for those aged between 45 and 59 remained relatively stable. According to the study's statistics, the adjusted incidence of hemorrhagic strokes, accounting for age and ethnicity during the same period, decreased from 5.21 per 10,000 (95% CI, 4.36-6.24) to 4.30 per 10,000 (95% CI, 3.21-5.76) [5]. Every year, approximately 55,000 more women than men experience a stroke (GCNKSS, NINDS - Greater Cincinnati/Northern Kentucky Stroke Study, National Institutes of Neurological Disorders and Stroke) [6]. Women face a higher risk of stroke compared to men. In the FHS (Framingham Heart Study), the lifetime risk of

stroke among individuals aged 55 to 75 was 1 in 5 for women (95% CI, 20% - 21%) and approximately 1 in 6 for men (95% CI, 14% - 17%) [7]. Age-correlated incidence is significantly lower in women than in men in younger and middle-aged groups. However, these differences diminish, and in the most advanced age group, incidence rates for women become approximately equal to or even higher than those for men [8,9].

Upon the initiation of pathological pathways in the ischemic cascade, irreversible neuronal damage occurs in the ischemic core within minutes of onset [10]. The severity of the injury depends on the degree and duration of ischemia and the brain's ability to recover and repair [11].

Due to high intrinsic metabolic activity and increased concentrations of neuroexcitatory substances (such as glutamate), brain tissue is vulnerable to ischemia [12]. This vulnerability may result from in-situ thrombosis or occlusive embolic events, often stemming from atheromatous plaques at the carotid or aortic arch levels. Neurologic dysfunction can manifest within seconds to minutes, hours, or even days from the onset of the ischemic event.

2. Neuronal Vulnerability in Ischemic Cascade

2.1 Glutamate excitotoxicity

Excitotoxicity and calcium excess are major factors in the early stages of ischemic cell death. The pathway is glutamate-dependent, the most abundant neurotransmitter that accumulates in the extracellular space due to ion pump dysfunction and reuptake mechanisms [13]. Excess glutamate leads to prolonged stimulation of NMDA and AMPA ionotropic receptors, increasing the influx of calcium, sodium, and water at the neuronal level. Massive calcium influx activates catabolic processes mediated by proteases, lipases, and nucleases [14]. Additionally, the activation of Ca2+-dependent enzymes leads to the production of nitric oxide, arachidonic acid metabolites, acting as triggers for cell death. Oxidative phosphorylation becomes inefficient, followed by ATP depletion and the generation of reactive oxygen species (ROS), releasing calcium from the mitochondria, accelerating the series of events leading to cell death [15].

Despite laboratory studies demonstrating glutamate toxicity and the positive impact of new therapies in neuroscience, clinical trials aiming to inhibit NMDA and AMPA receptors have not demonstrated their benefits in treating stroke patients [16]. The NMDA-AMPA model does not account for calcium influx independent of glutamate secondary to oxygen and glucose deprivation [17]. Moreover, it cannot consider the death of other cell types such as astrocytes, vascular cells, and microglia in the ischemic context. The selective increase in synaptic receptors promotes neuronal survival, while the activation of extrasynaptic receptors promotes cell death, in part through the activation of protein kinase 1 [18].

The MAP kinase family plays a role through phosphorylation-dependent regulatory mechanisms, such as the NR2A and NR2B receptor subunits [19]. Under ischemic conditions, cell vulnerability is reduced by modifying postsynaptic proteins. Targeting glutamate receptors early would lead to decreased channel regulation by suppressing depolarization [20].

2.2. Calcium dynamics in ischemic conditions

The elevated calcium influx resulting from the overactivation of glutamate receptors, along with calcium ions released from mitochondria and other cellular deposits, is nonetheless insufficient to offset the intracellular calcium accumulation due to excitotoxic stimulation. Other ion channels and pumps activated during ischemia are also involved in calcium accumulation mechanisms, including the Na+/Ca2+ pump, hemichannels, volume-regulated anion channels, and TRP channels [21-24]. ASIC1a, activated by ischemia-induced acidosis, is implicated in calcium influx, with its inhibition exhibiting a neuroprotective effect [25]. As these channels are activated at a specific pH commonly encountered in ischemia, this could explain the worsening prognosis associated with the onset of acidosis. Disruption of the Na+/Ca2+ pump also leads to calcium accumulation. The prostaglandin E2 EP1 receptor plays a significant role in altering Na+/Ca2+ exchanges during the ischemic period, and inhibiting them has a neuroprotective effect [26,27].

2.3. Reactive Oxygen Species {ROS}

Numerous factors associated with cardiovascular risk contribute to an upsurge in the generation of reactive oxygen species (ROS), fostering both a systemic and cerebral pro-inflammatory environment [28]. The vascular origins of ROS encompass enzymes like NADPH oxidase, xanthine oxidase, mitochondrial enzymes, and nitric oxide synthase. The detrimental repercussions of oxidative stress on blood vessels often correlate with the biological inactivation of nitric oxide [29]. Consequently, the loss of nitric oxide's vasoregulatory effects on vascular endothelium leads to vasoconstriction, disrupting the regulation of microvascular flow [29].

This loss further extends to the anti-aggregating, anti-proliferative, and cell adhesion maintenance effects, resulting in platelet aggregation, leukocyte adherence to endothelial cells, and proliferation of smooth muscle tissue – pivotal stages in the inflammatory response

within blood vessels [30]. ROS amplifies inflammation by elevating blood-brain barrier permeability through heightened expression of vascular endothelial growth factor, cytokines, matrix remodeling enzymes, metalloproteinases, and proinflammatory genes [31]. Additionally, the alteration of vasomotor reactivity occurs through the inactivation of crucial enzymes in endothelial and smooth muscle cells, coupled with the activation of poly-ADP-ribose polymerase, leading to ATP depletion and dysfunction of ion-dependent channels at the vascular level [32].

Simultaneously, vascular inflammation induces an augmented production of ROS, establishing a cyclic pattern that significantly intensifies vascular damage. Plasminogen activation, triggered by oxidative stress and a pro-inflammatory state, facilitates matrix remodeling, smooth muscle cell migration, and intimal hyperplasia – factors that potentiate the development of atherosclerosis [33-35]. Oxidative stress and vascular inflammation stand as predominant pathways through which the detrimental impact on blood vessels is manifested.

3. The role of Extracellular Vesicles (EVs) in ischemic stroke

3.1. Prognostic biomarkers

Extracellular vesicles (EVs) have become a focal point of considerable interest in recent decades, particularly within the realm of biomarker research. Initially dismissed as cellular debris or casually labeled with terms such as 'placental mist,' their significance has evolved, and they are now recognized as crucial mediators of intercellular communication. The spectrum of nomenclature continues to expand annually, propelled by the advent of novel isolation techniques and advancements in methods for quantifying and visualizing extracellular vesicles [36]. While numerous investigations have delved into neuroprotective and recovery strategies following ischemic stroke, the available data on circulating biomarkers possessing prognostic and diagnostic value remains somewhat limited. Blood-based biomarkers have emerged as a focal point of intensive research and are widely acknowledged as valuable tools for predicting the prognoses and survival rates of individuals who have experienced a stroke [37]. Consequently, the utilization of readily accessible biomarkers, detectable through quantitative polymerase chain reaction (qPCR)-based techniques from biofluids, holds the potential to significantly enhance our ability to predict long-term sensorimotor recovery with a high level of accuracy [38].

EVs exhibit a unique molecular profile contingent upon their origin, reflecting the phenotypic composition of the donor cell to some extent. In the context of stroke, various cell types within the brain and circulatory system have been demonstrated to release EVs into the bloodstream. This phenomenon underscores the potential of EVs to serve as informative biomarkers, offering insights into the molecular characteristics of the cells involved in the pathophysiology of stroke [39].

In the context of acute ischemic stroke (AIS), research has highlighted the correlation between the shedding of endothelial cell-derived extracellular vesicles (EVs) and the severity, lesion volume, and overall outcomes of AIS [40]. Another study focused on AIS revealed a general increase in EV release from various blood and vascular compartment cells, including endothelial cells, platelets, erythrocytes, leukocytes, monocytes, lymphocytes, and neural precursor cells [41]. Additionally, platelet-derived EVs were found to be elevated in AIS subgroups with large artery atherosclerosis (LAA) and small vessel disease (cSVD) [42]. Specifically, platelet EVs were significantly increased in patients with AIS due to large vessel occlusion (LVO) and cSVD [43].

Intracerebral hemorrhage (ICH) studies indicated a distinct temporal profiling of EVs, with origins from endothelial cells, erythrocytes, and neutrophils, revealing dynamic changes over time [44]. Annexin V-positive EVs were reported to be elevated in ICH patients compared to controls, with variations depending on the specific origin of EVs, including endothelial, leucocyte, and erythrocyte EVs [45-46]. Another study supported the finding of increased annexin V-positive EVs in ICH compared to controls at admission [47].

The technical hurdles linked to the consistent isolation of extracellular vesicles (EVs), coupled with the intricate methodologies essential for their efficient analysis, could hinder the utilization of 'pure' EV populations as reliable biomarkers. While the discovery of biomarkers is crucial for enhancing diagnosis, monitoring, and predicting outcomes in neurodegenerative diseases, prioritizing reproducibility and user-friendliness is paramount [48].

Although extracellular vesicles (EVs) demonstrate promising value in terms of prognosis, there remains a critical need for more comprehensive studies to thoroughly assess the longitudinal and dynamic evolution of the plasmatic response. It is essential to exercise caution when interpreting the results, considering the intricate nature of EV interactions within the circulatory system. Further investigations with a focus on the temporal aspects of EV release and their correlation with the evolving clinical condition will contribute to a deeper understanding of their diagnostic and prognostic potential in the context of various medical conditions, including

stroke.

3.2. Treatment potential

Exosomes originating from multipotent mesenchymal stem cells (MSCs) have proven effective in preserving white matter integrity and fostering functional recovery in a murine model of subcortical hemorrhage, simulating hemorrhagic stroke [49]. Additionally, these MSC exosomes demonstrate the capability to alleviate brain injury and curb neuroinflammation after middle cerebral artery occlusion (MCAO) by inhibiting the differentiation of proinflammatory M1 microglia [50].

The infusion of extracellular vesicles (EVs) in stroke-afflicted mice reveals an activation of the TGF-β/Smad2/3 signaling pathway in the ischemic brain. Additionally, EVs enriched with TGF-β1, secreted by microglia preconditioned under oxygen-glucose deprivation (OGD), induce M2 polarization in resident microglia within the ischemic cerebral environment. This process may play a role in regulating the early inflammatory response in postischemic hemispheres [51].

Experimental investigations focusing on ischemic stroke have revealed that extracellular vesicles (EVs) possess immunomodulatory and neuroprotective properties. These findings suggest that EVs may play a role in stimulating neurogenesis and angiogenesis in the context of ischemic stroke [52].

In light of the promising outcomes observed in animal models, the exploration of extracellular vesicles (EVs) transitioned to human studies. This shift in focus reflects the growing interest and optimism surrounding the potential translational impact of EVs in the field of ischemic stroke.

In a phase I/II clinical trial, Bang et al. administered intravenous BM-MSC transplantation twice within 9 weeks after ischemic stroke to five patients. No deaths, recurrences, or post-transplant abnormalities were reported, and significant improvement in Barthel's Index was observed during the first year compared to controls [53]. In a 5-year study, Lee et al. administered two intravenous autologous BM-MSC doses to 85 post-ischemic stroke patients. The MSC group showed a substantially higher survival rate (72%) than the control group (34%), correlating with increased blood SDF-1 levels and reduced damage in the lateral ventricles [54].

4. Conclusion

In conclusion, the intricate pathophysiological processes underlying ischemic stroke involve a cascade of events, from excitotoxicity and calcium influx to oxidative stress and inflammation. Despite significant strides in understanding these mechanisms, translating preclinical findings into effective clinical interventions remains a formidable challenge. The multifaceted roles of extracellular vesicles (EVs) emerge as a promising avenue, with their potential to serve as both diagnostic biomarkers and therapeutic agents. While studies have elucidated the immunomodulatory and neuroprotective properties of EVs in animal models, their application in human subjects presents a new frontier in stroke research. The ongoing clinical trials and evolving methodologies for EV isolation and analysis underscore the dynamic nature of this field. As we navigate this scientific landscape, the integration of EVs into stroke management holds great promise, offering a glimpse into the future of more effective diagnostic strategies and innovative therapeutic interventions for ischemic stroke.

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VEZICULELE EXTRACELULARE ÎN ACCIDENTUL VASCULAR CEREBRAL ISCHEMIC: DE LA BIOMARKERI LA PERSPECTIVE TERAPEUTICE

Rezumat
Accidentul vascular cerebral ischemic este o problemă globală de sănătate, ce necesită o explorare exhaustivă datorită impactului său semnificativ. Această review investighează aspecte critice, în special excitotoxicitatea și alterarea homeostaziei calciului, care joacă roluri cheie în deteriorarea ireversibilă a neuronilor. Excitotoxicitatea, inițiată de căile glutamatului, subliniază urgența înțelegerii acestor mecanisme intricate. Se pune accentul pe alterarea calciului, un contribuitor cheie la moartea celulară ischemică. Diverse canale ionice, pompe și receptori sunt implicate în acest proces, subliniind necesitatea unei înțelegeri superioare. În acest context, vesiculele extracelulare (EV-uri) apar ca elemente cruciale în comunicarea intercelulară. Odată considerate reziduuri celulare, EV-urile prezintă acum un potențial promițător în terapia accidentului vascular cerebral. Cu toate acestea, se recunosc provocările legate de izolarea și analiza EV-urilor, subliniind importanța markerilor biologici reproductibili. Rolul EV-urile este demonstrate in ceea ce priveste neuroinflamația, neurogeneza și angiogeneza, însă complexittea lor în fiziopatologia accidentului vascular cerebral rămâne încă incomplete elucidată. În ultimii ani, tot mai multe studii oferă o perspectivă cuprinzătoare, poziționând EV-urile ca posibili candidați atât pentru markeri diagnostici, cât și pentru intervenții terapeutice în accidentul vascular cerebral ischemic.
Cuvinte cheie: accident vascular cerebral, veziculele extracelulare, biomarkeri.

EXPLORING DIVERSITY: MURINE MODELS FOR INDUCING SEPSIS IN PRECLINICAL RESEARCH

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ABSTRACT

Murine sepsis models play a crucial role in preclinical research, providing an essential platform for mimicking and understanding the complexity of sepsis pathogenesis. These models allow researchers to accurately reproduce critical aspects of the immune response and microbial interactions in a controlled environment. The use of endotoxins or the cecal ligation and puncture (CLP) method in murine models provides a systematic approach to studying the immune response, enabling the investigation of both the early and late phases of sepsis. The advantages of these models include reproducibility, control over experimental variables, and the ability to manipulate genetics to assess the specific role of genes. However, in selecting murine models for sepsis studies, careful consideration of ethical aspects, minimizing animal suffering, and maximizing scientific validity is crucial. In the context of future research, emerging technologies such as advanced imaging and personalized models represent promising directions to improve relevance and deepen our understanding of sepsis pathology at the molecular and functional levels.

Keywords: sepsis, CLP, LPS, murine models

Introduction

Sepsis investigation is critical when it comes to preclinical studies, considering its highly important and consequential impact on global public health (1, 2). It is a life-threatening syndrome which arises from the dysregulated host response to infection, and it remains a leading cause of mortality and morbidity worldwide (3). Sepsis elucidation pathogenesis requires a thoughtful exploration of its intricate molecular and cellular mechanisms, a task that is optimally approached in the controlled environment afforded by preclinical research (4). Murine models, owing to their biological similarities and genetic tractability, play a pivotal role in this investigative landscape (5). Murine models offer a controlled experimental platform to simulate various aspects of sepsis, while facilitating the systematic dissection of immune responses, microbial interactions, and host tissue dynamics (6). The purpose of this article is to comprehensively examine the diversity inherent in murine models utilized in preclinical sepsis research, thereby enriching our understanding of sepsis pathogenesis. Through a meticulous analysis of these models and their applications, this exploration aims to contribute to the refinement of experimental methodologies, ultimately enhancing the translational relevance of preclinical findings to the complex clinical landscape of human sepsis.

Importance of Murine Models in Sepsis Research. Advantages of Murine Models in Preclinical Sepsis Research

Murine models are indispensable tools in preclinical research, offering several advantages that significantly contribute to the understanding of sepsis pathogenesis. Firstly, murine models offer precise control over experimental conditions, facilitating the systematic manipulation of variables to elucidate specific aspects of sepsis progression (7). The genetic tractability of mice allows for the development of transgenic or knockout models, providing researchers with the capability to investigate the role of specific genes in sepsis susceptibility and outcomes (8). Moreover, the short reproductive cycle of mice allows generations of large cohorts, facilitating robust statistical analyses and enhancing the reproducibility of experimental findings (9). The anatomical and physiological similarities between murine and human immune systems further enhance the translational relevance of findings from murine models to clinical sepsis scenarios (10).

Relevance of Murine Models in Studying Sepsis Mechanisms

Murine models allow a controlled environment for the meticulous dissection of immune responses, from the initial recognition of pathogens to the downstream signaling cascades (4). For instance, the use of murine models has been instrumental in delineating the roles of various

immune cells, such as macrophages and neutrophils, in sepsis-induced tissue damage (11). Also, murine models allow the study of host-microbe interactions, helping with the identification of microbial factors that contribute to sepsis severity (12). The insights obtained from murine studies enhance our understanding of sepsis at a molecular level, offering the information for the development of targeted therapeutic interventions (13).

Limitations and Challenges Associated with Murine Models

Although murine models offer invaluable insights, it is essential to acknowledge their limitations and challenges in accurately recapitulating the complexity of human sepsis. One notable limitation is the inherent variability in murine immune responses, which may differ from those observed in humans (14). Additionally, murine models may oversimplify the diverse etiologies and comorbidities associated with human sepsis, potentially limiting the generalizability of findings (15). The artificial nature of experimental sepsis induction methods, such as cecal ligation and puncture (CLP) or endotoxin administration, may not fully replicate the heterogeneity of clinical sepsis scenarios (16). Furthermore, the ethical considerations surrounding the use of animal models in research necessitate a constant evaluation of the balance between scientific advancements and animal welfare (17). Addressing these challenges is crucial for the judicious interpretation of murine model data and the meaningful translation of research findings to the complex clinical landscape of human sepsis.

III. Diversity in Murine Sepsis Models

a. Endotoxin-Induced Models

Endotoxin-induced models represent a widely employed approach in murine sepsis research, aiming to simulate bacterial infection by administering lipopolysaccharide (LPS), which is an essential component of Gram-negative bacterial cell walls (6). This model leverages the ability of LPS to activate Toll-like receptors, though triggering a robust inflammatory response reminiscent of bacterial infections (6). The endotoxin-induced model offers multiple advantages, including reproducibility, controlled experimental conditions, but also the ability to investigate specific aspects of the host response to bacterial components (18). Variations in this model involve adjusting LPS dosages, administration routes, and timing, allowing researchers to modulate the intensity and duration of the inflammatory response (18). These variations enable the exploration of diverse facets of sepsis pathophysiology, from the early stages of immune activation to the later phases characterized by immunosuppression (19).

b. Cecal Ligation and Puncture (CLP)

The Cecal Ligation and Puncture (CLP) method stands as a gold standard in murine sepsis models, serving as a robust technique to induce polymicrobial sepsis by mimicking the clinical scenario of intra-abdominal infection (13). The CLP model involves the ligation and puncture of the cecum, leading to the release of fecal contents into the peritoneal cavity and subsequent systemic infection (13). The relevance of CLP in inducing polymicrobial sepsis is particularly significant, as it mirrors the complexity of clinical sepsis scenarios where multiple pathogens may contribute to the infection (13). Variations in CLP models include adjustments in cecal ligation tightness, puncture size, and the number of punctures, allowing for the modulation of sepsis severity and the investigation of distinct clinical phenotypes (14). These variations enhance the model's versatility and contribute to its widespread use in understanding the dynamic aspects of sepsis progression.

c. Other Models

In addition to endotoxin-induced and CLP models, alternative murine models offer unique insights into sepsis pathophysiology. For instance, models involving the infusion of live bacteria provide a more authentic representation of bacterial infections, allowing the study of microbial virulence factors and host responses in real-time (20). Alternatively, the peritoneal contamination and infection (PCI) model involves the introduction of fecal material into the peritoneal cavity, leading to a polymicrobial infection akin to clinical scenarios involving abdominal sepsis (6). These models offer specific advantages, such as mimicking the natural course of infection, thereby providing a holistic understanding of sepsis dynamics. Researchers can strategically choose from this array of murine models based on the specific aspects of sepsis they aim to investigate, fostering a comprehensive exploration of sepsis pathogenesis.

IV. Considerations in Murine Model Selection:

a. Ethical Considerations

The ethical implications of utilizing murine models in sepsis research are paramount, necessitating a careful balance between scientific advancement and animal welfare. Ethical concerns primarily revolve around the potential for pain, distress, and suffering experienced by animals during experimentation. Efforts to address these concerns involve stringent ethical review processes, ensuring that research protocols adhere to established guidelines and regulations (21). Moreover, refinements in experimental design, including the use of analgesics and anesthesia, aim to minimize discomfort and distress in murine subjects (21). Researchers are increasingly adopting the principles of the 3Rs (Replacement, Reduction, Refinement) to enhance the ethical dimensions of murine studies, emphasizing the pursuit of alternatives, reduction of animal numbers, and refinement of experimental procedures to maximize scientific validity while minimizing the impact on animal welfare (22).

b. Relevance to Human Sepsis

Assessing the translational relevance of murine models to human sepsis is crucial for the meaningful extrapolation of preclinical findings to clinical scenarios. While murine

models offer valuable insights, inherent differences between murine and human immune systems pose challenges in directly translating experimental outcomes (23). A comprehensive understanding of the similarities and disparities between murine and human sepsis is essential for refining model selection. Despite limitations, murine models remain indispensable for elucidating fundamental aspects of sepsis pathophysiology (17). Advances in genetic engineering and the development of humanized mouse models, wherein murine immune components are replaced with human counterparts, show promise in bridging the translational gap (12). However, the complexity of sepsis and the diverse patient populations affected pose ongoing challenges for achieving a direct correlation between murine models and the clinical manifestation of human sepsis (24). Future improvements in model design, incorporation of advanced technologies, and collaborative interdisciplinary approaches are imperative for enhancing the translational relevance of murine models in sepsis research.

V. Recent Advances and Innovations

Recent years have witnessed significant strides in advancing murine sepsis models, with novel techniques and groundbreaking studies contributing to the refinement of preclinical research. One noteworthy development involves the integration of advanced imaging technologies, such as intravital microscopy and bioluminescence imaging, allowing real-time visualization of immune responses and microbial dynamics within living organisms (25). These imaging modalities offer unprecedented insights into the spatial and temporal aspects of sepsis pathogenesis. Additionally, the incorporation of genetically modified murine strains with fluorescent markers enables the tracking of specific immune cell populations, providing a nuanced understanding of cellular dynamics during sepsis (26). Such technological innovations enhance the granularity of data obtained from murine models, fostering a more comprehensive comprehension of sepsis progression.

VI. Future Directions

The evolving landscape of murine sepsis models opens avenues for future research aimed at further refining and expanding our understanding of sepsis pathophysiology. One promising direction involves the development of personalized murine models that consider individual genetic variations and susceptibility factors (27). This approach could enhance the model's relevance to the heterogeneity observed in human sepsis. Furthermore, the integration of systems biology approaches, encompassing omics technologies and computational modeling, holds potential for unraveling the complex network interactions underlying sepsis (26).

VII. Conclusion

In conclusion, the exploration of murine sepsis models has been marked by recent advances that leverage cutting-edge technologies to enhance the precision and depth of preclinical investigations. These innovations contribute significantly to unraveling the complexities of sepsis pathogenesis, offering unprecedented insights into immune responses, microbial interactions, and therapeutic interventions. The imperative for continued research in refining and expanding murine models remains evident. By embracing emerging technologies, incorporating personalized approaches, and adopting systems-level analyses, the field is poised to overcome current limitations and bridge the translational gap between murine models and human sepsis. The ongoing commitment to rigorous, ethically sound, and technologically sophisticated research is paramount for advancing our understanding of sepsis and translating preclinical insights into meaningful clinical applications.

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EXPLORAND DIVERSITATEA: MODELE MURINE PENTRU INDUCEREA SEPSULUI ÎN CERCETAREA PRECLINICĂ

Rezumat

Modelele murine de sepsis reprezintă instrumente cruciale în cercetarea preclinică, oferind o platformă esențială pentru mimarea și înțelegerea complexității patogenezei sepsisului. Aceste modele permit cercetătorilor să reproducă cu precizie aspecte critice ale reacției imune și ale interacțiunilor microbienelor într-un mediu controlat. Utilizarea endotoxinelor sau a metodei de legare și perforare a cecumului (CLP) în modelele murine furnizează o abordare sistematică în studiul răspunsului imun, permitând investigarea fazelor timpurii și târzii ale sepsisului. Avantajele acestor modele includ reproducibilitatea, controlul asupra variabilelor experimentale și posibilitatea manipulării genetice pentru a evalua rolul specific al genelor. Cu toate acestea, în selectarea modelelor murine pentru studiul sepsisului, este crucială evaluarea atentă a aspectelor etice, minimizarea suferinței animalelor și maximizarea validității științifice. În contextul cercetării viitoare, tehnologiile emergente, precum imagistica avansată și modelele personalizate, reprezintă direcții promițătoare pentru îmbunătățirea relevanței și aprofundarea înțelegerii patologiei sepsisului la nivel molecular și funcțional.

Cuvinte cheie: sepsis, CLP, LPS, murine

A PSYCHIATRY PERSPECTIVE OF COVID-19 DISEASE IN PATIENT HOSPITAL ADMISSION

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Abstract:

The Covid-19 pandemic has posed a significant challenge to society and the medical system, particularly impacting patients with psychiatric conditions. Studies indicate a correlation between imposed restrictions and an increase in decompensations among these patients, while access to specialized services has been limited. The pandemic's effects on the mental health of the general population are still under investigation, with signals of increased anxiety and depression. The SARS-CoV-2 virus has neurotropic potential, emphasizing the need for essential interdisciplinary collaboration for the proper treatment of patients. This research examines changes in the accessibility of patients in the psychiatry ward during the pandemic, considering factors such as restrictions, types of pathologies, and socio-demographic characteristics of patients. Comparative data reveal significant shifts in the management of psychotic, manic, and depressive disorders, suggesting that patients with psychiatric conditions have faced difficulties due to reduced access to psychiatry clinics, and ambulatory services have been affected by the fear of potential Covid-19 infection. The elevated levels of anxiety and depression have been felt across the entire population.

Keywords: Covid-19, psychiatry admission, SARS-CoV-2

Introduction

In late December of 2019, an outbreak of mysterious pneumonia emerged in the Huanan Seafood Wholesale Market, located in Wuhan, Hubei, China. The symptoms included fever, dry cough, fatigue, and occasional gastrointestinal issues. This initial outbreak was first noticed in December 2019 and affected approximately two-thirds of the market's employees. The market was shut down on January 1, 2020, following an epidemiological notice issued by the local health authorities on December 31, 2019 [1,2].

However, during the subsequent month of January, the disease continued to rapidly spread, impacting thousands of individuals across various regions in China, including provinces like Hubei, Zhejiang, Guangdong, Henan, Hunan, as well as cities such as Beijing and Shanghai. The virus also made its way to other countries, including Thailand, Japan, the Republic of Korea, Vietnam, Germany, the United States of America, and Singapore. The first case in our country was reported on January 21, 2019. This outbreak was eventually identified as a new beta-coronavirus, labeled as the 2019 novel coronavirus (2019-nCoV), drawing parallels with the severe acute respiratory syndrome (SARS-2003) caused by another beta-coronavirus that had emerged 17 years earlier [3,4].

In 2003, a novel coronavirus was identified in southeastern China, specifically in Guangdong province, and it was named the SARS coronavirus, confirming Koch's principles of disease causation. This virus had a mortality rate ranging from 10% to 15%. Despite advancements in medical facilities, there is still no effective treatment or vaccine for SARS. Another outbreak, linked to a novel coronavirus, took place in the Middle East in 2012, sharing many characteristics with the 2003 outbreak. Both of these outbreaks were caused by coronaviruses, with MERS thought to have an intermediate host in the dromedary camel, resulting in a fatality rate of up to 37%.

Both SARS and MERS often present with non-specific initial clinical symptoms, although most patients exhibit fever and respiratory issues. Hospital staff who lack proper protection and have contact with infected patients are at risk of contracting the disease, leading to nosocomial infections. Additionally, cases of SARS, MERS, and COVID-19 related to travel have been documented. COVID-19 presents a significant risk to Taiwan due to global travel and the prevalence of tourism [2-9].

Epidemiology

The first laboratory-confirmed case of 2019-nCoV infection presented with symptoms on December 1, 2019, in Wuhan, China (as shown in Table I).1 Initially, reports emerged of an outbreak associated with a local market, the Huanan Seafood Market, involving at least 41 individuals.[10]

On December 31, 2019, the local health authority issued an "epidemiologic warning," and the market ceased its operations on January 1, 2020. A total of 59 suspected cases involving fever and a dry cough were referred to a designated hospital, namely the Jin Yin-tan Hospital. Among these cases, 41 patients had their suspected cases confirmed through next-generation sequencing or real-time reverse transcription-polymerase chain reaction (RT-PCR). It's worth noting that 27 out of these 41 patients (66 percent, 27/41) had a history of exposure to the Huanan Seafood

Market.[10]

However, there is an important caveat: the initial case on December 1 did not have a history of exposure to the Huanan Seafood Market, and additional cases began to emerge nine days later on December 10. In the subsequent days, a wave of cases spread from Wuhan to other parts of Hubei province. This virus then affected multiple cities and regions. One contributing factor may have been the increased travel associated with the Chinese Lunar New Year, which fell on January 25. On January 13, 2020, the first documented case outside of China was reported in Thailand. The illness, however, rapidly and widely disseminated. Not only were familial clusters observed, but outbreaks occurred on cruise ships as well. As of February 6, 2020, the World Health Organization (WHO) had reported a total of 28,276 confirmed cases and 565 fatalities worldwide, spanning at least 25 countries.[40] On January 30, 2020, the WHO declared a public health emergency of international concern (PHEIC). To mitigate the spread, stringent quarantine measures and fever surveillance systems were implemented. Initially, the mortality rates for hospitalized patients were estimated to be in the range of 11%–15% [10,11] but more recent data suggests a range of 2%–3%. Person-to-person transmission is highly likely through respiratory droplets and physical contact. Nosocomial infections have occurred in healthcare settings, underscoring the critical importance of infection control measures.

Clinical manifestations

Fever (83–98%), cough (76–82%), and shortness of breath (31–55%) are the most common clinical manifestations. Approximately 15% of patients may present with all three of these symptoms.[1, 9] Notably, conjunctival injection was not reported in the initial cases, and the occurrence of the disease in individuals under 18 years of age was rare. Following the onset of symptoms, the disease typically progresses with moderate severity, and the median time to the first hospitalization is around 7.0 days (ranging from 4.0 to 8.0 days). However, in roughly 39% of patients, the condition may progress to shortness of breath (around 8 days), acute respiratory distress syndrome (ARDS, approximately 9 days), and the need for mechanical ventilation (about 10.5 days). Patients with severe conditions may develop ARDS and experience rapid deterioration, eventually succumbing to multiple organ failures.[1,10] The initial death rate in the early series of hospitalized patients was estimated to be between 11% and 15%, but subsequent data indicate a lower rate of 2%–3%.[10,4-7]

SMELL AND TASTE DISORDERS

Anosmia and dysgeusia are commonly observed as early symptoms in individuals with COVID-19, with more than 80% of patients in one study reporting these issues. A meta-analysis of 83 studies involving over 27,000 individuals found that 48% of patients experienced olfactory dysfunction (with a 95 percent confidence interval of 41.2-54.5). These symptoms can manifest as the initial indicators of COVID-19, even in the absence of nasal congestion or discharge. However, they are seldom the sole manifestations of COVID-19 [12].

Patients with COVID-19 have been reported to exhibit MRI signal abnormalities in one or both olfactory bulbs, which can be resolved with follow-up imaging. Pathological findings in two autopsy cases revealed inflammatory infiltration and axonal destruction in the olfactory tracts, although it remains uncertain whether direct viral infection is the root cause. Instead of direct damage to olfactory neurons, transient anosmia might result from inflammatory changes in the sustentacular cells of the nasal epithelium. Limited evidence is available regarding the olfactory cleft of the nasal canals, based on an MRI study of 20 anosmia patients. Improvement in olfactory function was correlated with reduced blockage at the one-month follow-up [13].

Reliable evidence concerning the long-term prognosis is lacking. In one study, the average duration of symptoms was eight days among the 33% of affected patients who regained their olfactory function. In an Italian study of non-hospitalized patients with olfactory dysfunction, 83% reported complete recovery 37 days after the onset of symptoms. Full recovery was observed in 84% and 96% of 51 individuals with anosmia who underwent objective olfactory testing at four and eight months, respectively. These symptoms can also be triggered by other viral infections and various factors, with a separate explanation provided for their evaluation [14].

ENCEPHALOPATHY

Encephalopathy is prevalent among critically ill COVID-19 patients. Delirium was widespread in a cohort study of 2088 COVID-19 patients admitted to an intensive care unit, affecting 55% of them. In a study of 509 COVID-19 hospitalized patients, 31.8% experienced encephalopathy, and those with encephalopathy tended to be older (66 versus 55 years), had a shorter time from symptom onset to hospitalization (6 versus 7 days), were more likely to be male, and had more risk factors than those without encephalopathy. These risk factors included a history of any neurologic disorder, cancer, cerebrovascular disease, chronic kidney disease, diabetes, dyslipidemia, heart failure, hypertension, or smoking [15].

COVID-19 can manifest with encephalopathy as a primary symptom. Encephalopathy was found in 28% of 817 older individuals (median age 78 years) evaluated in the emergency department and diagnosed with COVID-19 infection. Thirty-seven percent of these patients did not exhibit classic COVID-19 symptoms such as fever or dyspnea. Risk factors for encephalopathy included older age, visual impairment, a history of Parkinson's disease or stroke, and prior use of psychoactive medications [16].

The 2019-nCoV virus can enter the host through the respiratory tract or mucosal surfaces, such as the conjunctiva. There is no evidence to support oral-fecal

transmission. The virus exhibits a specific affinity for human airway epithelial cells, and like SARS, it uses ACE2 as its cellular receptor. Nevertheless, the clinical variations and pathophysiology of the disease in humans are not yet fully understood. It remains unclear whether the virus can replicate in other parts of the body.

Diagnosis

COVID-19 typically presents as an acute viral respiratory tract infection, and it can be confused with other common viral pneumonias, including influenza, parainfluenza, adenovirus infection, respiratory syncytial virus infection, metapneumovirus infection, and atypical pathogens like Mycoplasma pneumoniae and Chlamydophila pneumoniae infections, among others. Therefore, it is crucial to inquire about a suspected patient's travel history and exposure when they return from an epidemic area. Commercial respiratory syndromic diagnostic kits, such as the Filmarray Respiratory Panel, which can identify multiple causative agents, may aid in achieving a rapid differential diagnosis. [11,9]

Laboratory diagnosis of COVID-19 should be performed in a well-equipped laboratory with biosafety level 3 containment. COVID-19 is classified as a fifth-category notifiable communicable disease in Taiwan, and cases must be reported to the Taiwan Centers for Disease Control (CDC) within 24 hours. COVID-19 is defined based on specific criteria, as of February 7, 2020, including clinical conditions, laboratory evidence, and epidemiological factors.

Clinical conditions must include either a febrile illness (with a temperature of 38°C or higher) or an acute respiratory infection with signs of pneumonia on clinical, radiological, or pathological examinations.

Laboratory conditions may involve clinical specimens that are isolated and identified as 2019-nCoV, or a clinical specimen that tests positive through RT-PCR.

Epidemiological factors should cover events occurring within 14 days before symptom onset, including travel history to epidemic areas, interaction with individuals suffering from fever or respiratory symptoms in the first-class epidemic area (Hubei, including Wuhan and Guangdong), prior travel to or residence in other regions of mainland China (including Hong Kong and Macau), or interaction with suspected or confirmed COVID-19 cases.

If a patient meets any of the criteria mentioned above, they should be reported to the CDC within 24 hours. Even if the initial laboratory report is negative, if a patient's symptoms persist without an identifiable cause, a second sample should be analyzed 24 hours later to rule out a false-negative result.

Laboratory diagnosis is typically carried out using real-time reverse transcription polymerase chain reaction (RT-PCR) targeting specific regions of viral RNA, such as the pan beta-CoV consensus E region, RdRp, or N region. Imaging studies like chest x-rays and computed tomography (CT) scans often reveal bilateral pneumonia (75–98%) with various patterns such as mottling and ground-glass opacity. Routine laboratory findings in the early stages of the COVID-19 epidemic are similar to those of common viral infections, including lymphopenia, prolonged prothrombin time, elevated D-dimer, liver enzymes (alanine aminotransferase), total bilirubin, and lactate dehydrogenase, with more severe abnormalities in intensive care unit cases. Leukocytosis may occur in cases of secondary bacterial infection. Physicians should consider the necessity of regular blood sampling and aspiration while taking precautions to minimize the risk of unintended exposure [11,12].

Treatment

As of now, there is no established therapy for COVID-19. The primary approach involves providing supportive and symptomatic treatment, which includes monitoring vital signs, maintaining oxygen levels, and addressing complications such as secondary infections or organ failure.

Numerous investigational treatments are being explored due to the potential severity of COVID-19:

Remdesivir: Gilead Sciences, Inc. is developing this new nucleotide analog prodrug, which is an experimental antiviral medication initially studied for the treatment of Ebola and SARS. A case report in the United States showed a reduction in viral loads and clinical improvement when remdesivir was administered on day 11 of the illness. However, randomized controlled studies are necessary to establish its safety and effectiveness in treating 2019-nCoV-infected individuals.

Convalescent therapy (plasma from recovered COVID-19 patients): This method involves passive immunization, which has been explored in research related to MERS. Potential treatment options include convalescent plasma, interferon-beta/ribavirin combination therapy, and lopinavir. Nevertheless, there is currently no experience with COVID-19, and no randomized controlled clinical studies have evaluated these treatments.

Antiviral drugs: Lopinavir/ritonavir and ribavirin have been used to treat SARS with apparent clinical benefits. In vitro studies have shown antiviral activity against the SARS-associated coronavirus. Notably, recent research found similarities between the unique insertions in the 2019-nCoV spike protein and HIV-1 gp120 and Gag [17].

Prevention

Given the lack of effective therapies for COVID-19, preventing infection and further transmission is crucial. The general public is advised against traveling to COVID-19 epidemic areas, primarily in China, including Wuhan, Hong Kong, and Macau, as well as contact with or consumption of wild animals. For individuals returning from epidemic areas within the last 14 days, self-monitoring of body temperature and symptoms is recommended. If compatible symptoms emerge, approved transportation should be used to avoid unprotected exposure.

Healthcare professionals should wear personal

protective equipment when caring for suspected or confirmed patients and follow proper procedures for its removal. Strict infection control measures should be implemented for high-risk medical procedures. If healthcare personnel are exposed to a patient's blood or bodily fluids without protection, the exposed area should be thoroughly cleansed. The patient's body temperature should be monitored for 14 days following the exposure. Confirmed cases should be isolated, preferably in a negative pressure isolation room or, alternatively, in a single room with good ventilation. Isolation may be discontinued if symptoms have improved for 24 hours, and two consecutive negative tests have been obtained. Deceased individuals should be either cremated or buried deeply.

Effective disinfection methods include the use of active ingredients like sodium hypochlorite (0.1–0.5%), 70% ethyl alcohol, povidone-iodine (iodine%), chloroxylenol (0.24%), 50% isopropanol, 0.05% benzalkonium chloride, and 1% cresol soap. For areas with blood or bodily fluid spills, a 1:10 dilution of 5.25% household bleach should be used for cleaning, following WHO recommendations for Ebola virus disinfection.

In summary, 2019-nCoV is a zoonotic illness with a relatively low to moderate fatality rate. Currently, there is no established treatment for the condition, and only supportive care is available. While various experimental studies are underway, the best approach to prevent a widespread outbreak is the strict implementation of infection control measures. Healthcare providers should consider the possibility of 2019-nCoV infection in patients with relevant travel or exposure history and presenting symptoms. Frontline healthcare workers should be well-versed in appropriate infection prevention strategies for suspected cases.

The period of the Covid-19 pandemic meant a real challenge for society and the medical system considering the multiple symptoms and forms of manifestation of the infection with the SARS CoV-2 virus, each specialty being involved in the diagnosis and treatment of this condition.

In multiple studies, the connection is made between the period of national restrictions and the increase in the number of decompensations of patients with various psychiatric conditions, but access to specialized services was limited.

The effects of the pandemic on the population are still under investigation, but several studies claim that among them would be the remaining of anxiety, depression in the general population, but also the decompensation of old cases of psychiatric pathology.

Coronaviruses can cause multiple neuropsychiatric complications, being neurotropic, and interdisciplinary collaboration is essential for the adequate treatment of patients.

We started from the hypothesis that the addressability of patients in the psychiatric ward changed during the pandemic, several factors being involved.

We compared the hospitalizations of the Craiova Psychiatry Clinics I and II from the period 01.03.2019-28.02.2022.

In the pre-pandemic year, 1845 patients were admitted to the II Psychiatry Clinic. The percentages by pathology were as follows: 4.44% Dementia, 18.75% Organic personality disorder, 23.74% Disorders from the spectrum of schizophrenia and other psychotic disorders, 5.8% Bipolar affective disorders and 46.07% Depressive disorders, and in Clinic I Psychiatry, from a total of 1886 patients, the percentages were as follows: 1.80% Dementia, 9.54% Organic personality disorder, 16.91% Schizophrenia spectrum disorders and other psychotic disorders, 5.51% Bipolar affective disorders and 53.98% Depressive disorders.

During the pandemic period, 01.03.2020 to 28.02.2021, Clinic II Psychiatry, being a Covid support ward, limited its admissions only to psychiatric emergencies and suspected/confirmed patients with Covid-19 infection, admitting 595 patients, and Clinic I Psychiatry 1133 patients. The percentages by pathology were as follows: 4.03% Dementia, 18.49% Organic personality disorder, 33.95% Disorders from the spectrum of schizophrenia and other psychotic disorders, 6.55% Bipolar affective disorders and 29.92% Depressive disorders, and in Clinic I Psychiatry – 1.85% Dementia, 8.91% Organic personality disorder, 22.69% Schizophrenia spectrum disorders and other psychotic disorders, 8.12% Bipolar affective disorders and 43.25% Depressive disorders.

In the period 01.03.2021- 28.02.2022, Clinic I Psychiatry had a total of 1127 hospitalizations, the percentages being as follows: 2% Dementia, 6% Organic personality disorder, 25% Disorders from the spectrum of schizophrenia and other psychotic disorders, 10% Disorders bipolar affective and 57% Depressive disorders.

Clinic II Psychiatry had 793 admissions, and the percentages by pathology were as follows: 5% Dementia, 18% Organic personality disorder, 32% Schizophrenia spectrum disorders and other psychotic disorders, 9% Bipolar affective disorders and 36% Depressive disorders.

It is observed that psychotic disorders and manic episodes in particular could not be managed in the outpatient setting, but also the increase in the percentage of depressed patients.

We conclude that psychiatric patients encountered difficulties by reducing access to psychiatric clinics, the addressability to outpatient services was also impacted by the fear of a possible Covid-19 infection, in conditions where the increased level of anxiety and depression was felt by the whole population.

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ADRESABILITATEA PACIENȚILOR CU AFECȚIUNI PSIHIATRICE ÎN CONTEXTUL PANDEMIEI COVID-19

Rezumat:

Perioada pandemiei Covid-19 a însemnat o adevărată provocare pentru societate și sistemul medical având în vedere multiplele simptome și forme de manifestare ale infecției cu virusul SARS CoV-2, fiecare specialitate fiind implicată în diagnosticarea și tratamentul acestei afecțiuni. În multiple studii se face legătura între perioada de restricții naționale și creșterea numărului de decompensari ale pacienților cu diverse afecțiuni psihiatrice, însă accesul la servicii de specialitate a fost limitat. Efectele pandemiei asupra populației sunt încă în investigare, dar mai multe studii susțin că printre acestea s-ar număra resterea anxietății, depresiei în populația generală, dar și decompensarea cazurilor vechi de patologie psihiatrică. Coronavirusurile pot da multiple complicații neuropsihice, fiind neurotrope, iar colaborarea interdisciplinară este esențială pentru tratamentul adecvat al pacienților. Am pornit de la ipoteza că adresabilitatea pacienților în secția de psihiatrie s-a modificat în perioada pandemiei, fiind implicați mai mulți factori. Am evaluat comparativ internările Clinicii II Psihiatrie Craiova din perioada 01.03.2019-28.02.2022, anterioară pandemiei, dar și perioada ce a inclus starea de urgență și alertă națională după tipul de patologii și informațiile socio-demografice ale pacienților, cât și după numărul de zile de internare. În perioada martie 2020 – februarie 2021 clinica a fost unitate suport covid, fiind limitat numărul de internări la urgențele psihiatrice și pacienții confirmați/suspecți Covid – 19 ce asociau patologie psihiatrică. Se observă că tulburările psihotice și episoadele maniacale, în special nu au putut fi gestionate în ambulatoriu, dar și creșterea procentului de pacienți depresivi. Concluzionăm că pacienții de psihiatrie au întâmpinat dificultăți prin reducerea accesului la clinicile de psihiatrie, adresabilitatea la serviciile de ambulatoriu a fost afectată și de teama unei posibile infecții cu Covid-19, în condițiile în care nivelul crescut de anxietate și depresie a fost resimțit de întreaga populație.

Cuvinte cheie: Covid-19, spitalizare, pishiatrie, SARS-CoV-2.

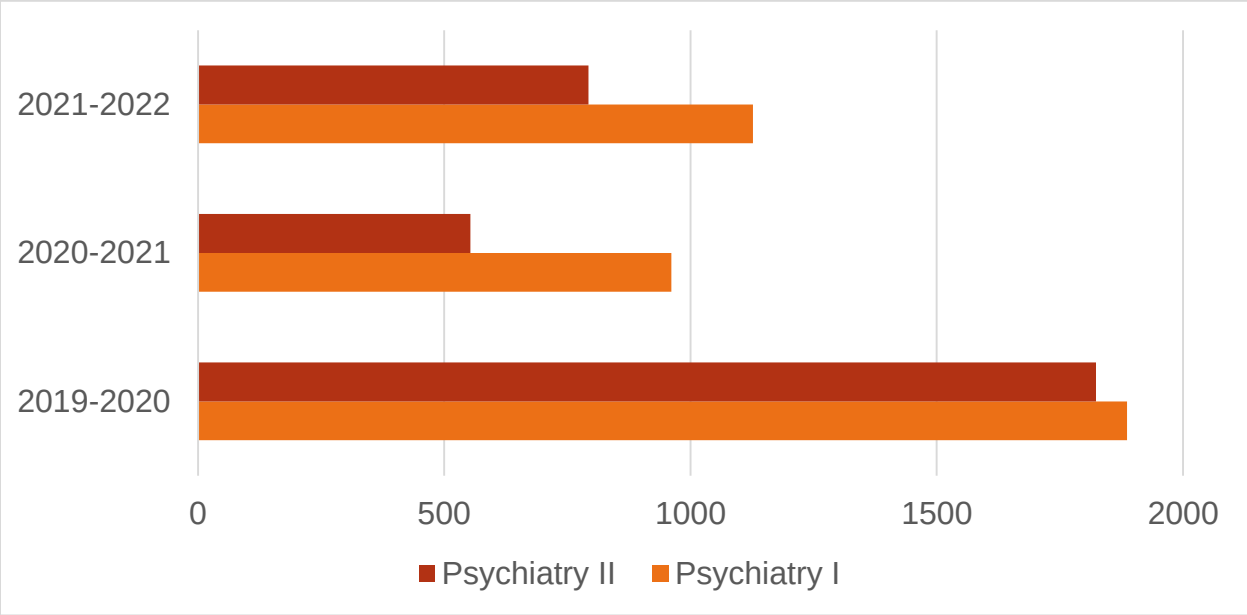

FIG. 1 Total number of admitted patients

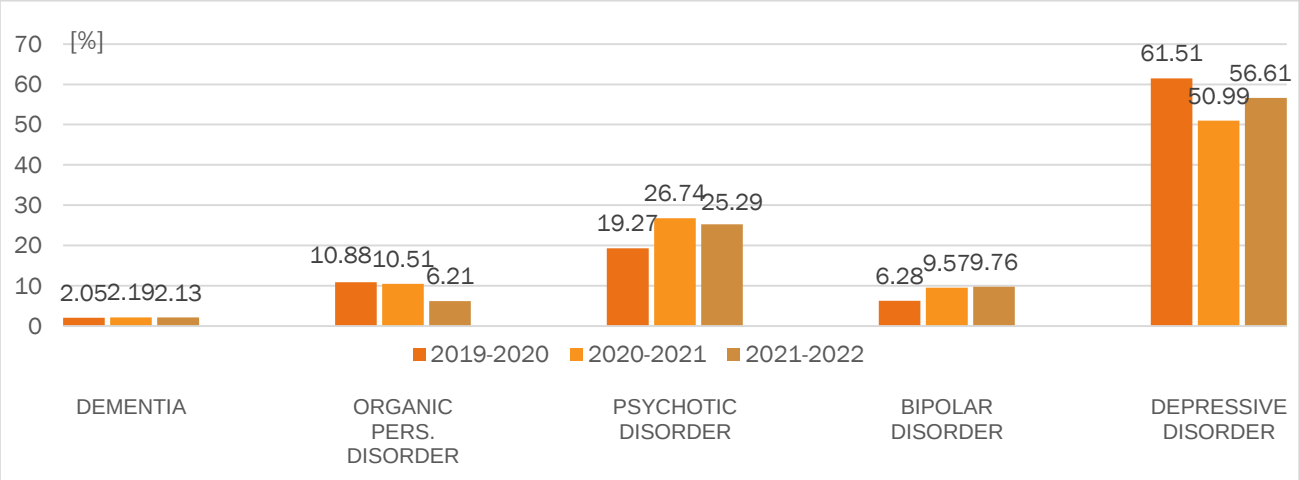

FIG. 2 Percentage of admitted cases by diagnosis in Psychiatry I Clinic

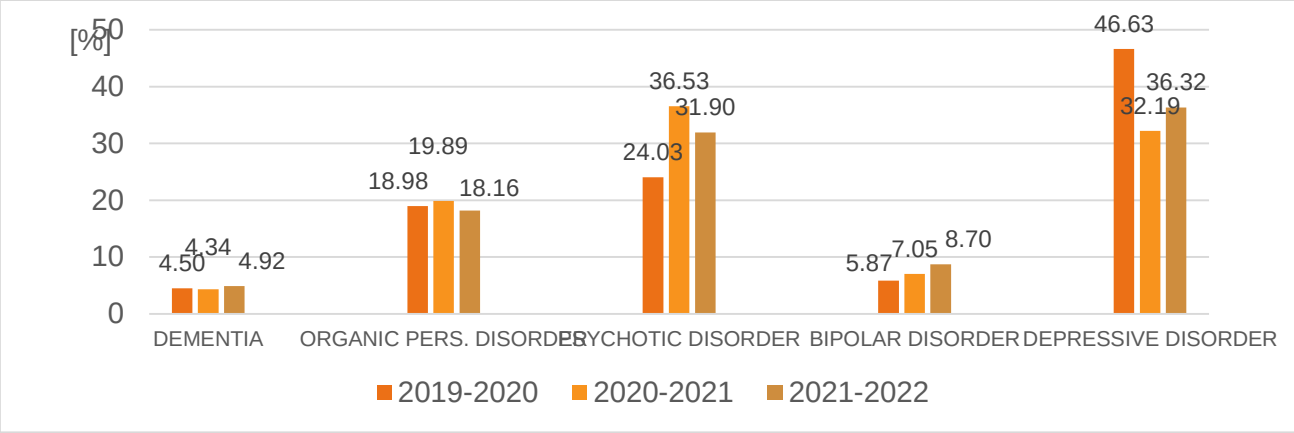

FIG. 3 Percentage of admitted cases by diagnosis in Psychiatry II Clinic

ALCOHOL MISUSE – DEMOGRAPHY, PREVALENCE AND COMPLICATIONS

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ABSTRACT

Alcohol abuse represents a pervasive global issue, with alcohol consumption being the most widespread among substances used for addictive purposes. This makes alcohol intoxication the leading cause of acute intoxication. On a global scale, the risk of developing harmful alcohol consumption patterns is 15% for men throughout their lifetime, approximately double that for women. The risk of developing alcohol dependence is lower, with a 10% risk for men and around 5% for women. The findings of this study lead to the conclusion that "Harmful Alcohol Use" encompasses a range of conditions, including alcohol abuse, alcohol dependence, alcohol use disorder, and alcoholism. It constitutes a significant contribution to the global burden of disease, being a major preventable cause of death and a prevalent psychiatric disorder.

"Harmful Alcohol Use" is a medical condition characterized by the diminished capacity of an individual to control or cease alcohol consumption, even in the face of adverse social, professional, or health consequences. Globally, alcohol consumption is responsible for 3.3 million deaths each year, contributing to disabilities and the overall poor health of millions of people. Harmful alcohol use accounts for 5.1% of the global disease burden.

In a study conducted on patients admitted to the Clinical Hospital of Neuropsychiatry in Craiova due to mental and behavioral disorders resulting from alcohol, several key findings emerged: the majority of admitted patients were men, representing 88% of cases. A significant portion of patients came from rural areas, constituting 78% of total admissions. The highest proportion of patients fell into the 45-54 years age group, representing 41% of cases. The most prevalent diagnosis among admitted patients was "withdrawal state with and without delirium," followed by "harmful alcohol consumption" and "psychotic disorder."

Keywords: alcohol consumption, psychiatric disorder, complications.

Introduction

Alcohol misuse is a widespread global issue. Among all substances used for addictive purposes, alcohol consumption is the most prevalent, making ethanol intoxication the primary cause of acute intoxication.

The prevalence of alcohol-related disorders varies with age, with the highest rates occurring in individuals aged 18-29 and the lowest rates (only 1.5%) in those over the age of 65 years. Approximately 3.6% of the population between the ages of 15 and 65 years experience an alcohol use disorder, with higher rates observed in developed countries compared to developing ones. Eastern European countries have the highest prevalence (10.9%), followed by America (5.2%) and Africa (1.1%). In Europe, Eastern and Northern

European countries exhibit the highest rates of alcohol-related disorders, while Mediterranean countries have the lowest rates.

On a global scale, the lifetime risk of developing harmful alcohol consumption patterns is 15% for men and approximately double that for women. The risk of developing alcohol dependence is lower, with a 10% risk for men and around 5% for women.

Numerous epidemiological studies conducted over periods ranging from 4 to 20 years have definitively established the variable nature of annual ethanol consumption, making it impossible to predict the potential trajectory of alcohol consumption over time. In urban adults aged 25-65, the complications resulting from excessive alcohol use rank alcoholism as the fourth leading cause of mortality in the United States. Alcohol-induced complications in the USA contribute to over 100,000 deaths each year.

In Romania, despite the absence of specific epidemiological data, a concerning trend of continuous growth in daily alcohol consumption is evident. An increase in unregulated alcohol production and the prevalence of consumption in increasingly larger quantities have also been observed. Privately produced alcoholic beverages in Romania frequently surpass the maximum allowable methanol limit (0.8%), with over 75% containing up to 5% methanol. These factors have led to the highest recorded mortality rate from ethanol-related liver cirrhosis in Europe.

The rates of alcohol-induced deaths and disabilities were estimated to be 3.8% and 4.6%, respectively.

Given this context, the implementation of proactive measures to reduce alcohol consumption in Romania is not just a recommendation but an imperative requirement. Without such measures, the World Health Organization (WHO) predicts that in the coming years, Romania will exceed an annual per capita consumption of 10 liters.

Onset, course, and prognosis of harmful alcohol use disorders

Generally, the initiation of alcohol consumption takes place during adolescence. It is widely acknowledged that genetic predisposition contributes to approximately 40-60% of the development of alcohol consumption disorders, with the remaining influence attributed to the interplay of environmental factors affecting the individual [1].

Another factor that significantly impacts the progression of the consumption pattern is the consumer's level of impulsivity. Individuals characterized by high levels of impulsivity tend to experience an earlier onset of consumption-related disorders and often exhibit more severe manifestations of these issues.

Typically, the peak level of alcohol consumption occurs in the age range of 18-22. Abusive consumption often begins or continues between the ages of 20 and 30, leading to alcohol addiction emerging around the age of 40. This extended journey, punctuated by multiple unsuccessful attempts to halt or regulate consumption, ultimately culminates in cumulative complications resulting from chronic abuse, often leading to death around the age of 60.

Maintaining sobriety for a duration exceeding one year is likely the most significant indicator of a potentially positive outcome. Although the long-term progression following comprehensive rehabilitation therapy has not been extensively studied, it has been observed that the most effective therapeutic outcomes are achieved through initial hospitalization in a specialized center for a relatively brief period (approximately 4-6 weeks), followed by the implementation of individualized long-term therapeutic interventions (lasting around 6-12 months) outside the hospital.

Chronic ethanol consumption leads to a multitude of morphological and physiological changes in the body, causing significant medical consequences. However, these consequences are sometimes reversible once alcohol consumption is ceased.

The harmful impact of ethanol on the liver can be attributed to its preferential utilization as an energy source by hepatocytes at the expense of lipids. This leads to the accumulation of lipids in the liver, subsequently released into the bloodstream, resulting in hepatic steatosis and hyperlipidemia. Even minimal alcohol consumption disrupts gluconeogenesis, affecting the conversion of carbohydrates into fats. This disruption accounts for the initial degenerative liver change, characterized by hepatic steatosis, followed by the development of ethanolic hepatitis, perivenular fibrosis, and ultimately, liver cirrhosis. When ethanol consumption is associated with any pre-existing liver condition, it invariably heightens liver toxicity and reduces the effectiveness of therapeutic interventions for the underlying pathology.

Alcohol consumption can facilitate the colonization of the stomach by the Helicobacter pylori bacterium, suggesting that alcohol consumption is not the primary factor leading to ulcer disease. Furthermore, alcohol consumption can exacerbate or prolong the healing process of pre-existing ulcers, disrupt the normal patterns of gastrointestinal peristalsis, contribute to the development of gastroesophageal reflux, and lead to hemorrhagic gastritis.

Additionally, ethanol is responsible for inducing structural, metabolic, and circulatory changes in the small intestine. It also contributes to the erosion of the intestinal villi, which, combined with increased intestinal motility, disrupts the absorption of nutrients. This can result in the development of hemorrhoids, often associated with portal hypertension.

Recurrent episodes of pancreatitis, marked by endocrine and exocrine pancreatic insufficiency, have the potential to progress to malabsorption and diabetes mellitus, or even death.

Cardiovascular complications in individuals who chronically consume ethanol stem from the direct toxic impact of ethanol on striated muscle fibers. This damage can result in myocardial inflammation, cardiomyopathy, left ventricular impairment, and the onset of cardiac arrhythmias. Typically, these cardiovascular complications manifest in approximately 25% of chronic ethanol users [2].

It is crucial to emphasize the importance of diligent cardiac monitoring during ethanol withdrawal, as preexisting cardiac conditions can deteriorate, and they may present with new signs and symptoms that differ from their usual clinical manifestation [2].

Hematological complications arising from the detrimental use of alcohol are quite diverse. To begin with, liver dysfunction results in sodium retention, which, in turn, increases plasma volume and leads to a reduction in hematocrit levels. Hemolytic anemia occurs due to the presence of macrocytes, schizocytes, and red blood cells displaying a "target" appearance in the bloodstream. These abnormalities in red blood cells are a consequence of alterations in the lipid components of the erythrocyte membrane stemming from liver dysfunction [3].

The occurrence of leukopenia, particularly lymphopenia, is responsible for the heightened susceptibility to infections observed in individuals who are chronic alcohol users [3].

At the pulmonary level, ethanol contributes to the development of obstructive sleep apnea by directly inhibiting the relaxation of the respiratory tract. Additionally, liver dysfunction can lead to the onset of respiratory alkalosis.

Liver and pancreatic impairment result in disruptions in carbohydrate metabolism. Ethanol consumption reduces tissue insulin response, while ethanol withdrawal reduces tissue insulin sensitivity, leading to fluctuations in blood glucose levels, which can be either too low or too high.

Depletion of essential ions such as K+, Na-, and Mg2+ disrupts the hydro-electrolytic balance and can trigger ethanolic ketoacidosis and lactic acidosis. These conditions often manifest clinically with symptoms like vomiting, nausea, abdominal pain, and abnormal blood glucose levels.

Alcohol also impacts the circadian rhythm, as it acts as a stimulant, causing daytime fatigue and insomnia while making it difficult to fall asleep and maintain sleep. Even moderate ethanol consumption in individuals who are not chronic drinkers can reduce REM sleep [4].

Acute alcohol intoxication and alcohol withdrawal

The colloquial term "drunkenness" actually refers to acute intoxication, which presents clinically with verbal and behavioral disinhibition, speech difficulties (dyslalia), emotional instability, and a state of euphoria. Alcohol overdose, on the other hand, happens when an individual ingests (either voluntarily or involuntarily) a quantity of alcohol exceeding what their body can handle. This overdose is characterized by symptoms such as coma, cyanosis (bluish skin discoloration), pulmonary edema, slowed breathing (respiratory depression), pinpoint pupils (miosis), lowered body temperature (hypothermia), low blood pressure (hypotension), cardiac arrhythmias, and profuse sweating.

Alcohol withdrawal typically occurs only in individuals who have developed physical alcohol dependence. It encompasses a combination of physical and psychological effects that arise after abruptly discontinuing alcohol consumption. The initial, uncomplicated withdrawal symptoms generally emerge around 72 hours after the cessation of drinking.

The first clinical signs, appearing in the early hours of withdrawal, include fever, high blood pressure (arterial hypertension), rapid heart rate (tachycardia), and tremors in the extremities. Within the following 24 hours, individuals may experience illusions, visual hallucinations (less commonly auditory ones), psychomotor agitation, anxiety, mental confusion, and worsening of symptoms associated with sympathetic nervous system activation. This clinical presentation is especially prevalent in individuals with preexisting comorbidities who have consumed significant quantities of alcohol.

One of the most concerning complications during the withdrawal process is known as delirium tremens. Despite therapeutic advances, this condition still carries an extremely high mortality rate, particularly within the first 48 hours. Delirium tremens is a medical-psychiatric emergency that necessitates specialized intensive care and is characterized by severe disturbances in mental function and overall bodily equilibrium [5].

Oneirism is linked to the occurrence of false recognitions, along with visual, auditory, olfactory, and gustatory hallucinations. It also involves experiences of allo-psychic phenomena, where individuals have perceptions related to external stimuli and temporal-spatial disorientation. In the context of delirium tremens, oneirism is distinctive due to the zoopsic nature of the hallucinations. This means that the hallucinations often involve animals, and what sets it apart is the patient's direct involvement or participation in the hallucinatory scenario, making the experience particularly vivid and intense [5].

The information was extracted by processing statistical data from the sanitary statistical forms, from the Clinical Hospital of Neuropsychiatry Craiova, and represents all the data and information related to the characteristics of patients admitted to the Psychiatry ward, who received diagnoses following the Manual of diagnosis and statistical classification of mental disorders, revision 5 (DSM-V) and Statistical Classification and International Classification of Diseases and Related Health Problems, Revision 10 (ICD-10). All information was taken in full respect of the patient's right to confidentiality. The phenomenon was analyzed throughout five years (2017-2021), so the designed study is a retrospective one. The total statistical collectivity analyzed is a dynamic type and is made up of the 6392 patients admitted to the Psychiatry ward who associate different psychiatric disorders, and the community partial statistics focusing on the 501 patients with mental and behavioral disorders induced by the harmful use of alcohol defines the main panel.

Analyzing the gender distribution of psychiatric disorders at the level of the general statistical community, a higher frequency of them was found among female patients (56.1%) compared to male patients (43.8%). However, within the main panel, the cast by gender of mental and behavioral disorders due to alcohol consumption is recorded as an important predominance of disorders among men. Percentage-wise wise it is a value of only 12% in the case of women and 78% in the case of men (Fig 1).

The female/male ratio of cases associated with mental and behavioral disorders due to alcohol consumption is 1:7. The next qualitative type statistical variable that was analyzed is represented by the distribution depending on the place of residence of the patients from the general and partial statistical collective respectively. Analyzing the distribution by the residential environment of patients with different types of conditions from the group of psychiatric disorders it was found that a percentage of 56.9% of cases come from the urban environment, and the remaining 43.1% come from the rural environment. In contrast to these data, in the case of patients with mental and behavioral disorders due to alcohol consumption (Figure 2) a percentage of 78.1% of the total cases as patients from rural areas, and the

remaining 21.9% are represented by patients who live in the urban environment. This phenomenon can be explained by the growth of unregistered production of alcohol in rural areas.

Analyzing the distribution of cases with different types of conditions from the group of psychiatric disorders, it was found that the age range between 55-64 years has the highest frequency of cases, with a percentage of approximately 33.55% of the total. This interval is followed by the group aged between 45-54 years (30.22%), 35-44 years (17.19%), 25-34 years (8.12%), 65-74 years (5.76%), 15-24 years old (2.9%), 75-84 years old (1.71%) and by the group over 85 years old in a percentage of 0.49% of the total. Conversely, the lowest frequency of cases, of only 0.01%, is found in the group with age between 5-14 years. We can state that the highest frequency of psychiatric conditions it is found in the group of patients aged between 55-64 years.

Following the analysis of the frequency by age group of mental and behavioral disorders due to alcohol consumption, it was found that most cases are registered in the age range between 45-54 years, in a percentage of approximately 40.6% of the total. This interval is followed by those aged between 35-44 years in a proportion of 21.8%, then by those aged between 55-64 years in a percentage of 15.6%. The age ranges between 25-34 years and 65-74 years each associate a percentage of about 15.6% of the total, and the age category between 65-75 years has the lowest percentage, only 3,10%. Thus we can affirm the fact that mental and behavioral disorders due to alcohol consumption have the highest frequency in the range of patients aged between 45-54 years.

Analyzing the distribution by year of the prevalence of mental and behavioral disorders induced by harmful use of alcohol, it was estimated that the share of cases in 2017 is 8.56%, in 2018 it is 7.13%, in 2019 it is 7.88%, in 2020 it is 10.29% and in 2021 it is 5.29%. Table I reports the dynamics of the absolute number of cases within the main panel. Thus from the obtained data it appears that the demonstrative statistical indicator for 2018 is 85.03%, for 2019 it is 113.38%, for the year 2020 it is 63% and for year 2021 it is 33.07%, all compared to the year 2017.

This was followed by the study of the frequency of different types of mental and behavioral disorders induced by the use of harmful alcohol. It was found that the state of withdrawal amounts to a proportion of 47.3%, followed by withdrawal complicated by delirium (26.1%), harmful use of alcohol (10.9%), psychotic disorder (7.7%), addiction syndrome (5.1%), non-specific mental disorders and behavior (0.9%), acute intoxication (1.3%), nonspecific mental and behavioral disorders (0.9%), and lastly, the amnesic syndrome (0.1%) (Fig 3).

Discussions. Conclusions

Alcohol consumption has historically been more prevalent among men, and this gender difference in alcohol use is a global phenomenon. In 2016, 56% of males (equivalent to 1.46 billion individuals) and 32% of females (0.88 billion) aged 15 and older consumed alcohol worldwide. In that year, alcohol was responsible for approximately 3 million deaths, accounting for 5% of all global deaths. Specifically, it resulted in 3.2 million deaths among men (8%) and 0.7 million deaths among women (3%). Gender disparities in alcohol use are present universally, but they can also vary from one country to another.

A study conducted at a neuropsychiatric clinic hospital from 2017 to 2021 found that, except 2019, a majority of admitted patients were men. Moreover, the percentage of men admitted with mental and behavioral disorders related to alcohol was significantly higher compared to women, with 88% of the admitted patients being men, while women constituted only 12% of the admissions.

The prevalence of Alcohol Use Disorders (AUDs) and Mental Health Disorders (MHDs) has been reported in many countries, and the WHO World Mental Health (WMH) Survey Initiative has facilitated comparisons of AUD prevalences across different nations to identify variations and commonalities. Looking at data from France, Germany, Italy, Romania, and Spain, the prevalence of past-year alcohol use, alcohol abuse, and alcohol dependence indicates variations in the number of individuals affected in each country. For instance, in France, there were 2894 cases, in Germany: 3555, Italy: 4712, Romania: 2357, and Spain: 5473.

When examining past-year DSM-IV alcohol use disorder in these countries among both men and women, the prevalence rates differ. For example, in France, 2.4% of men and 0.2% of women were affected, in Germany, the rates were 1.9% for men and 0.4% for women, in Italy, it was 0.3% for men and 0.1% for women, in Romania, 1.6% of men and 0.1% of women were affected, and in Spain, 1.4% of men and 0.1% of women were affected [6,7].

This study's findings lead to the conclusion that Alcohol Use Disorder (AUD) encompasses a range of conditions, including alcohol abuse, alcohol dependence, alcohol addiction, and alcoholism. It is a significant contributor to the global disease burden, a leading cause of preventable deaths, and a prevalent psychiatric disorder.

AUD is a medical condition characterized by an individual's impaired ability to control or cease alcohol consumption, even in the face of adverse social, occupational, or health consequences.

Globally, alcohol consumption is responsible for 3.3 million deaths each year, contributing to disabilities and overall poor health in millions of people. The harmful use of alcohol accounts for 5.1% of the global burden of disease.

In the study conducted on patients admitted to the psychiatric clinic hospital in Craiova due to mental and behavioral disorders resulting from alcohol, several key findings emerged:

- Gender Distribution: The majority of admitted patients were male, accounting for 88% of the cases.
- Rural Residence: A significant portion of the patients hailed from rural areas, constituting 78% of the total admissions.
- Age Group: The largest proportion of patients fell within the age group of 45-54, representing 41% of the cases.
- Most common diagnoses: The most prevalent diagnosis among the admitted patients was "withdrawal state with and without delirium", followed by "harmful use of alcohol" and "psychotic disorder".

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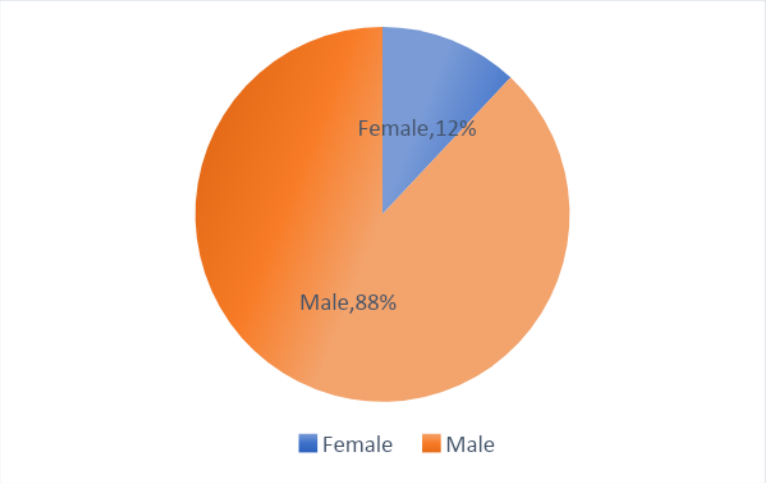

Fig 1. Percentage of patients admitted with alcohol disorders by sex

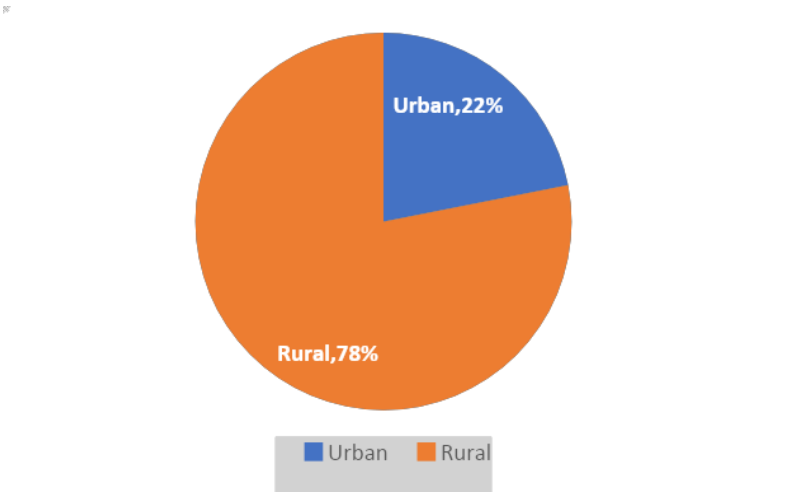

Fig 2. Distribution by environmental criteria

Year	No. of cases	Statistical index
2017	127	100 - base
2018	108	85.03
2019	144	113.38
2020	80	62.99
2021	42	33.07

Table I - Dynamics of the absolute number of cases

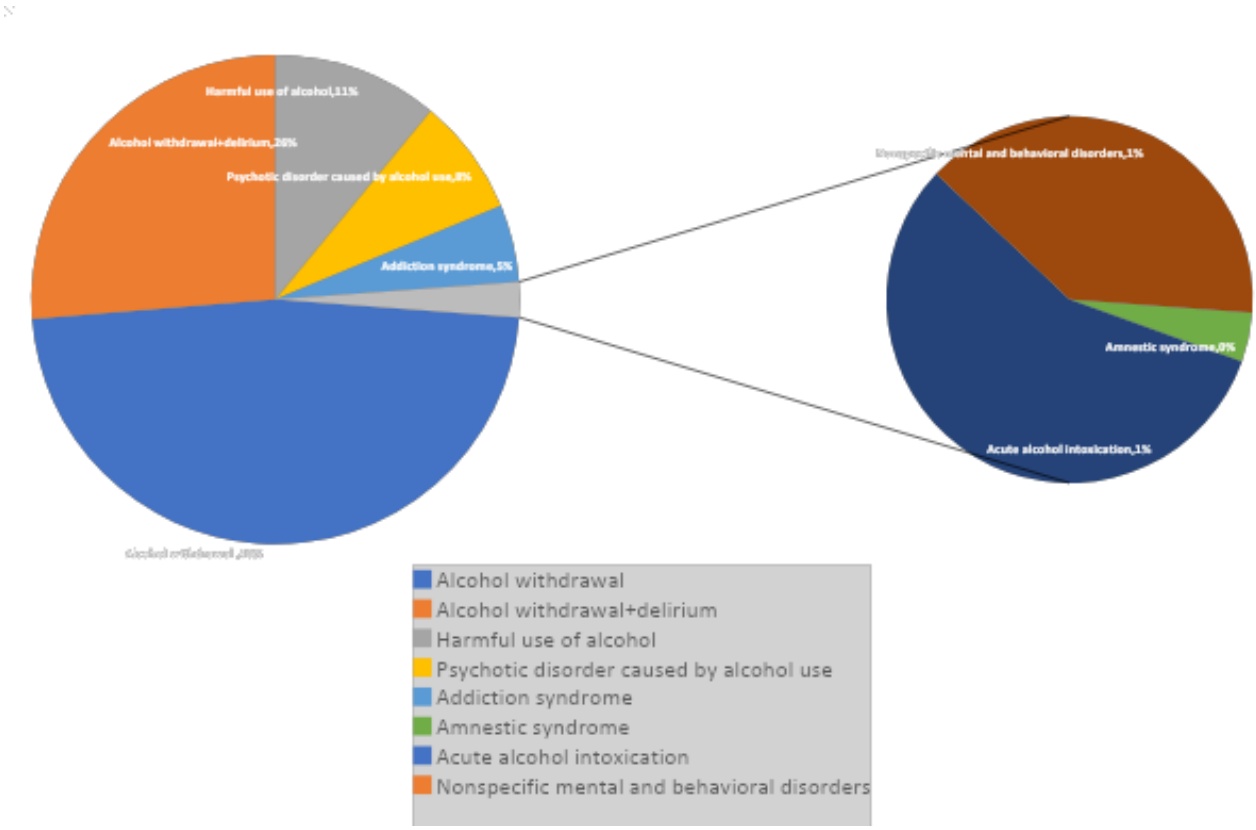

Fig 3. Percentage representation of the prevalence of different types of mental disorders and behavior due to alcohol consumption.

UZUL NOCIV DE ALCOOL, DATE DEMOGRAFICE, PREVALENȚĂ ȘI COMPLICAȚII

Rezumat

Abuzul de alcool este o problemă larg răspândită la nivel mondial. Dintre toate substanțele utilizate în scopuri de dependență, consumul de alcool este cel mai răspândit, făcând din intoxicația alcoolică cauza principală a intoxicației acute. La scară globală, riscul de a dezvolta modele dăunătoare de consum de alcool este de 15% pentru bărbați, pe parcursul vieții și este aproximativ dublu față de femei. Riscul de a dezvolta dependență de alcool este mai mic, cu un risc de 10% pentru bărbați și în jur de 5% pentru femei. Descoperirile acestui studiu conduc la concluzia că "Uzul nociv de alcool" cuprinde o serie de afecțiuni, inclusiv abuzul de alcool, dependența de alcool, dependența de alcool și alcoolismul. Este o contribuție semnificativă la povara globală a bolii, o cauză principală a deceselor care pot fi prevenite și o tulburare psihiatrică prevalentă. "Uzul nociv de alcool" este o afecțiune medicală caracterizată prin scăderea capacității unei persoane de a controla sau de a înceta consumul de alcool, chiar și în fața unor consecințe sociale, profesionale sau de sănătate adverse. La nivel global, consumul de alcool este responsabil pentru 3,3 milioane de decese în fiecare an, contribuind la dizabilități și la starea generală de sănătate precară a milioane de oameni. Consumul nociv de alcool reprezintă 5,1% din povara globală a bolii. În studiul efectuat pe pacienții internați în Spitalul Clinic de Neuropsihiatrie din Craiova din cauza tulburărilor psihice și comportamentale rezultate din alcool, au reieșit câteva constatări cheie: majoritatea pacienților internați au fost bărbați, reprezentând 88% din cazuri, o parte semnificativă a pacienţilor proveneau din mediul rural, constituind 78% din totalul internărilor, cea mai mare proporție de pacienți se încadrează în grupa de vârstă 45-54 de ani, reprezentând 41% din cazuri, diagnosticul cel mai răspândit în rândul pacienților internați a fost „starea de sevraj cu și fără delir", urmată de „consumul nociv de alcool" și „tulburarea psihotică".

Cuvinte cheie: consum de alcool, tulburare psihiatrica, complicatii.

EVALUATION OF POSSIBLE PSYCHOMETRIC CHANGES INDUCED BY THE PRACTICE OF PRAYER IN THE ORTHODOX CHRISTIAN TRADITION

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ABSTRACT

The present study investigates the possible psychological changes in the sphere of stress/anxiety induced by the practice of prayer (in the Orthodox Christian Tradition) - POCT, after an individual practice of 30 minutes daily, for 8 weeks. Participants (n=34) were divided into two groups (Study Group, n=16, average age 39.2 years and Control Group n=17, average age 38.6 years). The subjects of the Study Group – faithful Orthodox Christians without the practice of daily prayer, were trained, in a 3-hour session, with a view to the psycho-emotional, cognitive, spiritual and behavioural peculiarities of POCT. No intervention was performed on subjects in the Control Group. Before and after the interval of 8 weeks of daily practice of OCT, 30 min, five psychometric instruments were applied. Afterwards, the results obtained at the intra- and inter-group pre- and post-test evaluations were comparatively analyzed. The obtained results showed that POCT practice, according to the experimental scheme, did not produce statistically significant differences between the global scores on the Hamilton Anxiety Scale both recorded at SG in relation to CG, pre- and post-testing. For both groups of subjects there was a decrease in the Hamilton scale score, suggesting that, in the case of GS subjects, it cannot be said with certainty that the decrease in the Hamilton score occurred as a result of POCT practice. On the contrary, the statistically significant differences in CG subjects, post-test, regarding a9 item ("cardiovascular symptoms"), suggest that other factors may have contributed to improve the responses of CG subjects, which necessitates the introduction of additional conditions for more careful administration of POCT practice among SG subjects during the experimental interval. However, some significant results were obtained, regarding the SG subjects, at post-test, regarding item a3 ("fears/phobias"), of the Hamilton scale, suggesting that POCT practice according to the experimental plan could have reduced the intensity of fears/phobias among subjects in SG. Also, the change appearing in the post-testing SG regarding *a14* item ("appreciation of the anxious mood at the interview") is significant, suggesting a possible influence of the POCT practice. We also note, in the list of results, the averages of some coping mechanisms, which were significantly higher in SG compared to CG. These are e act ("active coping") and *e res* ("refrain/control/unwillingness") - among SG subjects at pre-test, and *e pos* ("positive interpretation and growth") and *e rel* ("religious approach/religious coping") at post-test compared to CG subjects, indicating a possible influence of POCT practice. The results need further studies that more closely correlate the changes in these items, but also in other psychometric ones, with those of a physiological nature.

Keywords: Orthodox Christian prayer, psychological changes in the sphere of stress/anxiety, coping strategies, fears/phobias, religious coping.

Introduction

Many studies have shown that various spiritual practices can reduce anxiety, mindfulness [1], MBI (Mindfulness-based interventions, in the case of students [2]), MBSR (Mindfulness Based Stress Reduction - in the case of students [3], or patients with depression [4]), cultivating self-compassion [5]. Also, practices such as self-compassion-centered mindfulness [6, 7], compassion training [2, 8] or MBSR [9] reduce stress. Along with these, other conscious techniques for reducing stress and anxiety, contemplative practices [10], ACT therapy - Acceptance and commitment therapy, DBT - Dialectical behaviour therapy, MBCT - Mindfulness-based Cognitive Therapy [11], or those that use progressive muscle relaxation, deep breathing, or sensory-guided imagery techniques [12]. Older meta-analyses [13, 14], but also recent ones [15, 16], have examined studies of this kind, emphasizing the therapeutic impact of practices of this kind.

The present study is part of a research that elaborated three studies on the possible psycho-metric and electro-physiological changes induced by the practice of Prayer in Orthodox Christian Tradition (POCT). The general objective of the present study is to highlight the psychological changes in the field of stress/anxiety induced by the practice of POCT. For this, we designed an experimental plan that provides for 30 minutes of daily practice, for 8 weeks, performed by the subjects of a study group (n=17), comparing the data provided by the psycho-metric evaluations, with those obtained in the case of the subjects of a control group (n=17), following the psychological changes in the field of manifestations related to stress/anxiety.

For this we have followed:

1. A statistical analysis of the psychological changes in the stress/anxiety sphere on the psychometric assessment tools used intra and inter-group (significant differences, correlations, etc.).

2. An interpretation of the significant results proposed "a priori" and highlighted in the basic analysis as well as other aspects observed during the analysis ("a posteriori").

2. For this study, we formulated the following working hypotheses:
3. Practicing POCT 30 minutes daily, for 8 weeks:
4. - significantly reduces the global score on the Hamilton Anxiety Scale, applied by the evaluator (Hypothesis 1.1);
5. - significantly reduces the GAD 7 score, obtained through self-assessment (Hypothesis 1.2);
6. - significantly reduces subjectively perceived stress on the Visual Analog Scale of Perceived Stress, obtained through self-assessment (Hypothesis 1.3)
7. - significantly reduces the Global Score on the perceived stress scale (PSS-10), applied by the evaluator (Hypothesis 1.4)
8. - strengthens coping mechanisms and increases the global COPE score, obtained through self-assessment (Hypothesis 1.5)

1. Participants and methods

The subjects of the present study were those who also participated in the study of physiological changes, published in *Physiology*, 2023, 1(104). We mean the following composition of SG and CG.

STUDY GROUP				CONTROL GROUP		
PT Code	Gender	Age		Age	Gender	PT Code
1	F	42		45	F	12
2	F	21		20	F	13
3	F	41		38	F	18
4	F	57		56	F	15
5	F	33		30	F	16
6	F	37		38	F	17
7	F	39		39	F	22
8	F	51		49	F	19
9	F	35		34	F	20
11	F	40		42	F	30
23	M	37		38	M	27
25	M	41		42	M	28
24	M	32		32	M	32
10	F	48		46	F	21
14	F	41		42	F	33
26	M	26		27	M	29
31	M	47		47		

Table I. Age and gender structure of CG and SG. The numbers in the table represent the subjects' work codes (PT code) used in the psychometric tests.

In order to highlight the possible psychological changes induced by POCT, we used psychometric measurement tools. The changes that occurred were highlighted by comparing the answers given by the GS subjects, pre- and post-test, but also the answers given by the SG subjects compared to those of the CG subjects, pre- and post-test.

The psychometric evaluation tools used in Study 1 were:
1. Hamilton scale for anxiety;
2. The GAD 7 (General Anxiety Disorder) test measures the level of anxiety symptoms;
3. Visual analogue scale of perceived stress, or visual analogue scale (VAC);
4. Perceived stress scale (PSS-10);
5. The COPE questionnaire targeting forms of coping with stress.

The working protocol in the application of psychometric instruments was as follows:

- Application of the Hamilton Anxiety Scale (HAM-A) by the psychiatrist (approx. 15 min.) – code A in the Database);
- Application of the GAD 7 Questionnaire – pencil and paper method, self-assessment (approx. 5 min.) - code B in the Database;
- Application of the Visual Analog Scale of Perceived Stress (approx. 2 min.) - code C in the Database;
- Application of the perceived stress scale by the psychiatrist (approx. 5 min.) - code D in the Database;
- Application of the COPE questionnaire - pencil and paper method, self-assessment (approx. 10 min.) - code

E in the Database.

(i) The Hamilton scale for anxiety is a tool for global assessment of anxiety or the severity of anxiety symptoms (such as mental tension, anxious mood) but also the somatic aspects that appear in anxiety (such as the bio-physiological changes associated with it) [17]. The Hamilton scale (14 items, corresponding to some symptoms of anxiety), is applied in the form of a semi-structured interview, the items being evaluated by qualified personnel (on a scale from 0 to 4, where 0 corresponds to the absence of the symptom, and 4 to a severe symptom and disabling) [17]. The Hamilton scale score ranges from 0 to 56, with panic disorders or generalized anxiety disorders corresponding to scores above 20, while scores below 20 indicate the absence of these disorders [17].

(ii) The GAD 7 test (General Anxiety Disorder – 7 – compiled by Robert L. Spitzer) contains 7 questions that assess the level of anxiety symptoms experienced by a person in the last two weeks. To these questions, the respondents choose as answers numbers from 0 to 3, which frame the assessments regarding how often they felt emotional problems (0 meaning "not at all", 1 meaning "a few days", 2 meaning "more than half the days" and 3 "almost daily", during the 14 days.)

(iii) The Visual Analogue Scale of Perceived Stress, or Visual Analogue Scale (VAS) is the visual representation of the level of stress a person thinks they are experiencing. The subjects represented, on an axis with values between 1 and 10, the answer to the question "How stressed do you feel?", represented in **Fig. 1**.

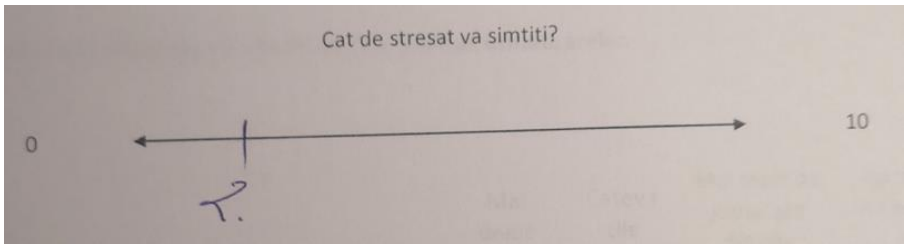

Fig. 1. Example of a visual analogue scale of perceived stress, where the subject, asked "How stressed do you feel?", graded and graphed level 2 out of 10.

(iv) Perceived Stress Scale (PSS-10) constructed by Cohen & all (1983) [18], is a 10-item questionnaire that assesses the degree to which a person perceives life as unpredictable, uncontrollable and difficult to manage during the past month. The subject is asked how often he felt a certain state, placing it through a numerical value between 0 and 4, 0 meaning "never", 1 meaning "almost never", 2 meaning "sometimes", 3 meaning "often", and 4 "very often".

(v) The COPE questionnaire, developed by Carver, Scheier and Weintraub (1989) [19], evaluates 15 forms of coping with stress, which are active or passive. The questionnaire includes 60 statements, each form of coping being evaluated through 4 items, with answers on a scale from 1 to 4, where the value 1 corresponds to the statement "I usually do not do this", 2 - for "I rarely do this" , 3 for "I sometimes do this", and 4 for "I often do this". For the evaluation, the score from each of the 4 items corresponding to each of the 15 coping mechanisms, mentioned below, is added.

2. Primary data

After the application of the questionnaires, which was done by a resident in psychiatry, the data were introduced in Excel format.

CODE	GENDER	AGE	EDU LEVEL	A1	A2	A3	A4	A5
1	2	42	7	1	1	1	1	1
2	2	22	5	1	1	2	0	0
3	2	43	7	1	1	1	2	1
4	2	58	8	1	1	0	0	0
5	2	33	7	1	2	3	1	0
6	2	37	8	2	2	3	0	0

Fig. 2. Extracted from the primary data summary table. In the first column are the codes associated with each

subject from GS and GC, from 1 to 33 (When applying the questionnaire, it was not known whether the subjects belonged to *GS* or *GC*). Next are the columns corresponding to gender, age, and education level for each subject, and the first five items (*A1-A5*) of the Hamilton scale, for which the subjects' responses are completed. The extracted fragment contains all responses completed by the subjects (with values between 0 and 4), for all subjects, at the time of pre-testing.

Next, after the columns intended for the Hamilton scale (A), columns *B1-B7*, *C*, *D1-D10*, *E1-E60* and respectively the corresponding codes for each of the 15 coping mechanisms assessed by the COPE questionnaire were introduced.

A = Hamilton Anxiety Scales
B = *GAD 7* Questionnaire – paper-pencil method, self-assessment
C = Visual analogue scales of perceived stress
D = Scale of stress perceived by the psychiatrist
E = *COPE* Questionnaire

B7	C	D1	D2
0	9	0	1
0	3	1	0
0	1	2	2
0	2	1	0

E59	E60	E POS	E MEN	E FOC	E
4	4	16	9	8	
4	3	14	7	8	
4	3	13	7	13	
4	4	16	8	7	

Fig. 3. Extracts from the primary data summary table, with four and six columns associated with answers B7, c, D1 and D2, respectively the last two answers from the COPE Test and the first three coping strategies *e pos*, *e men*, *e foc*, for subjects coded from 1 to 4. Their answers are between the values mentioned for each individual instrument, corresponding to the pre-testing moment.

The 15 coping strategies/mechanisms assessed by the COPE questionnaire and their corresponding items are:

Nr. crt.	code	Significance	Corresponding items
1.	*e pos*	Positive reinterpretation and growth	1, 29, 38, 59
2.	*e men*	Mental disengagement	2, 16, 31, 43
3.	*e foc*	Focus on and venting of emotions	3, 17, 28, 46
4.	*e use*	Use of instrumental social support	4, 14, 30, 45
5.	*e act*	Active coping	5, 25, 47, 58
6.	*e den*	Denial	6, 27, 40, 57
7.	*e rel*	Religious coping	7, 18, 48, 60
8.	*e hum*	Humour	8, 20, 36, 50
9.	*e beh*	Behavioural disengagement	9, 24, 37, 51
10.	*e res*	Restraint	10, 22, 47, 58
11.	*e emo*	Use of emotional social support	11, 23, 34, 52
12.	*e sub*	Substance use	12, 26, 35, 53
13.	*e acc*	Acceptance	13, 21, 44, 54
14.	*e sup*	Suppression of competing activities	15, 33, 42, 55
15.	*e pla*	Planning	19, 32, 39, 56

3. Statistical analysis tools

The descriptive statistical elements initially processed, on the variables to be analysed, revealed distributions that do not satisfy the condition of normality. This aspect, the small sample (N < 30, the Study Group having 17 subjects and the Control Group 16 subjects) and the type of analysed variables led to the choice of non-parametric tests in the statistical analysis [20].

The tools used for statistical analysis in the present study are non-parametric tests for the median (Wilcoxon signed-rank test), t-tests for paired data and the Mann-Whitney test for independent samples.

(a) The median and the average are two statistical indicators of central tendency for a set of statistical data. If the observed data have a small volume (usually below 30-40), tests for the average (called parametric tests) are not relevant, since the statistical assumptions under which they operate are not met. That is why, for the present situation, we used alternative tests (called non-parametric), for the median, especially since the latter is a better indicator of the central tendency of the data (compared to the mean), in case the data are asymmetric.

(b) The Wilcoxon signed-rank test is a nonparametric statistical test used to either test the median of a population based on a sample of data or to compare the medians of two populations using two matched samples. The test used here checks whether there are significant differences for the median value before and after the 8 weeks of personal experience (POCT).

(c) The t-test for paired data is a statistical method used to test whether the average difference between pairs of measurements (before and after the experiment) is zero or not. The test can be used when the data values are paired measurements and the distribution of differences between paired measurements should follow the normal distribution. (In the case of the present research, paired data refers to intra-group pre- and post-test data.)

(d) The Mann-Whitney test for independent samples is a tool applied to compare two independent samples when few data are available and when they do not follow a normal distribution.

Test results (equal medians or equal averages, as appropriate) are described by P-values (*Pv*), which represent the level of significance observed for each test. The *Pv* value is a probability calculated based on an appropriate statistic that describes how likely/plausible it is to have found a given set of observations if the null hypothesis (equal medians or equal means, as appropriate) were true. The result of a test is done by comparing the values of *Pv* and alpha (the theoretical significance level chosen by the user. By default/standard, alpha = 0.05, if *Pv* < alpha, then the hypothesis is accepted (the tested averages or medians are not equal.)

4. Statistical analysis and interpretation of results. Hamilton Anxiety Scale

The intra-group analysis was initially done on the global scores obtained and then refined, starting from the significant differences observed within the two groups, *SG* and *CG*. Intra-group analysis was performed with the Wilcoxon test, pre- and post-testing.

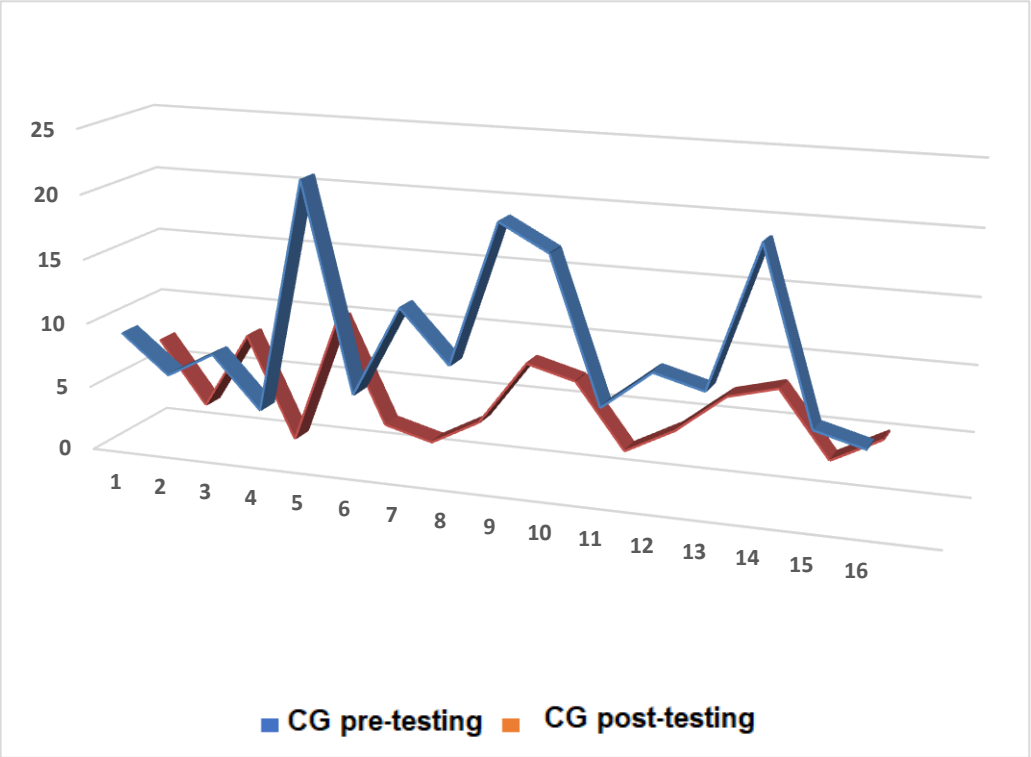

Fig. 4. Hamilton global score for CG, pre and post-testing.

Considering the values, it can be said that there are significant differences within *CG* pre- and post-testing on global Hamilton scale scores at P < 0.01 (p = 0.001, z = -3.423).

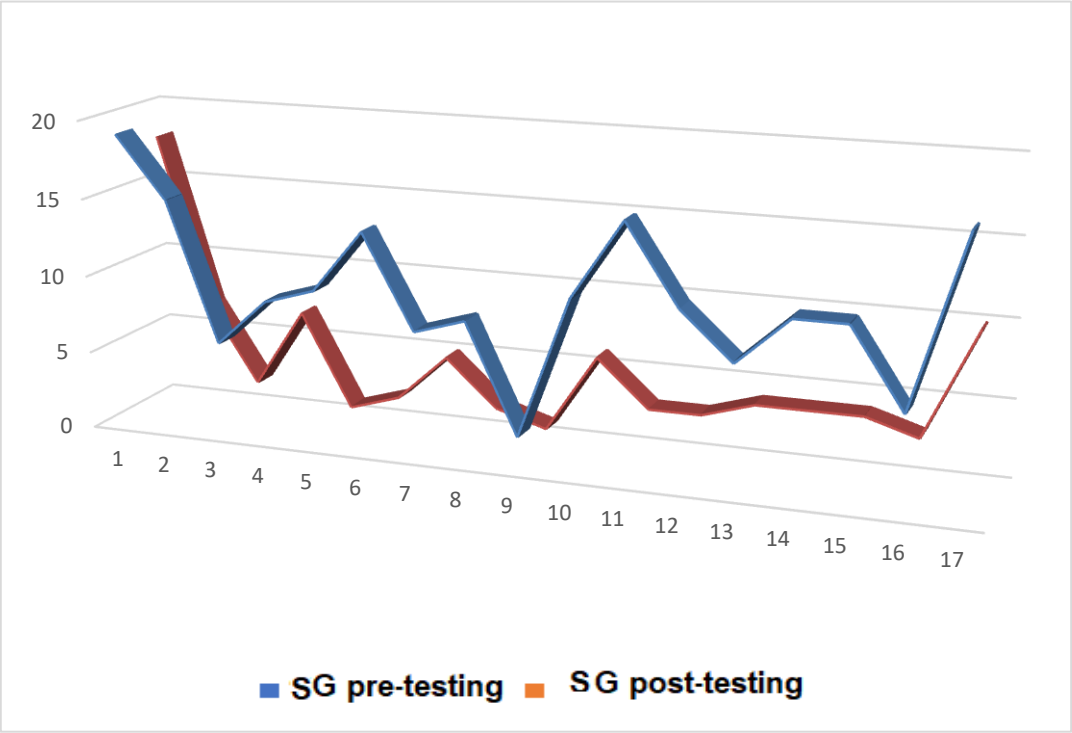

Fig. 5. Hamilton global score for SG, pre and post-testing.

We mention that, in order to verify these results, we transformed the two variables *sumA1* and *sumA2* into their sqrtsumA1 and sqrtsumA2 variants in order to obtain a distribution as normal as possible and to perform the t-test for paired samples, which would confirm the significance of the observed differences - *GC* pre-test/post-test P < P = 0.01, bilateral, df = 15, t = 6.689, *GS* pre-test/post-test P < P = 0.01, bilateral, df = 16, t = 7.177.

The inter-group analysis was performed with the Mann Whitney test for independent samples.

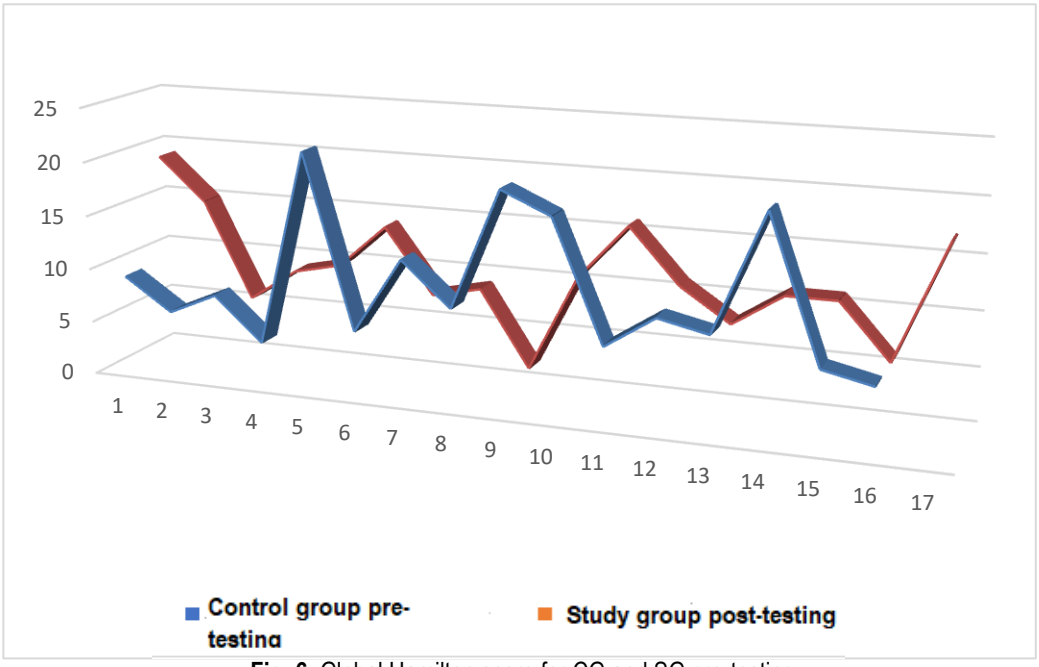

Fig. 6. Global Hamilton score for CG and SG pre-testing.

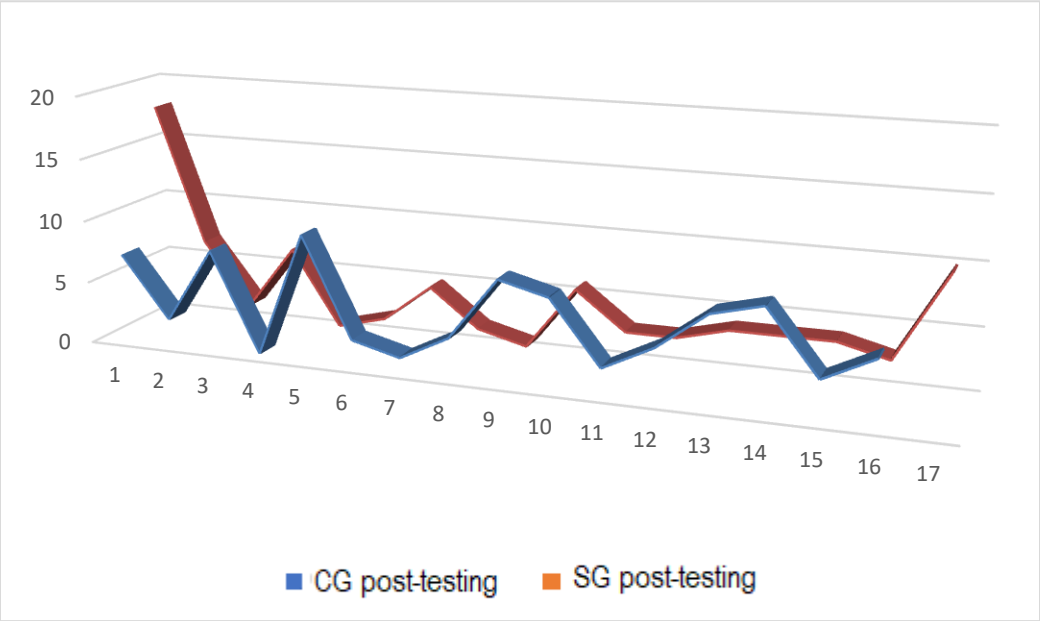

Fig. 7. Global Hamilton score for CG and SG post-testing.

We can say that there are no significant differences between the two groups both at the pre-testing ($p = 0.6 > 0.05$, $z = -0.525$) and at the post-testing ($p = 0.663 > 0.05$, $z = -0.436$).

We use the variables described above, sqrtsumA1 and sqrtsumA2, to perform the independent samples t-test. This confirms the non-significance of the observed differences in pre-test $P = 0.335 > P = 0.05$, two-sided, $df = 31$, $t = -0.40$ and post-test $P = 0.587 > P = 0.05$, two-sided, $df = 31$, $t = -0.53$.

We also present the variation in scores across the entire sample, pre- and post-testing.

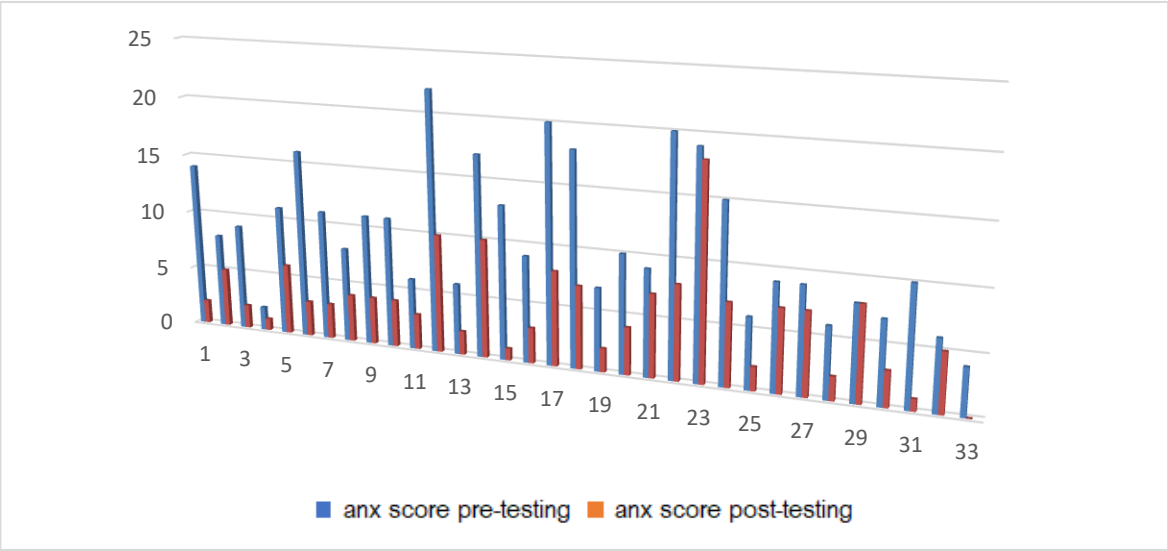

Fig. 8. Hamilton global scores on the entire sample pre- and post-testing

We notice that, although there are no significant differences between the global scores on the Hamilton scale between the two groups, pre- and post-testing, there are still statistically significant differences between the global scores recorded on both *SG* and *CG* pre- and post-testing. This similar evolution, of the subjects in *SG* and *CG*, by registering a decrease in the score in the Hamilton scale, does not highlight with certainty that the practice of POCT contributed to the decrease in the level of anxiety in SG. (In the discussion section we will analyse this situation.)

We have further thoroughly examined the existence of changes on the scale items, in the two groups, pre- and post-testing. The values of the 14 items, marked with "*a*", from *a1* to *a14*, were analysed. Table II shows the significant differences recorded. Significant differences are noticed in both groups on *a1, a2, a5, a6, a8 and a10*.

	Anxious mood (*a1*)	Difficulty in relaxing/muscle tension (*a2*)	Prosexo-mnesic difficulties (*a5*)	Depression (*a6*)	Changes of analysers (*a8*)	Breathing difficulties (*a10*)
Control group	P = 0,005 < P = 0,01, z = -2,804	P = 0,013 < P = 0,05, z = -2,496	P = 0,035 < P = 0,05, z = -2,111	P = 0,004 < P = 0,01, z = -2,889	P = 0,02 < P = 0,05, z = -2,333	p = 0,034 < P = 0,05, z = -2,121
Study group	P = 0,005 < P = 0,01, z = -2,81	P = 0,001 < P = 0,01, z = -3,307	P = 0,034 < P = 0,05, z = -2,121	P = 0,007 < P = 0,01, z = -2,714	P = 0,035 < P = 0,05, z = -2,111	*P* = 0,014 < 0,05, z = -2,46

Table II. Significant differences recorded between values of items *a1*, *a2*, *a5*, *a6*, *a8*, *a10*, among subjects in *SG* and *CG*, pre- and post-testing.

From the above mentioned data as well as from the analysis of the items where insignificant differences were recorded in both groups (*a4*, *a7*, *a11*, *a12*, *a13*), the relatively similar evolution of *SG* and *CG* can be noticed. For *SG*, the post-testing changes that appeared regarding item *a3* ("fears/phobias") and *a14* ("evaluating the anxious mood at the interview") are significant (Table III).

	Fears/phobias (*a3*)	Evaluating the anxious mood at the interview (*a14*)
Control group	P = 0,102 > P = 0,05, z = - 1,633	P = 0,414 > P = 0,05, z = - 0,816
Study group	*P* = 0,046 < P = 0,05, z = - 1,999	P = 0,002 < P = 0,01, z = - 3,162

Table III. Significant differences recorded between the values of the a3 item (fears/phobias) and a14 item (appreciation of anxious mood), among the subjects of SG, compared, pre- and post-testing.

It is significant that in the case of SG subjects the values of *a3* ("fears/phobias") decreased, which suggests a possible effect of POCT. This aspect could constitute a study hypothesis for a future research. The changes in the *a14* item ("evaluation of the anxious mood" at the interview, by the evaluator) are also important, but could have been biased by the assessment conditions.

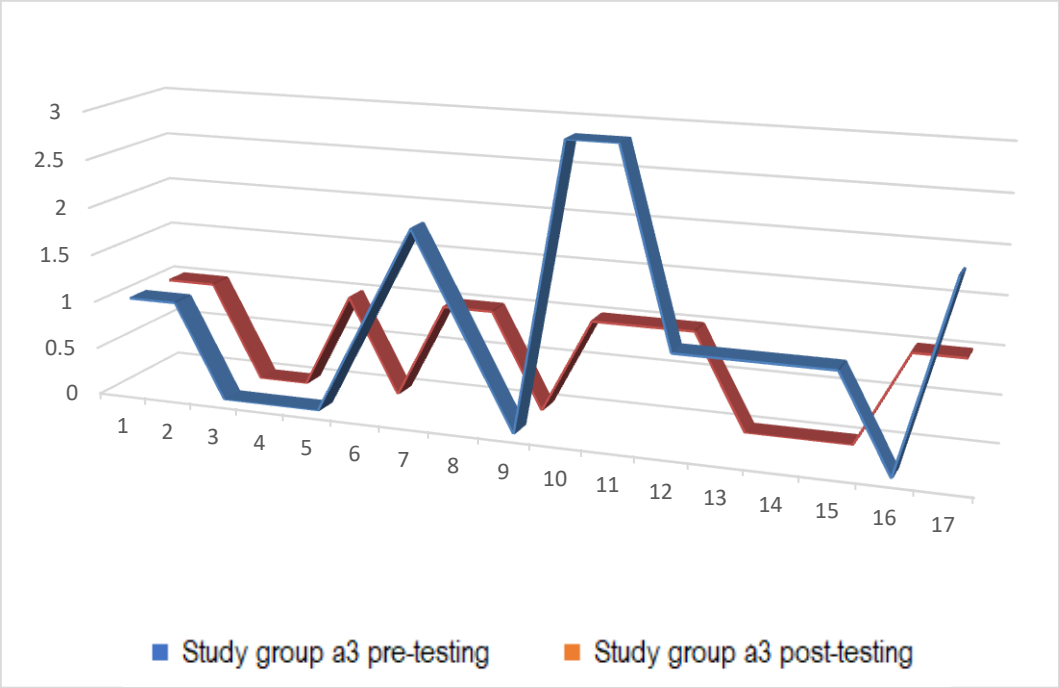

Fig. 9. Values of item *a3* ("fears/phobias") pre- and post-testing in *SG*.

At the same time, changes also appear in the *CG*, regarding the *a9* item ("cardiovascular symptoms") – Table IV.

	Cardiovascular symptoms (a9)
Control group (*CG*)	*P* = 0,021 < P = 0,05, z = - 2,31

Study group (*SG*)	*P* = 0,248 > P = 0,05, z = - 1,155

Table IV. Significant differences recorded between the values of the a9 item among *CG* subjects, comparatively, pre- and post-testing.

The fact that these significant differences also appear in *CG* - without the subjects having participated in an experimental intervention - suggests the possibility that other unknown factors, related to the plan of personal life, may have influenced the evolution. Also, good Hamilton scale scores recorded by subjects in the post-test *CG* could also be caused by the influence exerted by the subjects' experiences after participating in the stages of the study (preparatory interactions, general exposure to the project, EEG measurement sessions, pre and post-testing).

On the other hand, the fact that *SG* subjects do not register, post-testing, significantly greater changes in global scores on the Hamilton scale compared to *CG* subjects, could also be caused by the fact that significant changes in anxiety require a longer period of psychotherapeutic or drug intervention [21] (suggesting that for further improved research an experimental interval longer than 8 weeks could be introduced). Another possible reason for the significantly close scores of the *SG* and *CG* subjects, post-test, could be that the *SG* subjects failed - during the 8 weeks - to comply with the indications formulated for practicing POCT.

We can formulate the conclusion that the present research does not highlight changes in the experimental group that can be exclusively determined by the practice of POCT, changes being also reported in the case of *CG* subjects, so that other causes, such as the placebo effect, cannot be excluded or responses biased by laboratory conditions, during sessions dedicated to measurements.

5. Statistical analysis and interpretation of results. The GAD 7 test

The use of the *GAD 7* Test did not reveal significant changes between the Control Group and the Study Group, pre- and post-testing.

Intra-group, for both groups, analysis was performed with the Wilcoxon test for paired samples, without identifying significant differences, pre- vs. post-testing, between the two groups at P = 0.05.

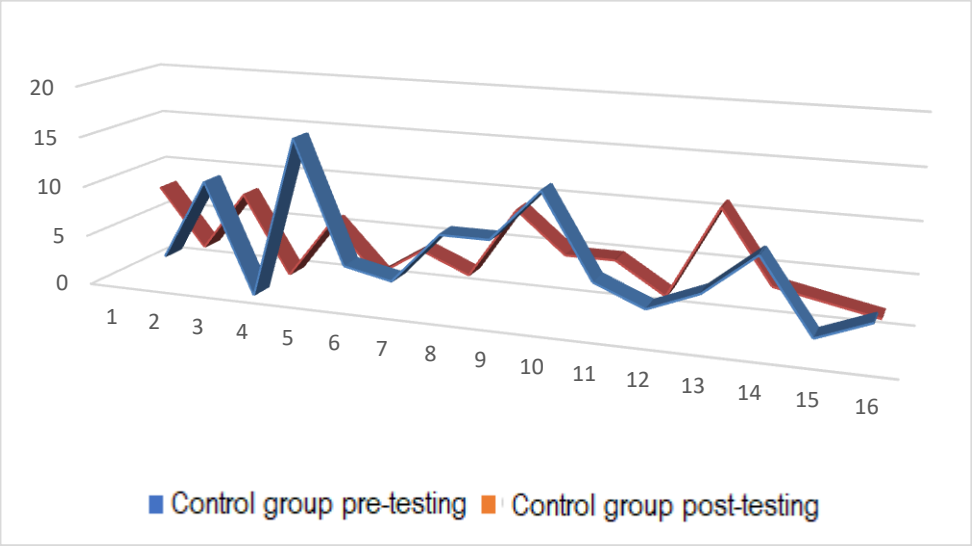

Fig. 10. Global *GAD 7* score for *CG* pre- and post-testing.

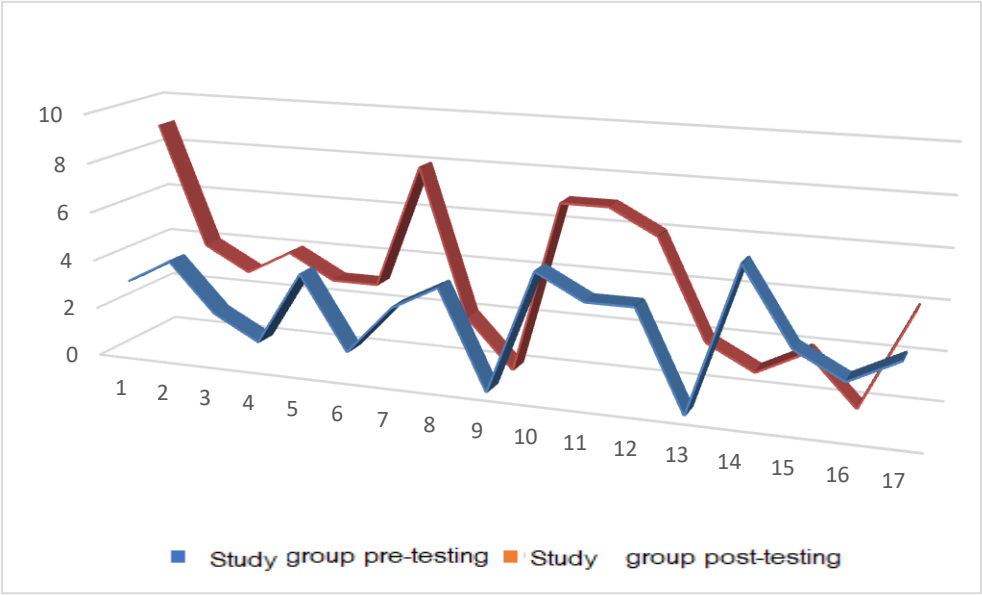

Fig. 11. Global *GAD 7* score of *SG*, pre- and post-testing.

Inter-group, however, the result is surprising: significant differences between the two groups *SG* and *CG* in the pre-test and the absence of these differences in the post-test were recorded.

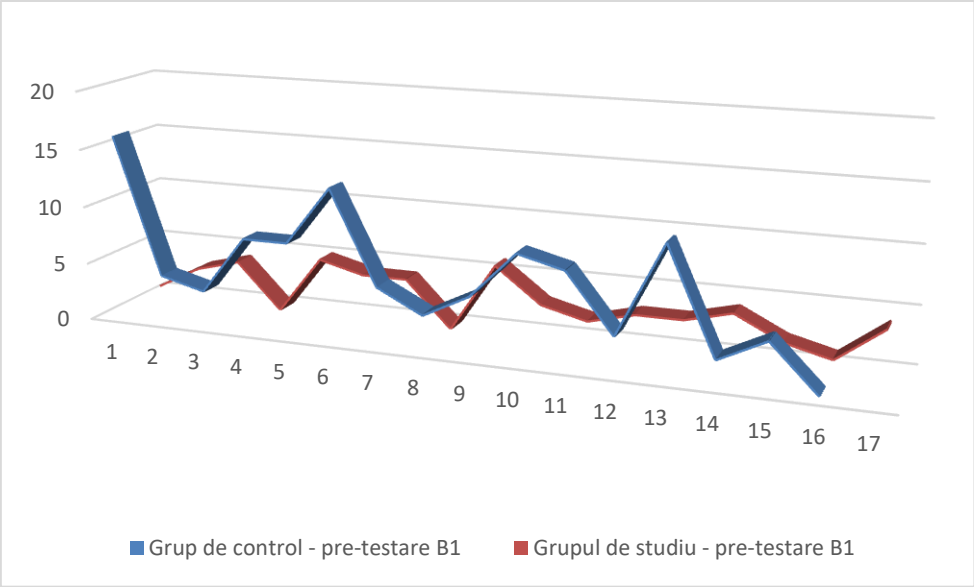

Fig. 12. *GAD 7* Scores for the *CG* vs. *SG* in pre-testing.

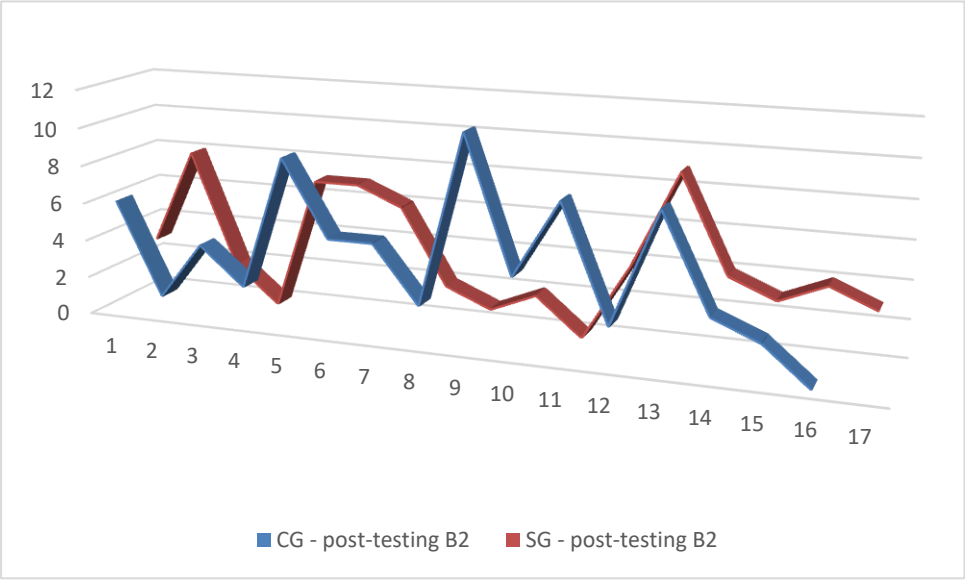

Fig. 13. *GAD 7* Scores for the *CG* vs. *SG* in post-testing.

The inter-group analysis, performed with the Mann-Whitney test for independent samples, revealed that the differences between *CG* and *SG* faded to non-significance in post-testing (at P = 0.05). The situation is novel from an experimental point of view, with the initial differences between the groups fading during the experimental period.

	Study group /Control group pre-testing	Study group /Control group post-testing
B1/B2	P = 0,012 < P = 0,05, z = -2,513	P = 0,586 > P = 0,05, z = -0,545

Table V. Significant differences in pre-testing between the Study Group and the Control Group - revealed by Mann-Whitney test for independent samples - fade in post-testing (at P = 0.05).

The maximum score in the Control Group in pre-testing was 16, and in post-testing was 11, with group average of 6.38 and 4.5, respectively. The maximum score in the Study Group in pre-testing was 6, and in post-testing was 9, with the group average of 2.88 and 3.82, respectively. Therefore, it can be concluded that the practice of POCT, in accordance with the chosen research plan, did not produce statistically significant changes in the global score of the *GAD 7* scale, and did not influence the intensity of the anxiety-type symptomatology explored with the help of this scale.

In pre-testing, in *SG*, the global values did not exceed the threshold of clinical intensity (>10), while in *CG* there were 3 initial values that exceeded this critical threshold, making it possible that this fact should have contributed to the significant differentiation of the two groups in the pre-testing. On the other hand, the fading of the differences between *SG* and *CG* in post-testing could be explained by the fact that *CG* subjects were influenced by the experiences of participating in the study regarding spiritual life. These changes, which may have occurred individually for some subjects in the *CG*, could have significantly influenced the data for the *CG* as a whole, as the number of subjects in the *CG* is small.

The conclusion of this analysis would be that the experimental design provided for the study failed to reveal changes brought about by POCT practice at the level of anxious symptomatology explored with the help of the *GAD* scale.

6. Statistical analysis and interpretation of results. Visual analogue scale

Analysis and interpretation of the data provided by the *Visual Analogue Scale of Perceived Stress* did not provide conclusive results. Intra-group, for both *CG* and *SG*, was performed with the Wilcoxon paired-samples test, pre- and post-testing, the differences being statistically insignificant.

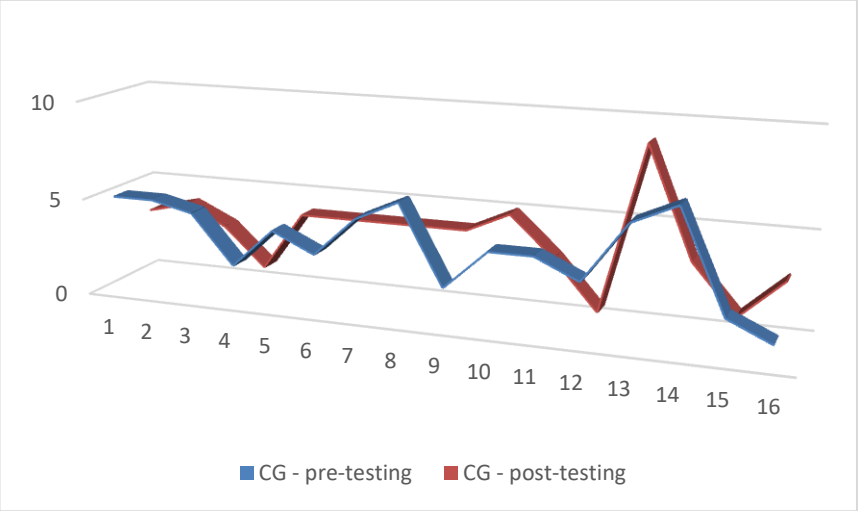

Fig. 14. Visual analogue scale for *CG* in pre/post-testing.

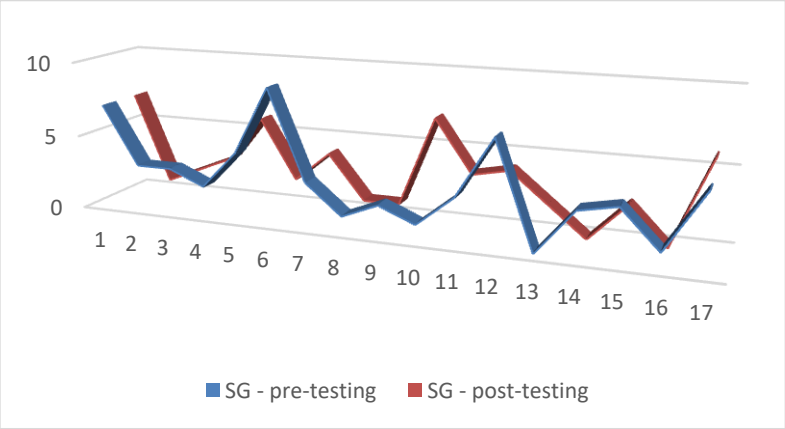

Fig. 15. Visual Analogue Scale for the *SG*, in pre/post-testing.

The Mann-Whitney analysis for independent samples, pre- and post-testing, was performed inter-group to assess differences between *SG* and *CG*, with inconclusive results.

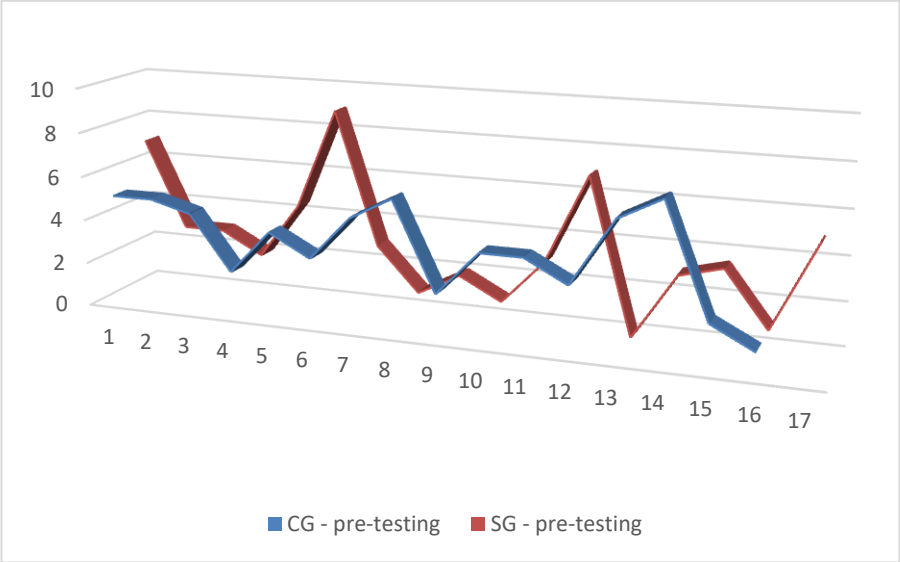

Fig. 16. Visual analogue scale for *CG* and *SG* in pre-testing.

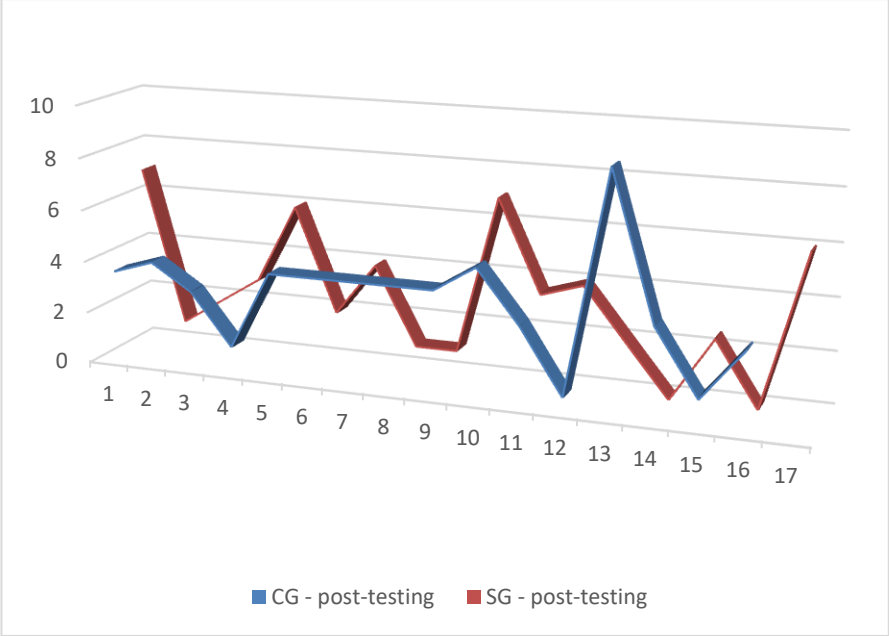

Fig. 17. Visual analogue scale for *CG* and *SG* in post-testing.

The conclusion, resulting from the use of this tool, is that the chosen experimental plan did not reveal significant changes produced by POCT regarding the level of subjectively felt (self-evaluated) stress.

7. Statistical analysis and interpretation of results. Perceived stress scale - evaluated by the psychiatrist

The data obtained regarding the *Perceived Stress Scale* - evaluated by the psychiatrist - did not provide conclusive results.

The intra-group analysis, done with the Wilcoxon test for paired samples, revealed statistically insignificant intra-group, pre- and post-testing differences for both *SG* and *CG* subjects.

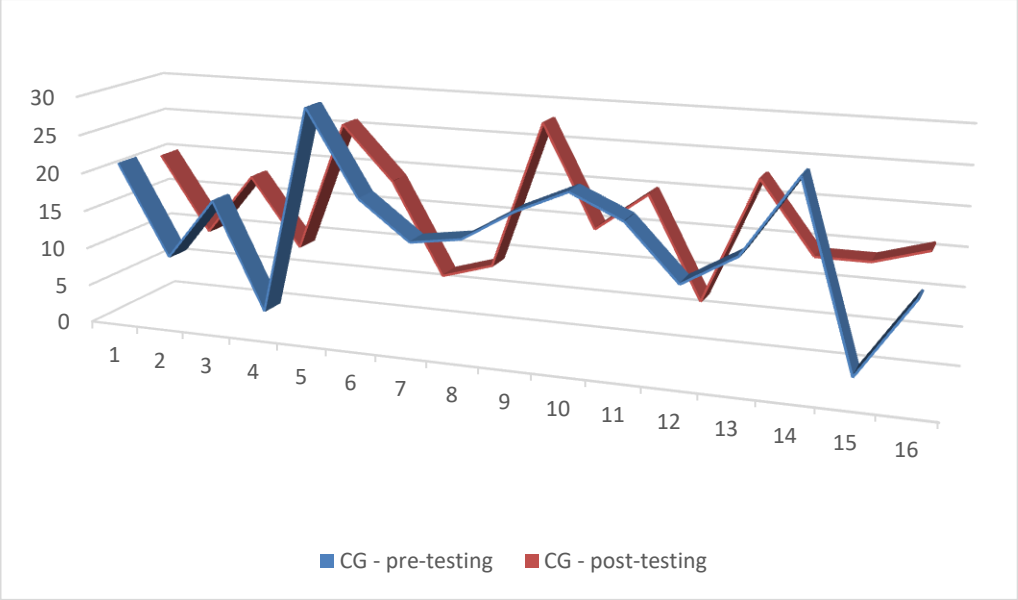

Fig. 18. *PSS-10* global score for *GC* subjects at pre- and post-testing.

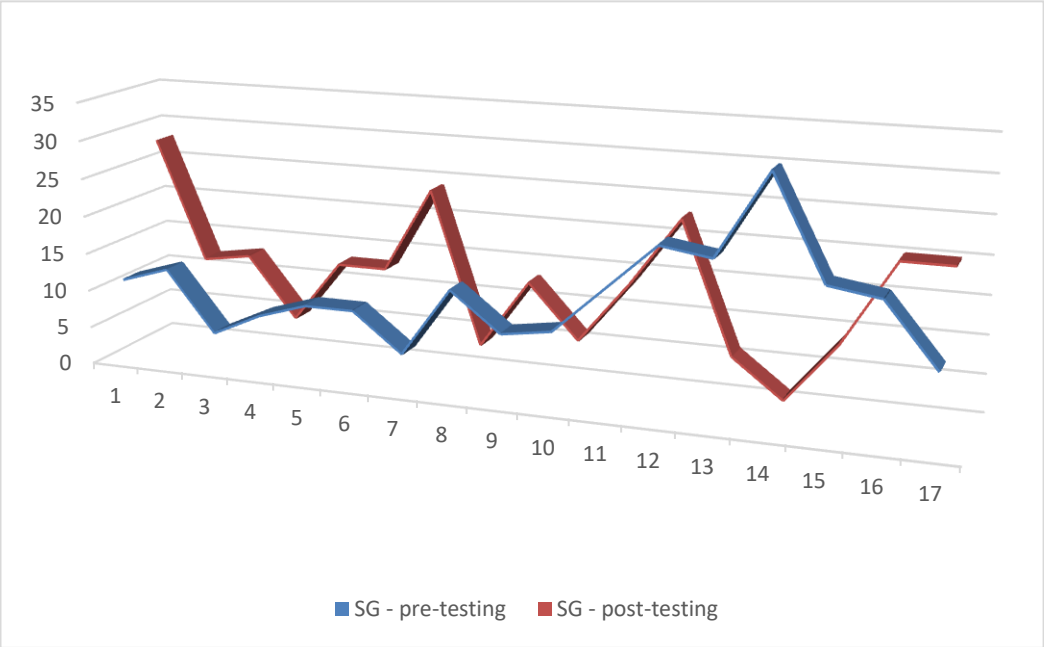

Fig. 19. *PSS-10* global score for *GS* subjects at pre- and post-testing.

The Mann-Whitney analysis for independent samples, pre- and post-testing, intra-group, was performed to assess differences between *SG* and *CG*.

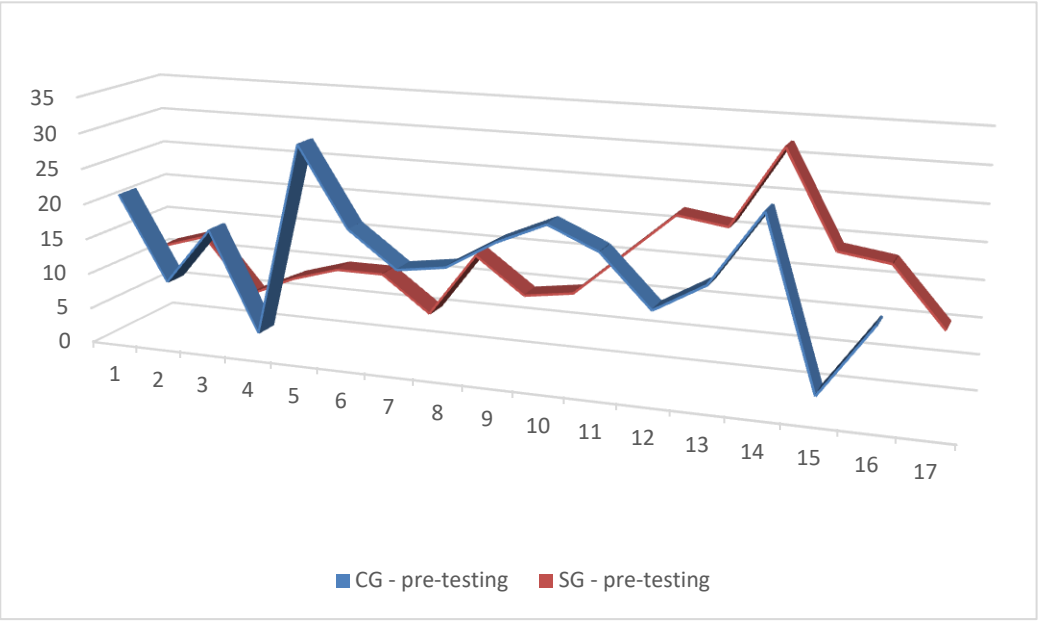

Fig. 20. *PSS10* global score for *CG* subjects vs. *SG* in pre-testing.

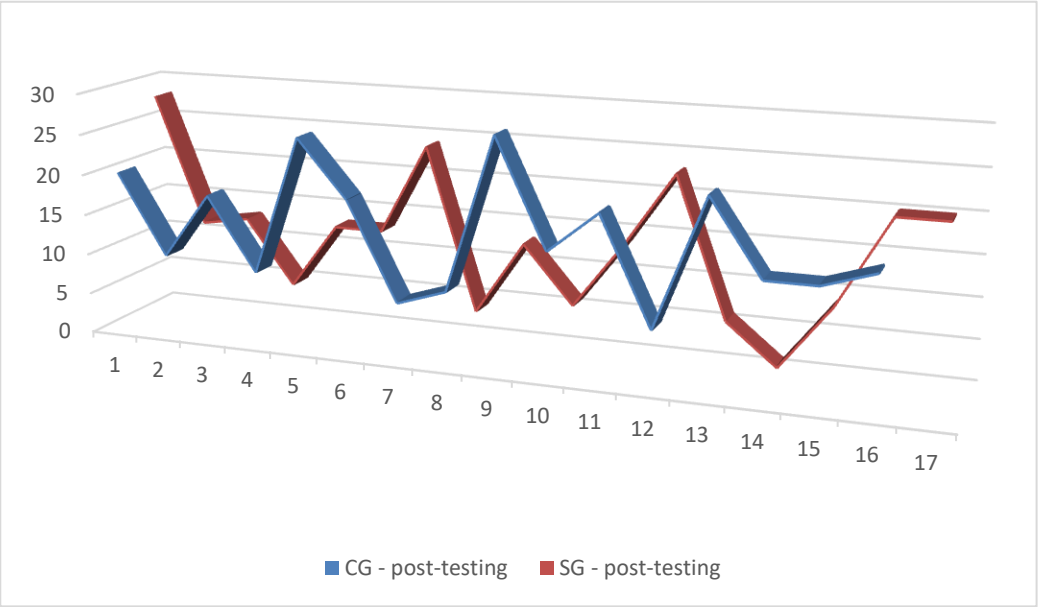

Fig. 21. *PSS10* global score for *CG* subjects vs. *SG* in post-testing.

The results obtained were inconclusive. The conclusion, resulting from the use of this tool, is that the chosen experimental plan did not reveal significant changes produced by POCT regarding the level of subjectively felt stress assessed by the *PPS-10*.

At the moment, we can formulate the conclusion that no change induced by the practice POCT in relation to the stress level was highlighted.

8. Statistical analysis and interpretation of results. The COPE test

Results of interest were obtained from the analysis of data corresponding to the *COPE* Test. In the first instance, there were no statistically significant differences in the intra-group analysis, which was performed using the Wilcoxon paired-samples test.

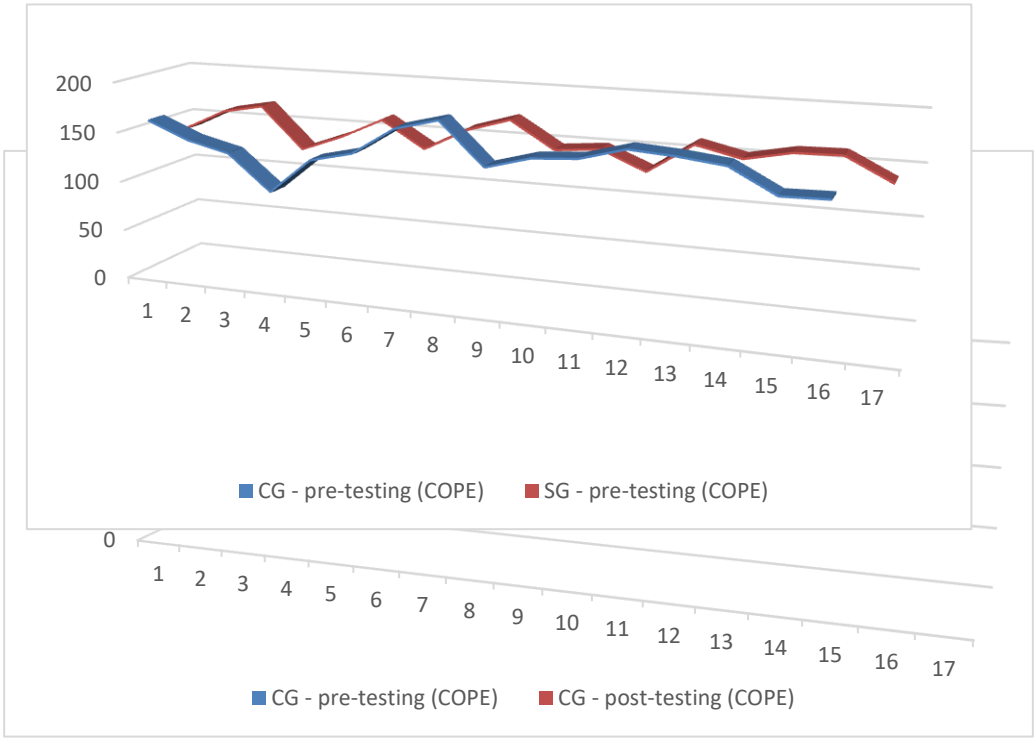

Fig. 22. The *COPE* global score for *CG* subjects at pre- and post-testing.

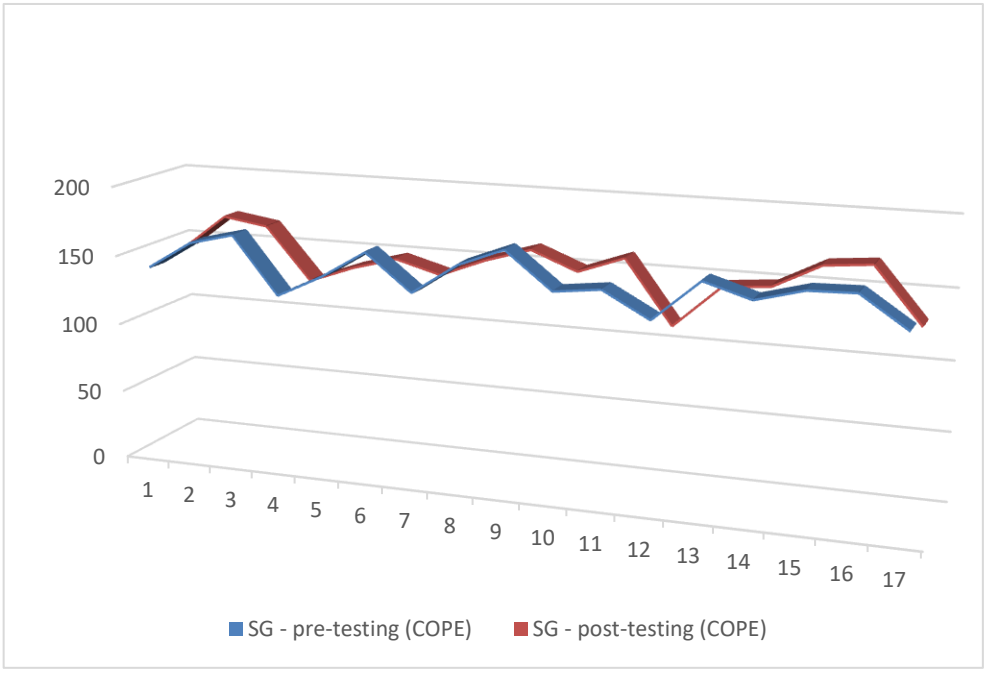

Fig. 23. *COPE* global score for *GS* subjects in pre- and post-testing.

Also, the inter-group analysis, performed with the Mann-Whitney test for independent samples, revealed statistically insignificant inter-group differences on global *COPE* scores.

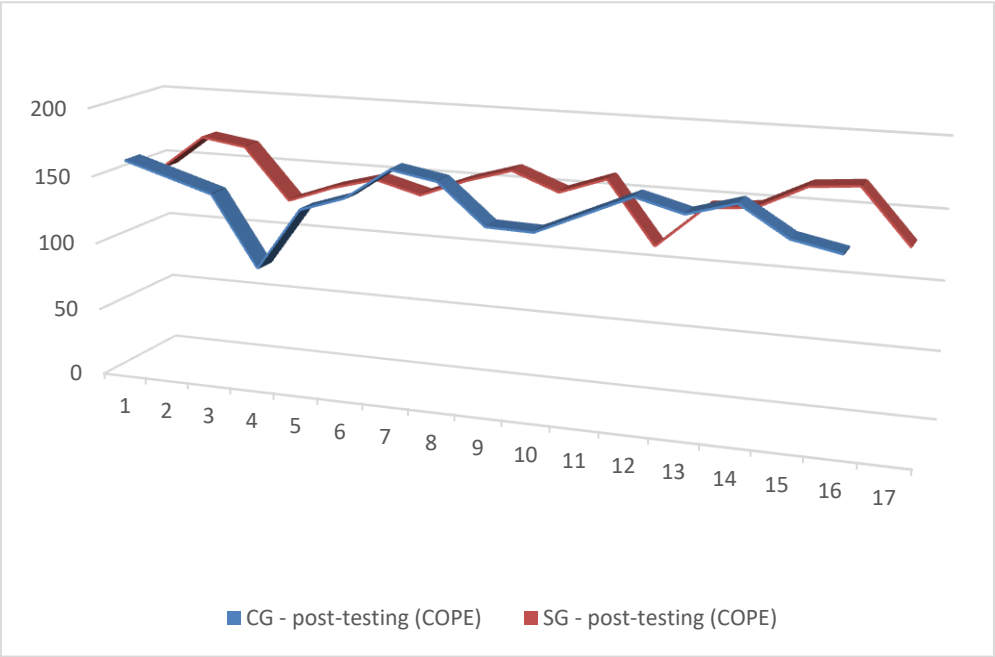

Fig. 24. *COPE* global score for *CG* subjects vs. *SG* in post-testing.

However, some significant aspects were highlighted. The averages of several coping mechanisms are higher in *SG* compared to *CG*:

- *e act* (the active coping approach) in the pre-testing;
- *e res* (control/restraint), in pre-testing;
- *e pos* (positive interpretation and growth), post-testing;
- *e rel* (religious approach/religious coping), post-testing.

	e act pre-testing	*e res* pre-testing	*e pos* post-testing	*e rel* post-testing
Control group/Study group	P = 0,025 < P = 0,05, z = -2,256	P = 0,041 < P = 0,05, z = -2,067	P = 0,023 < P = 0,05, z = -2,276	P = 0,035 < P = 0,05, z = -2,112

Table VI. Coping items for which the averages are significantly higher in *SG* compared to *CG*.

In *CG* the average for *e act* ("active approach to coping") in pre-testing is 11.81, compared to *SG*, where the average is 14, which suggests that, before the testing period, the active approach as a coping mechanism is more present in *SG* than in *CG*. The situation can be explained by the fact that the subjects selected for *SG* had a preoccupation with Christian *S/RE*.

In *CG* the average for *e res* ("control/restraint/necessity") in pre-testing is 9.38, while in *SG* it is 11.35. We can therefore say that this coping mechanism is more present in subjects from *SG* compared to subjects from *CG*, in pre-testing. However, these differences are no longer as large in post-testing, the difference between the average values for e act resulting from the two groups diminishes, becoming statistically insignificant. The situation shows similarities with the evolution of responses on the Hamilton scale for comparative anxiety, for CG and SG subjects, in post-testing and pre-testing.

Regarding the average for *e pos* ("positive interpretation and growth") in post-testing for *CG*, there was an average of 12.25, while for *SG*, the value was 13.94, values that suggest an increased presence of this coping mechanism among subjects in *SG* compared to *CG*, a change that could come from the practice of POCT.

Similarly, the values for e rel ("religious approach/religious coping"), in post-testing, are significantly higher for subjects in *SG* than for those in *CG* (13.82 vs. 11.75), which suggests an increased presence of religious approach as a coping mechanism among *SG* subjects after the experimental interval, a situation to which POCT practice could have contributed.

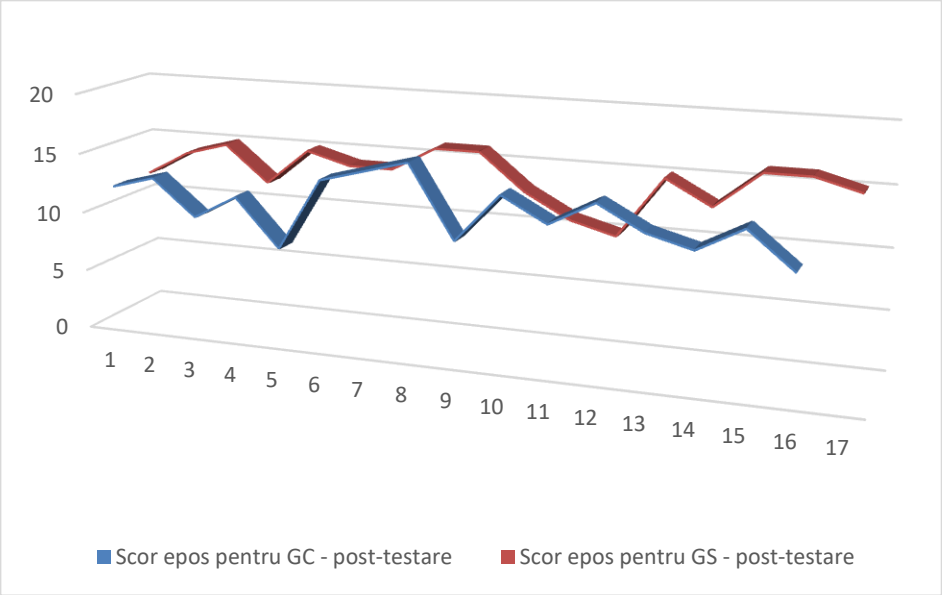

Fig. 27. The score is poor for subjects from *CG* vs. *SG* in post-testing

In *CG* the average in *e rel* 2 is 11.75 and in the *SG* it is 13.82.

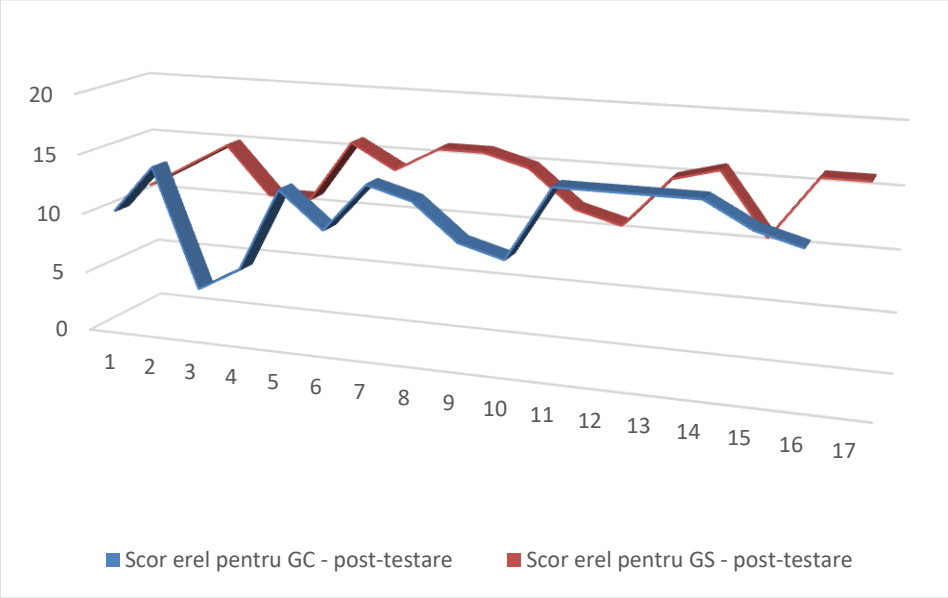

Fig. 28. The *e rel* score for subjects from *CG* vs. *SG* in post-testing

Although the study did not reveal a statistically significant influence of POCT practice regarding the global score on the *COPE* scale, however, in *SG*, in the post-test, changes in some values were observed regarding the two coping strategies, namely the positive interpretation and the religious approach.

9. Remarks

The obtained data also allow some correlations and some important remarks. There is a positive, statistically significant correlation between the Hamilton Anxiety Scale Global Score and the *GAD 7* global score (*B2*), high in value (Spearman's rho = 0.546), corresponding to a close relationship between the two variables, p=0.024<p=0.05), a result suggesting that the two vary in the same direction, confirming the decrease in anxiety.

Another statistically significant correlation (Spearman's rho=0.529) indicates a close connection between two other variables (p=0.029<p=0.05), namely *a3 pos* (item 3 of the Hamilton scale, corresponding to "fears/phobias") and the *GAD 7* global score, a result that indicates that the two vary in the same direction, the decrease in the value on item a3 correlating with the decrease in the intensity of the anxiety symptomatology evaluated by *GAD 7*, a coherent aspect in relation to the other obtained results.

The positive, statistically significant correlation (Spearman's rho = 0.492 - which indicates an average connection between two variables, p=0.029<p=0.05) is also noted, between the same *a3* item (item 3 on the Hamilton scale for "fears/phobias ") and the *PSS-10* global score post-testing, which tells us that the two vary in the same direction, the decrease in the intensity of fears/phobias correlates positively with the decrease in stress.

There is also a statistically significant negative correlation (Spearman's rho = - 0.547), indicating a close relationship between two other variables (p=0.029<p=0.05) e rel (the coping mechanism corresponding to the religious approach in the *COPE* test) post-testing and the *PSS-10* global score (*D2* in Appendix 10.4), which tells us that the two vary inversely proportionally, with perceived stress decreasing as the religious coping mechanism increases.

The results that we can consider the most important, (in relation to the significant statistical differences and the number of subjects in the experimental group, n = 17) - are those on *a3* post-testing (the fear/phobias item from the Hamilton scale), e2 post- testing (the positive interpretation type coping mechanism from *COPE*) and e *rel 2* ("the religious approach type coping mechanism from *COPE*"). We can support this interpretation with the assumption that even in the case of a small sample volume, which weakens the test results, the result remains significant because it shows an important effect size [149].

However, the changes recorded in the Study Group, in post-testing, in the two coping strategies positive interpretation (*e pos*) and religious approach (*e rel*) do not change the final conclusion, as no significant inter-group differences are recorded in post-testing. They can only provide a picture of the dynamics within the experimental group on the two coping mechanisms. Since neither did the overall scores significantly differ statistically speaking, nor did the two items vary significantly within the study group, the changes are not significant, but may represent a starting point for future research. This could consider the assessment of the possibility that POCT strengthens the belief-based coping mechanism and, likewise, the positive construal mechanism.

10. Discussions

Numerous studies have highlighted the beneficial impact of Christian prayers on anxiety and stress levels.

One study, which focused on subjects with a depressive episode (according to DSM-IV-TR criteria), is significant from the point of view of the experimental design, for the present paper. The subjects (ages 18, 60 women and one man) participated in an intervention that included one hour of prayer each week for six weeks. The prayer was led by a cleric, with distance, avoidance of touching and personalized texts adapted to the specific needs of the subjects. Measurements revealed, by applying the *Hamilton Depression Scale* and the *Hamilton Anxiety Scale*, a reduction in stress and anxiety levels compared to the Control Group ($p < 0.01$ in all cases), with the status quo still valid even one month after the intervention [22].

Other studies highlight similar influences determined by the practice of Christian prayers, in coping strategies [23-26], but also on the general mood (in the case of doctors and patients, for example) [28].

A study, published in 2017, used the *COPE* test to assess changes following a spiritual intervention. Possible links between stress coping strategies, spirituality and social support among patients with rheumatoid arthritis (*RA*) were examined using the Mini-COPE Inventory (n = 250). The results showed that stress coping strategies (return to religion, acceptance), spirituality and social support influenced the perception of pain among the participants [27], a fact also highlighted in the present study.

Other studies highlight similar influences determined by the practice of Christian prayers, in coping strategies [23-26], but also on the general mood (in the case of doctors and patients, for example) [28].

In relation to these results, but also to the conclusions of the present study, it can be mentioned that the decrease in the level of anxiety and stress determined by the practice of POCT could also be related to the way the relationship with the Supreme Being is understood and felt, on a personal level , in the case of Christian subjects, prayer being a way of dialogue with God, of entrusting the believer to God. This aspect is present in a study published in 2012, which took into account the state of teaching the subject of God (often found in religious people) and its influences on the mood of practitioners, using the *State Anxiety Inventory* (*STAI*). Regression analysis of the data provided by the *STAI*, applied to a sample of 460 people (including 306 women), showed that the posture of submission to God was inversely related to

stress, meaning that *S/RE* influences health, in the sense of lower stress levels [29].

11. Limits of the study

The present study has several significant limitations.

(a) Recruitment of subjects

Some of the limitations of the present research are related to the difficulties of recruiting the participating subjects, this aspect being found in the inventory of limitations in all three studies carried out in the present research.

In general, the selection of participants for the scientific study of ES/R is difficult. The experimental interval, the independent variable and the number of subjects), difficulties aimed at the composition of *CG* and *SG*, are also present in this research:

- The unusual topic in the field of empirical research in the field of medical sciences;
- The recruitment of subjects for *SG*, in the present research, was made, due to necessity, from among the faithful who are not practitioners, but willing to participate in the study;
- *CG* subjects were chosen from among people who are interested in the spiritual dimension of life (to be willing to participate in the presentation meetings and all stages of the experimental plan) but who do not systematically practice any kind of spiritual exercises (mindfulness, yoga, meditation, or other practices), in order not to introduce random variables into the experimental plan;
- Identification of subjects to express their agreement to participate, for a research that provides for a long experimental interval of 8 weeks, in which daily tasks are provided, for a duration of 30 minutes.

Constraints of this kind led to limitations in the configuration of *SG* and *CG*. The group of participants in this research, 33 subjects, was made up of 24 women (73%) and 9 men (27%). For this reason, the ratio of women/men in *SG* and *CG* does not reflect the known ratio at the population level, respectively 49%/51%. Applying the binomial test to the entire sample shows us that men are significantly less, relative to their proportion in the population at $p=0.005<p=0.05$. Applied, distinctly, to the two *SG* and *CG*, the binomial test shows that the proportion of men differs significantly in CG relative to the existing proportion in the population ($p=0.032<p=0.05$), but does not differ significantly in *SG* relative to the same proportion existing in the population ($p=0.061<p=0.05$), this is due to the numerical inequality of the groups (*CG* = 16, *CG* = 17), the groups being a little numerous.

(b) Administration of the independent variable

Other limitations of the present research stem from the administration of the independent variable of task. On the one hand, the experimental task provided for the *SG* subjects could have affected the quality of task performance (independent variable) contributing to inconclusive results:

- The frequency provided for the fulfillment of the task (daily);
- Duration of the task (30 min, without interruption);
- The interval provided for the administration of the task-independent variable (8 weeks);
- The indications for the performance of the task, which aimed to cover the spiritual, cognitive and psycho-emotional criteria of POCT, which increase the degree of difficulty of the requirements for the execution of the task by the *SG* subjects:
- Choosing a quiet space;
- Preliminary interventions, to prepare an appropriate state for POCT (calming, profile reading);
- The best possible attention to the text of the prayers read, during the prayer.

On the other hand, the conditions of the experimental plan (which aimed to carry out the prayer in private, in private space, in privacy) did not allow the supervision of the subjects in the *SG* by the specialized staff, excluding the exchange of email messages, during the experimental interval.

(c) Administration of psychometric instruments

The used scales assess the level of anxiety, but the instruments used do not distinguish between state anxiety and anxiety as a personality trait. Since SG and CG are not numerous, it is possible to use personality tests, which could highlight whether the increased level of anxiety stems from the personality profile. As a possible way to improve future studies, in addition to the anxiety assessment scales, the *STAI - State Trait Anxiety Inventory* - a test to assess the level of anxiety at a given moment (state anxiety), and the general tendency, could be used of being anxious (trait anxiety) [30], or using the Big Five Inventory, for the assessment of personality traits related to the tendency to be anxious [31].

Other limitations of the study may have arisen from the use of the English version of the Hamilton Anxiety Scale, even though it was administered by specialist staff. (Currently there is an authorized translation, which is used on the population of Romania, as an integral part of the *CES* (clinical evaluation system), cited at the beginning of this chapter.)

We saw that the evolution of Hamilton scores between pre and post-testing in the two groups was in some

situations similar and that in other respects the *CG* subjects had a favourable evolution without having an experimental task, which suggests that other unknown factors could have influenced the evolution of conditions in subjects in *SG* and *CG*, including placebo, especially since the experimental interval was considerable.

An important part of these limitations could be eliminated or significantly diminished if in further studies a number of volunteers, clerics were introduced to participate in prayer with the subjects from *SG*, together (as in the mentioned study) [22], for a more careful management of the conditions for the personal experience of POCT. On the other hand, an experimental design that provides prayer together with a cleric, for each of the *SG* subjects is difficult to administer, requiring more volunteers but also more supervision, to control the variables (which could come from the interpersonal relationships of the volunteers clerics and *SG* subjects).

Another intervention, for increased quality of task performance by SG subjects, could be provided by a more thorough instruction, during several meetings, further detailing the practical coordinates of POCT, as well as an interval designed to accommodate participants with POCT (1-2 weeks), before the experimental interval.

Finally, since the visual stress scale (by self-assessment) did not provide useful data, in future research on changes induced by POCT, this instrument could be abandoned.

Conclusions

In relation to the proposed working hypotheses, the results of this study are as follows:

Hypothesis 1: Practicing *OCP* daily, for 30 minutes, for 8 weeks, significantly reduces the global score on the Hamilton Anxiety Scale, administered by the evaluator.

Result: The practice of *OCP*, according to the experimental scheme, did not produce statistically significant differences between the global scores recorded both in *SG* in relation to *CG*, pre and post-testing. For both groups of subjects there was a decrease in the Hamilton scale score, suggesting that, in the case of *SG* subjects, it cannot be said with certainty that the decrease in the Hamilton score occurred as a result of POCT practice.

The analysis of the values of the 14 items, *a1* - *a14*, revealed significant differences in both groups, pre and post-testing, regarding *a9* ("anxious mood"), *a2* ("difficulties relaxing/muscle tension"), *a5* ("difficulties prosexo-mnesis"), *a6* ("depression"), *a8* ("changes on analyzers"), *a10* ("breathing difficulties"). There were also statistically significant differences in *CG* subjects only, who, in post-testing, showed changes in the *a9* item ("cardiovascular symptoms"), suggesting that other factors may have contributed to the improvement in *CG* subjects' responses.

The change appearing in the *SG* in post-testing regarding the a3 item ("fears/phobias") is significant, suggesting that POCT practice after the experimental design could have reduced the intensity of fears/phobias among the subjects in the *SG*. This result could be a starting point for a future study to evaluate the ways in which the practice of POCT changes the intensity of fears/phobias. Thus, we could further explore whether the modification of this item in the SG correlates with the decrease in the salivary cortisol level, an aspect that would strengthen the conclusion related to the effect of prayer on the decrease in the intensity of fears/phobias.

Also, the change in *SG* in post-testing regarding the *a14* item ("evaluation of anxious mood at the interview") is also significant, suggesting a possible influence of the POCT practice, but a future study is needed to check whether the variation of *a14* could be biased by the evaluator [32].

Hypotheses 2, 3, and 4 - POCT practice every day, 30 minutes daily, for 8 weeks:

- significantly reduces the *GAD 7* score, obtained through self-assessment;
- significantly reduces subjectively perceived stress on the Visual Analogue Scale of Perceived Stress, obtained through self-assessment. The hypothesis was not confirmed;
- significantly reduces the Global Score on the perceived stress scale (*PSS-10*), applied by the evaluator.

Result: working hypotheses 2, 3 and 4 were invalidated.

Hypothesis 5: Practicing *POCT* every day, 30 minutes daily, for 8 weeks strengthens coping mechanisms and increases self-reported *COPE* global score.

Result: Intra-group analysis (Wilcoxon test) for *CG* and *SG* did not reveal statistically significant differences post-testing, compared to pre-testing. Inter-group analysis (Mann-Whitney test for independent samples) revealed no statistically significant differences in post-testing *COPE* global scores.

However, the means of some coping mechanisms were significantly higher in *SG* compared to *CG*. Increased averages in *e act* ("active approach to coping") and *e res* ("control/unwillingness") – among *SG* subjects at pre-testing stem from subjects being faithful, preoccupied with the Christian *S/R* sphere. (It remains to investigate the fact, that at the end of the intervention, the difference between the average values for e act corresponding to the two groups decreases, a dynamic similar to the evolution of the subjects - in the pre and post-testing interval - discussed in relation to the evolution of anxiety evaluated with Hamilton scale.)

The increased averages among *SG* subjects for *e pos* ("positive interpretation and growth") and *e rel* ("religious approach/religious coping") in post-testing compared to *CG* subjects indicate a possible influence of *POCT* practice. Having no significant inter-group differences, in post-testing, these increased averages do not significantly change the final conclusion but highlight the dynamics of *SG* in the experimental interval, on the two coping mechanisms. We cannot consider this change significant because the global scores did not significantly differ statistically, nor did the two items

vary significantly within the SG, but this relevant aspect can represent a starting point for future research. *POCT* practice is expected to strengthen the belief-based coping mechanism and, along with it, the positive interpretation mechanism.

The experimental plan in this study did not reveal a significant influence of the POCT practice on the global score on the COPE scale, but it was revealed that the *SG* subjects have, in post-testing, higher averages on two coping strategies, the positive interpretation and the religious approach respectively.

The results that we may consider as the most important, (in relation to the significant statistical differences and the number of subjects in the experimental group, n=17) refer to *a3* ("fears/phobias") on the Hamilton scale, *e pos* (corresponding to the coping mechanism "positive interpretation" from *COPE*) and *e rel* (item for "religious approach" from *COPE*), for *SG* subjects, post-testing, even if the sample is small, as such a result could show the size of the important effect [149].

Further research could examine whether the practice of *POCT* strengthens coping strategies based on positive interpretation and religious approach and causes a decrease in anxiety.

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EVALUAREA POSIBILELOR MODIFICĂRI PSIHOMETRICE INDUSE DE PRACTICAREA RUGĂCIUNII ÎN TRADIȚIA CREȘTINĂ ORTODOXĂ

REZUMAT

Studiul de față investighează posibilele modificări psihologice în sfera stresului/anxietate induse de practica rugăciunii (în tradiția creștin ortodoxă) - POCT, după o practică individuală de 30 de minute zilnic, timp de 8 săptămâni. Participanții (n=34) au fost împărțiți în două grupuri (Grupul de studiu, n=16, vârsta medie 39,2 ani și Grupul de control n=17, vârsta medie 38,6 ani). Subiecții din Grupul de studiu - creștini ortodocși credincioși, fără practicarea rugăciunii zilnice, au fost instruiți, într-o sesiune de 3 ore, în vederea particularităților psiho-emoționale, cognitive, spirituale și comportamentale ale POCT. Nu s-a efectuat nicio intervenție asupra subiecților din Grupul de control. Înainte și după intervalul de 8 săptămâni de practică zilnică a POCT, de 30 de minute, au fost aplicate cinci instrumente psihometrice. Ulterior, au fost analizate comparativ rezultatele obținute la evaluările pre și post-test intra și inter-grupuri. Rezultatele obținute au arătat că practica POCT, conform schemei experimentale, nu a produs diferențe semnificative statistic între scorurile globale la scala de anxietate Hamilton, ambele înregistrate la SG în raport cu CG, pre și post-test. Pentru ambele grupe de subiecți s-a înregistrat o scădere a scorului scalei Hamilton, ceea ce sugerează că, în cazul subiecților din SG, nu se poate afirma cu certitudine că scăderea scorului Hamilton s-a produs ca urmare a practicii POCT. Dimpotrivă, diferențele semnificative din punct de vedere statistic la subiecții CG, post-test, în ceea ce privește un9 item ("simptome cardiovasculare"), sugerează că alți factori ar fi putut contribui la îmbunătățirea răspunsurilor subiecților CG, ceea ce impune introducerea unor condiții suplimentare pentru o administrare mai atentă a practicii POCT în rândul subiecților SG pe parcursul intervalului experimental. Cu toate acestea, s-au obținut unele rezultate semnificative, în ceea ce privește subiecții SG, la post-test, în ceea ce privește itemul a3 ("temeri/fobii"), din scala Hamilton, sugerând că practica POCT conform planului experimental ar fi putut reduce intensitatea temerilor/fobiilor în rândul subiecților din SG. De asemenea, modificarea apărută în SG post-test, în ceea ce privește itemul a14 ("aprecierea stării de anxietate la interviu") este semnificativă, sugerând o posibilă influență a practicii POCT. Remarcăm, de asemenea, în lista de rezultate, mediile unor mecanisme de coping, care au fost semnificativ mai mari în SG comparativ cu CG. Acestea sunt e act ("coping activ") și e res ("abținere/control/nevoință") - în rândul subiecților SG la pre-test, și e pos ("interpretare pozitivă și creștere") și e rel ("abordare religioasă/ coping religios") la post-test comparativ cu subiecții CG, indicând o posibilă influență a practicii POCT. Rezultatele necesită studii suplimentare care să coreleze mai îndeaproape modificările acestor itemi, dar și ale altora psihometrici, cu cele de natură fiziologică.

Cuvinte cheie: rugăciune creștin ortodoxă, schimbări psihologice în sfera stresului/anxietate, strategii de coping, frici/fobii, coping religios.