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Welcome to the Moldovan Medical Journal!

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

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ORIGINAL RESEARCHES

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Assessment of the nutritional pattern in frailty syndrome dependent institutionalized elderly

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Abstract

Background: Problems of nutritional status are widespread, of which malnutrition occurs more frequently in vulnerable groups of the population, especially in the low-income elderly, including the institutionalized elderly.
Material and methods: The study was performed on a group of 50 participants over the age of 65, from the nursing home; the nutritional status was assessed according to the Mini-Nutritional Assessment (MNA) score, the frailty syndrome (FS) was established based on Fried criteria and severity of FS – based on the Clinical Frailty Scale. The obtained data were statistically processed by using the "Statistica 6.0" software program. The difference was considered statistically significant with $p < 0.05$.
Results: The MNA score showed a positive correlation with reduced physical activity: r= 0.4*, between reduced physical activity and reduced walking speed: r= 0.66*, reduced physical activity and fatigue (lack of energy): r= 0.94* ($p \leq 0.05$). The cluster analysis revealed that against the background of the same food intake, body weight, mobility, neurological status can easily change, the phenomenon underlying the separation of the elderly in different clusters (1 and 2), while moderately low food intake in the last 3 months led to significantly reduced mobility, impaired neurological status (severe dementia or depression).
Conclusions: This study reported that the elderly in the nursing home have an increased risk of malnutrition, being associated with nutritional pattern, such as: involuntary weight loss, the presence of neuropsychiatric disorders, decline in food consumption, affecting the quality of life.
Key words: nutritional status, malnutrition, elderly.

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Introduction

Globally, there is an increase in the number of the elderly population, which will increase from 841 million in 2013 to over 2 billion by 2050, i.e. an increase from 11 to 22%, respectively [1]. The elderly are considered to be a vulnerable group of population, with an increased risk of co-morbidities, nutritional problems, frailty and geriatric syndromes [2]. Physical and social changes resulting from old age, polypharmacy, loss of autonomy and appetite are just some of the factors that influence the nutritional status of this social group of the population [3].

The study of nutrition in frailty syndrome is of particular interest to public health, as it investigates multiple aspects of human functionality: mobility, gait and balance, sarcopenia, physical activity, etc., along with the increase of medical and social resources resulting in considerable economic costs at the national level [5]. The consequences of malnutrition lead to social isolation, increased prevalence of infections, hospitalizations, institutionalizations, morbidity and mortality [4, 6, 7]. The prevalence of malnutrition in geriatric wards varies between 30 and 70%, and in nursing homes up to 74%, depending on demographic factors, educational level and assessment tools [8]. The indicators responsible for the diagnosis of malnutrition are: nutritional, anthropometric, biochemical parameters, cognitive status and associated diseases [9].

The author Wei K. pointed out that the interrelationship between malnutrition and frailty has common pathophysiological determinants and mechanisms, such as: reduction of adipose tissue, the presence of chronic inflammation, cognitive decline and functional disorders [10, 11]. Insufficient nutrient intake and involuntary weight loss aggravate the body's nutritional reserve against stressors [12].

There is currently no defining criterion for early identification of nutritional risk or malnutrition [13], but physicians, nurses and nutritionists use a wide range of tools for early assessment of the diagnosis of malnutrition in the geriatric population, such as: Mini-Nutritional Assessment (MNA) [9, 12], Geriatric Nutritonal Risk Index (GNRI) [14], Nutritional Risk Screening 2002 (NRS-2002) [15], Malnutrition Universal Screening Tool (MUST) [16], Short Nutritional Assessment Questionnaire (SNAQ) [17] and Nutrition Risk in the Critically Ill (NUTRIC) [18].

The European Society for Parenteral and Enteral Nutrition (ESPEN) recommends the evaluation of nutrition in the elderly by the MNA method [9, 12], being the most common and practical method for early estimation of nutritional deficiency and prevention of complications.

In residential homes or nursing homes, early identification of malnutrition plays a major role in the prophylaxis of nutrition in institutionalized elderly.

The purpose. This study aims to highlight and identify the main nutritional pattern aspects of the elderly patient from the nursing home by using statistical processing methods to identify valuable information indices and establish the associative links of nutrition through the cluster method.

Material and methods

The epidemiological study was the part of the Institutional Project 20.80009.8007.25 Frailty: diagnosis and prophylaxis in relation to the medico-psycho-social problems of the vulnerable elderly, with a positive opinion of the Ethics Committee No 51 of 16.06.2020, which included 50 elderly people between 65 and 93 years old, institutionalized at the Republican Asylum for the disabled persons and retirees. Participants were enrolled in research only after signing the informed consent to participate in the study.

All the elderly were examined according to the complex geriatric assessment (CGA), which included: physical status by assessment of autonomy – Activities of Daily Living / Instrumental Activities of Daily Living (ADLs and IADLs score) [19], assessment of gait and balance (Tinetti test) [20] and memory by test Mini-Mental State Examination (MMSE) [21, 22].

Data relating to ADLs and IADLs were assigned scores from 0 to 6 and 0 to 8, respectively; the highest of the score was the greater degree of functional independence for the parameters evaluated. Gait and balance disorders are among the most common causes of falls in older adults and often lead to injury, disability, loss of independence and limited quality of life, a score of 22 or less (total 28) indicates a patient at risk. For the analysis on MMSE scores, a minimum expected score of 24 was used for elderly individuals with normal activity of cognition, 20-23 – mild cognitive disorder, 10-19 – moderate cognitive disorder, 0-9 – severe cognitive disorder.

Nutritional status was determined by MNA (Mini-Nutritional Assessment) [9], it contains general data, anthropometric, dietary, self-assessment of nutritional status and health with 18 questions divided into two sections: screening with 6 questions, if the score is less than or equal to 11, the assessment is continued with 12 questions to get the malnutrition index. The maximum screening score is 14 points, if a score is obtained ≥12 – it is considered normal nutritional status, ≤11 points – malnutrition, and for evaluation – the maximum score is 16 points. The final MNA assessment shows the following scores: 24 – 30 normal nutritional status, 17-23.5 – risk for malnutrition and less than 17 – malnutrition.

The frailty syndrome (FS) was established by applying the Fried criteria and the severity of FS – by the Clinical Frailty Scale (CFS). The Fried frailty phenotype comprises five dimensions: involuntary weight loss (> 5% G / last year); decrease in the digito-palmar gripping force (clenching force of the fist) measured with a dynamometer, fatigue, normal walking speed low over the distance of 5 m (≤1 m/s), low level of physical activity [23, 24].

In the specialized works of the last years, the researchers reported the complexity of the frailty syndrome in the elderly population, mentioning the importance of the subtypes of frailty: functional – Groningen Frailty Index (GFI), The Short Physical Performance Battery (SPPB), Vulnerable Elders Survey (VES) – 13, SARC – F (A Simple Questionnaire to Rapidly Diagnose Sarcopenia) and biopsycho-social (Charlson Comorbidity Index) [25].

Data relating to GFI scores consists of 15 self-report items, a person is considered to be frail when the GFI sum score is 4 points or higher. The SPPB tool, establishes the physical activity of the elderly, the scores ≥10 out of 12 points correspond with higher levels of balance, lower body strength, mobility and physical function.

Vulnerable Elder's Survey (VES-13) includes 13 items relating to patient age, self-rated health, ability to perform six physical tasks and five items relating to function, the maximum score is 10 points and a cut-off of ≥3 denotes high-risk of experiencing future functional decline.

The components of SARC-F are: strength, assistance with walking, rise from a chair, climb stairs and falls, a score equal to or greater than 4 is predictive of sarcopenia and poor outcomes.

The Charlson Comorbidity Index is a method of categorizing comorbidities of patients based on the International Classification of Diseases (ICD) diagnosis codes found in administrative data, such as hospital abstracts data. Each comorbidity category has an associated weight (from 1 to 6), based on the adjusted risk of mortality or resource use, and the sum of all the weights results in a single comorbidity score for a patient. A score of zero indicates that no comorbidities were found. The higher the score, the more likely the predicted outcome will result in mortality or higher resource use.

In order to improve the testing systems, identify the informational value indices, elucidate the character variability, the dependence between various factors and classify the parameters of the elderly, it was necessary to use different

statistical methods that increase the degree of objectivity in interpreting the data. For this purpose, the data obtained from the program investigations were analyzed by methods of variational, correlational and cluster analysis.

The correlational analysis highlighted the correlation coefficient (r) which shows the degree of dependence between factors (0.0 ... 1.0) and the orientation of the dependence (+ or -) [26]. Cluster analysis of *k*-media [27, 28] highlighted the association of clinical manifestations (qualitatively assessed – absence/presence: 0/1) by dividing them into 3 clusters. The obtained results were processed in the STATISTICA 6.0 software package.

Results and discussion

The study was performed on a group of 50 participants, whose age exceeded 65 years, the average age of the group being 77.72±1.08 years. Regarding the distribution by age categories according to the Canadian Classification of the Elderly from 1998, the majority were elderly-mature (75-84 years) – 42%, followed by the young-elderly (65-74 years) – 40% and the old-elderly group (≥85 years) – 18%. The female sex predominated – 62% versus the male 38%. Most of them were from the central region of the country, constituting 84%, from the northern region of the country –6%, and from the southern – 8%. Referring to the place of residence of the elderly, a higher share was registered in urban areas – 74.62% versus rural areas – 25.38%.

According to the examination of the MNA nutritional score, 40±0.07% of the participants were at risk of malnutrition, 22±0.06% were malnourished and 38±0.07% had nutritional status within the norm (fig.1), and nutritional values after BMI revealed an average value of 27.77±0.78, muscle mass of 27.69±0.55, arm circumference – 28.36±0.56 and triceps fold thickness – 2.3±0.12.

The evaluation of the institutionalized elderly according to the frailty criteria registered the following average values: weakness measured by dynamometry – 70±0.06, fatigue – 78±0.06, reduced physical activity – 80±0.05, reduced walking speed – 76±0.06 and unintentional weight loss had 28±0.06% of the elderly (fig. 1). Thus, based on the frailty criteria, 16% of pre-fragile elderly people were diagnosed, who had one or two Fried positive criteria, 66% of fragile elderly people, who had three or more positive criteria, and 18% were robust patients.

CGA is an interdisciplinary approach to determine the medical, psychological and functional capabilities of a frail elderly person, with a view of developing a coordinated and integrated plan for prophylaxis and treatment [29]. Thus, according to the results of the geriatric and subtypes of frailty examination, it was established that the elderly included in the study showed functional, cognitive and social frailty, according to the researched items: ADL scores – 8.70±0.43 points, IADL – 7.84±0.67 points, Tinetti – 12.78±1.21 points, MMSE – 21.98±0.67 points, Charlson comorbidity – 6.02±0.33 points, Groningen frailty index – 7.50±0.59 points, sarcopenia SARC – F – 6.1±0.40 points and VES-13 – 9.0±0.96 points (fig. 2).

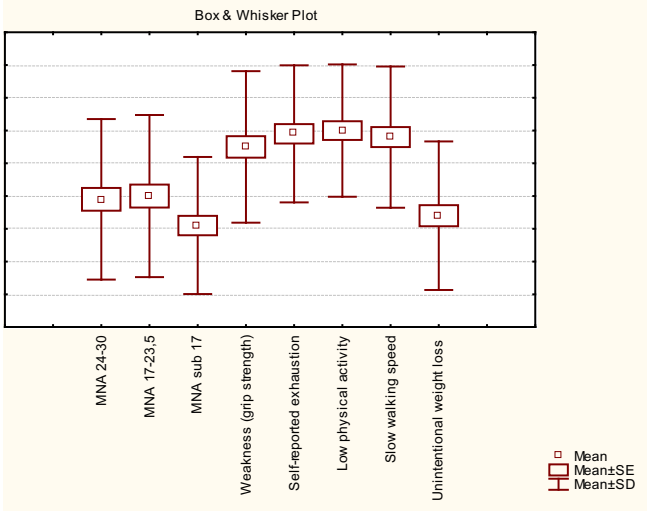

Fig. 1. Graphical representation of mean values of MNA score and Fried frailty

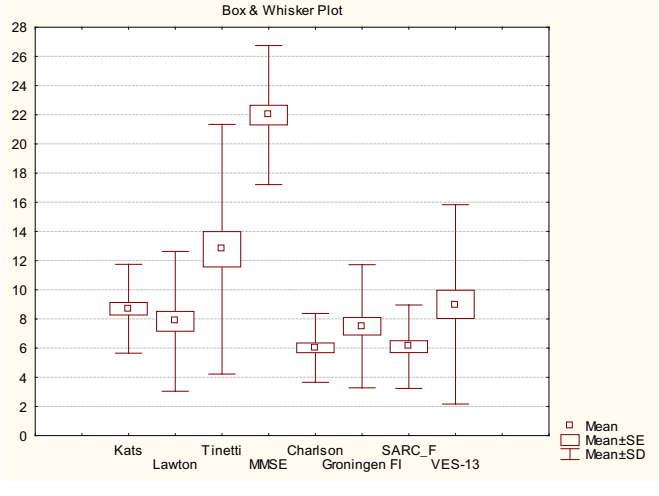

Fig. 2. Graphical representation of the average values of geriatric syndromes and instruments of frailty

Manifestations of pathological conditions are characterized not only by the presence of certain clinical indices, but also by their correlative associations [26]. Thus, significant dependencies were found, on the one hand, between malnutrition and frailty syndrome, on the other hand – between various clinical features. For example, there were high positive correlations between reduced physical activity and reduced walking speed: r= 0.66*, fatigue (lack of energy) and reduced physical activity: r= 0.94* (p≤0.05). It should be noted that the relatively low correlation coefficient (r) for the interrelationship risk of malnutrition –- reduced physical activity: r= 0.41* (p≤0.05), indicates that the dependencies found are a trend, rather than a strict relationship, which can be explained by the specificity of patients' response to the same mode of nutrition. The data obtained reveal that different clinical indices of malnutrition increase the risk of frailty of the elderly through various correlative dependencies.

The cluster analysis [28] by the centroid method of *k*-media established that the elderly groups, evaluated, separated into 3 clusters were divided according to the level and variability of the researched parameters. As members of cluster 1 were the elderly with numbers 16, 17, 20, 21, 26, 27, 29-33, 37, 39, 40, 42, 46; cluster 2 – 1-14, 23, 25, 43, 45, 47, 49; cluster 3 – 18, 19, 22, 23, 26, 28, 31-33, 44, 46, 48. The people in cluster 1 and 2 recorded the highest values of neuropsychiatric disorders, body mass index ≥23, food intake and body weight unchanged in the last 3 months, and those in cluster 3 showed values slightly above the 50% threshold for the aspect of mobility, the presence of psychological stress in the last 3 months, severe dementia or depression according to screenning (stage 1) of the MNA nutrition questionnaire (tab.1).

Table 1. Cluster analysis of nutritional screening (MNA) data of the association of clinical indicator frequencies

Cluster	Frequent indices in clusters (≥50%)
1, n=16	Unchanged food intake in the last 3 months – 81.25%; body weight unchanged in the last 3 months – 93.75%; mobility partially affected – 56.27%; psychological stress the last 3 months – 62.50%; neuropsychiatric disorders (mild dementia) – 100%; BMI ≥23 – 93.75%.
2, n=20	Unchanged food intake in the last 3 months – 100%; body weight unchanged in the last 3 months – 85.0%; unaffected mobility – 85%; unaffected neurological status – 90.0%; BMI ≥23 – 100%.
3, n=14	Moderately low food intake in the last 3 months – 57.14%; unknown body weight if it has changed in the last 3 months – 71.42%; mobility partially affected – 71.42%; psychological stress in the last 3 months – 57.14%; neurological status (severe dementia or depression) – 50.0%; BMI ≥23 – 78.57%.

Regarding the evaluation of nutrition (stage 2) according to the MNA nutrition questionnaire, members of cluster 1 were the elderly with numbers 1-14, 21, 40-43, 45, 47, 49, 50; cluster 2 – 15-17, 20, 24, 25, 27, 29, 30, 34, 35-39; cluster 3 – 18, 19, 22, 23, 26, 28, 31-33, 44, 46, 48. The people in cluster 1 recorded the highest values of self-care and daily diet with 3 meals per day or weekly protein intake (dairy, meat/fish), vegetables/eggs, polypharmacy, arm and leg circumference within the norm, cluster 2 presented the highest values of the daily diet of 3 meals per day or weekly intake of protein (dairy), vegetables/eggs, fluid intake per day (3-5 cups/day), and polypharmacy, self-sufficient and self-perception of nutritional status showed lower values, and cluster 3 demonstrated the highest values of self-care but with some difficulty in eating daily with 3 meals included daily or weekly intake of protein (dairy, vegetables/eggs), and pressure abrasions/ decubitus or skin ulcers showed the lowest values (tab. 2).

Table 2. Cluster analysis of nutritional status indices (MNA) and clinical indicator frequencies

Cluster	Frequent indices in clusters (≥50%)
1, n=23	Polymedication more than 3 drugs per day – 82.60%; pressure abrasions/decubitus or skin ulcers – 82.60%; diet with 3 meals per day – 100%, daily intake of protein: dairy – 95.65%, meat or fish – 91.30%, weekly intake (vegetables/eggs) – 95.65%, daily intake of fruits or vegetables – 60.87%, fluid consumption (≥5 cups / day) – 56.52%; food at own choice – 95.65%; self-perception of nutritional status unaffected – 86.95%; self-assessment of health: good – 52.17%; arm circumference ≥22 (cm) / leg ≥31 (cm) – 100%.
2, n=15	Polymedication more than 3 drugs per day – 60%; diet with 3 meals a day – 100%, daily protein intake: dairy – 100%, weekly intake (vegetables / eggs) – 93.33%, fluid intake (3-5 cups / day) – 80.0%; food at own choice – 66.66%; self-perception of nutritional status: no idea – 80%; arm circumference ≥22 (cm) – 93.33% and leg circumference ≤31 (cm) – 53.33%.
3, n=12	Pressure abrasions / decubitus or skin ulcers – 58.33%; diet with 3 meals per day – 100%, daily protein intake: dairy – 100%, weekly intake (vegetables / eggs) – 100%; food at own choice but with some difficulty – 100%; self-assessment of nutritional status: being insecure – 100%; arm circumference ≥22 (cm) – 83.33% and leg circumference ≥31 (cm) – 91.66%.

This study identified groups with specific care requirements that would reduce the limitations and loss of quality of life resulting from MNA and frailty score. A good functioning of nutritional status is related to self-assessment and should take priority over primarily clinical and medical approaches. Studies have shown that functional losses can be reversed through training for ADLs and IADLs, with positive consequences for self-perception of health [27].

Also it must be noted that the presence of subtypes of frailty, such as: Groningen Frailty Index, The Short Physical Performance Battery, Vulnerable Elders Survey, A Simple Questionnaire to Rapidly Diagnose Sarcopenia and Charlson Comorbidity Index should be taken into account when planning care actions, as this is an important parameter in overall geriatric assessment [25].

Conclusions

1. Following a broad approach, the study highlighted that the MNA nutritional score is a useful and effective method for identifying malnutrition among the elderly population in the nursing homes in the Republic of Moldova.

2. According to the cluster analysis (*k*-average method) of the MNA test stage of screenning and evaluation, the best ability to differentiate the parameters of the nutritional pattern presented: unaffected food intake and lack of weight loss in the last 3 months, neuropsychiatric disorders, polypharmacy, presence of pressure abrasions, provision of 3 meals per day with an intake of protein, vegetables, fluids

more than 5 cups a day and self-assessment of nutritional status as being without nutritional problems.

3. It was found that against the background of the same food intake, body weight, mobility, neurological status, can remain virtually unchanged or change slightly, the phenomenon underlying the separation of the elderly into different clusters (1 and 2), while moderately low food intake in the last 3 months has led to significant disorders of mobility, neurological status (severe dementia or depression).

4. The results obtained characterize the profile of institutionalized elderly and can be used as a basis for the development of effective strategies aimed at reducing functional dependence, self-assessment of nutritional status and quality of life.

References

1. Dawson A, Dennison E. Measuring the musculoskeletal aging phenotype. Maturitas. 2016;93:13-17. doi: 10.1016/j.maturitas.2016.04.014.
2. De Labra C, Maseda A, Lorenzo-López L, et al. Social factors and quality of life aspects on frailty syndrome in community-dwelling older adults: the Verisaúde study. BMC Geriatr. 2018;18(1):66-75. doi: 10.1186/s12877-018-0757-8.
3. Duppen D, Van der Elst MCJ, Dury S, et al. The social environment's relationship with frailty: evidence from existing studies. J of App Gerontol. 2019;38(1):3-26. doi: 10.1177/0733464816688310.
4. Fabrício-Wehbe SC, Rodrigues RA, Haas VJ, et al. Association of frailty in hospitalized and institutionalized elderly in the community-dwelling. Rev Bras Enferm. 2016;69(4):691-696. doi: 10.1590/0034-7167.2016690411i.
5. Tabue-Teguo M, Simo N, Gonzalez-Colaço HM, et al. Frailty in elderly: a brief review. Geriatr Psychol Neuropsychiatr Vieil. 2017;15(2):127-137. doi: 10.1684/pnv.2017.0670.
6. Abd Aziz NAS, Teng NIMF, Abdul Hamid MR, et al. Assessing the nutritional status of hospitalized elderly. Clin Interv Aging. 2017;12:1615-1625. doi: 10.2147/CIA.S140859.
7. Boulos C, Salameh P, Barberger-Gateau P. Malnutrition and frailty in community dwelling older adults living in a rural setting. Clin Nutr. 2016;35(1):138-143. doi: 10.1016/j.clnu.2015.01.008.
8. Sellier C. Malnutrition chez la personne âgée, dépister et prendre en charge [Malnutrition in the elderly, screening and treatment]. Soins Gerontol. 2018;23(133):12-17. French. doi: 10.1016/j.sger.2018.06.003.
9. Corish CA, Bardon LA. Malnutrition in older adults: screening and determinants. Proc Nutr Soc. 2019;78(3):372-379. doi: 10.1017/S0029665118002628.
10. Boulos C, Salameh P, Barberger-Gateau P. Social isolation and risk for malnutrition among older people. Geriatr Gerontol Int. 2017;17(2):286-294. doi: 10.1111/ggi.12711.
11. Wei K, Nyunt MSZ, Gao Q, et al. Frailty and malnutrition: related and distinct syndrome prevalence and association among community-dwelling older adults: Singapore longitudinal aging studies. J Am Med Dir Assoc. 2017;18(12):1019-1028. doi: 10.1016/j.jamda.2017.06.017.
12. Lorenzo-López L, Maseda A, de Labra C, et al. Nutritional determinants of frailty in older adults: a systematic review. BMC Geriatr. 2017;17(1):108-121. doi: 10.1186/s12877-017-0496-2.
13. Diekmann R, Winning K, Uter W, et al. Screening for malnutrition among nursing home residents – a comparative analysis of the mini-nutritional assessment, the nutritional risk screening, and the malnutrition universal screening tool. J Nutr Health Aging. 2013;17(4):326-331. doi: 10.1007/s12603-012-0396-2.
14. Bouillanne O, Morineau G, Dupont C, et al. Geriatric Nutritional Risk Index: a new index for evaluating at-risk elderly medical patients. Am J Clin Nutr. 2005;82(4):777-783. doi: 10.1093/ajcn/82.4.777.
15. Kondrup J, Rasmussen HH, Hamberg O, Stanga Z; Ad Hoc ESPEN Working Group. Nutritional risk screening (NRS 2002): a new method based on an analysis of controlled clinical trials. Clin Nutr. 2003;22(3):321-336. doi: 10.1016/s0261-5614(02)00214-5.
16. Stratton RJ, Hackston A, Longmore D, et al. Malnutrition in hospital outpatients and inpatients: prevalence, concurrent validity and ease of use of the 'malnutrition universal screening tool' ('MUST') for adults. Br J Nutr. 2004;92(5):799-808. doi: 10.1079/bjn20041258.
17. Kruizenga HM, Seidell JC, de Vet HC. Development and validation of a hospital screening tool for malnutrition: the short nutritional assessment questionnaire (SNAQ). Clin Nutr. 2005;24(1):75-82. doi: 10.1016/j.clnu.2004.07.015.
18. Heyland DK, Dhaliwal R, Jiang X, et al. Identifying critically ill patients who benefit the most from nutrition therapy: the development and initial validation of a novel risk assessment tool. Crit Care. 2011;15(6):268. doi: 10.1186/cc10546.
19. Ćwirlej-Sozańska AB, Sozański B, Wiśniowska-Szurlej A, et al. An assessment of factors related to disability in ADL and IADL in elderly inhabitants of rural areas of south-eastern Poland. Ann Agric Environ Med. 2018;25(3):504-511. doi: 10.26444/aaem/81311.
20. Rivolta MW, Aktaruzzaman M, Rizzo G, et al. Evaluation of the Tinetti score and fall risk assessment via accelerometry-based movement analysis. Artif Intell Med. 2019;95:38-47. doi: 10.1016/j.artmed.2018.08.005.
21. Larner AJ. Mini-Mental State Examination: diagnostic test accuracy study in primary care referrals. Neurodegener Dis Manag. 2018;8(5):301-305. doi: 10.2217/nmt-2018-0018.
22. Kelaiditi E, Cesari M, Canevelli M, et al. Cognitive frailty: rational and definition from an (I.A.N.A./I.A.G.G.) international consensus group. J Nutr Health Aging. 2013;17(9):726-734. doi: 10.1007/s12603-013-0367-2.
23. Rockwood K, Song X, MacKnight C, Bergman H, et al. A global clinical measure of fitness and frailty in elderly people. CMAJ. 2005;173(5):489-495. doi: 10.1503/cmaj.050051.
24. Fried LP, Tangen CM, Walston J, et al. Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci. 2001;56(3):146-156. doi: 10.1093/gerona/56.3.m146.
25. Checa-López M, Oviedo-Briones M, Pardo-Gómez A, et al; FRAILTOOLS consortium. FRAILTOOLS study protocol: a comprehensive validation of frailty assessment tools to screen and diagnose frailty in different clinical and social settings and to provide instruments for integrated care in older adults. BMC Geriatr. 2019;19(1):86. doi: 10.1186/s12877-019-1042-1.
26. Curtis EA, Comiskey C, Dempsey O. Importance and use of correlational research. Nurse Res. 2016;23(6):20-25. doi: 10.7748/nr.2016.e1382.
27. Winiarti S, Yuliansyah H, Purnama AA. Identification of toddlers' nutritional status using data mining approach. Int J Adv Comput Sci Appl. 2018;9(1):164-169. doi: 10.14569/ IJACSA.2018.090122.
28. Tseng GC. Penalized and weighted K-means for clustering with scattered objects and prior information in high-throughput biological data. Bioinformatics. 2007;23(17):2247-2255. doi: 10.1093/bioinformatics/btm320.
29. Welsh TJ, Gordon AL, Gladman JR. Comprehensive geriatric assessment – a guide for the non-specialist. Int J Clin Pract. 2014;68(3):290-293. doi: 10.1111/ijcp.12313.

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FLV conceptualized the study, designed the research, collected and interpreted the data, drafted the first manuscript; GS conducted the laboratory work and revised the manuscript critically; AP collected data and revised the manuscript critically; AN conducted the management work and revised the manuscript critically; EC and IS collected the data. All the authors revised and approved the final version of the manuscript.

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Ethics approval and consent to participate

The research was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (protocol No 51 of June 16, 2020).

Conflict of Interests

The authors have no conflict of interests to declare.

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Clinical and treatment aspects in chronic hypertrophic rhinitis in children

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Abstract

Background: Chronic hypertrophic rhinitis is a common problem in childhood and adolescence and has a negative impact on both physical, social and psychological well-being. Chronic hypertrophic rhinitis in the prevalence of children is 20-55%, and in the last 5 years this index is increasing.
Material and methods: The study included 65 patients, aged 5 to 18 years, being hospitalized in the ENT department of the *Emilian Cotaga* Clinic of the Institute of Mother and Child, Chisinau, diagnosed with rhinitis complicated chronic hypertrophic over the years January 2018 – December 2020. The study was conducted by retrospective analysis of complicated data from clinical observation sheets and documents accompanying the data. Treatment methods studied: laser coagulation, electrocoagulation, radiofrequency, mucotomy. In most cases, lasercoagulation was chosen.
Results: Nasal permeability in children with chronic hypertrophic rhinitis is altered in most cases. The most frequently affected age groups were 10-14 years with a rate of 61.53%, the majority being from urban living environment in 63.08%. The most representative clinical signs and symptoms according to this study are difficult nasal breathing in 96.93% and mouth breathing – 89.24%. From the indicated surgical methods, laser coagulation of the nasal cornets was performed in 47.7% of cases. It proved to be more effective and less invasive compared to electrocoagulation performed in 44.6% of cases and partial mucotomy in 4.62%.
Conclusions: From the data obtained in the study, we opted for laser coagulation of the lower nasal horns, which has a faster healing period, shorter postoperative complications, greater acceptability from the doctor and patient.
Key words: chronic hypertrophic rhinitis, treatment, laser coagulation.

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Introduction

Chronic hypertrophic rhinitis is a common problem in childhood and adolescence and has a negative impact on both physical, social and psychological well-being [1]. The prevalence of chronic hypertrophic rhinitis in children (CHRC) is 20-55%, and in the last 5 years this index is increasing. Epidemiological data on chronic hypertrophic rhinitis estimate that more than 200 million people worldwide suffer from it. In the pediatric population, they showed a prevalence of 16.1% by the age of 5 years and a prevalence of 42.3 % by the age of 14 [2]. The term "chronic rhinitis, hypertrophic" defines a state of congestion of the mucous membrane and underlying tissues of the nasal concha so that it reduces considerably inferior nasal airway with the functional consequence of constant nasal obstruction [3].

Although chronic hypertrophic rhinitis in children (CHRC) is a fairly common nosological entity in children, its symptoms are often subtle and nonspecific and this can lead to overlooking the diagnosis [4]. Usually the diagnosis of chronic hypertrophic rhinitis in children is based on symptoms that evolve for more than three months and some abnormalities determined by endonasal examination or imaging examination [5]. Patients with RCHC often receive several types of medications, decongestants, oral antibiotic treatments that can change the clinical presentation, especially in the early stages of the disease [6].

The initial management of RCHC is medical, with goals that include reducing inflammation, improving drainage, and eradicating pathogens. Drug treatment is the first line of approach to the treatment of chronic rhinitis. Depending on the type, duration and severity of symptoms, drug treatment in chronic hypertrophic rhinitis may use the following classes of drugs:

- Nasal decongestants – can be used in both topical and oral form;
- H1 anti-receptor antihistamines, used in patients with allergic rhinitis;
- Intranasal or oral corticosteroids;
- Cromone: sodium cromoglycate and nedocromil sodium;
- Anticholinergics: ipratropium bromide;
- Antileukotrienes;
- Specific immunotherapy, used in allergic rhinitis;
- Intramucosal injection of cortisone [7-9].

In case of inefficiency of drug treatment, surgical treatment is considered [8, 9]. Surgical treatment is reserved for cases where the correct and complete medication performed during 3 consecutive months is ineffective or in irreducible hypertrophy of the nasal cones, when surgical treatment is applied as a first-line method [10].

The specialty literature describes multiple surgical techniques to reduce the size of the nasal cones, the choice which depends on several factors: the type of hypertrophy, the experience and habit of the surgeon, the technical equipment of the clinic where the surgery is performed and, last but not least, the costs of intervention [11]. In case of reducible nasal corneal hypertrophy, several techniques are available, such as lateralization of the nasal cornet with or without submucosal vasotomy, mucotomy (partial, total, submucosal, anterior), electrocautery, diathermic coagulation, radiofrequency coagulation, vaporization or / and laser coagulation [10, 11].

A simple method of restoring nasal permeability is to lateralize the cornets. The procedure consists of fracturing and lateral mobilization of the lower nasal cornet in a controlled manner using a blunt instrument. The technique is often used in combination with other procedures. The technology is quite safe and easy to use, however in pronounced irreducible hypertrophy, it may not be sufficient [1].

Cauterization of the nasal horns by radiofrequency is another method of treatment of chronic hypertrophic rhinitis. It provides for the use of high frequency energy, with the aim of coagulating the tissues and thus reducing the volume of the cornet and reducing nasal obstruction. The radio frequency is generated by a specially designed device. The electric current is transmitted through an electrode to the tissues producing energy release, evaporation of cell fluid, which results in tissue coagulation and narrowing of the nasal cones.

The maximum effect occurs 7-14 days after the procedure. The technique has a number of advantages, namely: it eliminates the risk of bleeding and thus the need for nasal tamponade, excludes the need for general anesthesia in adults, so the method falls within the requirements of *ex officio* surgery [12, 13].

Microdebrider mucotomy is a beneficial alternative in chronic hypertrophic rhinitis because it has the advantage of reducing the hypertrophied and hyperplastic tissue of the lower cornet under direct visualization, without bone lesions and without deep thermal effect [14].

The mucotomy technique with the microdebrider can be done with local anesthesia, by infiltration, or with general anesthesia, depending on the size of the nasal cornet hypertrophy, the existing comorbidities, but also the patient's wishes. The entire tissue of the hypertrophied cornet is resected and with predilection of the posterior third and the tail of the inferior nasal cornet, which are then aspirated on the aspiration system of the apparatus. The results of cornet surgery with the microdebrider prove to be very good, with favorable long-term functional results [2, 14].

Electrocoagulation was the first method recorded for the RCHC treatment and has constantly evolved in its practice. This method consists in decreasing the size of the inferior nasal cone by cauterization due to the volumetric reduction of the nasal cone due to the retraction following the healing process that follows. The technique can be performed by local anesthesia (adults only) or general, under direct endoscopic visualization and usually requires nasal tamponade [14]. The long-term results of this technique are controversial because the recurrence rate of cornet hypertrophy is high [6, 10].

Cornetotomy or partial cornetectomy is performed in advanced hypertrophies of the nasal cornet when hyperplastic changes of connective tissue and turbine bone occur. Cornetotomies are performed submucosally by creating two flaps: medial and lateral, and removing excess connective and bone tissue. It is indicated in the hypertrophy of the head and body of the nasal cornet, less often in the muriform hypertrophy of the cornet tail. If done technically correctly, there is practically no open area of the wound and a significant volumetric reduction of the nasal cone is obtained. The procedure has been applied in several studies, which have shown a lasting positive effect on nasal breathing [4, 11, 15].

Regarding the extensive resections of nasal cones, it should be mentioned that at present they belong to the field of the past, having a predominantly historical interest, due to various complications – atrophic rhinitis, "empty nose" syndrome, which has generated new scientific research and research to make the surgical approach much more cost-effective. Thus, the concept of minimally invasive endonasal surgery was developed, which provides for the maximum preservation of the pituitary mucosa – the noble element of the nasal fossae [15-17]. Minimally invasive surgery involves surgical maneuvers, which are related to the limited ablation of pathological tissues with the maximum possible respect for healthy skin and surrounding tissues. This type of surgery has clearly superior advantages over radical surgery, because the repair processes are evolving much more favorably, in connection with the considerable reduction of surgical trauma.

Material and methods

The study included 65 patients, aged 5 to 18 years, being hospitalized in the ENT department of the *Emilian Cotaga* Clinic with the diagnosis of chronic hypertrophic rhinitis, during January 2018 – December 2020. This study was achieved through retrospective analysis of complicated data from clinical observation sheets and documents accompanying the data.

Laser coagulation of the nasal horns

Several types of lasers are known, but in nasal cornet surgery the most common are the Diode laser, the Potassium-Titanium-Phosphate (KTP) laser and the CO_2 laser. In the first two types, the conduction of the laser beam is performed through fiber and direct contact with the tissues of the nasal horns, which is an advantage. Another difference be-

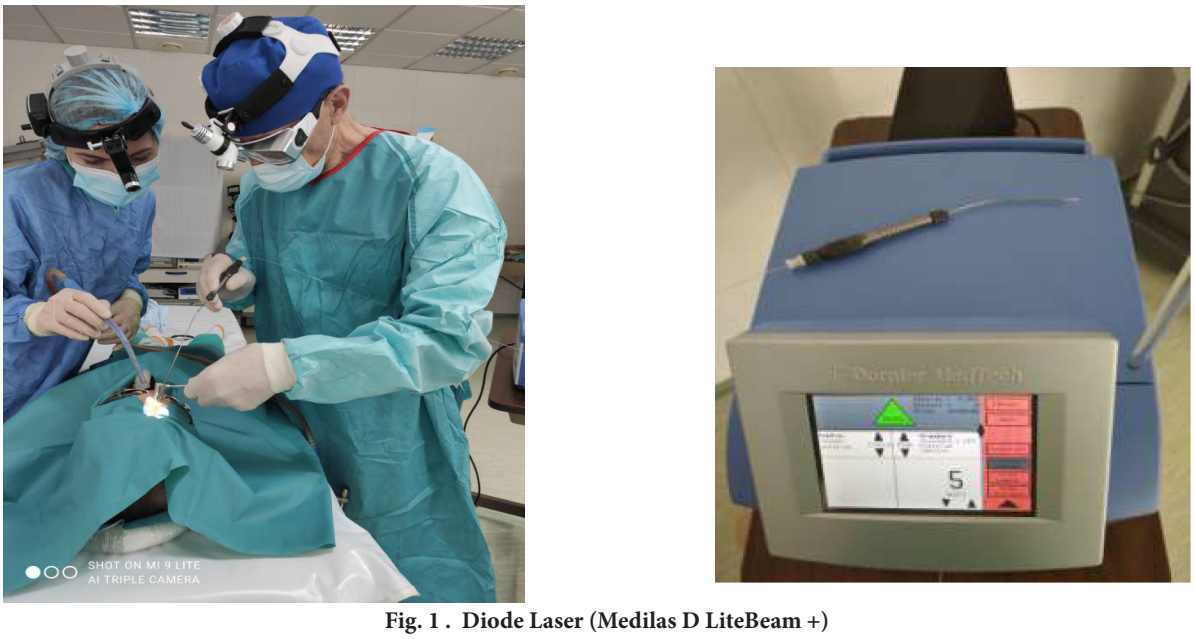

Fig. 1 . Diode Laser (Medilas D LiteBeam +)

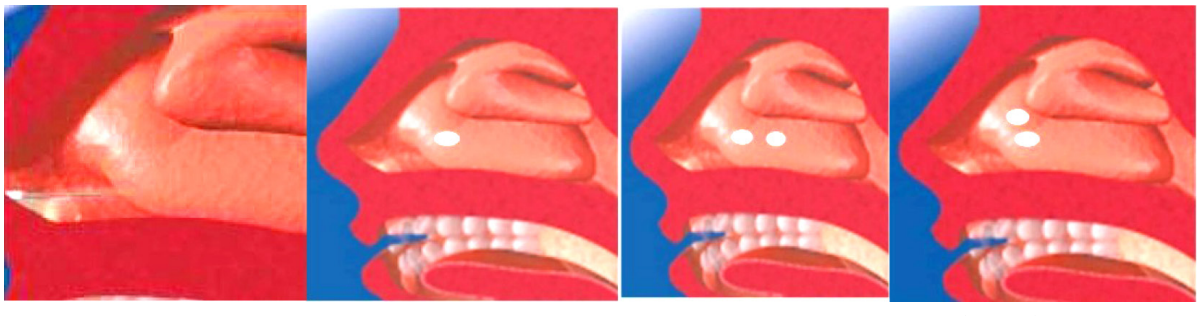

Fig. 2. Laser application techniques in chronic hypertrophic rhinitis in children (CHRC)

tween the first types and the CO_2 laser is the wavelength of the emitted light and its ability to penetrate deep. The effect of the wave is strictly on the mucosa of the lower nasal cornet, the laser having no effect on the skeleton.

In the case of diode laser intervention (fig. 1), the volume of the lower nasal cone decreases by the retraction of the loose tissue secondary to the thermal lesions of the tissues. The CO_2 laser produces necrotic debris and fibroepithelial reshaping, which replace the vaporized mucosa [18].

The application of choice is the submucosal one, and the important condition is the use of the endoscopic technique (fig. 2). The advantage of this technique is the absence of bleeding of the operative field and the need for nasal tamponade, minimal damage to the mucosal surface of the nasal cone, low reduction of possible disturbance of mucociliary clearance and the possibility of bleeding and scarring in the postoperative period. The operation in adults can be performed under local anesthesia.

Results and discussion

A number of risk factors were analyzed that could lead to the development of chronic hypertrophic rhinitis and to the worsening of the evolution of the subsequent diagnosis.

One of the main factors is the distribution according to the age of the patients, thus there was a higher addressing of patients aged 10-14 years with a rate of 61.53%, followed by those aged 5-9 years with a rate of 24.62%, then being followed by those aged 15-18 with a rate of 13.85%. The results are shown in fig. 5.

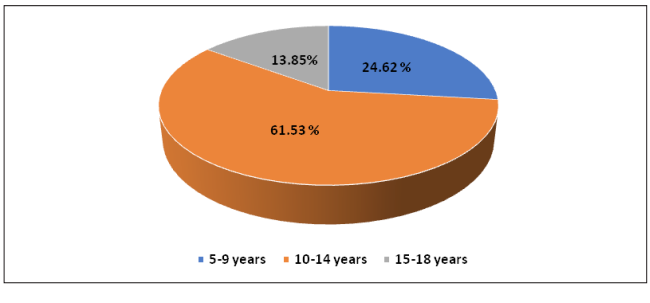

Fig. 5. Distribution of patients by age groups

Among the social factors that can influence the evolution of RCHC is the living environment of patients. Thus, according to figure 6, the number of patients is higher in the urban living environment – 41 cases, with an incidence rate of 63.08%, and in the rural living environment – 24 cases with a rate of 36.92%.

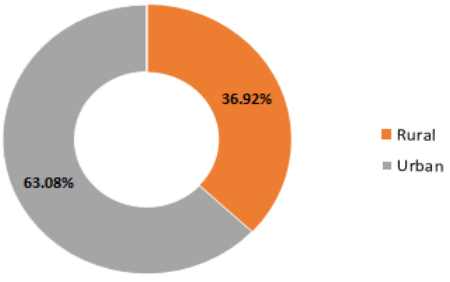

Fig. 6. Distribution of the number of cases depending on the living environment

Based on the data obtained from the study on a group of 65 patients, it is observed according to fig. 7, the main symptoms were: difficult nasal breathing in 96.93% and mouth breathing – 89.24%. Other symptoms presented were: nasal voice in 83.08%, fatigue in 78.47%, snoring in 70.77%, sero-mucous secretions in 66.16%, hyposmia in 49.24% and headache in 32.31% cases.

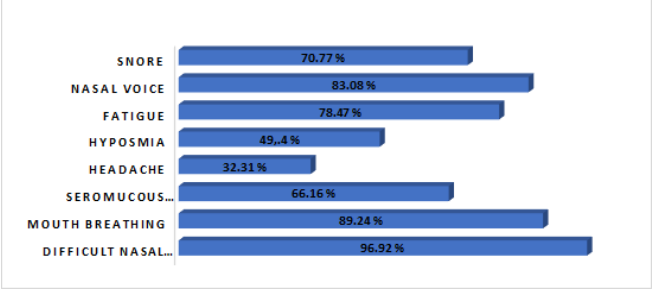

Fig. 7. Distribution of data depending on the symptoms presented

From the multitude of methods listed in chronic hypertrophic rhinitis in children (CHRC) surgery, in 31 cases, laser coagulation with a rate of 47.7% was chosen. The diode laser was used under general anesthesia, according to the method developed in the clinic, aseptic conditions were observed. Electrocoagulation applied in 29 cases at a rate of 44.62%, and 3 cases – 4.62% for the partial mucotomy, and in other two cases – 3.06 % another method was chosen (fig. 8).

One of the first studies on laser reduction of hyperplastic lower nasal turbinates were performed by Lenz et al., who treated over 2000 patients with nasal obstruction

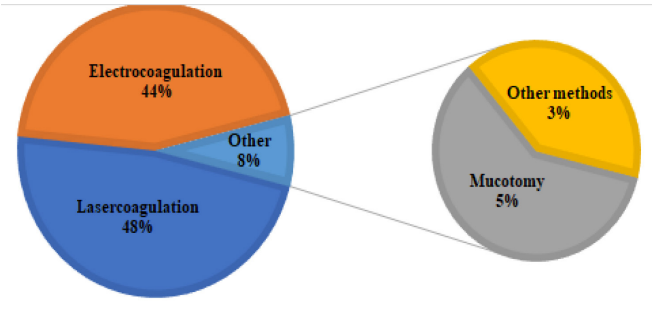

Fig. 8. Distribution of cases depending on the surgical method performed

due to vasomotor rhinitis [18]. In 80% of patients there was an improvement in nasal breathing and a completely free breathing after a period of about 10 years. Similar results were demonstrated by Levine HL. in a trial using laser coagulation in 29 patients suffering from allergic or vasomotor rhinitis [1]. After a monitoring period of 6 ± 12 months, 81% of patients described a subjective improvement in nasal breathing, less nasal congestion [19].

Reduction of hypertrophied nasal cornets by diode laser is safe, minimally invasive and effective in relieving the symptoms associated with chronic hypertrophic rhinitis. To date, there are only a few reports in the literature on mussel treatment. Min et al. treated 53 patients with diode laser vasomotor rhinitis (810 nm) [5]. The results on nasal obstruction were very satisfactory. In another investigation, 30 patients treated endoscopically with a diode laser (940 nm) were able to achieve an improvement in nasal respiration by 85% [3]. DeRowe et al. noticed that no more than 41% of patients received improved postoperative nasal breathing [8].

Hoffmann et al. performed the laser treatment of the lower turbines using the continuous wave diode laser [12]. The laser light was applied through endonasal endoscopes containing a laser channel. A power of 3W was established, and the laser tip was slowly pulled into contact from the rear end to the front end of the flow turbine, producing white areas of coagulation (”whitening”) of tissue [19]. Additional treatment was performed on hyperplastic tissue in important aerodynamic areas. Seventy-two patients were treated with this method, all noticing an improvement in nasal breathing after six months. Side effects and complications of laser surgery were not described in this study [20]. Compared to most of these treatment modalities, laser surgery of the nasal obstruction is a minimally invasive procedure with a controllable ablation of the hypertrophied tissue causing fewer complications (e.g. , bleeding, dryness, synechiae and pain). The application is easy to make, offering a cost-effective and time-saving method for reducing hyperplastic turbines. Moreover, laser treatment appears to be as effective or even more effective for reducing hyperplastic turbines than most conventional surgical techniques, such as mucotomy, electrocautery, radiofrequency [15]. More invasive (radical) operating methods, such as lower turbinoplasty, submucosal turbinectomy, lateral fracture, and partial and total turbinectomy have the advantage of partially removing large hyperplastic parts of the turbinates, leading to a reduction in obstructive tissue and better results. However, these methods have a higher invasiveness, a longer recovery and much higher costs than laser therapy [4]. Although the total turbinectomy produced a substantial increase in the level of nasal air, it also led to the destruction of the cilia, atrophy of the mucous membrane and chronic nasal infections, with a reduction in the moisturizing capacity and the nasal mucosa.

Submucosal diathermy was first used by Neres FE. [3] in 1907 and developed rapidly in the following years. Beck IC. led the 1930s by the unipolar method [14] and Hurd LM.

introduced bipolar diathermy in 1931 [20]. The F. target submucosal diathermy is current-induced scarring and obliteration in the submucosa of the lower nasal horns. The results of therapy after submucosal diathermy are evaluated very differently in the literature. The short-term results are good and exceed 80%, with a higher success of therapy in non-allergic patients [9]. However, if the monitoring period is longer, the results of the therapy are clearly worse [2]. A retrospective study showed that after 2 years, only 36% of patients treated were satisfied with the outcome of treatment [19].

From the data obtained in the study, we opted for laser coagulation of the lower nasal horns, which has a faster healing period, shorter postoperative complications, greater acceptability from the doctor and patient.

Conclusions

Nasal permeability in children with chronic hypertrophic rhinitis is altered in most cases. The most frequently affected age groups were 10-14 years with a rate of 61.53%, the majority being from urban living environment – 63.08%. The most representative clinical signs and symptoms according to this study are difficult nasal breathing in 96.93% and mouth breathing in 89.24%.

From the indicated surgical methods, laser coagulation of the nasal cornets was performed in 47.7% of cases. It proved to be more effective and less invasive compared to electrocoagulation performed in 44.6% of cases and partial mucotomy in 4.62%.

References

1. Levine HL. Lasers in endonasal surgery. Otolaryngol Clin North Am. 1997;30(3):451-455.
2. Hellings PW, Klimek L, Cingi C, Agache I, Akdis C, Bachert C, et al. Non-allergic rhinitis: position paper of the European Academy of Allergy and Clinical Immunology. Allergy. 2017;72(11):1657-1665. doi: 10.1111/all.13200.
3. Neres FE. Voltaic turbinal puncture for the relief of intumescent and hypertrophic rhinitis. J Am Med Ass. 1907;44.
4. Komshian SR, Cohen MB, Brook C, Levi JR. Inferior turbinate hypertrophy: a review of the evolution of management in children. Am J Rhinol Allergy. 2019;33(2):212-219. doi: 10.1177/1945892418815351.
5. Min YG, Kim HS, Yun YS, Kim CS, Jang YJ, Jung TG. Contact laser turbinate surgery for the treatment of idiopathic rhinitis. Clin Otolaryngol. 1996;21(6):533-536. doi: 10.1111/j.1365-2273.1996.tb01105.x.
6. Coste A, Yona L, Blumen M, Louis B, Zerah F, Rugina M, et al. Radiofrequency is a safe and effective treatment of turbinate hypertrophy. Laryngoscope. 2001;111(5):894-899. doi: 10.1097/00005537-200105000-00025.
7. Beard S. Rhinitis. Prim Care. 2014;41(1):33-46. doi: 10.1016/j.pop.2013.10.005.
8. DeRowe A, Landsberg R, Leonov Y, Katzir A, Ophir D. Subjective comparison of Nd:YAG, diode, and CO_2 lasers for endoscopically guided inferior turbinate reduction surgery. Am J Rhinol. 1998;12(3):209-212. doi: 10.2500/105065898781390145.
9. Lippert BM, Werner JA. Treatment of the hypertrophic inferior turbinate. HNO. 2000;48(3):170-181. doi: 10.1007/s001060050029.
10. Lopatin AS, Gromov VP. Ostryi i hronicheskii rinosinusit: etiologiia, patogenez, klinika, diagnostika i printsipy lecheniia [Acute and chronic rhinosinusitis: etiology, pathogenesis, clinical picture, diagnosis]. Moscow; 2014. 70 p. Russian.
11. Larrabee YC, Kacker A. Which inferior turbinate reduction technique best decreases nasal obstruction? Laryngoscope. 2013;124(4):814-815. doi: 10.1002/lary.24182.
12. Hoffmann JF, Parkin JL. Rhinologic applications of laser surgery. Otolaryngol Clin North Am. 1990;23(1):19-28.
13. Veit JA, Nordmann M, Dietz B, Sommer F, Lindemann J, Rotter N, Scheithauer MO. Three different turbinoplasty techniques combined with septoplasty: Prospective randomized trial. Laryngoscope. 2016;127(2):303-308. doi: 10.1002/lary.26264.
14. Beck JC. Pathology and intramural electrocoagulation of the inferior turbinate. Ann Otol Rhinol Laryngol. 1939;39:349-363.
15. Sommer F, Lindemann J, Scheithauer MO, Hoffmann TK, Veit JA. Nasenmuschelchirurgie [Nasal turbinate surgery]. HNO. 2017;65(5):443-456. doi: 10.1007/s00106-017-0349-5. German.
16. Lal D, Corey JP. Acoustic rhinometry and its uses in rhinology and diagnosis of nasal obstruction. Facial Plast Surg Clin North Am. 2004;12(4):397-405. doi: 10.1016/j.fsc.2004.04.002.
17. Nease C, Krempl GA. Radiofrequency treatment of turbinate hypertrophy: a randomized, blinded, placebo-controlled clinical trial. Otolaryngol Head Neck Surg. 2004;130(3):291-299. doi: 10.1016/j.otohns.2003.11.003.
18. Lenz H, Sicklinger A. Endonasal laser surgery: a 20 years experience report. Indications and procedure. Laser Med. 1997;13:120.
19. Tsilochristou O, Kyriakakou M, Manolaraki I, Lakoumentas J, Tiligada E, Maragkoudakis P, et al. Detection of local allergic rhinitis in children with chronic, difficult-to-treat, non-allergic rhinitis using multiple nasal provocation tests. Pediatr Allergy Immunol. 2019;30(3):296-304. doi: 10.1111/pai.13021.
20. Hurd LM. Bipolar electrode for electrocoagulation of the inferior turbinate. Arch Otolaryngol. 1931;13:442.

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Authors' contributions

EG developed the first manuscript; MM interpreted the data and critically revised the manuscript. PA interpreted the statistical data. LD reviewed the clinical data. All authors reviewed the material and approved the final version of the manuscript.

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Ethics approval and consent to participate

The study was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (protocol No 20.80009.8007.14 of 17.04.2021). Informed consent was obtained from all study participants.

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Smoking as a method of coping for medical workers

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Abstract

Background: This article analyzes the professional stress at healthcare professionals (HCP), related to their job. Health care providers can serve as role models in promoting healthy lifestyles; however, HCP commitment to help individuals to quit smoking may be influenced by their own smoking behavior.
Material and methods: In order to determine the level of perceptual stress, the Perceived Stress Questionnaire (PSQ) was applied, PSQ developed by Levenstein et al. In order to determine the level of addiction to smoking, the Fagerstorm nicotine addiction test was applied. The research was attended by 210 subjects from 5 medical institutions: doctors, nurses, and resident doctors. Confidentiality is maintained.
Results: The doctors accumulated 32% on the scale of severe dependence, 8% moderate dependence and only 9% mild dependence, after calculating the score and interpreting the data. The resident doctors have accumulated the maximum score on the scale of mild dependence, 7% moderate dependence and only 3% severe dependence, which speaks of the fact that they are the youngest category of medical workers at the beginning of their careers and still do not have good defense mechanisms. The nurses, who represent 9% of the entire group of subjects, accumulated 0% on the scale of severe dependence, 8% moderate dependence and 1% mild dependence.
Conclusions: Comparative data on smoking dependence levels in doctors of 32% according to the perceived stress level 71% confirm this. Stressed health workers are more likely to smoke, have different social problems and are males. The results of research have shown that the prevalence of smokers among health care professionals is high (51%), implying the ineffectiveness of the current government anti-tobacco strategy. Introducing new pillar – Harm Reduction in National Antismoking Action Plan, along with prevention, cessation and protection from second-hand smoking, can improve current smoking situation in general. It would be helpful to implement training programs about Tobacco Harm Reduction for healthcare workers to improve their ability in smoking cessation counselling techniques to provide active support to their patients.
Key words: stress, medical workers, smoking, tobacco harm reduction.

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Introduction

Smoking is the action of "breathing (something) in, inhaling tobacco smoke from a cigarette or pipe; to drink (tobacco), to stink" [1]. The term "smoking" appeared only at the end of the seventeenth century, and until then this action was called "dry drunkenness". The name "nicotine" comes from the name of the French ambassador to Portugal, Jean Nicot, who had received as a gift from some sailors to America some tobacco leaves. He later brought tobacco to the French royal court, from where it later spread throughout Europe [2].

Although everyone knows that smoking causes cardiovascular and respiratory diseases, even cancer, and legislative restrictions have been imposed to combat this addiction, smoking remains one of the daily actions of people on all continents. Globally, the number of smokers has exceeded 1 billion [3], and although in countries with a high standard of living it seems to be declining, globally this figure continues to rise. The worst side is that this habit is addictive, and trying to quit smoking is very difficult, and in most cases leads to withdrawal, the quite excruciating body's condition.

The word "tobacco" comes from the island of Tobago in the Antilles archipelago, where this plant was cultivated, being called "petum" by the natives. The Aztecs and Incas used tobacco mainly during religious ceremonies and it was used as an offering to the gods in purification rituals, which allowed priests or religious leaders to detach themselves from the clarity of mind in order to communicate with the divinity or induce spiritual visions. The tobacco also relieved their hunger and helped them cope better with fatigue [2].

At the beginning of its introduction in Europe, tobacco was a treat only for the nobility, later it began to spread among the needy. This is because smoking tools have started to evolve. If at first the tobacco was crumbled and inhaled or was chewed. Then the pipe was invented, then the cigar, and later the cigarettes. The first cigarette was made in the mid-1800s, and in 1881 the first cigarette maker appeared in

America. At the beginning of the twentieth century, when the industrial revolution reduced the cost of cigarettes, this habit became common among women. In the interwar period, large cigarette companies were hugely successful in manufacturing more than 300 billion cigarettes a year. By the middle of the twentieth century, half of Europe's adult population was already smokers and tobacco was conquering the planet.

The total number of smokers globally is on the rise. Currently nearly 1 billion people have this habit, according to a study quoted by bbc.co.uk. Although smoking is becoming less popular in many parts of the world, the total number of smokers is on the rise, according to a study conducted by the Institute for Health Metrics and Evaluation (IHME) at the University of Washington, United States, published in JAMA. In 2012, 967 million people smoked every day, compared to 721 million in 1980, according to data collected from 187 countries. Currently, approximately 3 out of 10 men (31%) and 1 out of 20 women (6%) smoke daily, compared to 4 out of 10 men (41%) and 1 out of 10 women (10%) in 1980 [4].

Smoking in the Republic of Moldova

In August 2011, the number of smokers worldwide was estimated at 1 billion [3]. By 2030, it is likely that 10 million people will die from smoking [2]. A turning point in the evolution of the tobacco industry is considered the entry into force on February 27, 2005 of the Framework Convention on Tobacco Control, the first mandatory comprehensive global health treaty [5]. The main provisions of the Convention concern protection against exposure to tobacco smoke; labeling; advertising; liability; illicit trade; rules on tobacco products and taxation. In 2007, Moldova also ratified this Convention, committing itself to comply with the recommendations on smoking cessation [6].

According to a 2010 global survey conducted by the World Health Organization, about 600000 people die each year from passive smoke, a third of who are children who are exposed to cigarette smoke at home [7]. The passive smoking can also cause hearing loss. The World Health Organization declared May 31 "World No Tobacco Day" in 1987, precisely to make smokers aware of the dangers of using tobacco, not only for themselves but also for the society in which they live. The smoking is the most important risk factor for chronic obstructive pulmonary disease.

The study on smoking, conducted by Magenta Consulting on a sample of 1505 respondents, highlighted the fact that our smokers are addicted.

Thus, a smoker from Moldova:

- Smokes on average 17.9 cigarettes per day (67% increase by 12 persentage points compared to 2012) – almost a pack.
- Knows about the harm of smoking, but does not worry that smoking will affect his health in the future.
- Is more reluctant to ban smoking in public places (addiction defense position).

21% of survey respondents smoke daily, and 1% smoke less than daily. Interestingly, by analyzing the socio-demographic profile of the smoker and the non-smoker, Magenta Consulting researchers found that smokers read less often, work more often outdoors, have fewer children and are exposed to cigarette smoke at home and at work to a greater extent [8]. The specialists say that within the stationary IMSP Republican Narcology Dispensary, during 2018, over 460 people received professional counseling to quit smoking, and 305 people received drug treatment for tobacco addiction. More than 3000 smokers received counseling assistance on smoking cessation provided by narcologists from territorial health institutions. Also, the general practitioners provided primary counseling assistance on smoking cessation to more than 156000 smokers. In 2018 alone, out of 500 tobacco addicts who turned to help, 305 quit smoking.

The data of a study conducted by WHO in Moldova show that about 25% of the population of our republic smoke, hence the fact that almost 44% are men and 5.6% - women. The data also show that Moldovans start smoking at the average age of less than 18 years [9].

In 2020, this campaign aimed to make young people aware of how dangerous tobacco products are and to prevent the tobacco industry from handling tobacco products. The experts warn that more and more young people smoke hookah, considering it less dangerous, or use menthol cigarettes to mask the use of cigarettes in front of parents, and nicotine from using e-cigarettes is no less dangerous for children's health, it also leads to addiction later. Flavored cigarettes only increase the number of children using tobacco, so that 81% of young people who have ever used tobacco products started using a flavored product. In this context, in the Republic of Moldova, from May 20, 2020, it is forbidden to place on the market tobacco products with a characteristic menthol flavor. In 2020, along with Armenia, Austria, Finland, Ireland and the United Kingdom, the Republic of Moldova was designated by the World Health Organization as one of the best implementers of tobacco control policies [10].

In 2020, the Deputy Director of the National Agency for Public Health, Ion Salaru, claimed that "smoking is one of the worst public health problems in the Republic of Moldova, because every second smoker dies of a disease caused by this addiction, being fit for work". The consumption of any tobacco product is a major risk factor affecting over 30% of cancer deaths, 90% of lung cancer deaths, 25% of coronary heart disease deaths, 85% of chronic obstructive pulmonary disease deaths and 25% of deaths from cerebrovascular disease. Also, smoking severely affects pregnant women, causing premature births and low birth weight, and increases the risk of sudden infant death syndrome in newborns. As a result, out of 38000-39000 people who die annually, over 4500-4700 (about 14%) die from diseases caused by tobacco use, including over 60% of people of working age, the mortality among men being 2 times higher than women. Since August 2020, in order to reduce the advertising and promotion of tobacco products, the visible display of tobacco

products and related products, devices and accessories for their use, recharging or heating in commercial spaces accessible to the public was prohibited. The provisions of Article 25(5) of Law 278/2007 took effect from August 15, 2020 in order to reduce their promotion among young people. The law was on tobacco control concerning the prohibition of visible exposure of tobacco and related products, devices and accessories for their use, recharging or heating in commercial spaces accessible to the public. Between August 17 and September 25, 2020, several unannounced checks were organized, establishing that 90% of the audited economic agents complied, and sanctions were applied until they fully complied with the legal provisions [11].

Stressors and explanatory-interpretative models of organizational stress

According to the concept of the Canadian scientist Hans Selye [12], launched in 1936, ***stress is the non-specific reaction of the body to any request,*** a concept that quickly became established in the international language. Thus, this notion is quickly imposed in everyday circulation. At the same time, it refers to the real and the imaginary stress, which must be well differentiated.

The dictionary of social psychology defines the term **psychic stress** as a state of tension, straining and discomfort, caused by emotional agents, with negative significance, frustration or depression of states of motivation (needs, desires, and aspirations), difficulty or the impossibility of solving some problems [13].

In the current conditions of life, we have another form of stress that occupies a leading place in these classifications, namely **organizational stress**, or **stress at work**. It is the most harmful result, having a negative impact, but also a positive one, both physically and mentally. At the base of the organizational stress, there are 2 stressors of a psychological nature:

1. Role conflict (when a person is subject to requests between the manager and the co-workers)
2. Ambiguity of the role (when a person does not have enough information to perform their function)

The workplace stressors include:

1. Stressors at executive and managerial level (role overload, very high responsability towards employees)
2. Operational stressors (unpleasant or even dangerous working conditions)
3. General stress factors (interpersonal conflicts, intergroup quarrels)

The effects of stress in general could be grouped into 5 main categories :

- **Subjective effects**: anxiety, aggression, indifference, fatigue, malaise, decreased self-confidence, nervousness, feelings of loneliness;
- **Behavioral effects**: impulsive behavior, emotional disorders, predisposition to mistakes, alcohol and coffee abuse, tendency to eat and /or smoke excessively;
- **Cognitive effects**: decreased ability to make rational decisions, lack of concentration, decreased attention, hypersensitivity to criticism, mental blockages;
- **Psysiological effects**: increase in pulse, blood pressure, blood sugar, dry mouth, cold sweats;
- **Organizational effects**: low efficiency, isolation, lack of job satisfaction, absenteeism, reduced responsibility, decreased loyalty to the organization, resignations.

The most common effects of stress felt on the employee and the organisation:

1. The fatigue distinctive for the organizational stress:
   a) Alarm phase (iritability, susceptibility...),
   b) Agitation phase (lack of trust in others, a kind of malice towards others...),
   c) Exhaustion phase (depression, isolation...).
2. Overworking (as a form of chronic fatigue).
3. Fluctuation of staff.
4. Alcoholism.

Smoking as a strategy of overcoming the occupational stress of the medical staff

Smoking is considered by smokers as a method of combating stress, thereby adding another excuse to quit smoking. This habit can also reduce stress. The ensemble of studies to date suggest that under certain conditions nicotine can act as an anxiolytic and an antidepressant, but that following chronic use, adaptations to nicotine can occur resulting in increased anxiety and depression following withdrawal [14].

Nicotine effects on mental state may vary. First, by causing the release of glucose from the liver and adrenaline (epinephrine) from the adrenal medulla, it induces excitation. From a subjective point of view, reported by smokers, this manifests itself in relaxation, calmness and liveliness. A mildly euphoric state has also been reported, due to endorfin euphoria. Decreased appetite and increased metabolism can result in weight loss in some smokers [15].

Scientists from the University of New York have found that nicotine compensates for brain defects in schizophrenia. It is no coincidence that 88% of Americans with this disease smoke, and a lot [16].

Despite the addiction of nicotine, it still has a positive property. But such it is not for mentally healthy people, but for people suffering from schizophrenia – nicotine increases their mental activity. Following such a report from researchers in the United States, the medical community released a drug with an effect similar to nicotine to avoid the health damage from smoking [17].

There are doctors among the smokers. Why? They are humans too. The main reasons for smoking in the present study were relaxation and fighting stress, and the cultural misconception of tobacco as a "socializer or helper" under some psychologically stressful conditions is indicated. The latter indicates the influence of cultural and environmental factors as well as personal or individual handicaps [18].

The doctors, the people who are best informed about the harm of smoking, are no exception to this habit. They also smoke, because here prevails the ordinary man, not the doctor. The doctors still believe that smoking a cigarette can relieve you of stress at work, as they are always in contact with different people. A doctor, when asked why he smokes,

will always say that he does it by habit, that he is very nervous, that he relaxes, and, of course, that it is a habit that will not last long and he can give it up whenever he wants. Also, he will be the one who will not recommend smoking to other people, saying that this addiction brings enormous damage to health. A doctor will always ask his patient if he smokes to find out the cause of the disease, or if smoking has worsened the patient's health, and will ask him to give up this habit, listing all the negative effects of harmful substances from the smoke to the body. But, then why does a doctor smoke? The doctors' attitude towards smoking does not differ significantly from the position of ordinary people despite the colossal baggage of knowledge, experience and work practice. The most interesting thing is that just doctors who smoke could answer the questions which are the pathologies tobacco smoking could lead to. According to some data, the doctors spend a lot of time to initiate measures to combat smoking, but they will still smoke. The average age of initiation of smoking among the doctors is 21 years. Thus, many of them start smoking since adolescence or after they enter the university [18].

So why the doctors smoke, according to their opinion:

- The medicine occupies leading positions if we classify professions according to their complexity;
- The mental and emotional stress if you have to prescribe treatments to people and are afraid of making mistakes;
- The increased flow of patients lately with a multitude of difficult situations;
- The abundance of documents to be completed, instead of actual work;
- In order to calm down after realization of professional duty (e.g. difficult operation);
- For the ride, socialization, so that you do not distinguish yourself from friends/colleagues/superiors who smoke;
- The negative influence of the family in which the closest relatives smoke;
- The desire to try smoking;
- The pleasure from smoking;
- The stress during working hours;
- The recreation.

Although there are many ways to combat stress at work and find peace of mind, such as walking to work, reading books in your free time, listening to music, they are not taken into account. As an excuse, it is called lack of time, while smoking a single cigarette for 2-3 minutes calms your nerves and calms you down. Like any person, the doctor will start to feel stressed shortly after smoking a cigarette but will always find other excuses. Although doctors know best the negative effects of smoking to the body by detecting pathologies associated with tobacco use, they smoke.

Many of them start smoking since university, because the professor-doctor smokes, his medical colleagues smoke, because many medical professionals smoke and they are absolutely healthy, because they want to feel equal in front of them. Many of them, like other people in different fields, think that they can quit smoking at any time, which unfortunately does not happen, because they have also formed a habit of smoking during a short break, after breakfast, or in a stressful situation. Of course, medical activity is associated with great psycho-emotional stress due to daily communication with a large number of patients, each coming not only with his health problems, but also with his negative load sometimes, the abundance of documents, clinical situations that require much physical and emotional effort, and in the process of treatment there are also negative patient-doctor relationships due to aggression, or even hatred from some patients, or even colleagues, situations that increase the level of stress, and it leads to smoking. All this leads in time to the **burnout syndrome**, from where later doubts can arise regarding the correctness of choosing the profession, and some of them see tobacco use as a quick treatment [19].

However, why do some doctors smoke and others do not? Because some people also have an unconscious feeling of superiority over their colleagues, with the illusion that they can quit smoking at any time, that they have complete control over their health, but they forget that from a biological point of view, he is the same person as the others, and nicotine has the same euphoric effects on everyone. The doctors oppose smoking, because the highest mortality rate is noted among them, however they do not stop smoking themselves: at the entrance to the hospital, dental clinics, etc. By trusting people in white robes, a false trust is formed in the benefits that tobacco can bring to the body. In order to prevent smoking, in China, for example, smoking doctors are deprived of the right to practice medicine. The doctors' recommendations regarding smoking cessation are viewed with reluctance, because those who are so much against smoking after the job or even during job smoke. And the worst thing is that among the doctors who smoke there are not only men, but also women [20].

And because doctors are humans, and they understand very well the harm that tobacco and nicotine do to the body, they should also fight this addiction through the same methods they recommend to their patients: a balanced lifestyle characterized by physical activity, intake of nutrients from fruits and vegetables, social interactions beneficial to the spirit, positive attitude towards oneself and those close to them, proper nutrition, ensuring 8 hours of peaceful sleep every night, combating stress through methods other than smoking.

Nicotine is not the cause of the disease, combustion is

Nicotine is found naturally in the tobacco plant. Once inhaled, the substance is absorbed through the lungs directly into the bloodstream, from where it is transported through the body to tissues and organs, including the brain. Here, the nicotine binds to certain receptors and triggers the release of dopamine and other neurotransmitters. Thus, the nicotine can stimulate but also affect short-term brain functions, such as emotions, learning ability and memory. Finally, prolonged exposure to nicotine increases the body's

tolerance to this substance and becomes addictive to the point where smoking cessation becomes extremely difficult to achieve.

Nicotine is addictive. According to a report by the Royal College of Physicians [21], nicotine increases heart rate and blood pressure and has a number of local irritating effects, but is not carcinogenic. There are three deadly conditions associated with smoking – lung cancer, which results mainly from direct exposure of the lungs to carcinogens in cigarette smoke, chronic obstructive pulmonary disease caused by irritating and inflammatory effects of smoke, and cardiovascular diseases, which are caused by the effects of smoke on vascular clotting and walls of blood vessels. None are caused primarily by nicotine.

In practical terms, as Mike Russell argued in the 1970s, "smokers smoke for nicotine, but are killed by tar" [22]. And the health effects of this tar are quite serious:

Very short-term negative effects. A few minutes after the first smoke:

- Heart rate accelerates;
- Laryngeal tissue becomes irritated;
- Carbon monoxid enters the blood;
- Carcinogens enter the lungs immediately;
- Air is polluted;
- Eyes may be irritated;
- Skin temperature drops.

Medium and long-term effects:

- Smoking is responsible for 80-90% of chronic bronchopneumopathy;
- Smoking is responsible for 85% of bronchopulmonary cancers;
- Smoking is responsible for 30% of deaths from cardiovascular disease;
- Carbon monoxide in cigarette smoke increases the level of cholesterol in the blood, which eventually leads to its deposition on the walls of blood vessels;
- Smoking increases the risk of cancer of the lips, tongue, salivary glands, mouth, larynx, esophagus and pharynx;
- Gastric cancer is alo associated with smoking;
- Smoking represents a risk factor, for bladder, kidney, cervical and pancreatic cancer;
- There is a strong addictive relationship between smoking and leukemia;
- In women who smoke, menopause sets in on average 5 years earlier than in non-smokers;
- Smoking can cause hormonal dysfunctions in reproductive system;
- Risk of osteoporsis is increased in women who smoke;
- Sperm mobility is slow in men who smoke.

Although many people remain relentless when it comes to quitting smoking, there are also among those who every morning when they wake up aim to quit this bad habit. Over the years, authorities in several countries have proposed measures to combat tobacco use, to reduce the number of smokers, by banning smoking in public places, printing slogans about the damage of nicotine to health even on cigarette packs, etc. Because, the number of smokers is still growing, they feel compelled to find alternative methods to regular cigarettes, especially since it has been shown that not so much nicotine is harmful to health as the process of burning tobacco. Thus, among the anti-smoking strategies, a series of nicotine-based products (patch, chewing gum, snus, electronic cigarettes, tobacco heaters) are proposed for those who have not been able to quit smoking permanently. Of course, complete smoking cessation would be the best alternative, because only that would eliminate the negative effects of smoking on health [23]. Hence, the dilemma of doctors is what to propose to humanity: quit smoking or alternative methods.

For example, some people smoke, being convinced that this habit relieves them of stress. Although, this is only a momentary effect, just as a simple cigarette does not drive away the problems we face, but, on the contrary, creates other health problems. Jean Gehricke [24], from the University of California has shown that nicotine does have calming effects on angry people, and they tend to smoke even more, becoming addicted to smoking. Others smoke because they have tried the effect of nicotine on the body in adolescence, and later became addicted.

Material and methods

At the first stage, the Perceived Stress Questionnaire (PSQ), developed by Levenstein and his collaborators [25], was applied as a research tool and aims to determine the level of perceived stress. The test contains 30 items, and the subjects were asked to read each item in turn, awarding the corresponding score by choosing a single answer for each item: almost never – 1 point, sometimes – 2 points, often – 3 points, usually – 4 points. For questions number 1, 7, 10, 13, 17, 21, 25, 29 – the score is reversed: almost never – 4 points, sometimes – 3 points, often – 2 points, usually – 1 point. The score between 30 and 120 allows falling into one of the 3 categories: 30-59 – low stress level; 60-89 – moderate stress level; 90-120 – high stress level. There is no time limit for this test.

The sample was formed of 210 medical workers aged between 20 and 61 years. The medical workers were divided into 3 categories: doctors, resident doctors and nurses.

Analysing the data in the table above, we see that resident doctors have obtained the highest percentage of intense stress, which speaks of the fact that they are still at the beginning of their career, showing some anxiety about the decisions made, not yet having well-formed coping mechanisms. The nurses follow with 86%, which speaks about the emotional and physical load they face, direct contact with the patient and the large volume of work. Doctors accumulated 71%, a lower percentage, which can be explained by the fact that they have a higher level of resilience due to work experience and seniority (fig. 1).

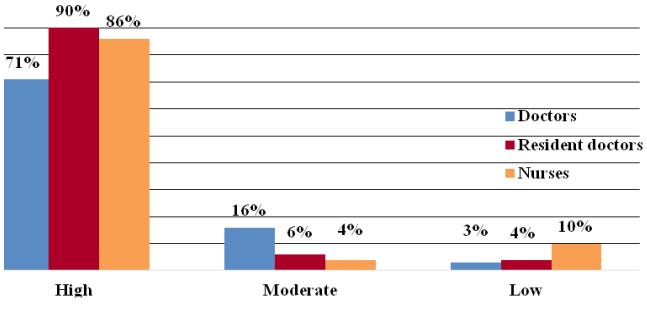

Fig. 1. Distribution of the total score for stress perception levels in medical workers

At the second stage, the Fagerström Test for Nicotine Dependence was applied. The purpose of the test is to determine the level of nicotine dependence. The test was applied only to smokers (108 subjects). It contains 6 questions, each question having three answer options. Subjects were asked to choose only one of the three options. The score and interpretation were calculated according to the scale: 7-10 points – high dependence, 4-6 points – moderate dependence, 0-3 points – low dependence. The subjects had no time limit, and confidentiality was maintained (tab. 1).

Table 1. Smoking dependence according to the categories of medical workers

Category of medical workers	Total number	High dependence	Moderate dependence	Low dependence
Doctors	55	32%	8%	9%
Resident doctors	44	3%	7%	32%
Nurses	9	0%	8%	1%

Following the results obtained, we can say that people with smoking dependence do not do this for pleasure but in order to avoid the discomfort caused by resistance, and medical workers start smoking more intensely when they accumulate internal tension (caused by a complicated intervention or when they had a busier working day, not to mention medical errors (malpractice) accompanied by remorse and guilt complexes (fig. 2).

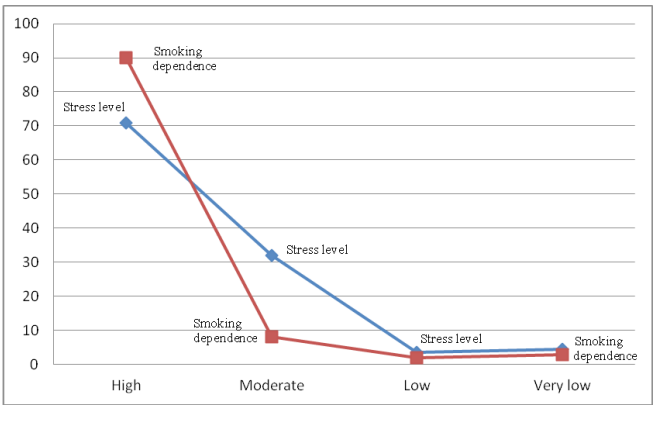

Fig. 2. Comparative data on the level of smoking dependence according to the level of perceived stress

It goes without saying that in the face of such a threat, any medical professional creates his/her own defence mechanisms (increasing the number of coffee drinks, alcohol consumption, sedatives, the number of cigarettes smoked) which are harmful forms of coping with stress they face.

Alternative smoking cessation solutions include:

- Electronic cigarettes;
- Chewing gum with nicotine;
- Nicotine patch;
- Nicotine nasal sprays;
- Snus;
- Tabacco heaters;
- Behavioral counselling;
- Financial rewards for quitting smoking.

Electronic cigarette – is a product designed to work with nicotine or other substances in the form of vapors. Its role is to mimic the experience of real smoking, in conditions less harmful to health. In many cases it is also used to quit smoking. It can overcome withdrawal symptoms when someone tries to quit smoking. In addition, e-cigarette users can choose between low-nicotine cartridges or even nicotine-free liquids [26].

Tobacco Heating System – or THS heats a specially formulated tobacco material using an electronically controlled heater to temperatures below that necessary to initiate combustion. The controlled heating of the tobacco material vaporizes water, glycerol (an aerosol former added to the tobacco material), nicotine an aerosol that is not smoke, and that is fundamentally different in origin, chemical and physical composition to cigarette smoke. As the tobacco material in the Tobacco Stick is heated and not burned, the aerosol generated contains on average 90 to 95% lower levels of harmful and potentially harmful constituents compared to the mainstream smoke of a standard cigarette [27].

Nicotine gum is used in the treatment of nicotine addiction by satisfying the exaggerated demand for nicotine and the symptoms of smoking cessation. [36]. Nicotine withdrawal occurs when the body no longer receives nicotine from tobacco. By administering nicotine gum, the body receives a low dose of nicotine, and decreases the risk of withdrawal symptoms, helping to quit smoking more easily. This gum contains 2/4 mg/pc of nicotine, compared to 6-8 mg compared to a cigarette. Nicotine is absorbed more slowly when chewed (90% in 20-30 minutes). It is recommended to use for 3-6 months. After a year of treatment, combined with other methods of therapy, 30% managed to quit smoking. Without psychotherapy and medical advice, success is low [28].

Nicotine patch is a patch that sticks to the skin, which releases nicotine into the body through it. It is used in nicotine replacement therapy (NRT) and is considered along with nicotine chewing gum one of the safest NRTs available for the treatment of tobacco use disorders. It is usually worn for 16-24 hours and can be removed during sleep. Research has shown that NRT in combination with cognitive behavioral therapy can improve smoking quitting rates in pregnant women. Nicotine patches are also being studied to help relieve the symptoms of postoperative pain and to treat early

dementia, anxiety, depression and inattention in subjects with attention deficit hyperactivity disorder [28].

Nicotine nasal spray – acts on the need to smoke in 60 seconds. It is used to help a person when he wants. This is a type of treatment called Nicotine Replacement Therapy (NRT). By using this spray, the human body continuously receives a small amount of nicotine for a short period of time. It is less harmful than smoking. This is because it does not contain tar, carbon monoxide or other toxins from cigarette smoke [28].

Snus – is a type of smokeless tobacco product. It is a crushed wet tobacco, which is placed between the upper lip (less often – lower) and the gum for a long time – from 30 to 60-70 minutes (according to the manufacturers, from 5 to 30 minutes). In this case, nicotine from the tobacco enters the body. Snus belongs to smokeless tobacco (this group also includes dry and wet snuff, chewing tobacco, etc.). It is characterized as a type of wet snuff with high nicotine content and a low content of carcinogens. It has been known in Sweden since 1637. It is produced and consumed mainly in this country (which is why it is often called Swedish snus). The EU (with the exception of Sweden) has banned the sale of snus since 1992, although its use is not restricted. Snus is legally sold in Sweden, Hungary, Denmark and Norway [29].

Behavioral counseling – permanent smoking cessation becomes more productive when you have someone close to whom you can talk and who can support you. These people include professionals from among doctors, psychologists, people who have already given up smoking, relatives [30].

Financial rewards for quitting smoking – when a person receives a sum of money just to quit smoking, he also receives psychological satisfaction. This reward can consist of cash or vouchers, offered by the managers of the institutions in which the person operates, if this institution aims to combat this vice, or even by the government, as a method of combating tobacco.

Although more and more people are trying to quit smoking, every second smoker returns to the old habit in 3-7 days, claiming that he has not found a good reason to quit smoking, does not have the will or feels very stressed because of smoking quitting. Sudden smoking quitting is quite difficult, and about 95% of those who try to quit smoking once and for all alone, without the help of a doctor's conciliation, soon return to the old habit [26].

Discussion

Veronica Gasca conducted a similar study on a sample of 61 people, represented by medical workers from the Chisinau Municipal Public Health Centre and the National Public Health Centre. The applied questionnaire included several groups of symptoms. The intellectual symptoms caused by stress specified by medical workers referred to making difficult decisions (27.9%), often being distracted (26.2%) and concentrating around a single problem (24.6%). Among the behavioural symptoms caused by stress, the respondents more frequently highlighted the chronic lack of time (42.6%) and little time of communication with family and friends (26.2%). A share of 21.3% of respondents felt increased distress and anxiety, which refers to emotional symptoms. Among the physiological symptoms, more frequently the medical workers felt fatigue (42.6%), had problems from the circulatory system (27.9%) and headaches or aches in different parts of the body with indeterminate character (26.2%). An impressive number of health workers is affected by occupational stress, which has adverse health consequences [31].

Occupational stress ranks second in the hierarchy of occupational health problems in EU countries, after osteomuscular disorders. A pilot study of the European Agency for Safety and Health at Work, conducted in 2001, shows that over 25% of Western Europeans working in healthcare field have stress problems. And among them, the most affected are doctors in the primary care sector. He/she is most often required by situations to act effectively. The family doctor bears, like everyone else, the stressors existing in society.

Smoking is one of the oldest habits in the world and one of the strongest factors that leads to serious consequences of illness. Trying to prevent or stop smoking at an early stage is much easier than when it becomes addictive. Many people who are addicted to smoking do so out of pleasure but to avoid the discomfort caused by resistance.

Smoking kills one-third to one-half of the total number of smokers each year, which is the leading cause of illness and mortality in the world. One problem is that smoking is also widespread among medical workers who start smoking more intensely when they accumulate internal tension (caused by a complicated intervention or when they have had a busier work day, not to mention the medical errors (malpractice) accompanied of remorse and guilt complexes).

It goes without saying that in the face of such a threat, any medical professional creates his own defense mechanisms (increasing the number of coffee drunk, alcohol consumption, sedatives, the number of cigarettes smoked) which are harmful ways to deal with in the front of stress.

Health care professionals are familiar with the harmful effects of smoking and passive smoking. But in fact they usually smoke in front of their patients and kids [32]. Almost all of the examinees (198) agree with the claim that the medical health professionals have an ethical responsibility to warn pregnant women who smoke of the potentially harmful effects smoking can have on the fetus during pregnancy. Those are results similar to other studies where physicians showed willingness for a conversation with a pregnant woman about adverse effects of smoking and advising about cessation [33].

The great number of physicians completely agrees that healthcare professionals have an ethical responsibility to warn smokers of the harmful effects of smoking. However, health care professionals who smoke feel discomfort in situations when they are supposed to counsel their patients about smoking cessation and the harmfulness of the tobacco smoke [34].

They have difficulties in creating transfer/countertransference relationship with patients in the smoking prevention [35]. Problems can also be caused by the lack of motivation of the health care professionals due to previous negative

experiences when patients didn't accept the advice given by them regarding the use of tobacco products for cessation.

To prevent such issues in the future and minimize negative experience, healthcare workers should be informed about Tobacco Harm Reduction (THR) concept.

THR is a public health strategy to lower the health risks to individuals and wider society associated with using tobacco products. It is an example of the concept of harm reduction, a strategy for dealing with the use of drugs, and reducing smoking is vital to public health.

Clearly then, traditional tobacco control interventions elaborated in the WHO Framework Convention on Tobacco Control (FCTC) are not enough. Tobacco harm reduction (THR) policies therefore should be regarded as complementary rather than inimical to reducing the global death and disease from smoking [36].

The glimmer of hope is that some countries have taken a more inclusive approach to THR as part of the overall strategy towards a smoke-free world.

Recent published data from the Global Burden of Disease Study – Age-standardised prevalence of current smoking tobacco use in 2019 and percentage change in age-standardised prevalence of current smoking tobacco use in 1990–2019 among individuals aged 15 years [36] reveal that the largest decreases in age-standardised prevalence of smoking tobacco use were observed in Brazil – 73.4%, Norway – 53.5%, Denmark – 49.3%, Iceland – 49.7%, Japan – 32.6%.

All these countries are members of WHO FCTC and succefuly implemented all necessery tobacco control strategies (increasing taxation, display ban, public places smoking restrictions and etc) but not only. At the same time they accepted THR concept and introduced it in legislation in different way:

Brasil – e-cigarettes are allowed as smoking cessation devices. In Norway and Denmark – traditionally use snus. In 2018, the Norwegian Public Health Institute issued a report evaluating harm reduction as a strategic element of tobacco control policy and assessing the pros and cons of the availability of less risky nicotine-containing alternatives compared to cigarettes. The institute concluded that preventing access to less risky nicotine-containing products can paradoxically entrench the position of the most hazardous product, i.e., cigarettes [37].

Iceland – a study from Iceland's Directorate of Health shows that smoking in Iceland is on the decline, with evidence that the use of e-cigarettes is contributing to a decrease in the consumption of traditional cigarettes. The study indicates that last year daily smoking fell to 9 percent of population, a drop of 5 percent in three years, while daily e-cigarettes use reached 4 percent.

Japan – Cummings et al. also reported that from 2011 to 2016, sales of cigarettes declined by about 2% to 4% annually, while a decline of 13% in cigarette sales occurred in 2017, 12% in 2018, and 9% in 2019, indicating a much more significant decline over this period. Between 2015 and 2019, the period when THS was introduced in the market, total cigarette sales dropped by 34%. It is likely that the introduction of HTPs in Japan has caused a significant decline in cigarette sales [38].

The best thing that adult smokers can do to improve their health is to quit tobacco and nicotine use altogether. For smokers who don't quit, the health impacts of cigarette smoke can be greatly reduced if they switch completely to a less harmful alternative and stop smoking.

Conclusions

The results of this study reveal that smoking dependence is directly related to the level of perceived stress. Comparative data on smoking dependence levels in doctors of 32% according to the perceived stress level 71% confirm this. Stressed health workers are more likely to smoke, have different social problems and are males. Physicians and nurses, as a part of the health care system, play an equally important role in the prevention and education of patients about the harmfulness of smoking. The results of research have shown that the prevalence of smokers among health care professionals is high (51%), implying the ineffectiveness of the current government anti-tobacco strategy. Health care professionals, who should inform others about the harmfulness of the tobacco smoke, are not fully aware to what extent smoking is harmful both to them and to people who surround them. Introducing new pillar – Harm Reduction in National Antismoking Action Plan, along with prevention, cessation and protection from second-hand smoking, can improve current smoking situation in general. It would be helpful to implement training programs about Tobacco Harm Reduction for healthcare workers to improve their ability in smoking cessation counselling techniques to provide active support to their patients. Regulatory bodies should give medical workers possibility to develop healthy coping strategies to prevent and overcome occupational stress such as: an extensive process of introspection; examining the lifestyle, one's own motivations, priorities; development of strategies for stress control; application of time management strategies; use of systematic physical training for effort capacity.

References

1. Doll R. Uncovering the effects of smoking: historical perspective. Stat Methods Med Res. 1998;7(2):87-117. doi: 10.1177/096228029800700202.
2. Charlton A. Medicinal uses of tobacco in history. J R Soc Med. 2004 Jun;97(6):292-6. doi: 10.1258/jrsm.97.6.292.
3. Mishra A, Chaturvedi P, Datta S, Sinukumar S, Joshi P, Garg A. Harmful effects of nicotine. Indian J Med Paediatr Oncol. 2015;36(1):24-31. doi: 10.4103/0971-5851.151771.
4. Ng M, Freeman MK, Fleming TD, et al. Smoking Prevalence and Cigarette Consumption in 187 Countries, 1980-2012. JAMA. 2014;311(2):183-192. doi: 10.1001/jama.2013.284692.
5. Zhou SY, Liberman JD, Ricafort E. The impact of the WHO Framework Convention on Tobacco Control in defending legal challenges to tobacco control measures. Tob Control. 2019;28(Suppl 2):s113-s118. doi: 10.1136/tobaccocontrol-2018-054329.
6. The Republic of Moldova, The Parliament. Legea nr. 124 din 11.05.2007 pentru ratificarea Convenției-cadru a Organizației Mondiale a Sănătății privind controlul tutunului [LAW No 124 of 11.05.2007 for the ratification of the Framework Convention of the World Health Organization on tobacco control]. Monitorul Oficial al Republicii Moldova. 2007;(78-81):art. 362. Romanian.

7. Oberg M, Jaakkola MS, Woodward A, Peruga A, Prüss-Ustün A. Worldwide burden of disease from exposure to second-hand smoke: a retrospective analysis of data from 192 countries. Lancet. 2011 Jan 8;377(9760):139-46. doi: 10.1016/S0140-6736(10)61388-8.
8. Magenta Consulting. Cercetare Magenta: Fumătorii fumează și mai mult, iar numărul nefumătorilor crește [Magenta Research: Smokers smoke even more, and the number of non-smokers increases] [Internet]. Chisinau; 2014 [cited 2021 Apr 9]. Available from: https://consulting.md/rom/statistici-si-publicatii/cercetare-magenta-fumatorii-fumeaza-si-mai-mult-iar-numarul-nefumatorilor-creste. Romanian.
9. World Health Organization. Tobacco Control Fact Sheet: The Republic of Moldova. Geneva: WHO; 2016.
10. Ministry of Health, Labour and Social Protection of the Republic of Moldova. Încurajăm pe toți să educăm și să creăm o generație fără tutun [We encourage everyone to educate and create a tobacco-free generation] [Internet]. Chisinau: The Ministry; 2020 [cited 2021 Apr 9]. Available from: https://msmps.gov.md/comunicare/incurajam-pe-toti-sa-educam-si-sa-cream-o-generatie-fara-tutun/. Romanian.
11. Salaru I. Fiecare al doilea fumător moare de o boală provocată de acest viciu [Every second smoker dies from a disease caused by this addiction] [Internet]. Chisinau; 2021 [cited 2021 Jun 3]. Available from: http://amtbuiucani.md/2021/06/02/ion-salaru-fiecare-al-doilea-fumator-moare-de-o-boala-provocata-de-acest-viciu/. Romanian.
12. Tan SY, Yip A. Hans Selye (1907-1982): founder of the stress theory. Singapore Med J. 2018;59(4):170-171. doi: 10.11622/smedj.2018043.
13. Monroe SM, Cummins LF. Stress: psychological perspectives. In: Wright JD, editor. International Encyclopedia of the Social and Behavioral Sciences. 2nd ed. Oxford: Elsevier; 2015. p. 583-587. https://doi.org/10.1016/B978-0-08-097086-8.25038-1.
14. Picciotto MR, Brunzell DH, Caldarone BJ. Effect of nicotine and nicotinic receptors on anxiety and depression. Neuroreport: 2002 July 2;13(9):1097-1106. doi: 10.1097/00001756-200207020-00006.
15. Jiloha RC. Biological basis of tobacco addiction: Implications for smoking-cessation treatment. Indian J Psychiatry. 2010;52(4):301-307. doi: 10.4103/0019-5545.74303.
16. Lucatch AM, Lowe DJE, Clark RC, Kozak K, George TP. Neurobiological determinants of tobacco smoking in schizophrenia. Front Psychiatry. 2018;9:672. doi: 10.3389/fpsyt.2018.00672.
17. Kutlu MG, Parikh V, Gould TJ. Nicotine addiction and psychiatric disorders. Int Rev Neurobiol. 2015;124:171-208. doi: 10.1016/bs.irn.2015.08.004.
18. Mahfouz AA, Shatoor AS, Al-Ghamdi BR, Hassanein MA, Nahar S, Farheen A, Gaballah II, Mohamed A, Rabie FM. Tobacco use among health care workers in Southwestern Saudi Arabia. BioMed Res Int. 2013;2013:960292. https://doi.org/10.1155/2013/960292.
19. Vogt F, Hall S, Marteau TM. General practitioners' and family physicians' negative beliefs and attitudes towards discussing smoking cessation with patients: a systematic review. Addiction. 2005 Oct;100(10):1423-31. doi: 10.1111/j.1360-0443.2005.01221.x.20.
20. Pinget C, Martin E, Wasserfallen JB, Humair JP, Cornuz J. Cost-effectiveness analysis of a European primary-care physician training in smoking cessation counseling. Eur J Cardiovasc Prev Rehabil. 2007 Jun;14(3):451-5. doi: 10.1097/HJR.0b013e32804955a0.
21. Royal College of Physicians. Nicotine without smoke: Tobacco harm reduction. London: RCP; 2016. 191 p.
22. Fiore M, Baker T. Reduced-nicotine cigarettes – promising regulatory pathway. N Engl J Med. 2015;373(14):1289-1291. doi: 10.1056/NEJMp1509510.
23. Baumeister RF. Addiction, cigarette smoking, and voluntary control of action: Do cigarette smokers lose their free will? Addict Behav Rep. 2017;5:67-84. doi: 10.1016/j.abrep.2017.01.003.
24. Gehricke JG, Potkin SG, Leslie FM, Loughlin SE, Whalen CK, Jamner LD, Mbogori J, Fallon JH. Nicotine-induced brain metabolism associated with anger provocation. Behav Brain Funct. 2009;5:19. doi: 10.1186/1744-9081-5-19.
25. Fliege H, Rose M, Arck P, Walter OB, Kocalevent RD, Weber C, Klapp BF. The Perceived Stress Questionnaire (PSQ) reconsidered: validation and reference values from different clinical and healthy adult samples. Psychosom Med. 2005 Jan-Feb;67(1):78-88. doi: 10.1097/01.psy.0000151491.80178.78.
26. Rahman MA, Hann N, Wilson A, Mnatzaganian G, Worrall-Carter L. E-cigarettes and smoking cessation: evidence from a systematic review and meta-analysis. PLoS One. 2015;10(3):e0122544. doi: 10.1371/journal.pone.0122544.
27. Jaccard G, Tafin Djoko D, Moennikes O, Jeannet C, Kondylis A, Belushkin M. Comparative assessment of HPHC yields in the Tobacco Heating System THS2.2 and commercial cigarettes. Regul Toxicol Pharmacol. 2017 Nov;90:1-8. doi: 10.1016/j.yrtph.2017.08.006.
28. Balbani AP, Montovani JC. Methods for smoking cessation and treatment of nicotine dependence. Braz J Otorhinolaryngol. 2005;71(6):820-826. https://doi.org/10.1016/S1808-8694(15)31254-4.
29. Clarke E, Thompson K, Weaver S, et al. Snus: a compelling harm reduction alternative to cigarettes. Harm Reduct J. 2019;16(1):62. https://doi.org/10.1186/s12954-019-0335-1.
30. Hosford RE. Behavioral counseling – a contemporary overview. Counseling Psychologist. 1969;1(4):1-33. doi: 10.1177/001100006900100402.
31. Gasca V. Simptomele stresului ocupațional la lucrătorii medicali [Symptoms of occupational stress of medical workers]. In: SUMPh *Nicolae Testemitanu*. Collection of scientific summaries of students, residents and young researchers. Chisinau: Medicina; 2015. p. 268. Romanian [cited 2021 May 9]. Available from: http://repository.usmf.md/bitstream/20.500.12710/2263/1/SIMPTOMELE_STRESULUI_OCUPATIONAL_LA_LUCRATORII_MEDICALI.pdf
32. Duaso M, Duncan D. Health impact of smoking and smoking cessation strategies: current evidence. Br J Community Nurs. 2012;17(8):356-63.
33. Glover M, Paynter J, Bullen C, Kristensen K. Supporting pregnant women to quit smoking: postal survey of New Zealand general practitioners and midwives' smoking cessation knowledge and practices. N Z Med J. 2008;121(1270):53-65.
34. Lancaster T, Fowler G. Training health professionals in smoking cessation. Cochrane Database Syst Rev. 2000(3):CD000214. http://dx.doi.org/10.1002/14651858.
35. Abdullah AS, Stillman FA, Yang L, Luo H, Zhiyong Z, Samet JM. Tobacco use and smoking cessation practices among physicians in developing countries: a literature review (1987–2010). Int J Environ Res Public Health. 2014;11(1):429-55. doi: 10.3390/ijerph110100429.
36. GBD 2019 Tobacco Collaborators. Spatial, temporal, and demographic patterns in prevalence of smoking tobacco use and attributable disease burden in 204 countries and territories, 1990–2019: a systematic analysis from the Global Burden of Disease Study 2019. Lancet. 2021;S0140-6736(21)01169-7. https://doi.org/10.1016/S0140-6736(21)01169-7.
37. Norvegian Ministry of Health and Care Services. Evaluation of harm reduction as a strategic element in tobacco work. Oslo: The Ministry; 2017.
38. Cummings KM, Nahhas GJ, Sweanor DT. What is accounting for the rapid decline in cigarette sales in Japan? Int J Environ Res Public Health. 2020;17(10):3570. doi: 10.3390/ijerph17103570.

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Author's contribution
LG conceptualized the idea, conducted literature review, collected the data, interpreted the data, and wrote the manuscript.

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The knowledge and practices of family doctors in counselling obese patients

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Abstract

Background: Promoting a healthy lifestyle is one of the basic pieces in the family medicine activity. In order to formulate and / or adjust some approaches in clinical practice of the family doctor in the Republic of Moldova, it was necessary to analyse the knowledge and practices of the family doctor regarding the obese patients counselling.
Material and methods: Cross-sectional study was based on primary data collection. The study sample included 316 family doctors. The descriptive analysis was expressed in absolute values, percentages and standard errors, means and standard deviation of the mean. Statistical significance was considered to be $p < 0.05$.
Results: Family doctors feel best prepared in counselling patients on stress management (75.9 ± 2.40%), prescribing healthy diets (71.2 ± 2.55%), prescribing physical activity (71.0 ± 2.55%), cerebrovascular accident (CVA) prevention (68.9 ± 2.60%). They feel less prepared in counselling patients with regard to weight control (67.5 ± 2.63%).
Conclusions: Managing obesity in primary health care remains indispensably linked with the clinical practices of family doctors. Research has shown that family doctors are less prepared in counselling patients regarding weight control than regarding other important components of obesity management. According to the study, it was found that male family doctors, family doctors over the age of 45, those who work in the primary health care providers located in the rural area, those who have a working experience of over 21 years, and those who serve sectors with a population of up to 1500 people feel more prepared in counselling obese patients.
Key words: obesity, primary health care, family doctor.

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Introduction

Promoting a healthy lifestyle is an important piece of an efficient management of obesity among the population and, but not least, one of the basic elements of the family medicine activity. The literature presents numerous evidences that support the critical role of primary health care in health promotion and disease prevention, including significant contributions of the family doctor approaches in overweight management among patients, but at the same time there is evidence that obesity screening and counselling is not a common practice in primary health care [1, 2].

In order to formulate and / or adjust some approaches in clinical practice of the family doctor in the Republic of Moldova, the aim was to analyse the knowledge and practices of the family doctor regarding the obese patients counselling.

Material and methods

Descriptive, cross-sectional study was based on primary data collection. Inclusion criteria for the study: 1) family doctor working in a public or a private primary health care provider in the Republic of Moldova; 2) the presence of the informed consent agreement for participation in the study. The study sample was calculated based on selective irrevocability and included 316 family doctors. Primary data were collected through interview structured in a questionnaire and accompanied by informed consent. In order to ensure data confidentiality, the information collected was anonymised and encrypted. Statistical analysis was performed using SPSS / *Statistical Package for Social Sciences for Windows* / version 20.

The descriptive analysis was expressed in absolute values, percentages and standard errors, means and standard deviation of the mean. Statistical significance was considered to be $p < 0.05$. For the analysis of the capacities and practices of the family doctor in counselling obese patients there were taken as a basis the answers offered by the participants at the study. Thus, based on the score of the answers provided (from 0-*not at all* to 5-*excellent*) we determined the average score obtaining the value 3.4 ± 0.7DS, which would correspond to the answer between "*moderate*" and "*good*". Therefore, we considered high counselling capacities at the average value higher than 3.5 and lower counselling capacities at the average value lower than 3.4.

Results

Of the 316 family doctors who participated in the study, the majority had a mean age of 48.0±0.7, the youngest family doctor was 28 years old, and the oldest family doctor was 72 years old.

Depending on the location of the primary health care provider where the family doctors work, 50.6 ± 2.81% were from the rural area, 49.4 ± 2.81% – from the urban area (p> 0.05). In 88.0 ± 1.82% of cases the family doctors were females, and in 12.0 ± 1.82% of cases the family doctors were males (p <0.05). The length of working experience in the primary health care field was on average 23.0 ± 0.8 years, the shortest length of working experience was 1 year and the highest length was 55 years. On average, the population served by a family doctor was 1725 inhabitants, the smallest served sector had a population of 450 people and the largest of 4500 people. In 97.5 ± 0.88% of cases, family doctors indicated that obese patients are under surveillance in the served sector.

According to the results family doctors feel best prepared in counselling patients on stress management (75.9±2.40%; n=240)), healthy diets (71.2±2.55%; n=225), physical activity and exercise prescription (71.0±2.55%; n=224), cerebrovascular accident prevention (68.9±2.60%; n=218). They feel less prepared in counselling patients with regard to weight control (67.5±2.63%; n=213) (fig. 1).

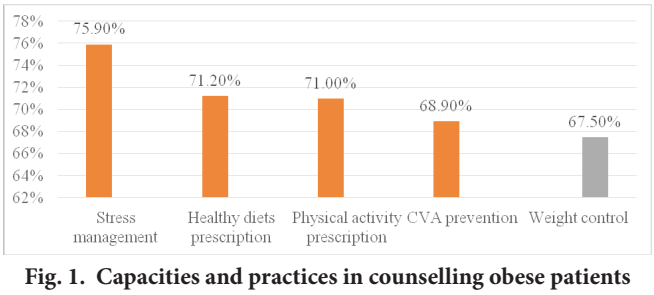

Fig. 1. Capacities and practices in counselling obese patients

The data provided in tab. 1 show that male family doctors, family doctors over the age of 45, those who work in the primary health care providers located in the rural area, those who have a work experience of over 21 years, and those who serve sectors with a population of up to 1500 people feel more prepared in counselling obese patients.

Table 1. Capacities and practices of family doctors in counselling obese patients in relation to demographic parameters

Variable	Sample	Capacity of counselling		Value p
		Low *Mean ≤ 3.4*	High *Mean ≥ 3.5*	
	Abs. (%)	Abs. (%)	Abs. (%)	
Sex				
Male	38 (12.0)	14 (36.8)	24 (63.2)	p<0.05
Female	278 (88.0)	120 (43.2)	158 (56.8)	p<0.05
Age group				
28-44 years	120 (38.0)	61 (50.8)	59 (49.2)	p>0.05
45-55 years	103 (32.6)	41 (39.8)	62 (60.2)	p<0.01
56 years and older	93 (29.4)	32 (34.4)	61 (65.6)	p<0.01
Location of PHC provider				
Urban	156 (49.4)	72 (46.2)	84 (53.8)	p>0.05
Rural	160 (50.6)	62 (38.8)	98 (61.2)	p<0.01
Length of working experience				
Up to 20 years	133 (42.1)	67 (50.4)	66 (49.6)	p>0.05
21 years and over	183 (57.9)	67 (36.6)	116 (63.4)	p<0.01
Served sector population				
Up to 1500 people	53 (16.8)	19 (35.8)	34 (64.2)	p<0.01
More than 1501 people	263 (83.2)	115 (43.7)	148 (56.3)	p<0.01

Discussion

Although globally there are numerous evidences that support the importance of primary health care in health promotion and overweight prevention activities, there are also another proofs suggesting that screening and obesity counselling is not a common practice in primary care. And a reason for the latter is that in medical practices, in the most common cases, the time spent with the patient during the visit is insufficient. Several studies refer to the disproportion between the number of patients visits and the time provided for them, especially for obese patients [3-5]. This is due to the fact that during visits, doctors usually advise on ongoing, current and / or urgent health problems and less on screening and counselling related to overweight and obesity [6]. A study was conducted in the USA in order to estimate the time required for counselling on healthful diet (8.2 minutes) and additionally for counselling on physical activity (4 minutes) [7]. In this context, it is to be emphasized that in order to provide adequate treatment and counselling schemes to patients who are overweight and obese, the time given to them needs to be increased [8]. With regard to the time allocated for consultations and which would require the inclusion of preventive and curative services on overweight and obesity, several studies address the subject of additional remuneration of doctors [9-12], one of the reasons being that the additional time required for the provision of preventive and counselling services during visits will be offset by the time required for consultation visits to other patients [10].

Conclusions

1. Managing obesity in primary health care remains indispensably linked with the clinical practices of family doctors.

2. Research has shown that family doctors are less prepared in counselling patients regarding weight control

than regarding other important components of obesity management.

3. According to the study, it was found that male family doctors, family doctors over the age of 45, those who work in the primary health care providers located in the rural area, those who have a working experience of over 21 years, and those who serve sectors with a population of up to 1500 people feel more prepared in counselling obese patients.

References

1. Sturgiss E, Elmitt N, Haelser E, van Weel C, Douglas KA. Role of the family doctor in the management of adults with obesity: a scoping review. BMJ Open. 2018;8(2):e019367. doi: 10.1136/bmjopen-2017-019367.
2. Cochrane L, Olson C, Murray S, Dupuis M, Tooman T, Hayes S. Gaps between knowing and doing: understanding and assessing the barriers to optimal health care. J Contin Educ Health Prof. 2007;27(2):94-102. doi: 10.1002/chp.106.
3. Kraschnewski J, Sciamanna C, Stuckey H, et al. A silent response to the obesity epidemic: decline in US physician weight counseling. Med Care. 2013;51(2):186-192. doi: 10.1097/MLR.0b013e3182726c33.
4. Davis N, Wildman R, Forbes B, Schechter C. Trends and disparities in provider diagnosis of overweight analysis of NHANES 1999-2004. Obesity. 2009;17:2110-2113. doi: 10.1038/oby.2009.129.
5. McAlpine D, Wilson A. Trends in obesity-related counseling in primary care: 1995-2004. Med Care. 2007;45(4):322-329. doi: 10.1097/01.mlr.0000254575.19543.01.
6. Abbo E, Zhang Q, Zelder M, Huang E. The increasing number of clinical items addressed during the time of adult primary care visits. J Gen Intern Med. 2008;23(12):2058-65. doi: 10.1007/s11606-008-0805-8.
7. Yarnall K, Pollak K, Ostbye T, et al. Primary care: is there enough time for prevention? Am J Public Health. 2003 Apr;93(4):635-41. doi: 10.2105/ajph.93.4.635.
8. Bocquier A, Verger P, Basdevant A, et al. Overweight and obesity: knowledge, attitudes, and practices of general practitioners in France. Obes Res. 2005;13(4):787-95. doi: 10.1038/oby.2005.89.
9. Devlin R, Sarma S. Do physician remuneration schemes matter? The case of Canadian family physicians. J Health Econ. 2008;27(5):1168-1181. doi: 10.1016/j.jhealeco.2008.05.006.
10. Ayres C, Griffith H. Perceived barriers to and facilitators of the implementation of priority clinical preventive services guidelines. Am J Manag Care. 2007;13(3):150-155.
11. Gosden T, Forland F, Kristiansen I, et al. Capitation, salary, fee-for-service and mixed systems of payment: effects on the behaviour of primary care physicians. Cochrane Database Syst Rev. 2000;(3):CD002215. doi: 10.1002/14651858.CD002215.
12. Sharma A, Kushner R. A proposed clinical staging system for obesity. Int J Obes. 2010;33(3):289-295. doi: 10.1038/ijo.2009.2.

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Authors' contributions
AT developed the first manuscript; OL interpreted the data and critically revised the manuscript. AC interpreted the statistical data. All the authors reviewed the material and approved the final version of the manuscript.

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No competing interests were disclosed.

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Brain imaging of children with postischemic seizures

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Abstract

Background: Arterial ischemic stroke is a rare disease found in the pediatric population, with an incidence of 2-13 out of 100000 children per year. Children develop seizures more often in the initial phase of stroke compared to adults.
Material and methods: There have been included children examined by brain MRI hospitalized in the department of neurology (early aged children), Hospital of Mother and Child Health Care, with a diagnose of ischemic stroke, with an acute focal neurological syndrome installed. The children not having an imagistic confirmation of arterial ischemic stroke were excluded.
Results: The study lot was composed of 78 children, who corresponded to all the inclusion criteria for this study (Tab.1). The children were examined by 1.5T and 3T MRI with multiplanar assessment of brain structures. In cases with acute / subacute stroke specific diffusion sequences (DWI and ADC) were applied to determine the type of stroke, approximate installation period. Ischemic brain lesions on MRI are presented by areas of cytotoxic edema in acute/ subacute cases, in chronic / late cases by the appearance of changes such as lacunar defects, areas of encephalomalacia.
Conclusions: The vast majority of children with brain lesions on MRI have convulsions with early onset and have developed recurrent seizures after the acute phase of stroke.
Key words: ischemic stroke, MRI, brain.

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Introduction

Arterial ischemic stroke is a rare disease found in the pediatric population, with an incidence of 2-13 out of 100000 children per year. According to the evaluation of studies in the literature [1-3] a correlation was observed between the early onset of seizures and the arterial ischemic stroke, them being diagnosed in 19% up to 44% after stroke. Children develop seizures more often in the initial phase of stroke compared to adults [1, 3]. MRI neuroimaging is very informative for establishing the diagnosis of stroke. **The aim of the study:** evaluation of brain ischemic lesions in children with seizures.

Material and methods

There have been included children examined by brain MRI hospitalized in the department of neurology (early aged children), Hospital of Mother and Child Health Care, with a diagnose of ischemic stroke, with an acute focal neurological syndrome installed. The children not having an imagistic confirmation of arterial ischemic stroke were excluded.

The types of seizures are defined after the International League Against Epilepsy criteria. Early-onset seizures were defined as seizures that appear up to 7 days after the arterial ischemic stroke. Late-onset seizures appear later than 7 days after the onset of the ischemic stroke, and post arterial ischemic stroke epilepsy can be defined by the presence of more than 2 seizures that emerged after the acute phase of arterial ischemic stroke (longer than 1 month).

In order to get the most truthful results the t student test was applied for comparing continuous variables and the $\chi 2$ test for discrete variables. We have used the Kaplan-Meier estimator for determining the cumulative incidence of previously unprovoked seizures. The log-rank test was used in order to compare the risk of appearance of subsequent unprovoked seizures at children with and without early-onset seizures. Values with $p<0.05$ were considered statistically significant values.

Results

The study lot was composed of 78 children, who corresponded to all the inclusion criteria for this study (tab.1). Early-onset seizures were present at 20 children out of 78 (25.6 %), the average age of debut being 1.4±1.9 years, the

Table 1. Characteristic of patient

Seizures			With early onset		With late onset		
	№	N (%)	№	N (%)	№	N (%)	P
Number of patients	78	(100)	20	(25.6)	58	(74.4)	
Age of onset			1.4 ±1.9		3.2± 6.2		<0.001
Risk factors							
Cardiac Disease	23	(29.5)	7	(35.0)	16	(27.6)	0.58
Coagulopathy	20	(25.6)	6	(30.0)	14	(24.1)	0.77
Vascular pathologies	19	(24.4)	4	(20.0)	15	(25.9)	0.77
Infections	11	(14.1)	6	(30.0)	5	(8.6)	0.03
Vasculites	7	(9.0)	0	(0)	7	(12.1)	0.18
Methabolic Disease	6	(7.7)	3	(15.0)	3	(5.2)	0.17
Hemathologic Disease	2	(2.6)	0	(0)	2	(3.4)	1.0
Cortical involvment							
Cortical involvment	56	(71.8)	19	(95.0)	37	(63.8)	0.01
Bylateral localization	15	(19.2)	5	(25)	10	(17.2)	0.52
General State							
Focal neurological impairment	66	(84.6)	12	(60)	54	(93.1)	0.001
Alteration of consciousness	18	(23.1)	9	(45.0)	9	(15.5)	0.01
Duration of the Study							
Average duration (month)	55.6±48.4		73.0±52.2		49.0±45.6		0.62

children being younger than the ones who were not diagnosed with this type of seizures, 3.2± 6.2 years, p<0.001. Out of them, for 20 children (90%), the seizures were the first manifestations of the clinical picture.

The repartition of this convulsive access after the structure was as follows: focal seizures diagnosed at 15 children (75%), 3 children had generalized seizures (15%) and in 2 cases the debut could be determined neither as focal nor as generalized (10%). Out of all analyzed cases the frequent (daily) seizures were determined at 12 children (60%). Cases with only one seizure at the debut of the ischemic stroke were noted at 5 children (25%) and 3 children have developed status epilepticus.

In the study group, in the EEG picture, the following modifications were noted: slow diffusion of the route in 8 cases (40%), focal slowing in 7 cases (35%) and epileptiform activity in 5 cases (25%).

Children who had developed early-onset seizures, in comparison with those who had late-onset seizures have a predisposition to infections as a risk factor for arterial ischemic stroke (30 % vs 8.6 %, p=0.03), more frequent cortical affectations (95 % vs 63.8 %, p=0.01), and knowledge disturbances from the beginning (45% vs 15.5%, p=0.01). The neuronal focal shortage was more frequent at children with late-onset seizures (60% vs 93.1%, p=0.001).

The study duration was 53.7±48.8 months. The early-onset seizures did not influence the mortality after the acute phase of arterial ischemic stroke (0% versus 8.6%, p=0.32).

During 4.5 years, 13 children (65%) out of the group with early-onset seizures and 5 children (8.6%) out of the ones without early-onset seizures were diagnosed with unprovoked seizures. During all these years, the cumulative incidence of the development of the repeated seizures in the both groups was 52.5% and 3.5% for 1 year after the onset, 52.5% and 6.2% for 2 years after the onset and 72.3% and 14.4% in 4.5 years. The children from the early-onset seizures group have developed a more significant risk of the appearance of repeated seizures during the years after the arterial ischemic stroke, in comparison with those with late-onset seizures (p<0.001). The majority (67%) of the subsequent repeated crises had their onset during the first post-arterial ischemic stroke year vs 27% after 2 years after the evolution.

The children were examined by 1.5T and 3T MRI (Siemens Avanto and Skyra) with multiplanar assessment of brain structures. In cases with acute / subacute stroke specific diffusion sequences (DWI and ADC) were applied to determine the type of stroke, approximate installation period [4].

Diagnostic efficiency can be improved by administering

the intravenous contrast agent. In children younger than 7-8 years, sedation was performed during the investigation, of about 15-20 minutes with the involvement of a team of radiologist and anesthesiologist available for this procedure.

Stroke in children has significant neurological deficits. Ischemic brain lesions on MRI are presented by areas of cytotoxic edema in acute / subacute cases, in chronic / late cases by the appearance of changes such as lacunar defects, areas of encephalomalacia (fig. 1).

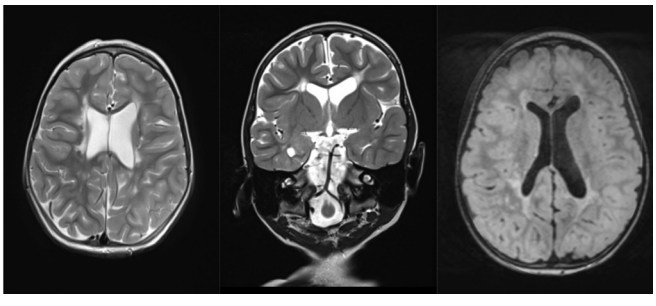

Fig. 1. MRI pictures (TW2ax, cor and FLAIR ax) characteristic for areas of chronic postischemic encephalololomalacia of bilateral periventricular white matter, with ex-vacuo dilatation of the lateral ventricles

More frequently, the bilateral (fig. 2) or unilateral involvement of the basal nuclei is attested (fig. 3). In the sequences sensitive to the determination of hemorrhage (SWI type), there are hemeosiderin inclusions in the ischemic region.

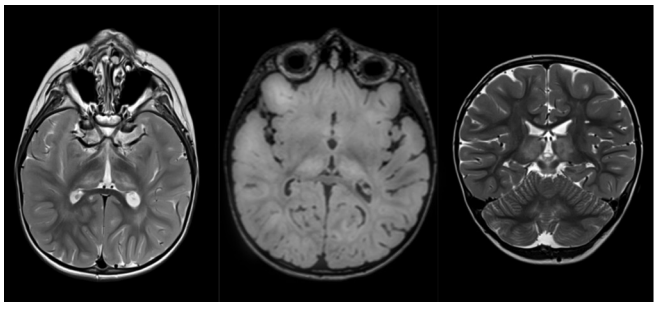

Fig. 2. MRI pictures (TW2ax, FLAIR ax and TW2cor) characteristic for ischemic stroke with involvement of the bilateral thalamus and periventricular white matter

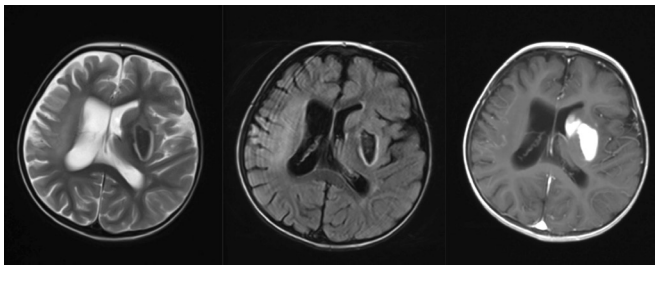

Fig. 3. MRI pictures (TW2, FLAIR and TW1 postcontrast) characteristic for arterial ischemic stroke in the teritory of the arteria cerebri media (left)involving left basal ganglias. Atrofic-glyotic modifications in the fronto-temporal lobes (right)

Discussion

In this study there was made a conclusion that the frequency of children who have presented early-onset seizures after the arterial ischemic stroke is 25.6%, in comparison with other studies that present values starting with 19% up to 44% [1, 5]. Also in the existing studies it is proved that the incidence of this kind of seizures is higher amongst the children than amongst the adults [3, 6]. In the given study this fact was confirmed through the higher incidence of seizures among the younger children, in comparison with those who are older.

As the result of the study a connection between early-onset seizures and post-arterial ischemic stroke epilepsy was determined. The cumulative incidence of the development of late-onset seizures was of 72.3% for 4.5 years of follow-up. Children with early-onset seizures have developed post-arterial ischemic stroke epilepsy in 60% of the cases. In the existent studies it was determined that children with post-arterial ischemic stroke seizures were more likely to have epilepsy in the future, the fact confirmed in this study too. The aim of this study was to determine the risk factors for the recurrence of the post-arterial ischemic stroke debut strokes in the future, but there were not found any statistically significant differences linked with the age or the sex of the children implied in this study or with the usage of the antiepileptic means in a prophylactic purpose. A lot of the existent studies have shown that the antiepileptic drugs used in the initial phases of the arterial ischemic stroke with the purpose of stopping the acute seizures do not reduce the risk of the development of epilepsy [7, 8]. In addition, the previous studies concerning the adult population have proved that the usage of antiepileptic medicines in the initial phases, in order to prevent the recurrence of seizures is not necessary [7, 8, 9]. However, it is necessary to mention that the therapeutic tactics is different in the adult vs pediatric population and additional studies are required for proving this hypothesis.

Conclusions

In the course of this study, it was found that the vast majority of children with brain lesions on MRI have convulsions with early onset and have developed recurrent seizures after the acute phase of stroke. There are many challenges and complications in the evaluation of these children, being often the need for sedation to obtain informative images.

It is necessary to study further the relationship between seizures and the risk of developing post-stroke epilepsy, as well as to develop an effective method of preventing subsequent recurrent seizures.

References

1. deVeber GA, MacGregor D, Curtis R, Mayank S. Neurologic outcome in survivors of childhood arterial ischemic stroke and sinovenous thrombosis. J Child Neurol. 2010;15(5):316-324. doi: 10.1177/088307380001500508.

1. Thurman DJ, Beghi E, Begley CE, et al. Standards for epidemiologic studies and surveillance of epilepsy. Epilepsia. 2011;52(Suppl 7):2-26. doi: 10.1111/j.1528-1167.2011.03121.x.
2. Zahuranec DB, Brown DL, Lisabeth LD, Morgenstern LB. Is it time for a large, collaborative study of pediatric stroke? Stroke. 2015;36(9):1825-9. doi: 10.1161/01.STR.0000177882.08802.3c.
2. Manea M, Golea G, Mălăescu R. [Arterial ischemic stroke in children]. Revista de Neurologie și Psihiatrie a Copilului și Adolescentului din Romania [Rom J Child Adolesc Neurol Psychiatr]. 2014;17(3):27-38. Romanian.
3. Ferguson PL, Smith GM, Wannamaker BB, Thurman DJ, Pickelsimer EE, Selassie AW. A population-based study of risk of epilepsy after hospitalization for traumatic brain injury. Epilepsia. 2010;51(5):891-898. doi: 10.1111/j.1528-1167.2009.02384.x.
4. Beslow LA, Abend NS, Gindville MC, et al. Pediatric intracerebral hemorrhage: acute symptomatic seizures and epilepsy. JAMA Neurol. 2013;70(4):448-54. doi: 10.1001/jamaneurol.2013.1033.
5. Lossius MI, Ronning OM, Slapo GD, Mowinckel P, Gjerstad L. Post-stroke epilepsy: occurrence and predictors – a long-term prospective controlled study. Epilepsia. 2011;46(8):1246-1251. doi: 10.1111/j.1528-1167.2005.57904.x.
6. Fullerton HJ, Wu YW, Sidney S, Johnston SC. Risk of recurrent childhood arterial ischemic stroke in a population-based cohort: the importance of cerebrovascular imaging. Pediatrics. 2007;119(3):495-501. doi: 10.1542/peds.2006-2791.
7. Leone MA, Tonini MC, Bogliun G. Risk factors for a first epileptic seizure after stroke: a case control study. J Neurol Sci. 2009;277(1-2):138-142. doi: 10.1016/j.jns.2008.11.004.

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Authors' contribution

MM conceptualized the idea, conducted literature review and wrote the manuscript; CC collected and interpreted the data. Both authors approved the final version.

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Ethics approval and consent to participate

The study was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (protocol No 69 of March 21, 2017). An informed consent from all participants in the study was obtained.

Conflict of interests

No competing interests were disclosed.

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Cytokine storm in severe patient COVID-19 and curative plasmapheresis

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Abstract

Background: In the intensive care unit, the curative plasmapheresis technique is widely applied among severe patients, who show signs of both endogenous and exogenous intoxication. The impact of isovolemic isooncotic curative plasmapheresis on the status of hemoleukogram and immunological markers has not been studied yet.
Material and methods: The study is retrospective, observational. The medical records of 34 COVID-19 patients from the COVID intensive care units of *Timofei Mosneaga* Republican Clinical Hospital who benefited from isovolemic, curative isooncotic plasmapheresis were analyzed. There were 25 complete, analyzed data sets. General data were recorded: preplasmapheresis, postplasmaphesis hemoleukogram.
Results: Isovolemic, isooncotic plasmapheresis, performed in patients with severe COVID-19, SARS-CoV2 showed effects of improving the status of laboratory indices that denote the evolution and favorable prognosis of the disease. The activity of the cytokine storm decreased in both groups of patients, both in those with a mean age of up to 60 years and in those over 60 years of age, with p = 0.001. Patients over 60 years of age had a marked improvement in serum ferritin index, compared to patients in the age group up to 60 years, in the period after plasmapheresis, p = 0.043. Analogously, the level of C-reactive protein was analyzed, which clearly decreases in patients <60 years of age, compared to those> 60 years of age, p = 0.037
Conclusions: Isovolemic, isooncotic plasmapheresis is a procedure performed in severe COVID-19 (SARS-CoV-2) patients to improve the status of the cytokine storm. Moreover, the performance of the procedure in different age groups induced an improvement in the satiety of hemoleukogram and proinflammatory indices.
Key words: plasmapheresis, cytokine storm, immune status, hemoleukogram.

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Introduction

Curative plasmapheresis is a method of related therapy applied in different pathological conditions in order to remove from the body pathological products of different genesis [1]. The mechanism of elimination and biotransformation of the pathological substrate is the key to the detoxification effect. The correction of the rheological status is achieved by the mechanism of decreasing blood viscosity, increasing plasticity and decreasing the aggregation of blood cells [2]. Immunocorrection is due to the elimination of immune complexes, cytokines, immunocompetent cells thus achieving the "unblocking" of the immune system [3]. In the intensive care unit, the technique of curative plasmapheresis is widely applied among severe patients, who show signs of both endogenous and exogenous intoxication [4].

COVID-19 is a systemic pathology with a significant impact on the hematopoietic system and the immune system [6, 8, 9, 14]. Lymphopenia can be considered a laboratory indicator of major significance for the prognosis of COVID-19 patients with the evaluation of serum ferritin. The neutrophil / lymphocyte ratio and the maximum platelet / lymphocyte ratio may also have prognostic value in determining severe cases [3].

Some authors recommend close monitoring of leukocyte count, lymphocyte count, platelet count, and serum ferritin as markers for determining the potential prognosis for a critical disease [12, 13].

No references were found to the impact of plasmapheresis on the status of the hemoleukogram and the cytokine storm in COVID-19 (SARS-CoV-2) patients in the intensive care unit. In this regard, the aim of the study was to evaluate the status of hemoleukogram and proinflammatory markers in severe patients in the Intensive Care Unit with the diagnosis of COVID-19 [7, 10 11].

Material and methods

The study is retrospective, observational. The medical records of 34 COVID-19 patients from the COVID intensive care units of *Timofei Mosneaga* Republican Clinical Hospital who benefited from isovolemic, curative isooncotic plasmapheresis were analyzed. Duration of data collection: April-May 2020. The participants eligible to be included

in the study were 34 patients. There were 25 patients with complete data sets containing fully analyzed data sheets.

The indications for the plasmapheresis procedure were: elevated values of serum ferritin, C-reactive protein, lymphocytopenia.

The inclusion criteria in the study were:

- Adult patient (≥18 years), beneficiary of curative plasmapheresis;
- Patient diagnosed with COVID-19 in intensive care.

The exclusion criteria from the study were:

- Incomplete standardized data sheet.

Statistical analysis

The numerical values of the parameters were numbered in the Excel table, after which – imported into the statistical analysis software GraphPad Prism version 8. The data are presented in the form of absolute and relative values, or average and interval 95% reliable average. Applied statistical tests: One way ANOVA, Simple T test. P <0.05 was considered statistically significant.

Results

The dynamics of the laboratory parameters, measured before and after the plasmapheresis session is presented in table 1.

Analysis of hemoleukogram data and proinflammatory markers up to plasmapheresis vs the period after plasmapheresis, in patients up to 60 years of age and over 60 years, shows in both age groups a positive dynamics of the results of laboratory indices. Of note is the result for serum ferritin, which in the general group shows a significant decrease in mean values with n = 1093.7 to n = 564.1.

The results shown in table 1 demonstrate an improvement in immune status, with an increase in the number of lymphocytes in the whole group from 7% to 12.69% and in age groups from 9% to 15% (over 60 years), from 6% to 11% (under 60 years).

Discussion

The activity of the cytokine storm decreased in both groups of patients: with an average age of up to 60 years and in over 60 years of age.

In the series of data with values of laboratory parameters, it was observed that in some patients the results of the analyses decreased faster, and in others – slower. Thus, the results were analyzed in patients depending on the presence of comorbidities, which according to the literature influence the severity prognosis of COVID-19, SARS-CoV2.

Bolagholi S. et al. [4] noticed that plasmapheresis has a beneficial impact on prognosis of COVID-19, but there is no evidence about the influence of the comorbidities on the dynamics of the process.

Patients in the study who had concomitant diabetes mellitus, the outcome of hemoleukogram indices and proinflammatory markers were evaluated to determine the influence of concomitant pathology on plasmapheresis efficacy. Blood glucose values were not investigated in the patients included in the study. The patient with diabetes mellitus was evaluated as a criterion.

The number of leukocytes, C-reactive protein, serum ferritin, and lymphocytes in patients with diabetes mellitus after isovolemic isooncotic plasmapheresis, while maintaining hemodynamic and respiratory protective status, were evaluated and the result showed an improvement in this proinflammatory status of patients after plasmapheresis.

Table 1. Dynamics of laboratory parameters before and after isovolemic isooncotic plasmapheresis

Parameter	Preplasmapheresis	Postplasmapheresis	t	p
	(n=)	(N=)		
PCR (whole batch) mg/dl	118.91	55.3	2.11	0.0001
PCR sublot over 60 years	135.71	38.13	1.451	0.0011
PCR sublot under 60 years	108.41	66.04	2.34	0.005
Leukocytes (whole group)	13.86	13.16	3.4	0.0001
Leukocytes sublot over 60 years	11.49	13.02	3.43	0.0005
Leukocytes sublot under 60 years	15.34	13.26	3.91	0.0017
Lymph (whole group)	7%	12.69%	1.41	0.0001
Lymph sublot over 60 years	9%	15%	1.29	0.001
Lymph sublot under 60 years	6%	11%	1.52	0.0003
Monocytes (whole lot)	4.08	3.25	1.61	0.0001
Monocytes sublot over 60 years	3.42	3.2	1.84	0.0467
Monocytes sublot under 60 years	4.08	3.2	1.65	0.0002
Ferritin (whole lot)	1093.73	765.35	2.35	0.0001
Ferritin it sublot over 60 years	613.08	564.1	2.27	0.04
Ferritin sublot under 60 years	1394.15	891.13	2.498	0.0005

Similarly, the patients in the study who had concomitant hypertension were analyzed from a statistical point of view, which according to previous research is also a reserved prognostic indicator in case of severe evolution of COVID-19.

During the research, the results of hemoleukogram indices and proinflammatory markers were analyzed in patients with concomitant hypertension for determining the degree of influence of the concomitant condition on the efficacy of plasmapheresis. The T-student test presented a statistically significant result with p = 0.045, with the improvement of the laboratory indicators analyzed among patients with hypertension, although the evolution of the dynamics of laboratory analyses is noticeable to be slow. Similar to the group of patients with diabetes, no different blood pressure values were analyzed, and the results of the indices after performing the plasmapheresis procedure. Reference value showed the presence or absence of pathology of hypertension in the patient. There was a positive evolution of proinflammatory markers and hemoleukogram in this group of patients, significant both clinically and statistically.

In the research, similar to other subjects, it was determined that the age of over 60 increases the predictability of mortality and morbidity for COVID-19. As reference markers of the cytokine storm in the study, the following were selected: C-reactive protein, serum ferritin and lymphocytes. Thus, one of the objectives was to analyze the correlation of different age groups with the evolution of the above-mentioned indices at the stage of pre-plasmapheresis and post-plasmapheresis for establishing the existence of the correlation between the plasmapheresis procedure and the results of the laboratory indices.

Rodríguez-Morales A., Cardona-Ospina J. et al. [5] in their meta-analyses determined that the impact of comorbidities on the prognostic of the COVID-19, in ICU patients is huge, and the damage as well. This study brings the high-light of a procedure that may improve the outcome in that group of patients. More studies are needed to be continued for better analyses.

Thus, four groups of patients were statistically analyzed. The indicator analyzed was the value of C-reactive protein. There is a decrease in C-reactive protein levels in post-plasmapheresis patients, both in those up to 60 years of age and after 60 years. It is also worth noting that the level of C-reactive protein clearly decreases in patients <60 years of age, compared to those> 60 years of age. Although among those of >60 years a positive dynamics is maintained at the stage after plasmapheresis.

The represented analysis is statistically significant, with p value = 0.037. And the improvement of this marker for both age groups after performing the isooncotic, isovolemic plasmapheresis procedure emphasizes the usefulness of the procedure itself in COVID-19 patients in the intensive care unit.

Patients over 60 years of age showed a marked improvement in indices compared to patients in the age group up to 60 years, in the period after plasmapheresis. At the same time, the study demonstrates the efficiency of achieving isovolemic isooncotic plasmapheresis among patients with different age groups. The analysis has statistical significance, with p value = 0.041. The result is all the more clinically significant as it correlates with the C-reactive protein indicator, and the hemodynamic and respiratory stability of patients during and after the plasmapheresis procedure.

Conclusions

Isovolemic, isooncotic plasmapheresis is a procedure performed in severe COVID-19 (SARS-CoV-2) patients to improve the status of the "cytokine storm". Moreover, performing the procedure in different age groups induced an improvement in the satiety of hemoleukogram and proinflammatory indices. And the presence of concomitant diseases did not influence the effectiveness of the results after the realization of isovolemic, isooncotic plasmapheresis.

References

1. Zhou B, She J, Wang Y, Ma X. Utility of Ferritin, Procalcitonin, and C-reactive Protein in severe patients with 2019 Novel Coronavirus Disease. ResearchSquare. 2020. Epub 2020 April. doi: 10.21203/rs.3.rs-23645/v1.
2. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Feb 15;395(10223):507-513. doi: 10.1016/S0140-6736(20)30211-7.
3. Lalueza A, Ayuso B, Arrieta E, et al. Elevation of serum ferritin levels for predicting a poor outcome in hospitalized patients with influenza infection. Clin Microbiol Infect. 2020;26(11):1557.e9-1557.e15. doi: 10.1016/j.cmi.2020.02.018.
4. Balagholi S, Dabbaghi R, Eshghi P, et al. Potential of therapeutic plasmapheresis in treatment of COVID-19 patients: Immunopathogenesis and coagulopathy. Transfus Apher Sci. 2020;59(6):102993. doi: https://doi.org/10.1016/j.transci.2020.102993.
5. Rodríguez-Morales A, Cardona-Ospina J, Gutiérrez-Ocampo E, et al. Clinical, laboratory and imaging features of COVID-19: a systematic review and meta-analysis. Travel Med Infect Dis. 2020;34:101623. doi: 10.1016/j.tmaid.2020.101623.
6. Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 Novel Coronavirus-infected pneumonia in Wuhan, China. JAMA. 2020;323(11):1061-1069. doi: 10.1001/jama.2020.1585.
7. Senjo H, Higuchi T, Okada S, Takahashi O. Hyperferritinemia: causes and significance in a general hospital. Hematology. 2018;23(10):817-822. doi: 10.1080/10245332.2018.1488569.
8. Liu Y, Gayle A, Wilder-Smith A, Rocklov J. The reproductive number of COVID-19 is higher compared to SARS coronavirus. J Travel Med. 2020 Mar 13;27(2):taaa021. doi: 10.1093/jtm/taaa021.
9. Wu F, Zhao S, Yu B. A new coronavirus associated with human respiratory disease in China. Nature. 2020;579(7798):265-269. doi: 10.1038/s41586-020-2008-3.
10. Slaats J, Ten Oever J, van de Veerdonk F, Netea M. IL-1beta/IL-6/CRP and IL-18/ferritin: distinct inflammatory programs in infections. PLoS Pathog. 2016;12(12):e1005973. doi: 10.1371/journal.ppat.1005973.
11. Kernan K, Carcillo J. Hyperferritinemia and inflammation. Int Immunol. 2017;29(9):401-409. doi: 10.1093/intimm/dxx031.
12. Sanaei Dashti A., Alizadeh S., Karimi A, et al. Diagnostic value of lactate, procalcitonin, ferritin, serum-C-reactive protein, and other biomarkers in bacterial and viral meningitis: a cross-sectional study. Medicine (Baltimore). 2017;96(35):e7637. doi: 10.1097/MD.0000000000007637.

13. Kawamata R, Yokoyama K, Sato M, et al. Utility of serum ferritin and lactate dehydrogenase as surrogate markers for steroid therapy for Mycoplasma pneumonia. J Infect Chemother. 2015;21(11):783-9. doi: 10.1016/j.jiac.2015.07.009.

14. Eurosurveillance Editorial Team. Note from the editors: World Health Organization declares novel coronavirus (2019-nCoV) sixth public health emergency of international concern. Euro Surveill. 2020;25(5):200131e. doi: 10.2807/1560-7917.ES.2020.25.5.200131e.

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Author's contribution
VR conceptualized the idea, conducted literature review, collected the data, interpreted the data, and wrote the manuscript.

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Treatment effectiveness evaluation of dental caries as a factor affecting patients' quality of life

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Abstract

Background: The study of the patient's quality of life is an important condition for assessing the effectiveness of the treatment and the clinical condition of patients after treatment, in addition to objective diagnostic methods. The purpose of the study was to investigate the dynamics of changes in the quality of life of dental patients suffering from caries of the contact areas of the lateral group of teeth, after treatment in accordance with the established plan for the use of various matrix systems, in the study groups.
Material and methods: The study involved 147 patients: 48 patients of the 1st experimental group; 54 patients of the 2nd experimental group; 45 patients of the 3rd experimental group.
Results: In the course of the study, it turned out that such a criterion as the quality of life is an informative dynamic indicator, the value of which depends on the method of treatment used.
Conclusions: In the course of the study, the effectiveness of the use of a separation-retaining device for the restoration of the lateral group of teeth during the treatment of caries in the contact areas of the teeth was assessed as a factor influencing the quality of life of patients.
Key words: dental caries, quality of life of the patient, treatment.

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Introduction

Determination of the quality of life of dental patients, as a set of factors that determine the physical, psychological, emotional, social state of the patient based on his subjective perception, serves as a sufficient basis for the characteristics of the treatment. When considering the issue of not only the quality of care provided to the patient, but also the long-term results of the therapy, they take into account the general negative impact on the physiological parameters and the most important functions of the patient – his physical, psychological, emotional and social state [1]. Thus, the purpose of this study was to investigate the quality of life of patients during treatment.

The ability to obtain accurate information about the indicators of the quality of life of a patient, a group of patients, allows one to give a reliable assessment of the quality of life of society in its various sections in dynamics, determining the effectiveness of numerous reforms and programs aimed at increasing the level of well-being and improving the quality of life of the population [2].

Therefore, to assess the severity of the disease and the effectiveness of treatment, only objective clinical criteria and laboratory and instrumental data are not enough, since their interpretation is based on the subjective perception of a specialist and his idea of health. The methodological reasonableness of the quality of life provides the possibility of integrating this parameter into the system for evaluating the effectiveness of treatment methods, which increases the reliability of assessing the patient's condition. Such a medical approach meets the most important principle of the clinical work of a doctor – "treat the patient, not the disease" [2].

The questionnaires of the quality of life, to the greatest extent, allowing getting a holistic idea of the patient's well-being, remain the main tools for assessing the "holistic situation of the disease through the eyes of the patient". The main method for assessing the quality of life is the use of standardized questionnaires. In addition to questionnaires for determining the general medical condition of a patient, there are highly specialized options, such as the "Oral Health Impact Profile" OHIP14 [3-4]. With the localization of the carious process on the contact areas of the lateral

group of teeth, a patient suffers comparable to other severe chronic dental diseases, such as periodontitis, diseases of the oral mucosa [5]. Patients experience embarrassment about the appearance of their mouth, irritation about their appearance, a feeling of self-doubt during a conversation, eating. Patients are most concerned about the reaction of the causative teeth to temperature stimuli and the feeling of bad breath, which indicates their physical suffering [1]. Suspiciousness, anxiety, emotional instability, irritability are manifested in patients with dental caries, which negatively affect the quality of life [2]. It is noticed that the decrease in the quality of life indicators depends on the duration of the course of dental caries, untimely referral to specialists and the clinical severity of the process.

The ability to obtain accurate information about the indicators of a person's quality of life allows one to give a reliable assessment of the patient's condition in dynamics, determining the effectiveness of the therapy. The concept of "quality of life" is the basis of a new paradigm for understanding the disease and determining the effectiveness of treatment methods [4].

Material and methods

During the research, the aim was to evaluate the effectiveness of the use of various matrix systems for the restoration of the lateral group of teeth during the treatment of caries in the contact areas of the teeth, as a factor affecting the quality of life of patients [6]. A total of 147 patients were involved: 48 of them were included in the 1st group, who were treated by restoration technique using a retaining ring, a sectional matrix and a wedge; 54 patients of the 2nd group, who had a fixation system made of nickel-titanium (Ni-Ti) alloy with an adaptive working surface, instead of a fixing ring; the remaining 45 patients of the 3rd group received a separation-holding device for the restoration of the lateral group of teeth [7]. Patients of all groups received treatment in accordance with clinical guidelines (treatment protocols) for the diagnosis of dental caries approved by Resolution No 18 of the Council of the "Dental Association of the Russian Federation" dated September 30, 2014 – anesthesia, initiation of the tooth surface for treatment (removal of supra and subgingival teeth deposits), preparation of hard tooth tissues, removal of affected dentin, installation of the matrix system and wedges, etching and bonding the dental cavity, layer-by-layer restoration of the surface of missing tissues, removal of auxiliary devices, grinding, polishing, occlusal checking [5, 8, 9]. When presenting the results of evaluating interventions, the indicators recommended by the editors of the journals Evidence-Based Medicine, ACP Journal Club, adopted in evidence-based medicine were calculated [10]. The statistical significance of differences for testing the hypothesis about the relationship of two qualitative characteristics in the groups of subjects was calculated by the methods of nonparametric statistics using the χ2-square test with Yates' correction or Fisher's test (if the numbers in the cells of the 2x2 contingency table are less than 5). The sample parameters given in the tables have the following meaning: M – mean, s – standard (root-mean-square) deviation, m – mean error, n – volume of the analyzed subgroup, p – achieved level of significance. The critical value of the significance level was taken equal to 5%. Data analysis was carried out using the SAS 8 and SPSS 11 software packages. To identify the dependence of the treatment on the psychoemotional and physical state of patients in dynamics, a survey was conducted using a specialized validated questionnaire of quality of life "Profile of the impact of dental health" OHIP-14 RU, which contains 14 questions reflecting the impact of the patient's dental status on everyday life. The choice of this questionnaire was carried out in accordance with the requirements of International Association for the Assessment of Quality of Life (IQOLA) and Good Clinical Practice (GCP), and was mediated by a specific clinical situation. Five options were offered from "very often" to "never" and are rated from 5 to 1 points, respectively. Further, the points were summed up, and according to the result obtained, the level of quality of life of patients was determined, where 14-28 is a good level of quality of life, 29-56 is satisfactory, 57-70 – unsatisfactory quality of life.

Results and discussion

Patients were questioned before treatment, then after 1, 3, 6 and 18 months. As a result of the survey, were received 5 indicators of quality of life for each of 147 patients. Thus, an array of data from 735 questionnaires was formed. Statistical processing of the information obtained made it possible to calculate the average indicators for the quality of life index in each clinical group. Changes in the indicators of the quality of life index over time for each of the groups of patients with dental caries of the contact surfaces of the lateral group of teeth are reflected in table 1. The value of statistical significance according to the calculations showed the same value of $p > 0.05$ for all groups and terms of the study.

Dynamics of quality of life indicators, the difference in indicators before and after treatment in patients with the use of a separation-holding device for the restoration of the lateral group of teeth (group I), a fixation system made of nickel-titanium alloy with an adaptive working surface (group II) compared with patients in the group (III) and restoration using a retaining ring – provides a visual display of the effectiveness of using modern matrix systems in the treatment of dental caries, not only for a satisfactory clinical result, but also to improve the general condition of patients.

According to the results of the study, it was found that the level of quality of life in the studied groups during the treatment had a unidirectional tendency to improve 1 month after the treatment (fig. 1). However, the results in the 1st clinical group differed markedly from the other 2 groups, which almost identically achieved the level of "good" quality of life in patients. Subsequently, was observed a rapid decline in the quality of life in the first group and a gradual discrepancy between the data in the second and third groups according to the results of the questionnaire after 3 months. According to the results of the survey of patients 6 months after treatment, data were obtained indicating changes in

Table 1. Indicators of the quality of life index of patients during the study

Groups	Indicators (M±m)					
	OHIP 14					
	Before treatment	1 month later	3 months later	6 months later	18 months later	Differences between indicator before and after treatment
Group I (n=48)	63.29±0.62	35.98±0.37	46.46±0.24	61.72±0.16	69.71±0.14	-6.42±0.52
Group II (n=54)	62.7±0.59	16.07±0.35	18.92±0.25	30.08±0.16	37.12±0.15	25.58±0.34
Group III (n=45)	63.39±0.80	16.21±0.46	17.79±0.31	19.79±0.18	20.77±0.19	42.62±0.61

the quality of life of patients of the 1st group to values "unsatisfactory", which practically corresponded to the figures "before treatment". In the 2nd group, the indicators were the boundary data of "good" and "satisfactory" quality of life. The best result, according to the study of the quality of life, was formed in the third study group. The indicators in the third group revealed a "good" level of quality of life of dental patients. The situation after the examination of patients 18 months after the treatment carried out also confirms the tendency formed in each of the clinical groups towards the dynamic development of the state of the quality of life.

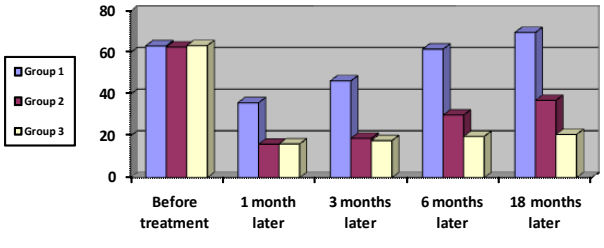

Fig. 1. Dynamics of changes in the quality of life of dental patients of various clinical groups

Conclusions

Thus, in the process of studying the results of the questionnaire survey of patients before and after the treatment, the dependence of the level of quality of life depending on the treatment performed was shown. The use of the retaining ring in the 1st group was found to be untenable, since it allowed achieving a "satisfactory" result only a month after the treatment, after which the indicator began to worsen rapidly. The use of a fixation system made of nickel-titanium (NI-TI) alloy with an adaptive working surface has finally allowed achieving a "satisfactory" result due to the modern technology. At the same time, having disadvantages in the form of the need for additional tools for work, the doctor was forced to carry out additional manipulations during the treatment process, which, most likely, influenced the manifestations of negative results in the form of complications that affected the quality of life of patients of the 2nd group. As noted, in the group with the use of a separation-holding device for the restoration of the lateral group of teeth of our design, the level of quality of life was as stable as possible and was within the "good" grade. This state of patients in the 3rd group proves the effectiveness of the tool that was developed in terms of the level of treatment provided in the long-term period.

References

1. Gil'miiarov EM, Arnautov BP, inventors. Separatsionno-uderzhivaiushchee ustroistvo dlia restavratsii bokovoi gruppy zubov [Separation and retaining device for the restoration of the lateral group of teeth]. The Russian Federation №153071. 2015 Jun 5. Russian.
2. Baker F, Intagliata J. Quality of life in the evaluation of community support systems. Eval Program Plann. 1982;5(1):69-79. doi: 10.1016/0149-7189(82)90059-3.
3. Straus ShE, Richardson VS, Glatseio P, Heins BR. Meditsina, osnovannaia na dokazatel'stvakh [Evidence-based medicine]. Moscow: GEOTAR-Media; 2010. 322 p. Translation from English by Vlasov VV. Russian.
4. Shpigel' AS, Belousova LG, Haikin MB. Printsipy dokazatel'noi meditsiny kak metodologicheskaia osnova priniatiia klinicheskikh i upravlencheskikh reshenii v stomatologii [Principles of evidence-based medicine as a methodological basis for the adoption of clinical and managerial decisions in dentistry]. Izvestiia Samarskogo Nauchnogo Tsentra Rossiiskoi Akademii Nauk [Bull Samara Sci Cent Russian Acad Sci]. 2015;17(1-3):778-780. Russian.
5. Dubova MA, Salova AV, Hiora ZhP. Rasshirenie vozmozhnostei esteticheskoi restavratsii zubov. Nanokompozity [Expanding the possibilities of aesthetic dental restoration. Nanocomposites]. Sankt Petersburg; 2005. 144 p. Russian.
6. Novik AA, Ionova TI. Rukovodstvo po issledovaniiu kachestva zhizni v meditsine [Guidelines for research on quality of life in medicine]. 2nd ed. Moscow; 2007. 320 p. Russian.
7. Gazhva SI, Guluev RS, Gazhva IuV. Kachestvo zhizni patsientov s zabolevaniiami polosti rta (obzor literatury) [The quality of life in paitents with diseases of oral cavity (review of literature)]. Sovremennye Problemy Nauki i Obrazovaniia [Mod Probl Sci Educ]. 2012;(4):25-27. Russian.
8. Gil'miiarov EM. Monoportsionnaia tekhnika v posteriornoi restavratsii zubov [Monoportioning technique in posterior dental restorations]. Dental magazine. 2013;(9/117):84-86. Russian.
9. Kotelnikov GP, Shpigel' AS. Sistema vnedreniia printsipov dokazatel'noi meditsiny – neobkhodimyi i obiazatel'nyi komponent povysheniia kachestva obrazovaniia v usloviiakh modernizatsii zdravookhraneniia [System of introduction and realization of principles of evidence-based medicine as a necessary and mandatory element of improvement of quality of education in the context of health care modernization] Meditsinskoe Obrazovanie i Professional'noe Razvitie [Med Educ Prof Dev]. 2012;(1):43-50. Russian.
10. Lobovkina LA, Romanov AM. Algoritm esteticheskoi restavratsii perednikh i bokovykh zubov [Algorithm for aesthetic restoration of anterior and lateral teeth]. Moscow: Medpress-Inform; 2008. 47 p. Russian.

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MP conceptualized the idea and revised critically the manuscript, SC completed the final text, KG wrote the first manuscript, SP collected data, EP conducted literature review, CP revised the final text. All the authors approved the final version of the manuscript.

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Ethics approval and consent to participate
The study was approved by the Research Ethics Committee of Dental Association of the Russian Federation (Resolution No 18 of 30.0.2014). An informed consent from all participants in the study was obtained.

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Preliminary results of the cohort study of cognitive impairment associated with Parkinson's disease

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Abstract

Background: Cognitive impairment (CI) is frequent in Parkinson's disease (PD). CI patients have particular features. These are preliminary data of a cohort study of Moldovan patients with incident Parkinson's disease.
Material and methods: 65 out of 111 consecutive PD patients (mean age 64.87 ± 7.69 y.o.; disease duration 50.21 ± 38.61 mo.; 48 women (43.2%), 63 men (56.8%)) underwent Montreal Cognitive Assessment (MoCA) test. Cognitive status graded as: (1) normal and (2) impaired cognition.
Results: There were similar: ages (65.79 ± 7.13 vs 62.17 ± 12.21 y.o.), onset ages (61.44 ± 7.61 vs 57.00 ± 12.95 years), disease duration (49.63 ± 36.78 vs 66.00 ± 26.48) months), levodopa (574.58 ± 129 vs 249.55) and agonists doses (5.19 ± 3.02 vs 1.05 ± 0.05) and Beck scores (8.13 ± 6.21 vs 7.4 ± 3.85), in groups. CI was present in 59 (90.8%) patients; more frequent in patients with cardiovascular risk factors (91.7%, p> 0.05), symmetrical Parkinsonism (93.2%, p> 0.05), and in first disease symptom bradykinesia patients (93.8%, p> 0.05). Upper / Lower Asymmetry Index were lower in CI patients, all lower type patients (p> 0.05) having CI. MoCA scores correlated with Unified Parkinson's Disease Rating Scale in ON fase (UPDRSon), (r = -0.320, p <0.022), and red flags number (r = -0.590, p < 0.006).
Conclusions: Cognitive impairment is more expectable in akinetic, symmetric and lower type Parkinsonism, also in patients with cardiovascular risk factors, with probable PD, and a more motor impairment.
Key words: Parkinson's disease, cognitive impairment.

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Introduction

Cognitive impairment (CI) is frequent in Parkinson's disease (PD) even in early stages and over 75% of PD patients may develop dementia over time [1]. The combination of Lewy pathology and amyloid pathologies is the most robust pathological substrate of PD dementia [2]. Cognitive impairment has been found to be associated with multiple neurotransmitter transmission deficiencies, including dopamine and acetylcholine, indicating a widespread neurotransmitter dysfunction in PD-related dementia [3], but cholinergic deficits due to degeneration of the *nucleus basalis* of Meynert have been the most involved in this process [4]. Cognitively impaired patients have particular clinical features – older age, postural instability-gait disorder phenotype, psychiatric symptoms (psychosis, hallucinations, and depression), sleep disorders, rapid eye movement (REM) sleep behavior disorder, and diurnal sleepiness); all these factors have been associated with PD cognitive worsening [5]. PD patients with comorbid cardiovascular disorders have an extra risk of developing gait and balance disorders and dementia [5]. A study has shown that PD patients with concurrent cerebral microangiopathy fall more frequently and more often have a disturbed pull test than PD patients with normal CT findings [6]. There is a strong association between the severity of age-related vascular changes as white matter lesions and the severity of gait and motor compromise, probably because white matter lesions may interfere with central processing of sensory-motor signals, leading to impaired gait and postural responses, and resulting in an increased frequency of falls [7].

Material and methods

These are preliminary data of a cohort study of Moldovan patients with incident Parkinson's disease. Diagnosis of PD was based on widely acknowledged criteria [8]. Structured interview on complaints, medical history and family history of cardiovascular, neurological and psychiatric diseases and drug history was applied; general neurological and medical examinations were conducted. Severity of parkinsonism and disability were assessed by the Modified Unified Parkinson's Disease Rating Scale (MDS-UPDRS) [9]. Cognitive impairment was ascertained by Montreal Cognitive Assessment (MoCA) Scale [10]. Patients were subclassified at baseline into two groups according to MoCA scores: (1) normal cog-

nition PD patients and (2) cognitively impaired (CI) PD patients. The data analysis was performed via statistical program StatDirect, using descriptive, variation, and correlational analysis. Student's t tests or Mann-Whitney tests were used as appropriate. P values less than 0.05 were considered statistically significant.

Results and discussion

The study comprised 111 consecutive PD patients. The mean age in the cohort was 64.87 ± 7.69 y.o. Patients had mean disease duration of 50.21 ± 38.61 months. Forty-eight patients (43.2%) were women and 63 patients (56.8%) were men.

Sixty-five out of 111 patients underwent MoCA test. The Montreal Cognitive Assessment has adequate psychometric properties as a screening instrument for the detection of mild cognitive impairment or dementia in Parkinson's disease, in particular for language fluency and visuospatial tests, that are more prevalent in PD [10]. According to their MoCA scores, our PD patients were divided in: (1) normal cognition (control group) and (2) cognitive impaired (CI) patients, with a cut-off of 26/30 [10].

Fifty-nine patients (90.8%) of the cognitively tested patients had a MoCA score below 26 and were assigned to the cognitive impaired (CI) group.

Cognitive dysfunction is prevalent in PD. Approximately 10% of a PD population develop dementia per year and the relative risk for developing dementia in PD compared to non-PD subjects ranges from 1.7 - 5.9 [11]. In Buter T.C. et al. study from a total of 233 PD patients, 140 patients (60%, 95% CI 54% to 66%) had developed dementia by the end of the study period, but the cumulative incidence of dementia steadily increased with age and duration of PD to 80% - 90% by the age of 90 years; women lived with PD longer than men and spent more years with dementia [12].

The age of CI patients (65.79 ± 7.13 y.o.) in this study was slightly higher, but similar to those of normal cognition patients (62.17 ± 12.21 y.o., p>0.05). It is known that aging is a risk factor for mild cognitive impairment [11] and the strongest predictive factor of dementia in PD patients [11]. An argument of association of CI and age comes from a functional RMI study [13] which proves age-related reduction of hub function in the medial prefrontal cortex. The results of a study [11] provide, that having mild CI, was associated with: older age at assessment, older age at disease onset, male gender, depression, more severe motor symptoms, and advanced disease stage.

In the present study PD onset ages (61.44 ± 7.61 vs 57.00 ± 12.95 years, p>0.05) and disease duration (49.63 ± 36.78 vs 66.00 ± 26.48 months, p>0.05) in CI patients were similar to normal cognition patients.

Levodopa equivalent daily doses (574.58 ± 129 vs 249.55 mg, p>0.05) and agonists doses (5.19 ± 3.02 vs 1.05 ± 0.05 mg, p>0.05) were slightly higher, but without statistic difference between study and control group. Levodopa (L-dopa) is the gold standard treatment for Parkinson's disease and the use of L-dopa in patients with Parkinsonism with dementia does not adversely affect cognitive function. Molloy S.A. et al. also established in their study, that the mean daily dose of L-dopa was larger in patients with PD dementia (672 (451) mg) than in those with Parkinson's disease (407 (197) mg, p=0.02) with normal cognition [14]. The explanation may be more motor impairment associated with CI in PD.

The most common clinically relevant neuropsychiatric symptoms in PD are apathy (38.9%), depression (34.5%), and anxiety (23.8%) [15]. Depressive symptoms in Parkinson's disease correlate with impaired global and specific cognitive performance [16]. When screened for depression, patients with CI of this study, had insignificantly higher Beck scores than patients without CI (8.13 ± 6.21 vs 7.4 ± 3.85, p>0.05). As usual, depressive symptoms correlate with global cognitive performance, but naming, verbal memory, and language are the most susceptible affected cognitive domains [16].

The present research revealed that CI was more frequent in patients with cardiovascular risk factors (according to QRISK3 list [17]). So, 91.7% of patients having one or more vascular risk factors, had emphasized CI on MoCA test, vs 80.0% of those not reporting vascular risk factors (p> 0.05). According to recent data, a third of all dementia cases may be preventable, due to cardiovascular risk factors, and trials targeting primarily cardiovascular risk factors show improved cognitive function in people at risk [18]. In both sexes, 10-year absolute risk of all-cause dementia increases with increasing age, number of apolipoprotein E (APOE) ε4 alleles, number of genome-wide association studies (GWAS) risk alleles, and presence of cardiovascular risk factors [18].

Forty-one of our patients (93.2%) with symmetrical Parkinsonism (according to their right/left asymmetry index) had CI (vs 18p (85.7%, p> 0.05). In patients reporting bradykinesia as the first disease symptom, 30 patients (93.8%) developed CI, in contrast to 23 patients (85.2%), reporting tremor as the first disease symptom (p> 0.05).

Alves G. et al. found that in nearly all dementia cases, dementia was preceded by PIGD (postural instability gait disorder) or a kinetic dominant or by the transfer from tremor-dominant (TD) to PIGD type PD. The odds ratio for dementia was 56.7 (95% CI: 4.0-808.4; P = 0.003) for patients changing from TD or indeterminate subtype to PIGD subtype, and 80.0 (95% CI: 4.6-1400.1; P = 0.003) for patients with persistent PIGD subtype (compared to patients with persistent TD or indeterminate subtype). Patients with TD subtype at baseline become demented only when they developed PIGD subtype; dementia did not occur among patients with persistent TD subtype of Parkinsonism [19].

Upper / Lower Asymmetry Indexes (0.60 ± 0.37 vs 2.4 ± 0.97, p> 0.05) were lower in CI patients indicating a relative symmetric involvement of limbs.

According to the "α-Synuclein Origin and Connectome Model of Parkinson's Disease Motor Asymmetry", "body-first" patients at onset (initial origin of α-Synuclein pathology in peripheral nervous system spreading bilateral

to brain) already have a larger, more symmetric burden of α-Synuclein pathology, which in turn promotes symmetric faster disease progression and accelerated cognitive decline [20].

In the current study, patients with predominant involvement of lower limbs, according to their superior/inferior asymmetry index, were assigned as lower type Parkinsonism patients. All lower type Parkinsonism patients of this study (15 – 100%) developed CI, in contrast to control (18 – 85.6%), p>0.05.

Estimates of the frequency of lower type Parkinsonism, indicating a vascular origin vary greatly worldwide: from 3% to 6% of all Parkinsonism cases [21]. Attention and memory impairment were found in 73.5% of patients with vascular Parkinsonism after mean disease duration of 4.4±3.4 years in Colosimo C. et al. study [22].

MoCA and Unified Parkinson's Disease Rating Scale in ON fase (UPDRSon) scores in this study were inversely correlated – patients with more severe motor impairment had lower cognitive scores, hence more expressed cognitive impairment ($r = -0.320$, $p < 0.022$).

According to a recent study, female gender and education supported cognitive function in PD, while age and depression scores were inversely correlated with overall cognition; and UPDRS had little influence on cognition; the conclusion of that study being that education, aging, comorbidity, and gender may impact cognition more than overall disease severity in PD [23]. However, another study on 2327 PD patients with a disease duration of over 20 years, found that demented PD patients exhibited more severe motor features compared to non-demented PD patients [24]. Memory impairment was most common (13.3%; 11.6–15.3), followed by visuospatial (11.0%; 9.4–13.0) and attention/executive ability impairment (10.1%; 8.6–11.9); (11.3% 9.7–13.1) were classified as nonamnestic single-domain MCI, 8.9% (7.0–9.9) as amnestic single-domain, 4.8% (3.8–6.1) as amnestic multiple-domain, and 1.3% (0.9–2.1) as nonamnestic multiple-domain. MoCA scores are lower in PD, particularly for language fluency and visuospatial tests [10].

There were recorded the presence and number of red flags, according to MDS clinical diagnostic criteria for Parkinson's disease [25]. The number of red flags also correlated with MoCA scores. number ($r = -0.590$, $p < 0.006$), indicating that a lower degree of certainty of Parkinson's disease diagnosis is associated with a more pronounced cognitive deficit.

Conclusions

According to the presented data, patients with Parkinson's disease and cognitive impairment, displayed no difference regarding ages, onset ages and disease duration compared to the controls. Cognitive impairment was more prevalent in akinetic PD phenotype, as well as in symmetric and lower type Parkinsonism. Patients having cardiovascular risk factors more frequently had cognitive impairment as well; and cognitive dysfunction was associated with more pronounced motor impairment.

References

1. Santangelo G, Vitale C, Picillo M, et al. Mild Cognitive Impairment in newly diagnosed Parkinson's disease: a longitudinal prospective study. Parkinsonism Relat Disord. 2015;21(10):1219-1226. doi: 10.1016/j.parkreldis.2015.08.024.
2. Smith C, Malek N, Grosset K, et al. Neuropathology of dementia in patients with Parkinson's disease: a systematic review of autopsy studies. J Neurol Neurosurg Psychiatry. 2019;90(11):1234-1243. doi: 10.1136/jnnp-2019-321111.
3. Silbert LC, Kaye J. Neuroimaging and cognition in Parkinson's disease dementia. Brain Pathol. 2010;20(3):646-653. doi: 10.1111/j.1750-3639.2009.00368.x.
4. Mattila PM, Röyttä M, Lönnberg P, et al. Choline acetyltransferase activity and striatal dopamine receptors in Parkinson's disease in relation to cognitive impairment. Acta Neuropathol. 2001;102:160-166. doi: 10.1007/s004010100372.
5. Painous C, Marti MJ. Cognitive impairment in Parkinson's disease : what we know so far. Res Reviews Parkinsonism. 2020;10:7-17. https://doi.org/10.2147/JPRLS.S263041.
6. Ebersbach G, Sojer M, Müller J, et al. Gleichgewichtsstörungen bei idiopathischer Parkinson-Erkrankung: Der Einfluss zerebrovaskulärer Komorbidität [Dysequilibrium in Parkinson's disease: influence of cerebrovascular comorbidity]. Nervenarzt. 2002;73:162-165. doi: 10.1007/s00115-001-1234-7. German.
7. Baezner H, Blahak C, Poggesi A, et al. Association of gait and balance disorders with age-related white matter changes: the LADIS study. Neurology. 2008;70(12):935-942. doi: 10.1212/01.wnl.0000305959.46197.e6.
8. Postuma RB, Poewe W, Litvan I, et al. Validation of the MDS clinical diagnostic criteria for Parkinson's disease. Mov Disord. 2018;33(10):1601-1608. doi: 10.1002/mds.27362.
9. Goetz CG, Tilley BC, Shaftman SR, et al. Movement Disorder Society-Sponsored Revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): scale presentation and clinimetric testing results. Mov Disord. 2008;23:2129-2170. doi: 10.1002/mds.22340.
10. Kasten M, Bruggemann N, Schmidt A, et al. Validity of the MoCA and MMSE in the detection of MCI and dementia in Parkinson's disease. Neurology. 2010;75(5):478-479. doi: 10.1212/WNL.0b013e3181e7948a.
11. Aarsland D, Kurz MW. The epidemiology of dementia associated with Parkinson's disease. J Neurol Sci. 2010;289(1-2):18-22. doi: 10.1016/j.jns.2009.08.034.
12. Buter TC, van den Hout A, Matthews FE, et al. Dementia and survival in Parkinson's disease: a 12-year population study. Neurology. 2008;70(13):1017-1022. doi: 10.1212/01.wnl.0000306632.43729.24.
13. Nagano-Saito A, Bellec P, Hanganu A, et al. Why is aging a risk factor for cognitive impairment in Parkinson's disease? - A resting state fMRI study. Front Neurol. 2019;10:267. doi: 10.3389/fneur.2019.00267.
14. Molloy SA, Rowan EN, O'Brien JT, et al. Effect of levodopa on cognitive function in Parkinson's disease with and without dementia and dementia with Lewy bodies. J Neurol Neurosurg Psychiatry. 2006;77(12):1323-1328. doi: 10.1136/jnnp.2006.098079.
15. Hommel ALAJ, Meinders MJ, Lorenzl S, et al. The prevalence and determinants of neuropsychiatric symptoms in late-stage Parkinsonism. Mov Disord Clin Pract. 2020;7(5):531-542. doi: 10.1002/mdc3.12968.
16. Fernandez HH, See RH, Gary MF, et al. Depressive symptoms in Parkinson's disease correlate with impaired global and specific cognitive performance. J Geriatr Psychiatry Neurol. 2009;22(4):223-227. doi: 10.1177/0891988709335792.
17. Hippisley-Cox J, Coupland C, Brindle P. Development and validation of QRISK3 risk prediction algorithms to estimate future risk of cardiovascular disease: prospective cohort study. BMJ. 2017;357:1-21. doi: 10.1136/bmj.j2099.
18. Juul Rasmussen I, Rasmussen KL, Nordestgaard BG, et al. Impact of cardiovascular risk factors and genetics on 10-year absolute risk of dementia: risk charts for targeted prevention. Eur Heart J. 2020;41(41):4024-4033. doi: 10.1093/eurheartj/ehaa695.
19. Alves G, Larsen JP, Emre M, et al. Changes in motor subtype and risk for incident dementia in Parkinson's disease. Mov Disord. 2006;21(8):1123-1130. doi: 10.1002/mds.20897.

20. Borghammer P. The α-Synuclein Origin and Connectome Model (SOC Model) of Parkinson's disease: explaining motor asymmetry, non-motor phenotypes, and cognitive decline. J Parkinsons Dis. 2021;11(2):455-474. doi: 10.3233/JPD-202481.
21. Vale TC, Barbosa MT, Caramelli P, et al. Vascular Parkinsonism and cognitive impairment: literature review, Brazilian studies and case vignettes. Dement Neuropsychol. 2012;6(3):137-144. doi: 10.1590/S1980-57642012DN06030005.
22. Colosimo C, Morgante L, Antonini A, et al. Non-motor symptoms in atypical and secondary Parkinsonism: the PRIAMO study. J Neurol. 2010;257(1):5-14. doi: 10.1007/s00415-009-5255-7.
23. Lin SJ, Baumeister TR, Garg S, et al. Cognitive profiles and hub vulnerability in Parkinson's disease. Front Neurol. 2018;9:1-13. doi: 10.3389/fneur.2018.00482.
24. Szeto JYY, Walton CC, Rizos A, et al. Dementia in long-term Parkinson's disease patients: a multicentre retrospective study. NPJ Park Dis. 2020;6:2. doi: 10.1038/s41531-019-0106-4.
25. Postuma RB, Berg D, Stern M, et al. MDS clinical diagnostic criteria for Parkinson's disease. Mov Disord. 2015;30(12):1591-1601. doi: 10.1002/mds.26424.

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AC conceptualized the idea, conducted literature review, wrote the manuscript, revised and finalized the text.

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The importance of matrix metalloproteinases in the prognosis of acute ischemic stroke patients

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Abstract

Background: Ischemic stroke is one of the leading causes of mortality and disability worldwide. Numerous studies were performed to assess the risk of clinical deterioration of acute ischemic stroke patients, including the risk of haemorrhagic transformation. The complexity of cerebral ischemia pathology raised the possibility of a multitude of candidate-molecules to be studied as stroke biomarkers. The blood brain barrier integrity biomarkers have shown promising results both in fundamental and clinical studies. Matrix metalloproteinases have been extensively analysed and gave encouraging results for predicting unfavourable neurological outcome, including the risk for haemorrhagic transformation. Matrix metalloproteinase-9 plays a crucial role in the disruption of the blood-brain barrier following focal cerebral ischemic stroke. Elevated matrix metalloproteinase-2 levels are responsible for the degradation of tight junction proteins, basal lamina and neuronal injury after ischemia, and may contribute to infarction and hemorrhagic volume. The review provides an overview of matrix metalloproteinases' role in the prognosis of acute ischemic stroke patients, regarding the stroke outcome and the risk of haemorrhagic transformation.
Conclusions: Matrix metalloproteinases, especially gelatinases, are extensively studied for their predictive value in ischemic stroke evolution. Matrix metalloproteinase-2 and matrix metalloproteinase-9 correlate with stroke severity and haemorrhagic transformation in acute ischemic stroke, but large validation studies are needed for practical translation. Future studies should focus on developing a biomarker panel for predicting outcomes in stroke patients.
Key words: cerebrovascular accident, matrix metalloproteinases, stroke outcome.

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Introduction

Ischemic stroke (IS) is one of the leading causes of mortality and disability worldwide, with an important increase in low-middle income countries for the last 20 years [1]. Moreover, stroke has the tendency to affect younger people in the less developed countries with consecutive impact on economic and social levels. There are numerous targets to improve in the management of ischemic stroke, starting with the population awareness about stroke and emergency service priority for such patients, continuing with the necessity of comprehensive stroke centres for acute/24h diagnosis and acute treatment, and finishing with appropriate long-term rehabilitation centres for in- and outpatients [2].

Given the limited therapeutic options for the acute ischemic stroke patients – chemical and mechanical thrombolysis, the multitude of exclusion criteria, and the limited time-window, only one in five patients (according to international data) or even less – one in ten patients (according to national data, in hospitals with stoke units), will receive specific treatment for cerebral ischemia [3]. These little encouraging data can become even worse when regarding the possible complications of thrombolysis, including haemorrhagic transformation (HT) [3-7]. HT of ischemic stroke represents the bleeding in the infarcted area of the brain after IS. It can occur in up to 70% of cases of cerebral ischemia – by radiologic and morphologic studies, with a various percentage of symptomatic HT (HT associated with worsening of the neurological status) – from 1 to 20% [8].

Numerous studies were performed to assess the risk of clinical deterioration of acute IS patients, including the risk of HT [9]. Various parameters were analysed, including clinical manifestations, radiologic features, laboratory factors, in a separate way, either in combined tools – scores [10-12].

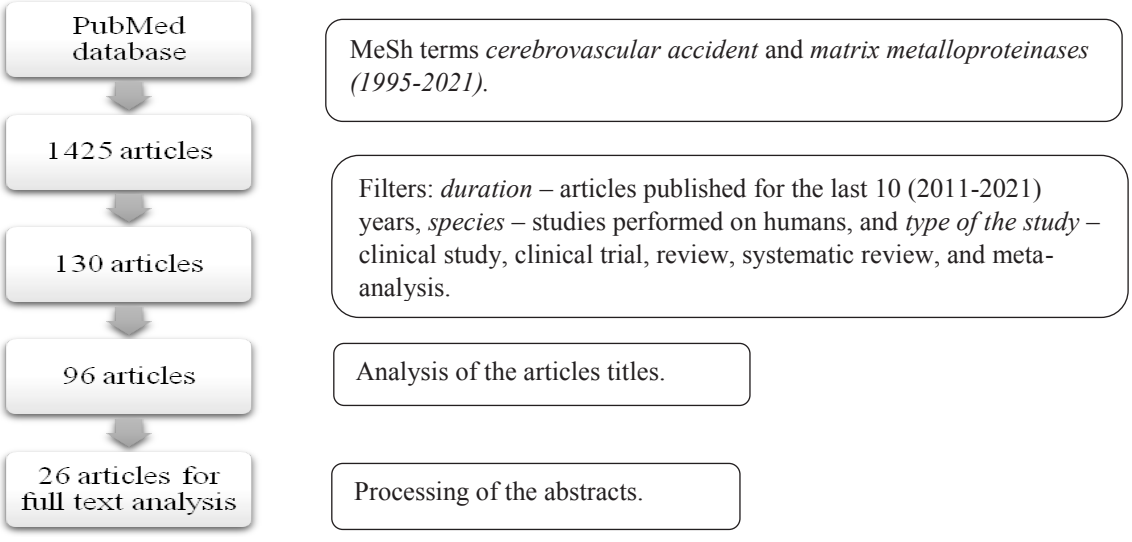

Fig. 1. Algorithm of literature research

Given the acute onset of cerebral ischemia and the limited time for diagnosis, a high percentage of the researches focused on finding the "gold-molecule" able to identify and/ or predict the evolution of patients with IS, similar to the cardiac troponins – so-called cerebral infarction biomarker [13]. *Biomarkers* are considered "signatures" of different biological processes, able to identify, stratify, predict the evolution of the assessed events.

The complexity of cerebral ischemia pathology raised the possibility of a multitude of candidate-molecules to be studied as stroke biomarkers. A PubMed search of stroke and biomarkers key words reveals an important number of studies and publications in the field (more than 15 thousand), starting with 1 article in the 80-s to up to 1662 papers in 2020. The studied molecules can be classified by the target process in the stroke pathology, but also, even more important for clinical activity, by the potential use of the biomarker.

The classification according to the affected pathway in the IS pathogenesis includes molecules related to: inflammation/oxidative stress, vasoreactivity, coagulation/ fibrinolysis disorders, and to the brain-blood barrier (BBB) disruption. The potential clinical use of the stroke biomarkers includes the following categories: prediction of stroke risk, determination of stroke mechanisms, diagnosis of cerebral vein thrombosis, prediction of infarct volume, prediction of stroke outcome, estimation of in-hospital complications risk, determination of the response to therapy [13, 14].

Regarding the response to therapy and stroke outcome, the BBB integrity biomarkers have shown promising results both in fundamental and clinical studies. Among them, matrix metalloproteinases (MMPs) have been extensively analysed and gave good results both for predicting the answer to treatment, and also for unfavourable neurological outcome, including the risk for haemorrhagic transformation [13].

The **aim** of this research was the review of matrix metalloproteinases' role in the prognosis of acute ischemic stroke patients, regarding the stroke outcome and the risk of haemorrhagic transformation.

Material and methods

The online research using the PubMed database has been conducted. There have been used the MeSh terms: cerebrovascular accident and matrix metalloproteinases, obtaining the initial number of 1425 articles for the combined searched terms (1995-2021). To restrain the number of the studies, there have been applied the following filters: duration – articles published within the last 10 years (2011-2021), species – studies performed on humans, and type of the study – clinical study, clinical trial, review, systematic review, and meta-analysis. The reasoning for the applied filters was to reveal the most recent researches, with the highest clinical application and feasibility. The selection revealed 130 articles. Individual analysis of the articles titles was thereafter done, with consecutive exclusion of the articles focusing on other cerebrovascular pathologies (e.g. cerebral sinuses thrombosis, subarachnoid haemorrhage), articles in other languages than English, studies of the different diagnosis tools (e.g. laboratory devices). For the remaining 96 articles, abstracts were processed, with final selection of 26 articles for full text analysis (fig. 1).

Nine supplementary articles on complementary data for our research – stroke epidemiological data, blood-brain barrier structure – were included.

Results and discussion

The sudden blockage of a cerebral vessel leads to immediate and delayed pathological processes linked with oxygen and glucose delivery failure – a cascade of ischemia-induced pathological events, that ultimately lead to irreversible neuronal injury – neuronal death [7, 15]. At each stage of the IS pathogenesis, numerous molecules are involved with sequential plasma increase/decrease, making them potential

biomarkers for stroke. As biomarkers, the molecules should be easily measurable, specific and sensitive [16].

Emphasising the most important steps in the cerebral ischemia, the acute energy failure caused by vessels blockage will determine at first a burst of excitotoxicity (by activation of N-methyl-aspartate receptors and calcium channels), which leads to the activation of inflammatory response (synthesis of pro-inflammatory cytokines as tumor necrosis factor α (TNF-α), interleukine 1β (IL-1β), activation of neural nitric oxide (NO) synthase, reactive oxygen species synthesis, oedema and finally neurons death (apoptosis). TNF-α and IL-1β synthesis will trigger a second line cytokine expression with release of interleukine (IL-6) and chemokine CXCL-8. These pro-inflammatory agents can increase the C-reactive protein level and start the expression of numerous matrix metalloproteinases [1, 16, 17].

Matrix metalloproteinases are a family of zinc-dependent endoproteases with multiple roles in tissue remodelling and degradation of various proteins in the extracellular matrix (ECM): collagen, proteoglycans, elastin or fibronectin. MMPs promote inflammatory response, cell proliferation, migration, and differentiation. Among MMPs, gelatinases (MMP-2 and MMP-9) are the most investigated enzymes, given the high prevalence and widespread distribution in different tissues (e.g. endothelium, intima, vascular adventitia, fibroblasts, platelets, macrophages, neutrophils, brain, heart, lungs, liver, kidney, breast, uterus, placenta, ovary, testis, prostate, tooth enamel, skin, keratinocytes) [16]. Therefore, MMP-2 and MMP-9 are studied in various medical fields. In neurology, gelatinases were investigated related to stroke, multiple sclerosis, Alzheimer disease, Parkinson disease, neuroinfections, brain tumors and others [16-18].

Characteristics of matrix metalloproteinases

In the early 1960s, MMPs were first identified by Gross and Lapiere as enzymes with collagen proteolytic activity that causes extra-cellular matrix protein degradation during resorption of the tadpole tail [5,13]. MMPs are calcium-dependent zinc-endopeptidases, which are expressed as inactive zymogens with a pro-peptide domain (pro-MMPs) that must be removed for MMP activation [18-20].

At present, time, in vertebrates, there are 28 members in the MMP family, at least 23 are expressed in human tissues [6, 21], and 14 of those – in the blood vessels. Based on the variations in MMPs biochemical structure and affinity to substrates, they were classified into several groups including [16, 17]:

1. Collagenases (MMP-1, MMP-8, MMP-13, MMP-18),
2. Gelatinases (MMP-2, MMP-9),
3. Stromelysins (MMP-3, MMP-10, MMP-11),
4. Matrilysins (MMP-7, MMP-26),
5. Membrane-type matrix metalloproteinases (MMP-14, MMP-15, MMp-16, MMP-17, MMP-24, MMP-25),
6. Other MMPs (MMP-12, MMP-19, MMP-20, MMP-21, MMP-23, MMP-27, MMP-28).

The majority of MMPs are produced and secreted from cells in the inactive form (proenzymes) with consecutive activation in the final active form within the extracellular matrix [16]. After activation, their activity is regulated mainly by natural tissue inhibitors of MMPs (TIMPs) that can bind to the active side and block the substrate availability. To date, four types of tissue inhibitors of MMPs are known, numbered from 1 to 4. Each TIMP can inhibit multiple MMPs with different efficacies [16, 17, 20]. Among four known TIMPs, TIMP-1 has the highest affinity to MMP-9, and TIMP-2 for MMP-2 [1, 22].

The role of gelatinases in the progress of IS

The plasma concentration of gelatinases varies during IS, both in the acute and later phases of cerebral ischemia [1, 16].

The concentration of MMP-9 is elevated in serum during acute phase of all types of ISs, starting from 12h up to 48h after stroke onset, and are increasingly correlated with neurological deterioration [23]. Among stroke patients, the cardioembolic stroke results in the highest MMP-2 concentrations compared to other types of stroke [16].

MMP-9 could have a prognosis value for ischemic stroke occurrence, as shown by a recent study conducted on patients with attrial fibrilation [24]. From 268 unique biomarkers, only 6 were most strongly associated with subsequent ischemic stroke/systemic embolism, among them matrix metalloproteinase-9.

The following main functions/processes are influenced by gelatinases activity during IS [16, 17, 21, 25, 26]:

1. Brain-blood-barrier (BBB) destruction – in addition to cleavage of collagen type IV, MMP-9 is able to digest occludin and claudin – essential components of tight junction proteins (TJPs) in the BBB. Finally, the high activity of MMP-9 in the blood within the acute phase of IS increases the risk of secondary bleeding within the ischemic focus. Moreover, the appearance of active enzyme form in plasma, as a result of rtPA administration, augments the risk of intracranial bleeding.

2. Inflammatory answer – TNF-α and IL-6 can activate the expression of MMP-9 which is involved into further activation of IL-1β and CXCL-8.

3. Formation of glial scar – mechanism of the second gelatinase, MMP-2.

MMP-9 plays a crucial role in the disruption of the BBB following focal cerebral ischemic stroke. Elevated MMP-2 levels were responsible for the degradation of tight junction proteins, basal lamina and neuronal injury after ischemia, and may contribute to infarction and hemorrhagic volume [1, 15, 27]. MMP-2 deficiency reduced the incidence of hemorrhage in the cortex in mouse [6]. Fundamental studies on mice with early reperfusion suggested that MMP-2 deficiency as well as MMP-2 and MMP-9 double deficiency were more protective than MMP-9 deficiency alone against HT after the early stages of ischemia and reperfusion [1].

The pro-inflammatory action of the MMPs have been shown to correlate with the atherosclerotic plaques rupture, and therefore responsible for cardio- and cerebrovascular events. Given the MMP-2 action substrate (structural components of the sub-endothelium of medium and large-size arteries, like gelatin, fibronectin, laminin-1, type IV colla-

gen and elastin), its role in vascular remodeling, neutrophil and platelet activation, numerous studies have been conducted to analyze the borderline between physiologic and pathologic hemostatic answer at the levels of atherosclerotic plaques promoted by these enzymes [28]. For example, Lenti M. et al. have demonstrated that atherosclerotic plaques of patients undergoing carotid endarterectomy determine platelet activation due to their high content in active MMP-2, the effect being confirmed by abolishment after preincubation of platelets with MMP-2 inhibitors [28].

Gelatinases and HT

HT can occur in the natural evolution of ischemic stroke, but more frequently is a complication of rt-PA thrombolysis in the treatment of acute ischemic stroke.

Radiologically, HT is defined and divided into 4 subtypes: small petechial hemorrhagic infarction (HI1), confluent petechial hemorrhagic infarction (HI2), small parenchymal hemorrhage (PH1, < 30% of infarct, mild mass effect), and large parenchymal hemorrhage (PH2, > 30% of infarct, marked mass effect) [29]. Clinically, HT is divided in: asymptomatic HT (no clinical worsening on the National Institutes of Health Stroke Scale (NIHSS) score despite HTs), minor symptomatic HT (a 1 to 3-point increase in the NIHSS score), and major symptomatic HT (a ≥ 4-point increase in the NIHSS score) [29]. Despite the "asymptomatic" term, long-term prognosis is poorer in ischemic stroke patients with all clinical types of HT.

Structurally, HT of IS occurs when blood-brain barrier is disrupted. The BBB separates the brain parenchyma and the blood circulation, providing anatomical and physiological protection for the central nervous system, supplying nutrition for brain tissue, filtering harmful substances from the brain back to the blood and protecting the brain from toxic material in the blood [25, 29].

The rupture of BBB damages the whole neurovascular unit (NVU), which consists of the extracellular matrix, endothelial cells, astrocytes, neurons, and pericytes [5, 21, 29]. The main mechanisms of HT are considered related to proteolysis, oxidative stress and leukocyte infiltration.

HT can occur spontaneously, especially in case of cerebral embolism, either induced by anticoagulants, thrombolytic therapy and endovascular procedures [23, 30]. All these situations are associated with an increased degradation of the extracellular matrix components by proteolytic enzymes, particularly MMPs, which aggravates brain edema and enhances brain damage.

Different studies applied MMP-9/TIMP-1 ratio as an indicator of MMP-9 activity *in vivo*. The proteolytic activation of MMP-9 is conducted by active forms of other metaloproteinases as MMP-2 or MMP-3. The activation of MMP-9 facilitates the BBB destruction and secondary bleeding within the ischemic focus [22, 31]. MMP-2 and MMP-9 have different temporal expression and action in the post-stroke period. It was established that MMP-2 is involved in the initial phase of BBB opening (maximum at 3h), while MMP-9 is more active in the delayed opening of the BBB after ischemic stroke [6].

Given the higher incidence of HT after thrombolytic treatment with rt-PA, numerous fundamental studies were conducted to elucidate the mechanisms underlying this phenomenon. Thus, it was established that when NVU is impaired, rt-PA may cross the brain and activate endogenous tPA signaling pathways associated with HT. t-PA mostly affects the BBB through various plasminogen independent mechanisms, such as the overexpression of MMP-2, -3 and -9 and activation/cleavage of lipoprotein receptor related protein or platelet-derived growth factor receptor alpha [15, 29].

Both fundamental and clinical studies have found an elevation of gelatinases MMP-2 and MMP-9 in the ischemic brain/plasma of IS patients within the first 24h, correlating with cerebral infarction extension, worse outcome and HT. Preclinical studies demonstrated that MMP-2 plays a key role in the initial opening of the BBB after cerebral ischemia by degradation of tight junction proteins, collagen, and occludin. According to literature data, the most extensively studied gelatinase in relation to IS and HT, MMP-9 can serve as independent predictor of HT after tPA administration [5, 22].

A systematic review (Ramos-Fernandez M. et al., 2011) analyzed 22 clinical studies, including 3289 patients, to evaluate the role of MMP-9 plasma level in acute ischemic stroke. According to this analysis, plasma MMP-9 level, measured before the administration of thrombolytic therapy in acute stroke patients, accurately predicts the development of hemorrhage [32].

Importantly, an analysis of non-selected series of patients has shown that an MMP-9 value ≥140 ng/mL within 24h of stroke onset had a high negative predictive value for future HT. The data suggest that a lower plasma level of MMP-9 is associated with a low risk of HT [23, 32, 33].

Experimental studies strengthen the correlation of both MMP-2 and MMP-9 with HT, with even a higher association of MMP-2 with HT, given its action mechanism by degradation of tight-junction proteins and the basal lamina, BBB disruption and neuronal injury [23].

The fundamental studies raised the idea of MMPs inhibition for improving clinical outcome of IS patients. Numerous molecules have been analyzed in clinical settings to inhibit gelatinases, including minocycline, (4-phenoxyphenylsulfonyl) methylthiirane (known as SB-3CT), lentiviral-mediated MMP-9 gene silencing, recombinant TIMP-1 in its native form, PLGA (poly lactic-co-glycolic acid) nanoparticles [5], majority of them showing that inhibition of MMP-9 could be a therapeutic strategy for acute ischemic stroke treatment, but with a limited time-window after which MMP inhibitors have negative impacts on stroke patients [6, 12].

Stroke severity and functional outcome versus MMPs levels

Stroke severity and final infarct volume are among the most important factors associated with functional outcomes in patients after IS. The most available and widely used tools are those clinical – NIHSS score and radiological – CT and

MRI protocols and scores. While their sensibility and specificity are high, not all hospitals, especially from rural areas, have 24h available radiological service. Therefore, the use of blood biomarkers to predict stroke patient's evolution could be a good supplement for the clinical assessment and treatment rationale in these cases.

Different biomarkers show good correlation with stroke clinical severity and infarct size, and may be useful for predicting poorer outcomes [23, 31, 32, 34].

In a review on MMPs' role in BBB breakdown during acute ischemic stroke, Lakhan S. et al., 2013, have determined that cerebral infarct size is reduced in mice deficient in MMP-9 or after treatment with MMP inhibitor [1].

MMP-2/-9 levels, when collected within 20h of stroke onset, showed a direct, significant correlation with both initial and final stroke severity, as measured by the NIHSS and infarct size. When collected earlier (within 6h of stroke onset), MMP-9 was the only predictor of infarct volume measured as a diffusion lesion [1, 23].

Consecutively, in an Italian study [31], including 327 tPA-treated patients, MMP-9 circulating level variation proved association, independent of major clinical determinants, with symptomatic HT or death. The MMP-9 serum levels correlated with the NIHSS values prior and after thrombolytic treatment.

In an extensive review by Turner R. et al., 2016, the authors highlithed that, in terms of long-term outcomes, MMP-9 was associated with a poor neurological outcome at 3 months post-stroke and hyperacute levels of MMP-9 correlated with worse Rankin outcome at 3 months post-stroke. The review mentions one single study to report that both MMP-2 and MMP-9 levels correlated with clinical severity and the extent of the infarct [21].

Iemolo F. et al., 2016, realized a prospective evaluation of a panel of blood biomarkers to assess their value in acute stroke prognosis. The panel included the following molecules: Brain Natriuretic Peptide, D-Dimers, Matrix-Metalloproteinase-9, and S100β protein generating a Multimarker index of these values. The outcome of the study was 120-day mortality. Among 244 patients included in the analysis, 161 (66.0 %) had an increase of biomarkers [34]. However, detailed statistical analysis failed to give significant correlations. The authors concluded that neither one marker nor combination of all markers was of significant benefit in acute stroke diagnostics, and DWI-MRI was the procedure with the highest diagnostic quality in case of acute cerebral ischemia.

Another study was conducted by Zhong et al., 2017, to prospectively investigate the association between serum MMP-9 levels and prognosis in patients with acute ischemic stroke using data from the China Antihypertensive Trial in Acute Ischemic Stroke (CATIS). This large study included 3.186 participants (2.008 men and 1.178 women). Study outcome data on death, major disability (modified Rankin Scale score ≥3), and vascular disease were collected at 3 months after stroke onset. The authors observed dose-response associations between higher MMP-9 levels in acute ischemic stroke and increased risk of major disability and death at 3 months after stroke onset, after adjustment for other established covariates [35].

Conclusions

It has been detected that the involvement of gelatinases into multiple physiological and pathological processes makes them an attractive target for investigations. Numerous researches revealed that both enzymes, MMP-2 and MMP-9 have their specific role during brain ischemia. Promising results are shown by both fundamental and clinical studies analyzing the predictive value for hemorrhagic transformation of gelatinases. Thus, MMP-2 and MMP-9 correlate with stroke severity and haemorrhagic transformation in acute ischemic stroke, but large validation studies for practical translation are needed.

Given the etiological heterogeneity of ischemic strokes, the variety of clinical manifestations, it is challenging to identify one single predictive biomarker for stroke outcome. Future studies should focus on developing a biomarker panel for predicting outcomes in stroke patients presenting with cerebrovascular accident.

References

1. Lakhan SE, Kirchgessner A, Tepper D, Leonard A. Matrix metalloproteinases and blood-brain barrier disruption in acute ischemic stroke. Front Neurol. 2013 Apr;4:1-15. doi: 10.3389/fneur.2013.00032.
2. Johnson W, Onuma O, Owolabi M, Sachdev S. Stroke: a global response is needed. Bull World Health Organ. 2016;94(9):634A-635A. doi: 10.2471/BLT.16.181636.
3. Manole E, Lisnic V, Groppa S, Costru-Tasnic E, Filioglo A, Odainic O, et al. Registrul RES-Q în Republica Moldova – primele rezultate naționale în cadrul unui proiect internațional [RES-Q registry in the Republic of Moldova – first national results within an international project]. [Bull Acad Sci Moldova. Sci Med]. 2017;(5):72-77. Romanian.
4. Berezowski V, Fukuda AM, Cecchelli R, Badaut J. Endothelial cells and astrocytes: a concerto en duo in ischemic pathophysiology. Int J Cell Biol. 2012;2012:176287. doi: 10.1155/2012/176287.
5. Wang W, Li M, Chen Q, Wang J. Hemorrhagic transformation after tissue plasminogen activator reperfusion therapy for ischemic stroke: mechanisms, models, and biomarkers. Mol Neurobiol. 2015 Dec 4;52(3):1572-9. doi: 10.1007/s12035-014-8952-x.
6. Sarvari S, Moakedi F, Hone E, Simpkins JW, Ren X. Mechanisms in blood-brain barrier opening and metabolism-challenged cerebrovascular ischemia with emphasis on ischemic stroke. Metab Brain Dis. 2020;35(6):851-68. doi: 10.1007/s11011-020-00573-8.
7. Li Y, Zhong W, Jiang Z, Tang X. New progress in the approaches for blood-brain barrier protection in acute ischemic stroke. Brain Res Bull. 2019;144:46-57. doi: 10.1016/j.brainresbull.2018.11.006.
8. Zhang J, Yang Y, Sun H, Xing Y. Hemorrhagic transformation after cerebral infarction: current concepts and challenges. Ann Transl Med. 2014;2(8):81. doi: 10.3978/j.issn.2305-5839.2014.08.08.
9. Sung SF, Chen SCC, Lin HJ, Chen YW, Tseng MC, Chen CH. Comparison of risk-scoring systems in predicting symptomatic intracerebral hemorrhage after intravenous thrombolysis. Stroke. 2013;44(6):1561-6. doi: 10.1161/STROKEAHA.111.000651.
10. Jha R, Battey TWK, Pham L, Lorenzano S, Furie KL, Sheth KN, et al. Fluid-attenuated inversion recovery hyperintensity correlates with matrix metalloproteinase-9 level and hemorrhagic transformation in acute ischemic stroke. Stroke. 2014 Apr;45(4):1040-5. doi: 10.1161/STROKEAHA.113.004627.
11. Kim Y, Kim YK, Kim NK, Kim SH, Kim OJ, Oh SH. Circulating matrix metalloproteinase-9 level is associated with cerebral white matter hyper-

intensities in non-stroke individuals. Eur Neurol. 2014;72(3-4):234-40. doi: 10.1159/000362876.
12. Chang JJ, Emanuel BA, Mack WJ, Tsivgoulis G, Alexandrov AV. Matrix metalloproteinase-9: dual role and temporal profile in intracerebral hemorrhage. J Stroke Cerebrovasc Dis. 2014;23(10):2498-505. doi: 10.1016/j.jstrokecerebrovasdis.2014.07.005.
13. Polivka J, Polivka J, Krakorova K, Peterka M, Topolcan O. Current status of biomarker research in neurology. EPMA J. 2016;7(1):14. doi: 10.1186/s13167-016-0063-5.
14. Lu G, He Q, Shen Y, Cao F. Potential biomarkers for predicting hemorrhagic transformation of ischemic stroke. Int J Neurosci. 2018;128(1):79-89. doi: 10.1080/00207454.2017.1349766.
15. Miao Y, Liao JK. Potential serum biomarkers in the pathophysiological processes of stroke. Expert Rev Neurother. 2014 Feb 13;14(2):173-85. doi: 10.1586/14737175.2014.875471.
16. Kurzepa J, Kurzepa J, Golab P, Czerska S, Bielewicz J. The significance of matrix metalloproteinase (MMP-2) and MMP-9 in the ischemic stroke. Int J Neurosci. 2014;124(10):707-16. doi: 10.3109/00207454.2013.872102.
17. Cui N, Hu M, Khalil RA. Biochemical and biological attributes of matrix metalloproteinases. Prog Mol Biol Transl Sci. 2017;147(617):1-73. doi: 10.1016/bs.pmbts.2017.02.005.
18. Romi F, Helgeland G, Gilhus NE. Serum levels of matrix metalloproteinases: implications in clinical neurology. Eur Neurol. 2012;67(2):121-8. doi: 10.1159/000334862.
19. Rempe RG, Hartz AMS, Bauer B. Matrix metalloproteinases in the brain and blood-brain barrier: versatile breakers and makers. J Cereb Blood Flow Metab. 2016;36(9):1481-507. doi: 10.1177/0271678X16655551.
20. Chang JJ, Stanfill A, Pourmotabbed T. The role of matrix metalloproteinase polymorphisms in ischemic stroke. Int J Mol Sci. 2016;17(8):1323. doi: 10.3390/ijms17081323.
21. Turner RJ, Sharp FR. Implications of MMP-9 for blood-brain barrier disruption and hemorrhagic transformation following ischemic stroke. Front Cell Neurosci. 2016;10:1-13. doi: 10.3389/fncel.2016.00056.
22. Gołąb P, Boguszewska-Czubara A, Michal K, Kurzepa J. The rtPA increases MMP-9 activity in serum during ischaemic stroke. Neurol Neurochir Pol. 2014;48(5):309-14. doi: 10.1016/j.pjnns.2014.07.012.
23. Maestrini I, Ducroquet A, Moulin S, Leys D, Cordonnier C, Bordet R. Blood biomarkers in the early stage of cerebral ischemia. Rev Neurol (Paris). 2016;172(3):198-219. doi: 10.1016/j.neurol.2016.02.003.
24. Hijazi Z, Wallentin L, Lindbäck J, Alexander JH, Connolly SJ, Eikelboom JW, et al. Screening of multiple biomarkers associated with ischemic stroke in atrial fibrillation. J Am Heart Assoc. 2020;9(24):e018984. doi: 10.1161/JAHA.120.018984.
25. Thomsen MS, Routhe LJ, Moos T. The vascular basement membrane in the healthy and pathological brain. J Cereb Blood Flow Metab. 2017;37(10):3300-17. doi: 10.1177/0271678X17722436.
26. Jin R, Yang G, Li G. Molecular insights and therapeutic targets for blood–brain barrier disruption in ischemic stroke: critical role of matrix metalloproteinases and tissue-type plasminogen. Neurobiol Dis. 2010;38(3):376-85. doi: 10.1016/j.nbd.2010.03.008.
27. Yang Y, Rosenberg GA. Blood-brain barrier breakdown in acute and chronic cerebrovascular disease. Stroke. 2011;42(11):3323-8. doi: 10.1161/STROKEAHA.110.608257.
28. Lenti M, Falcinelli E, Pompili M, Rango P De, Conti V, Guglielmini G, et al. Matrix metalloproteinase-2 of human carotid atherosclerotic plaques promotes platelet activation: correlation with ischaemic events. Thromb Haemost. 2014;111(6):1089-101. doi: 10.1160/TH13-07-0588.
29. Liu C, Xie J, Sun S, Li H, Li T, Jiang C, et al. Hemorrhagic transformation after tissue plasminogen activator treatment in acute ischemic stroke. Cell Mol Neurobiol. 2020 Oct 30. doi: 10.1007/s10571-020-00985-1.
30. Wang L, Wei C, Deng L, Wang Z, Song M, Xiong Y, et al. The accuracy of serum matrix metalloproteinase-9 for predicting hemorrhagic transformation after acute ischemic stroke: a systematic review and meta-analysis. J Stroke Cerebrovasc Dis. 2018;27(6):1653-65. doi: 10.1016/j.jstrokecerebrovasdis.2018.01.023.
31. Inzitari D, Giusti B, Nencini P, Gori AM, Nesi M, Palumbo V, et al. MMP9 variation after thrombolysis is associated with hemorrhagic transformation of lesion and death. Stroke. 2013;44(10):2901-3. doi: 10.1161/STROKEAHA.113.002274.
32. Ramos-Fernandez M, Bellolio MF, Stead LG. Matrix metalloproteinase-9 as a marker for acute ischemic stroke: a systematic review. J Stroke Cerebrovasc Dis. 2011;20(1):47-54. doi: 10.1016/j.jstrokecerebrovasdis.2009.10.008.
33. Castellanos M, Sobrino T, Millán M, García M, Arenillas J, Nombela F, et al. Serum cellular fibronectin and matrix metalloproteinase-9 as screening biomarkers for the prediction of parenchymal hematoma after thrombolytic therapy in acute ischemic stroke: a multicenter confirmatory study. Stroke. 2007;38(6):1855-9. doi: 10.1161/STROKEAHA.106.481556.
34. Iemolo F, Sanzaro E, Duro G, Giordano A, Paciaroni M. The prognostic value of biomarkers in stroke. Immun Ageing. 2016;13(1):1-6. doi: 10.1186/s12979-016-0074-z.
35. Zhong C, Yang J, Xu T, Xu T, Peng Y, Wang A, et al. Serum matrix metalloproteinase-9 levels and prognosis of acute ischemic stroke. Neurology. 2017;89(8):805-12. doi: 10.1212/WNL.0000000000004257.

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HPV+ and HPV- head and neck squamous cell carcinoma by analysis of tumor microenvironment

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Abstract

Background: Head and neck squamous cell carcinomas (HNSCCs) are particularly aggressive epithelial tumors, that affect more than half a million patients worldwide each year. They represent a multi-factorial group of tumors caused by: alcohol, tobacco, and human papillomavirus (HPV) infections. Over the last ten years the overall 5-year survival rate of HNSCCs remained ~40–50%, inspite of significant improvement in clinical outcome of many tumor types. There are recent data that claim how some of these cells fulfill a suppressive role in the antitumor immune response. It is interesting that new clinical studies demonstrated that HPV (+) HNSCCs were among tumors with the highest immune infiltrates, while HPV (-) presented a reduced number of immune infiltrating cells.

Conclusions: Recent researches prove that tumor microenvironment of HNSCC has an important role in tumor progression, aggressivity, metastasis process, in addition to genetic aberrations and molecular alterations of cancer cells. New researches in stromal composition of the HNSCC may be useful in understanding of mechanisms of different responses to therapy, also can be used as a target for therapeutic purposes. Cancer-associated fibroblasts and immune cells, as well as their products found in neck squamous cell carcinoma significantly influence the biological properties of this tumor. Smoking is one of the risk factors of occurrence of most HPV-associated tumors. Promoting smoking cessation should become an essential contributor to the treatment of cancer in all oncologic pathologies. In cases when patients can't quit smoking completely within the shortest possible period of time, doctors should focus on harm reduction strategies – tobacco harm reduction.

Key words: head and neck cancer, squamous cell, tumor microenvironment, smoking, tobacco harm reduction.

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Introduction

Approximately 90% of head and neck cancers are squamous cell carcinomas [1, 2]. Squamous cell carcinomas (SCC) are the frequent malignant tumor of oral cavity, being responsible for a high number of cancer-related deaths worldwide [3]. In Western Europe, most head and neck squamous carcinomas (HNSCCs) have as etiological factors alcohol and tobacco (for oral cancers), but infection with high-risk human papilloma viruses (HPVs) is also now determined as an important etiological factor particularly for oropharynx [4-6]. According to the report published by GLOBOCAN 2018 (global cancer statistics), more than 800000 new HNSCC cases are diagnosed each year. Currently, the majority of head and neck cancers are present with regionally advanced lymph node metastases at the time of diagnosis. The patients are often given the standard treatment options of surgery, radiotherapy, chemotherapy, or a combination of these interventions, but 40–60% of treated patients experience recurrence and are irresponsive to subsequent therapeutic interventions [7]. Therefore, despite the significant improvement in overall survival for patients with other tumor types, the 5-year rate of HNSCCs has not changed much over the past decade.

Discussion

The aim of this review is to present the role and effect of noncancerous cells and their crosstalks with cancer cells. Furthermore it will describe the metabolic changes in tumor microenvir-onment, the new data about how some of these cells accomplish a suppressive role in the antitumor immune response. Finally, it will show the clinical significance of all these factors according to the actual literature.

Overview on the tumor microenvironment (TME)

Head and neck squamous cell carcinoma is genetically a group of heterogeneous tumors. The tumor microenvironment represents an interactive, organized, and dynamic environment where cancerous cells as well as many different cellular and biochemical structures exist together and are continuously in contact and interact with each other [8]. Squamous cell carcinomas microenvironment includes lymphocytes, macrophages, dendritic cells, vascular cells, and stromal cells.

The cellular elements of the TME often develop abnormal phenotypes in a spoil response to the cancer. Genetic changes in the carcinoma cells, like alterations of TP53, NOTCH1, and specific gene expression profiles, contribute to derangements in cancer and microenvironment cells increased reactive oxygen species (ROS), overproduction of cytokines, and epithelial to mesenchymal transition [8].

Stromal Cell Contributions

Besides tumoral cells, miscellaneous stromal components: cellular or acellular provide promoting and maintaining of HNSCC invasion. Deposition of specific extracellular matrix (ECM) proteins (collagen IV, collagen XVII, fibronectin, and laminin) is enhanced in HNSCC tumors and serves as a chemo-attractant for HNSCC cells in various *in vitro* invasion assays [9]. As HNSCC tumors progress towards metastatic disease, non-tumor cell types from the associated stroma have been shown to have direct and indirect roles in facilitating HNSCC invasion [10].

1. Mast cells are part of the immune myeloid line, that mediate immune responses by granule exocytosis, releasing histamine, serine proteases, carboxypeptidase A, proteoglycans, prostaglandin D2, leukotriene C4, tumor necrosis factor (TNF)-α, IL-3, IL-4, IL-5, IL-6, IL-8, and IL-16. In advanced stages of HNSCC, mast cells accumulate in the tumor stroma, and their presence is directly correlated with increased angiogenesis [11]. Additionally, heparanase, an enzyme involved in cleavage and remodeling heparin sulfate proteoglycans from the extracellular matrix, accumulates at the HNSCC invasive front, and is a marker of poor prognosis for lymph node metastasis and tumor recurrence [12]. Mast cells, along with tumor infiltrating neutrophils, endothelial cells, and macrophages exhibit heparanase activity [13]. Because, mast cells also secrete large amounts of heparin, they are the type of cells that probably are in charge for invasion-associated heparanase activity in the tumor microenvironment. Their presences also stimulate HNSCC tumor neo-vascularization and dissemination to loco-regional lymph nodes.

2. Neutrophils are enlisted to the tumor microenvironment by pro-inflammatory signals, including IL-8, transforming growth factor (TGF)-β, IL-4, IL-10, IL-13 and TNF-α [14]. In tumor microenvironment, neutrophils secrete VEGF-A, stimulating neovascularization through endothelial cell recruitment and proliferation, which can be suppressed by anti-VEGF-A antibodies or angiostatin treatment [13, 14]. Also, neutrophil-derived HGF and MMP-9 facilitate tumor cell migration and invasion towards the newly formed vascular bed [14].

3. Macrophages play a direct role in immune supervision via endocytosis of pathogens and cellular debris [15]. Infiltration into the tumor microenvironment with tumor associated macrophage (TAM) relates to lymph node involvement, tumor stage, and extracapsular spread [16]. Once they arrive in the tumor microenvironment, they secret several paracrine signaling loops that drive tumor cell invasion and metastasis. Macrophages are able to stimulate and maintain the HNSCC invasive phenotype, serve to basement membrane breakdown and recruitment of other cell types into the tumor microenvironment.

4. Fibroblasts are involved in demoplastic respons in cancer, as they can break and modify a variety of ECM proteins including type I and IV collagens, laminin, and fibronectin [17, 18]. Some studies suggest that HNSCC stroma is enriched in infiltrating cancer-associated fibroblasts (CAFs) and their highest concentration is near the invasive front of the tumor. CAFs have some characteristics of myofibroblasts, like enhanced proliferation, motility and secretion of matrix metalloproteinase-2 and hepatocyte growth factor (HGF). They express cytokeratins, vimentin, and α-smooth muscle actin (SMA) [19, 20]. Secretion of matrix metalloproteinase leads to extracellular matrix degradation and remodeling, while HGF enhances HNSCC cell motility [9, 17, 21]. The final result of fibroblasts in the tumor microenvironment is permutation of extracellular matrix proteins, allowing fibroblasts to lead tumor cells into surrounding tissues or creates pathways in the stroma for invasive tumor cells which result in HNSCC metastatic progression [22].

Involvement of HPV Virus in Head and neck cancers

A lot of studies relate that human papillomaviruses (HPVs) is implicated in the pathogenesis of HNSCCs. HPV16 represent a high-risk is the predominant HPV type in some forms of head and neck cancers, and it account about 90% of HPV DNA-positive tumors detected [22]. While, excessive smoking and alcohol usage represent major risk factors for the majority HPV (-) HNSCCs cases.

HNC and Tobacco Use, Harm Reduction Strategies

Head and neck cancers are a heterogeneous group of tumors, responsible for more than 650000 cases and 330000 deaths per year worldwide, and one of the most important etiological factors is tobacco smoking [23]. The first contact organ for tobacco smoke is mucosal tissue of the upper respiratory tract and the upper part of the digestive tract. Smoke contains chemical compounds, cytotoxic to the upper airway tissue, and can cause significant DNA damage. Therefore,the mutagenicity of tobacco smoke cannot be completely excluded as a risk factor for HNC [24]. Tobacco smoke is composed of over 7000 chemicals, of which at least 69 are cancer-causing [25]. The biggest misconception is policymakers and community in general believes that nicotine is the cancer-causing culprit in cigarettes. Cigarette smoking and the many chemicals it exposes a person to, not nicotine itself, present the highest risk.

According to Cancer Research UK [26], nicotine is addictive but does not cause cancer. Most damage to health caused by smoking is due to other chemicals in smoke, such as benzo[a]pyrene, tobacco-specific nitrosamines, and benzene, which are the primary causes of smoking-related diseases [27]. The risk of developing cancer is always higher for tobacco smokers than for non-smokers. Electronic nicotine delivery system such as tobacco heating system (THS) for our country has become increasingly popular in the last 3 years and is considered less harmful than traditional tobacco products, due to the lower content of toxic and carcinogenic compounds [28].

THS – uses an electronically controlled mechanism to precisely heat specially designed tobacco sticks at operating temperatures well below combustion (less than 350°C). As a result, the generated aerosol is composed of mainly water, glycerin and nicotine while the levels of harmful and potentially harmful constituents are significantly decreased compared to cigarettes smoke [28, 29].

The Netherlands Institute of RIVM identified carcinogenicity impact of switching from smoking cigarettes to consuming heated tobacco, the risk of cancer was reduced by 10 to 20 times, depending on different types of cancer [30].

Harm Reduction as a solution for adult smokers

Harm reduction refers to a range of practical strategies aimed at lessening the negative social and physical consequences associated with particularly risky human behaviors. Harm reduction policies are supported by 84 countries worldwide, with 74 countries having explicit supportive reference to harm reduction in national policy documents.

Quitting smoking is one of the most effective public health measures and tobacco harm reduction can supplement tobacco control. Tobacco harm reduction remains a controversial topic in tobacco control. Tobacco harm reduction involves providing tobacco users who are unwilling or unable to quit using nicotine products with less harmful nicotine containing products for continued use. Some Public Health experts believe that these products have great potential to reduce mortality and morbidity among smokers who completely switch to them. Others believe that we will be addicting another generation to tobacco products [31].

Researchers investigate the tumor-stroma interplay in high-risk human papilloma virus positive HPV+ and HPV- head and neck cancers from patients diagnosed with HNSCC. Immunosuppression in HPV positive HNSCC, is significant increased by viral infection, which may explain why, these tumors for the most part develop within the immune tissue of tonsillar lymphoid follicles, an anatomic site that should favor immunologic antitumor response [32]. The immunosuppression produced by inflammatory cytokines maintains latent infection and favors tumor genesis, which is initiated when the viral DNA integrates into the host genome and drives genomic instability. Once infected and transformed by HPV, tumor cells activate additional mechanisms to escape the immune system by preventing exposure of tumor antigens and promoting apoptosis of effector T lymphocytes and down-regulation of NK cells [4]. New knowledge in immunological characteristics of HPV^+ and HPV^- HNSCCs may improve therapeutic targeting and immunotherapy strategies for different subtypes of HNSCCs.

Conclusions

Recent research proves that tumor microenvironment of HNSCC has an important role in tumor progression, aggressivity, metastasis process, in addition to genetic aberrations and molecular alterations of cancer cells. New researches in stromal composition of the HNSCC may be useful in understanding of mechanisms for different responses to therapy, also can be used as a targeted for therapeutic purposes. Cancer-associated fibroblasts and immune cells, as well as their products found in neck squamous cell carcinoma significantly influence the biological properties of this tumor. Smoking is one of the risk factors of occurrence of most HPV-associated tumors. Promoting smoking cessation should become an essential contributor to the treatment of cancer in all oncologic pathologies. In cases when patients can't quit smoking completely within the shortest possible period of time, doctors should focus on harm reduction strategies – tobacco harm reduction.

References

1. Dittmer C, Katalinic A, Mundhenke C, Thill M, Fischer D. Epidemiology of vulvar and vaginal cancer in Germany. Arch Gynecol Obstet. 2011;284(1):169-74. doi: 10.1007/s00404-011-1850-9.
2. Oliva M, Spreafico A, Taberna M, Alemany L, Coburn B. Immune biomarkers of response to immune-checkpoint inhibitors in head and neck squamous cell carcinoma. An Oncol. 2019;30(1):57-67. doi: 10.1093/annonc/mdy507.
3. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5): E359-86. doi: 10.1002/ijc.29210.
4. Lewis MP, Lygoe KA, Nystrom ML, Anderson WP, Speight PM, Marshall JF, Thomas GJ. Tumor-derived TGF-β1 modulates myofibroblast differentiation and promotes HGF/SF-dependent invasion of squamous carcinoma cells. Br J Cancer. 2004;90(4):822-832. doi: 10.1038/sj.bjc.6601611.
5. Ljokjel B, Haave H, Lybak S, Aarstad HH, Karlsdottir A, Vintermyr OK. The impact of HPV infection, smoking history, age and operability of the patient on disease-specific survival in a geographically defined cohort of patients with oropharyngeal squamous cell carcinoma. Acta Otolaryngol. 2014;134(9):964-73. doi: 10.3109/00016489.2014.927590.
6. Markopoulos AK. Current aspects on oral squamous cell carcinoma. Open Dent J. 2012;6:126-130. doi: 10.2174/1874210601206010126.
7. Alsahafi E, Begg K, Amelio I, Raulf N, Lucarelli P, Sauter T, Tavassoli M. Clinical update on head and neck cancer: molecular biology and ongoing challenges. Cell Death Dis. 2019;10(8):540. doi: 10.1038/s41419-019-1769-9.
8. Hassona Y, Cirillo N, Lim KP, Herman A, Mellone M, Thomas GJ, Pitiyage GN, Parkinson EK, Prime SS. Progression of genotype-specific oral cancer leads to senescence of cancer-associated fibroblasts and is mediated by oxidative stress and TGF-β. Carcinogenesis. 2013;34(6):1286-1295. doi: 10.1093/carcin/bgt035.
9. Iamaroon A, Pongsiriwet S, Jittidecharaks S, Pattanaporn K, Prapayasatok S, Wanachantararak S. Increase of mast cells and tumor angiogenesis in oral squamous cell carcinoma. J Oral Pathol. 2003;32(4):195-199. doi: 10.1034/j.1600-0714.2003.00128.x.
10. Steven MM, Scott AW. Tumor and stromal-based contributions to head and neck squamous cell carcinoma invasion. Cancers. 2015;7(1):382-406. doi: 10.3390/cancers7010382.
11. Jacobsen MR, Dongre H, Ahmed I, Tuljaurkar V, Pai PS, Patil A, Sapkota D, Johannessen AC, Filipovic N, Vaidya M. Development of a molecular diagnostic tool for more precise diagnosis of oral squamous cell carcinoma. Clin Cancer Res. 2017;23(23 Suppl):45. doi: 10.1158/1557-3265.AACRAHNS17-45.
12. Beckhove P, Helmke BM, Ziouta Y, Bucur M, Borner W, Mogler C, Dyckhoff G, Herold-Mende C. Heparanase expression at the invasion front of human head and neck cancers and correlation with poor prognosis. Clin Cancer Res. 2005;11(8):2899-2906. doi: 10.1158/1078-0432.CCR-04-0664.
13. Benelli R, Morini M, Carrozzino F, Ferrari N, Minghelli S, Santi L, Cassatella M, Noonan DM, Albini A. Neutrophils as a key cellular target

for angiostatin: Implications for regulation of angiogenesis and inflammation. FASEB J. 2002;16(2):267-269. doi: 10.1096/fj.01-0651fje.
14. Scapini P, Bazzoni F, Cassatella MA. Regulation of B-cell-activating factor (BAFF)/B lymphocyte stimulator (BLyS) expression in human neutrophils. Immunol Lett. 2008;116(1):1-6. doi: 10.1016/j.imlet.2007.11.009.
15. Canning M, Guo G, Yu M, Myint C, Groves MW, Byrd JK, Cui Y. Heterogeneity of the head and neck squamous cell carcinoma immune landscape and its impact on immunotherapy. Front Cell Dev Biol. 2019;7:52. doi: 10.3389/fcell.2019.00052.
16. Sawatsubashi M, Yamada T, Fukushima N, Mizokami H, Tokunaga O, Shin T. Association of vascular endothelial growth factor and mast cells with angiogenesis in laryngeal squamous cell carcinoma. Virchows Arch. 2000;436(3):243-248. doi: 10.1007/s004280050037.
17. Görkem E. Tumor microenvironment in head and neck squamous cell carcinomas. Turk Arch Otorhinolaryngol. 2015;53(3):120-127. doi: 10.5152/tao.2015.1065.
18. Liu Y, Hu T, Shen, J, Li SF, Lin JW, Zheng XH, Gao QH, Zhou HM. Separation, cultivation and biological characteristics of oral carcinoma-associated fibroblasts. Oral Dis. 2006;12(4):375-380. doi: 10.1111/j.1601-0825.2005.01207.x.
19. Barth PJ, Schweinsberg T, Ramaswamy A, Moll R. CD34 fibrocytes, alpha-smooth muscle antigen-positive myofibroblasts, and CD117 expression in the stroma of invasive squamous cell carcinomas of the oral cavity, pharynx, and larynx. Virchows Arch. 2004;444(3):231-234. doi: 10.1007/s00428-003-0965-1.
20. Ljokjel B, Lybak S, Haave H, Olofsson J, Vintermyr OK, Aarstad HJ. The impact of HPV infection on survival in a geographically defined cohort of oropharynx squamous cell carcinoma (OPSCC) patients in whom surgical treatment has been one main treatment. Acta Otolaryngol. 2014;134(6):636-45. doi: 10.3109/00016489.2014.886336.
21. Lim KP, Cirillo N, Hassona Y, Wei W, Thurlow JK, Cheong SC, Pitiyage G, Parkinson EK, Prime SS. Fibroblast gene expression profile reflects the stage of tumour progression in oral squamous cell carcinoma. J Pathol. 2011;223(4):459-469. doi: 10.1002/path.2841.
22. Curry JM, Sprandio J, Cognetti D, Luginbuhl A, et al. Tumor microenvironment in head and neck squamous cell carcinoma. Semin Oncol. 2014 Apr;41(2):217-34. doi: 10.1053/j.seminoncol.2014.03.003.
23. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424. doi: 10.3322/caac.21492.
24. Welz C, Canis M, Schwenk-Zieger S, Becker S, Stucke V, Ihler F, Baumeister P. Cytotoxic and genotoxic effects of electronic cigarette liquids on human mucosal tissue cultures of the oropharynx. J Environ Pathol Toxicol Oncol. 2016;35(4):343-354. doi: 10.1615/JEnvironPatholToxicolOncol.2016016652.
25. World Health Organization. Tobacco WHO Report [Internet]. Geneva: WHO; 2020- [cited 2020 Nov 12]. Available from: https://www.who.int
26. Cancer Research UK. Is vaping harmful? [Internet]. Oxford; 2020- [cited 2020 Nov 12]. Available from: https://www.cancerresearchuk.org/about-cancer/causes-of-cancer/smoking-and-cancer/is-vaping-harmful
27. Public Health England. Vaping in England: an evidence update including vaping for smoking cessation, February 2021 [Internet]. London: PHE; 2021- [cited 2021 Mar 19]. Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/962221/Vaping_in_England_evidence_update_February_2021.pdf
28. Schaller JP, Keller D, Poget L, et al. Evaluation of the Tobacco Heating System 2.2. Part 2: Chemical composition, genotoxicity, cytotoxicity, and physical properties of the aerosol. Regul Toxicol Pharmacol. 2016;81 Suppl 2:S27-S47. doi: 10.1016/j.yrtph.2016.10.001.
29. Bentley MC, Almstetter M, Arndt D, et al. Comprehensive chemical characterization of the aerosol generated by a heated tobacco product by untargeted screening. Anal Bioanal Chem. 2020;412(11):2675-2685 doi: 10.1007/s00216-020-02502-1.
30. Slob W, Soeteman-Hernández LG, Bil W, et al. A method for comparing the impact on carcinogenicity of tobacco products: a case study on heated tobacco versus cigarettes. Risk Anal. 2020 Jul;40(7):1355-1366. doi: 10.1111/risa.13482.
31. Hatsukami DK, Carroll DM. Tobacco harm reduction: past history, current controversies and a proposed approach for the future. Prev Med. 2020 Nov;140:106099. doi: 10.1016/j.ypmed.2020.106099.
32. Gaggioli C, Hooper S, Hidalgo-Carcedo C, Grosse R, Marshall JF, Harrington K, Sahai E. Fibroblast-led collective invasion of carcinoma cells with differing roles for RhoGTPases in leading and following cells. Nat Cell Biol. 2007;9(12):1392-1400. doi: 10.1038/ncb1658.

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Risk factors of SARS-CoV-2 infection

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Abstract

Background: Review is based on the biggest observational population study of all the risk factors of COVID-19 infection. The study was carried out in Royal College of General Practitioners, Oxford, and covering over 4 million of people. Were studied and analyzed risk factors as: age, sex and ethnicity, socioeconomic level, living space dimensions, rural-urban population, body mass index, smoker status, pregnancy, hypertension, chronic kidney disease, ischemic heart disease, chronic respiratory diseases including asthma, and chronic obstructive pulmonary disease, and type 1 and 2 diabetes. Patient variable with malignancy and immunocompromised status was separated due to the small number of patients in each group.
Conclusions: By September 2020 more than 28.000 articles had been published related to COVID-19 in less than 9 months, 211 new papers every day. Most of them had small population of the studies. In the investigated sample, it was found that increasing age, male sex, economic deprivation, urban location and black ethnicity were associated with higher chances of testing positive for SARS-CoV-2. Active smoking decreased the chance of a positive test. The review covers the most important subjects influencing the development of severe infection outcomes.
Key words: SARS-CoV-2, COVID-19, risk factors.

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Introduction

In late 2019 – early 2020, the world was hit by a pandemic caused by the virus causing Severe Acute Respiratory Syndrome (SARS-CoV-2), an infection also called COVID-19. This is a new coronavirus that was primarily reported to the World Health Organization (WHO) as a group of cases of viral pneumonia of unknown aetiology in Wuhan, China, on 31 December 2019. It is currently known that the cause of the disease is coronavirus (COVID-19), which affects both the upper and lower respiratory tract. On 30 January 2020, the WHO declared the outbreak of COVID-19 a global public health emergency. Since then, the infection has continued to spread around the world and was described by the WHO as a pandemic on 11 March 2020.

COVID-19 virus belongs to the family of single-stranded RNA viruses, some of which have been previously described to be responsible for the Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS). Although the symptoms and clinical presentation of COVID-19 are similar to SARS and MERS, the rate of spread is greater.

In the United Kingdom, the first cases were detected at the end of January 2020; the transmission among the population began at the end of that month. Initial reports from China, Italy and Spain clinically described the characteristics of people diagnosed with COVID-19 and the risk factors, which include old age, male sex, pre-existing cardiovascular diseases, high blood pressure and diabetes. However, the most recent researches to date have been done among in-patients with COVID-19, i.e., the risk factors for infection in the general population have not been directly evaluated.

The aim of the study was to evaluate the potential risk factors that could influence the spread, and change the management and prevention of the spread of this new type of virus.

The Research and Supervision Centre of the Royal College of General Practitioners in Oxford includes over 500 health centres in both urban and rural areas, covering over 4 million people.

Material

A cross-sectional study of patients in the Network of the Research and Surveillance Centre in Oxford, who were tested for SARS-CoV-2 between 28 January and 4 April 2020, was performed. Pseudonymized results were taken from electronic primary health care records. These data enabled estimating the living space sizes, the isolation level and rural-urban classification. Starting with the last week of January 2020, research and surveillance centres submitted nasopharyngeal smears for SARS-CoV-2 testing in patients with symptoms of influenza or respiratory infection.

The analytical specifics of RT-PCR assays for SARS-CoV-2 are greater than 95% and the analytical sensitivity of the assays is usually 90-95%, with high performance.

Patients registered with the research and surveillance centres as of 30 September 2019, and who had a record in their medical sheet reporting a positive or negative result for SARS-CoV-2 were only people with a positive test or negatively coded, and not those under suspicion.

Studied variables

The following independent demographic aspects were studied as factors: age, sex and ethnicity, using an ontology to maximize case identification; socioeconomic level using the English quintile index for multiple deprivation; living space dimensions based on the patient's pseudonymized address; and rural-urban division. The most recent record of the following clinical variables was included, which are similar to those associated with higher susceptibility to influenza: body mass index (BMI), smoker status, pregnancy, hypertension, chronic kidney disease, ischemic heart disease, chronic respiratory diseases, including asthma and chronic obstructive pulmonary disease, and type 1 and 2 diabetes. Patient variable with malignancy and immunocompromised status was created due to the small number of patients in each group. Malignancy was identified using the most recently recorded disease codes and prescriptions of prednisolone and antirheumatic drugs as a surrogate for immunosuppression with a positive test for SARS-CoV-2.

Statistical methods

Descriptive statistics and reported counts were used for categorical data and distribution measures for continuous data. Collinearity was checked by measuring the variation of the inflation factor for each covariate – all were considered within acceptable limits, with a maximum value below 2.0. New sensitivity analyses were performed using only complete cases, non-ethnic data imputed from the census data.

Results

Between 28 January and 4 April 2020, 587 patients with positive results on SARS-CoV-2 and 3215 with negative results were obsessed with the surveillance programme. The first positive case reported on 30 January 2020 reaching the number of 100 cases on 17 March 2020. In total, 2190 (57.6%) of 3802 patients were women and 2497 (65.7%) were white (tab. 1).

The data are n (%). SARS-CoV-2 = severe acute respiratory syndrome – coronavirus 2.BMI = body mass index.* The level of socio-economic deprivation was assessed at a practical level using the English Index of Multiple Deprivation Quintiles. † BMI categories were based on the WHO classification (normal weight 18.5-24.9 kg/m^2, overweight 25.0-29.9 kg/m^2, obese 30.0-39.9 kg/m^2, severely obese ≥40 kg/m^2)

In the univariate analysis, the chances of testing positive for SARS-CoV-2 were higher among the elderly, non-white men, and people living in more disadvantaged areas (tab. 2). The chances of a positive test were lower in households from two to four or five to eight people. The clinical factors in the univariate analysis, chronic kidney diseases, obesity, malignancy or immunocompromise, diabetes, chronic respiratory diseases, chronic heart diseases, and hypertension were associated with higher odd ratios of a positive SARS-CoV-2 test.

In case of active smoker, he has had a decreased level for a positive COVID test. In the multivariable analysis, adjusted for all other variables in tab. 3, male sex remained independently associated with SARS-CoV-2 positive testing. The age linked variable: children and people aged 40–64 years were at a lower risk (5.36, 3.28–8.76) compared to adult ones and people aged 75 years and older (5.23, 3.00–9.09) were at the highest risk. Black people were at a higher risk of testing positive for SARS-CoV-2 (4.75, 2.65-8.51), compared to white people. Were associated with higher odd ratios of SARS-CoV-2 test - urbanized areas (4.59, 3.57–5.90). In the adjusted analysis, people with chronic kidney disease were more likely to be tested positive for SARS-CoV-2 than those without it (1.91, 1.31–2.78), but without a significant association with other chronic conditions. Smoking cigarettes was associated with lower chances of a positive SARS-CoV-2 test result (adjusted OR 0-49, (95% CI) 0.34-0.71). Higher odd ratios of a positive test among obese people was found compared to those with normal weight (1.41, 1.04–1.91).

The review of the literature suggested that COVID-19 affected men aged between 30 and 65, and about half of the patients were over 50 years old. There is a higher risk of a similar positive SARS-CoV-2 test in men as in people aged over 40. It is known that the chance of a positive SARS-CoV-2 test increases with higher population density due to the increased social mix, which correlates with the finding of a high level of a positive test in urban areas.

Socially disadvantaged areas were found to be associated with a higher risk of other respiratory infections, and thus the risk of death from COVID-19 is higher in the poorest parts of the UK.

The decrease in the socio-economic level was associated with the increase of a positive test, independently of the area of the place of residence, urban location and smoking. No association was found between household size and the risk of SARS-CoV-2 positivity, despite previous data of a higher risk of transmission through household contacts. Social distancing measures could have influenced the transmission speed and areas. Some data raised concerns about the potential increased risk of complications associated with COVID-19 among black and Asian people, but there were few epidemiological studies that have assessed the risk by ethnicity. An analysis of 3370 people in the UK admitted to the ICU (Intensive Care Unit) with confirmed positive COVID-19 found that 402 (11.9%) were black, 486 (14.4%) were Asian and 2236 (66.4%) were white [35], compared to the national figures that were of 3.3%, 7.5% and 86.0%. These results were not adjusted for socio-demographic potential or clinical confusions [36].

Table 1. Demographic and clinical characteristics of the study cohort [50]

SARS-CoV-2 test result	Participants 3802	Pregnancy	
Negative	3215 (84.6%)	No	3742 (98.4%)
Positive	587 (15.4%)	Yes	60 (1.6%)
Missing data	0	Missing data	0
Age			
0–17	**499** (13.1%)	**BMI†**	
18–39	**666** (17.5%)	Normal weight	1296 (34.1%)
40–64	**1316** (34.6%)	Overweight	1095 (28.8%)
65–74	**557** (14.7%)	Obesity	680 (17.9%)
≥75	**764**(20.1%)	Morbid obesity	145 (3.8%)
Missing data	0	Missing data	586 (15.4%)
Sex			
Women	2190 (57.6%)	**Hypertension**	
Men	1612 (42.4%)	No	2708 (71.2%)
Missing data	0	Yes	1094 (28.8%)
Ethnicity		Missing data	0
white people	2497 (65.7%)		
Asian people	152 (4.0%)	**Chronic kidney diseases**	
black people	58 (1.5%)	No	3595 (94.6%)
others	81 (2.1%)	Yes	207 (5.4%)
Missing data	1014 (26.7%)	Missing data	0
Level of socio-economic deprivation*			
5 (less deprived)	1855 (48.8%)	**Diabetes**	
4	633 (16.6%)	No	3299 (86.8)
3	646 (17.0%)	Yes	503 (13.2)
1 and 2 (more deprived)	668 (17.6%)	Missing data	0
Missing data	0		
Number of people living in a common space		**Chronic heart diseases**	
1	824 (21.7%)	No	3202 (84.2%)
2-4	2341 (61.6%)	Yes	600 (15.8%)
5-8	408 (10.7%)	Missing data	0
≥9	135 (3.6%)		
Missing data	94 (2.5%)	**Chronic respiratory diseases**	
Population density		No	3544 (93.2%)
Rural	1986 (52.2%)	Yes	258 (6.8%)
Urban	1816 (47.8%)	Missing data	0
Missing data	0		
Smoker status		**Cancers or immune compromise**	
Non-smoker	1125 (29.6%)	No	3164 (83.2%)
Active smoker	413 (10.9%)	Yes	638 (16.8%)
Ex-smoker	1753 (46.1%)	Missing data	0
Missing data	511 (13.4%)		

Table 2. Univariable analysis of demographic and clinical risk factors for testing positive for SARS-CoV-2 [50]

	SARS-CoV-2 positive	Non-adjusted odd ratio (95% CI)	p value
			<0.0001
0–17	**23/499 (4.6%)**	**1 (ref)**	
18–39	**84/666 (12.6%)**	**2.98 (1.85–4.81)**	
40–64	**243/1316 (18.5%)**	**4.69 (3.00–7.28)**	
65–74	**88/557 (15.8%)**	**3.88 (2.40–6.25)**	
≥75	**149/764 (19.5%)**	**5.00 (3.18–7.90)**	
Sex			<0.0001
Women	**291/2190 (13.3%)**	**1 (ref)**	
Men	**296/1612 (18.4%)**	**1.47 (1.23–1.75)**	
Missing data			
Ethnicity			
White people	**388/2497 (15.5%)**	**1 (ref)**	
Asian people	**47/152 (30.9%)**	**2.43 (1.70–3.49)**	
Black people	**36/58 (62.1%)**	**8.90 (5.20–15.30)**	
Others	**20/81 (24.7%)**	**1.78 (1.10–2.90)**	
Missing data	**96/1014 (9.5%)**	**0.57 (0.45–0.72)**	
*Level of socio-economic deprivation**			<0.0001
5 (less deprived)	**143/1855 (7.7%)**	**1.00 (ref)**	
4	**112/633 (17.7%)**	**2.58 (1.97–3.36)**	
3	**135/646 (20.9%)**	**3.16 (2.45–4.10)**	
1 and 2 (more deprived)	**197/668 (29.5%)**	**5.01 (3.95–6.35)**	
The number of people living in a common space			<0.0001
1	**163/824 (19.8%)**	**1.00 (ref)**	
2-4	**320/2341 (13.7%)**	**0.64 (0.52–0.79)**	
5-8	**53/408 (13.0%)**	**0.61 (0.43–0.85)**	
≥9	**35/135 (25.9%)**	**1.42 (0.93–2.16)**	
Missing data	**16/94 (17.0%)**	**0.83 (0.47–1.46)**	
Population density			<0.0001
Rural	**111/1986 (5.6%)**	**1.00 (ref)**	
Urban	**476/1816 (26.2%)**	**6.00 (4.82–7.46)**	
Smoker status			<0.0001
Non-smoker	**201/1125 (17.9%)**	**1 (ref)**	
Active smoker	**47/413 (11.4%)**	**0.59 (0.42–0.83)**	
Ex-smoker	**303/1753 (17.3%)**	**0.96 (0.79–1.17)**	
Missing data	**36/511 (7.0%)**	**0.35 (0.24–0.51)**	
Pregnancy			0.0400
No	**583/3742 (15.6%)**	**1 (ref)**	
Yes	**4/60 (6.7%)**	**0.39 (0.14–1.10)**	
BMI †			<0.0001
Normal weight	**171/1296 (13.2%)**	**1 (ref)**	
Overweight	**198/1095 (18.1%)**	**1.45 (1.20–1.80)**	
Obesity	**142/680 (20.9%)**	**1.74 (1.36–2.20)**	
Morbid obesity	**26/145 (17.9%)**	**1.44 (0.91–2.27)**	
Missing data	**50/586 (8.5%)**	**0.61 (0.44–0.85)**	
Hypertension			<0.0001
No	**378/2708 (14.0%)**	**1 (ref)**	
Yes	**209/1094 (19.1%)**	**1.46 (1.20–1.75)**	
Chronic kidney diseases			<0.0001
No	**519/3595 (14.4%)**	**1 (ref)**	

Yes	**68/207 (32.9%)**	**2.90 (2.14–3.93**	
Diabetes			<0.0001
No	**473/3299 (14.3%)**	**1 (ref)**	
Yes	**114/503 (22.7%)**	**1.75 (1.40–2.20)**	
Chronic heart diseases			<0.0001
No	**451/3202 (14.1%)**	**1 (ref)**	
Yes	**136/600 (22.7%)**	**1.79 (1.44–2.20)**	
Chronic respiratory diseases			<0.0001
No	**529/3544 (14.9%)**	**1 (ref)**	
Yes	**58/258 (22.5%)**	**1.65 (1.21–2.25)**	
Oncological or immunocompromising diseases			<0.0001
No	**460/3164 (14.5%)**	**1 (ref)**	
Yes	**127/638 (19.9%)**	**1.46 (1.17–1.82)**	

Table 3. Multivariable analysis of risk factors for SARS-CoV-2 positive testing [50]

Age	**Non-adjusted odd ratios (95% CI)**	**<0.0001**
0–17	1 (ref)	
18–39	2.83 (1.69–4.74)	
40–64	5.36 (3.28–8.76)	
65–74	4.41 (2.52–7.69)	
≥75	5.23(3.00–9.09)	
Sex		<0.0001
Women	1 (ref)	
Men	1.55 (1.27–1.89)	
Missing data		
Ethnicity		
White people	1 (ref)	
Asian people	1.46 (0.94–2.29)	
Black people	4.75 (2.65–8.51)	
Others	1.71 (0.97–3.01)	
Missing data		
*Level of socio-economic deprivation**		
5 (less deprived)	1 (ref)	
4	1.51 (1.13–2.03)	
3	2.35 (1.78–3.11)	
1 - 2 (more deprived)	2.03 (1.51–2.71)	
The number of people living in a common space		0.4900
1	1 (ref)	
2-4	0.97 (0.77–1.23)	
5-8	0.86 (0.57–1.31)	
≥9	1.29 (0.80–2.07)	
Missing data		
Population density		
Rural	1 (ref)	
Urban	4.59 (3.57–5.90)	
Smoker status		
Non-smoker	1 (ref)	
Active smoker	0.49 (0.34–0.71)	
Ex-smoker	0.87 (0.69–1.10)	
BMI †		0.0090
Normal weight	1 (ref)	
Overweight	1.26 (0.99–1.61)	
Obesity	1.41 (1.04–1.91)	
Morbid obesity	1.28 (0.78–2.10)	
Missing data		
Hypertension		0.3100
No	1 (ref)	
Yes	0.89 (0.69–1.14)	
Chronic kidney diseases		
No	1 (ref)	
Yes	1.91 (1.31–2.78)	
Diabetes		0.8300
No	1 (ref)	
Yes	1.03 (0.78–1.36)	
Chronic heart diseases		0.1800
No	1(ref)	
Yes	1.21(0.92–1.60)	
Chronic respiratory diseases		0.8200
No	1(ref)	
Yes	1.04(0.72–1.50)	
Oncological or immunocompromising diseases		
No		0.9800
Yes	1 (ref)	
	1.01 (0.78–1.31)	

Discussion

The study has shown that black people were more likely to test positive for SARS-CoV-2 than white ones, which remained significant even after adjusting for comorbidities, such as high blood pressure and diabetes, whose prevalence is higher in black people. Other socio-economic factors that we did not examine, such as high-risk professions, level of education, income and barriers to health care, could have contributed to this association and should be explored as soon as possible [29, 30, 32, 33].

Regular reviews showed that people with COVID-19 who have chronic comorbidities, such as hypertension, diabetes and cardiovascular diseases are at high risk for severe COVID-19 evolution [38]. Risk factors for SARS-CoV-2 infection may be different, and no evidence was found of an association between these conditions and a positive SARS-CoV-2 test. It was detected that chronic kidney disease and obesity were associated with testing positive for SARS-CoV-2. Both factors – chronic kidney disease and obesity were associated with a higher risk of other respiratory infections, angiotensin-converting enzyme inhibitors, which are recommended as treatments for chronic kidney disease and were postulated to impact SARS-CoV-2 interactions on the host cell. However, there is no observational evidence to support this effect, and further analyses to investigate the relationship between drugs, chronic diseases, and SARS-CoV-2 positivity would be evaluated.

Previous studies reported that smoking is associated with a higher risk of hospitalization in intensive care unit or death among people with COVID-19 [46]. Yet, several studies have reported a low prevalence of smokers among people with COVID-19 [47, 48]. A Chinese study found that only 137 (12.6%) of 1085 patients with COVID-19 were current smokers, compared to the proportion of 27.7% of adult smokers in the general population, another analysis of cases in the Centres for Disease Control and Disease Prevention in the US detected that only 96 (1.3%) of 7162 COVID-19 cases were active smokers, compared to 13.7% of smokers in the general US population. However, it should be admitted that in these studies it is sometimes difficult to identify active smokers among patients with COVID-19.

The data obtained show that active smoking was associated with a decrease in the chances of having a positive COVID-19 test result. There are several plausible reasons for this result. Active smoking could affect the viral load of the nasopharynx and therefore affect the sensitivity of the RT-PCR test, rather than protection against actual infection, although this effect is not known in the RT-PCR test for influenza. Smokers are more likely to have a cough, which means they may also be more likely to be tested positive for SARS-CoV-2 than non-smokers, even if they are SARS-CoV-2 negative. This more frequent test could increase the proportion of smokers with a negative SARS-CoV-2 result in our sample, which would harm our results. However, the share of smokers in our study was low. In addition, former smokers and people with chronic lung disease would also be expected to cough more, but these groups were not more likely to be tested positive for SARS-CoV-2. Therefore, the relationship between smoking and SARS-CoV-2 infection should be further investigated. Nicotine in turn may decrease the sensitivity of angiotensin-converting enzyme receptors, the place used by SARS-CoV-2 for entering cells, although studies found an increase in the concentration of angiotensin-converting enzyme 2 in lungs among smokers and people with chronic obstructive pulmonary disease. The findings should not be used to conclude that smoking prevents SARS-CoV-2 infection or to encourage continued smoking, especially given the well-documented damage to overall health caused by smoking, there may be alternative explanations for these findings.

Although the study group of primary care patients is likely to be more similar to the general population than that of hospital-based studies, there is a risk of bias in selection, as the results may reflect groups of patients who were more likely to come for evaluation and be selected for SARS-CoV-2 testing in accordance with the guidelines. If certain groups (e.g., men, people in disadvantaged areas, non-smokers, and black people) may only come or be tested when they are in a more serious condition, those who were tested may be more likely to be positive for COVID-19. In contrast, groups with lower thresholds for presentation could be tested with less severe symptoms and therefore are more likely to be tested negative. Population surveys should provide consistent test levels by subgroups as far as possible to reduce the risk of selection bias. Although RT-PCR testing is the gold standard for SARS-CoV-2 Diagnosis, the overall sensitivity of the test in the clinic, may be reduced by factors, such as smear technique and time relative to the onset of symptoms. Therefore, some SARS-CoV-2 cases could have been missed, especially among patients with lower viral loads.

Additional data are needed to establish the epidemiology of SARS-CoV-2, especially with regard to emerging factors, such as ethnicity, deprivation, population density and smoking.

Conclusions

Primary health care network data provide important information on the epidemiology of SARS-CoV-2, although the study was limited by small sample and selection of patients who came for SARS-CoV-2 testing through routine health care services. It is necessary to continuously study the factors that influence the development of the epidemic, the severity of symptoms, and the conditions in which COVID infection spreads faster.

References

1. Lai CC, Shih TP, Ko WC, Tang HJ, Hsueh PR. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): the epidemic and the challenges. Int J Antimicrob Agents. 2020;55(3):105924. doi: 10.1016/j.ijantimicag.2020.105924.
2. Kinross P, Suetens C, Gomes Dias J, et al. Rapidly increasing cumulative incidence of coronavirus disease (COVID-19) in the European Union/European Economic Area and the United Kingdom,1 January to 15

March 2020. Euro Surveill. 2020;25(11):2000285. doi: 10.2807/1560-7917.ES.2020.25.11.2000285.
3. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72314 cases from the Chinese Center for Disease Control and Prevention. JAMA. 2020;323:1239-42. doi: 10.1001/jama.2020.2648.
4. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395(10229):1054-62. doi: 10.1016/S0140-6736(20)30566-3.
5. Lipsitch M, Swerdlow DL, Finelli L. Defining the epidemiology of Covid-19 - studies needed. N Engl J Med. 2020;382(13):1194-96. doi: 10.1056/NEJMp2002125.
6. Fleming DM, Miles J. The representativeness of sentinel practice networks. J Public Health (Oxf). 2010;32(1):90-96. doi: 10.1093/pubmed/fdp087.
7. Correa A, Hinton W, McGovern A, et al. Royal College of General Practitioners Research and Surveillance Centre (RCGP RSC) sentinel network: a cohort profile. BMJ Open. 2016;6(4):e011092. doi: 10.1136/bmjopen-2016-011092.
8. de Lusignan S, Correa A, Smith GE, et al. RCGP Research and Surveillance Centre: 50 years' surveillance of influenza, infections, and respiratory conditions. Br J Gen Pract. 2017;67(663):440-41. doi: 10.3399/bjgp17X692645.
9. Fleming DM. Weekly returns service of the Royal College of General Practitioners. Commun Dis Public Health. 1999;2(2):96-100.
10. Pebody RG, Whitaker H, Ellis J, et al. End of season influenza vaccine effectiveness in primary care in adults and children in the United Kingdom in 2018/19. Vaccine. 2020;38(3):489-97. doi: 10.1016/j.vaccine.2019.10.071.
11. Pebody RG, Warburton F, Andrews N, et al. Uptake and effectiveness of influenza vaccine in those aged 65 years and older in the United Kingdom, influenza seasons 2010/11 to 2016/17. Euro Surveill. 2018;23(39):1800092. doi: 10.2807/1560-7917.ES.2018.23.39.1800092.
12. Fleming DM, Durnall H. Ten lessons for the next influenza pandemic – an English perspective: a personal reflection based on community surveillance data. Hum Vaccin Immunother. 2012;8(1):138-45. doi: 10.4161/hv.8.1.18808.
13. de Lusignan S, Borrow R, Tripathy M, et al. Serological surveillance of influenza in an English sentinel network: pilot study protocol. BMJ Open. 2019;9(3):e024285. doi: 10.1136/bmjopen-2018-024285.
14. de Lusignan S, Lopez Bernal J, Zambon M, et al. Emergence of a novel coronavirus (COVID-19): protocol for extending surveillance used by the Royal College of General Practitioners Research and Surveillance Centre and Public Health England. JMIR Public Health Surveill. 2020;6(2):e18606. doi: 10.2196/18606.
15. de Lusignan S, Sherlock J, Ferreira F, O'Brien S, Joy M. Household presentation of acute gastroenteritis in a primary care sentinel network: retrospective database studies. BMC Public Health. 2020;20(1):445. doi: 10.1186/s12889-020-08525-8.
16. de Lusignan S, McGee C, Webb R, et al. Conurbation, urban, and rural living as determinants of allergies and infectious diseases: Royal College of General Practitioners Research and Surveillance Centre Annual Report 2016-2017. JMIR Public Health Surveill. 2018;4(4):e11354. doi: 10.2196/11354.
17. Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020;25(3):2000045. doi: 10.2807/1560-7917.ES.2020.25.3.2000045.
18. NHS England and NHS Improvement. Guidance and standard operating procedure: COVID-19 virus testing in NHS laboratories [Internet]. London: NHS England; c2020- [updated 2020 March 16; cited 2020 Apr 30]. Available from: https://www.england.nhs.uk/coronavirus/wpcontent/uploads/sites/52/2020/03/guidance-and-sop-covid-19-virustesting-in-nhs-laboratories-v1.pdf.
19. BioPortal. COVID-19 Surveillance Ontology [Internet]. Stanford: National Center for Biomedical Ontology; 2005- [cited 2020 Apr 30]. Available from: https://bioportal.bioontology.org/ontologies/COVID19
20. Department of Health and Social Care. Coronavirus (COVID-19): notice under reg 3(4) of the Health Service Control of Patient Information Regulations 2002 – general [Internet]. London: DHSC; 2020- [updated 2020 March 20; cited 2020 May 5]. Available from: https://www.gov.uk/government/publications/coronavirus-covid-19-notification-of-data-controllers-to-share-information/coronavirus-covid-19-notice-under-regulation-34-of-the-health-service-control-of-patient-information-regulations-2002-general.
21. Health Research Authority. Defining research table [Internet]. London: NHS England; c2018- [updated 2017 Oct; cited 2018 Feb 6]. Available from: http://www.hra-decisiontools.org.uk/research/docs/DefiningResearchTable_Oct2017-1.pdf.
22. Tippu Z, Correa A, Liyanage H, et al. Ethnicity recording in primary care computerised medical record systems: an ontological approach. J Innov Health Inform. 2017;23(4):920. doi: 10.14236/jhi.v23i4.920.
23. Department for Communities and Local Government. The English Index of Multiple Deprivation (IMD) 2015 – Guidance [Internet]. London: DCLG; 2020- [cited 2020 Apr 30]. Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/464430/English_Index_of_Multiple_Deprivation_2015_-_Guidance.pdf
24. World Health Organization. Physical status: the use and interpretation of anthropometry: Report of a WHO Expert Committee. Geneva: WHO;1995.
25. Buuren A, Groothuis-Oudshoorn K. Multivariate imputation by chained equations in R. J Stat Softw. 2011;45(3):1-67.
26. Mertens BJA, Banzato E, deWreede LC. Construction and assessment of prediction rules for binary outcome in the presence of missing predictor data using multiple imputation and crossvalidation: methodological approach and data-based evaluation. Biom J. 2020;62(3):724-41. doi: 10.1002/bimj.201800289.
27. Office for National Statistics. KS201EW: ethnic group [Internet]. London: The Office; 2011- [cited 2020 Apr 30]. Available from: https://www.nomisweb.co.uk/census/2011/ks201ew
28. Office for National Statistics. Census geography: An overview of the various geographies used in the production of statistics collected via the UK census [Internet]. London: The Office; 2011- [cited 2020 Apr 30]. Available from: https://www.ons.gov.uk/methodology/geography/ukgeographies/censusgeography
29. Yi Y, Lagniton PNP, Ye S, Li E, Xu RH. COVID-19: what has been learned and to be learned about the novel coronavirus disease. Int J Biol Sci. 2020;16(10):1753-66. doi: 10.7150/ijbs.45134.
30. Wang KW, Gao J, Wang H, et al. Epidemiology of 2019 novel coronavirus in Jiangsu Province, China after wartime control measures: a population-level retrospective study. Travel Med Infect Dis. 2020;35:101654. doi: 10.1016/j.tmaid.2020.101654.
31. Smith S, Morbey R, de Lusignan S, Pebody RG, Smith GE, Elliot AJ. Investigating regional variation of respiratory infections in a general practice syndromeic surveillance system. J Public Health (Oxf). 2020;fdaa014. doi: 10.1093/pubmed/fdaa014.
32. Office for National Statistics. Coronavirus (COVID 19) roundup [Internet]. London: The Office; 2011- [cited 2020 May 5]. Available from: www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/conditionsanddiseases/articles/coronaviruscovid19roundup/2020-03-26
33. Bi Q, Wu Y, Mei S, et al. Epidemiology and transmission of COVID19 in 391cases and 1286 of their close contacts in Shenzhen, China: a retrospective cohort study. Lancet Infect Dis. 2020;20(8):911-919. doi: 10.1016/S1473-3099(20)30287-5.
34. Pareek M, Bangash MN, Pareek N, et al. Ethnicity and COVID-19: an urgent public health research priority. Lancet. 2020;395(10234):1421-1422. doi: 10.1016/S0140-6736(20)30922-3.
35. Intensive Care National Audit & Research Centre. ICNARC report on COVID-19 in critical care [Internet]. London: ICNARC; 2020- [cited 2020 Apr 30]. Available from: https://www.icnarc.org/DataServices/Attachments/Download/c31dd38d-d77b-ea11-9124-00505601089b
36. UK Government. Population of England and Wales [Internet]. London: The Government; 2020- [cited 2020 Apr 30]. Available from: https://www.ethnicity-facts-figures.service.gov.uk/uk-population-by-ethnicity/national-and-regional-populations/population-of-england-andnwales/latest
37. Carson AP, Howard G, Burke GL, Shea S, Levitan EB, Muntner P. Ethic differences in hypertension incidence among middle-aged and older adults: the multi-ethnic study of atherosclerosis. Hypertension. 2011;57(6):1101-07. doi: 10.1161/HYPERTENSIONAHA.110.168005.
38. Yang J, Zheng Y, Gou X, et al. Prevalence of comorbidities and its effects in patients infected with SARS-CoV-2: a systematic review and meta-analysis. Int J Infect Dis. 2020;94:91-95. doi: 10.1016/j.ijid.2020.03.017.

39. McDonald HI, Thomas SL, Millett ER, Nitsch D. CKD and the risk of acute, community-acquired infections among older people with diabetes mellitus: a retrospective cohort study using electronic health records. Am J Kidney Dis. 2015;66(1):60-68. doi: 10.1053/j.ajkd.2014.11.027.
40. Su G, Trevisan M, Ishigami J, Matsushita K, Stålsby Lundborg C, Carrero JJ. Short- and long-term outcomes after incident pneumonia in adults with chronic kidney disease: a time-dependent analysis from the Stockholm Creatinine Measurement project. Nephrol Dial Transplant. 2020;35(11):1894-1900. doi: 10.1093/ndt/gfz119.
41. Zammit C, Liddicoat H, Moonsie I, Makker H. Obesity and respiratory diseases. Int J Gen Med. 2010;3:335-43. doi: 10.2147/IJGM.S11926.
42. Vaduganathan M, Vardeny O, Michel T, McMurrary JVJ, Pfeffer MA, Solomon SD. Renin–angiotensin–aldosterone system inhibitors in patients with Covid-19. N Engl J Med. 2020;382(17):1653-59. doi: 10.1056/NEJMsr2005760.
43. Mancia G, Rea F, Ludergnani M, Apolone G, Corrao G. Renin–angiotensin–aldosterone system blockers and the risk of Covid-19. N Engl J Med. 2020;382(25):2431-2440. doi: 10.1056/NEJMoa2006923.
44. Reynolds HR, Adhikari S, Pulgarin C, et al. Renin–angiotensin–aldosterone system inhibitors and risk of Covid-19. N Engl J Med. 2020;382(25):2441-2448. doi: 10.1056/NEJMoa2008975.
45. Mehta N, Kalra A, Nowacki AS, et al. Association of use of angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers with testing positive for coronavirus disease 2019 (COVID-19). JAMA Cardiol. 2020;5(9):1020-1026. doi: 10.1001 /jamacardio.2020.1855.
46. Vardavas CI, Nikitara K. COVID-19 and smoking: a systematic review of the evidence. Tob Induc Dis. 2020;18:20. doi: 10.18332/tid/119324.
47. Parascandola M, Xiao L. Tobacco and the lung cancer epidemic in China. Transl Lung Cancer Res. 2019;8(Suppl 1):S21-30. doi: 10.21037/tlcr.2019.03.12.
48. Chow N, Fleming-Dutra K, Gierke R, et al. Preliminary estimates of the prevalence of selected underlying health conditions among patients with coronavirus disease 2019 – United States, February 12 - March 28, 2020. MMWR Morb Mortal Wkly Rep. 2020;69(13):382-86. doi: 10.15585/mmwr.mm6913e2.
49. Godoy P, Castilla J, Soldevila N, et al. Smoking may increase the risk of influenza hospitalization and reduce influenza vaccine effectiveness in the elderly. Eur J Public Health. 2018;28(1):150-55. doi: 10.1093/eurpub/ckx130.
50. de Lusignan S, Dorward J, Correa A, et al. Risk factors for SARS-CoV-2 among patients in the Oxford Royal College of General Practitioners Research and Surveillance Centre primary care network: a cross-sectional study. Lancet Infect Dis. 2020;20(9):1034-1042. doi: 10.1016/S1473-3099(20)30371-6.

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Author's contribution
EC conceptualized the idea, conducted literature review, wrote the manuscript and revised the final text.

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Vulnerability in the elderly

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Abstract

Background: Aging process involves an increased risk for the development of vulnerability, because senescence is a process characterized by a multitude of changes that influence the living conditions and health of the individuals. In geriatrics, the term "vulnerability" implies a multidimensional aspect, among which, multimorbidity, functional incapacity, socio-economic and cognitive problems in the elderly. The main objective of the article is to systematize data from the literature through the analysis of the concept and prevalence of vulnerability, assessed by the score Vulnerable Elders Survey-13 (VES-13) in the elderly. For this purpose, publications from the database GoogleSearch, PubMed, Hinari, etc. were analyzed. The information was systematized, highlighting the main aspects of the contemporary vision of the last 5 years.A series of studies (USA, Brazil, etc.) revealed a high prevalence of vulnerability in the elderly according to the VES-13 score, it was estimated in respondents aged > 65 years, between 40-50% of cases were vulnerable people, with a score ≥ 3 p. The vulnerability of the elderly results from different conditions, correlated with each other, especially biological, social and genetic factors. There was a functional decline between 13 and 24% of cases, especially in the elderly over 75 years, and an association with health problems, mobility and low autonomy in over 50% of cases, with a poor quality of life and increased risk of institutionalization.
Conclusions: Vulnerability assessment measures are important for identifying older people at high risk of deteriorating health, which is an important target for interdisciplinary intervention.
Key words: elderly, vulnerability, Vulnerable Elders Survey-13 score.

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Introduction

The increase of the elderly population is considered one of the most significant demographic changes in recent years, which occurred quickly and abruptly and without an adequate monitoring of social and economic processes, so in the context of gerontology we explore the concept of vulnerability, defined as the condition of individuals or a group of people who, for some reason, have a reduced capacity for self-care and who may have difficulty for performing daily needs due to deficits in functionality, cognitive function, financial resources and/or other attributes [1].

Aging poses an increased risk for the development of biological or individual vulnerability of a socio-economic or psycho-social aspect, which interacts with socio-cultural processes and the cumulative effects of poor education, low income and poor lifelong living conditions. These conditions can have a significant negative impact on the elderly, in particular favoring the individual vulnerability strongly associated with bio-physiological components [2]. Although the terms "fragility" and "vulnerability" are widely used in the gerontological literature, they are not the same. In the study of this literature, the term vulnerability is considered as a concept that involves an increased risk of developing functional decline or death over a period of up to 2 years, in relation to the deterioration of biological and physiological factors, and it is considered to be associated with the term of pre-frailty, as a condition that may precede the state of fragility. In order to address adequately the term of vulnerability, it is necessary to analyze this concept and develop/adopt screening tools for the subsequent identification of vulnerable elderly people at different levels of health care. In geriatrics, the term "vulnerability" implies a multidimensional aspect with an increased risk of deteriorating quality of life, subsequent institutionalization and death. Therefore, in the context of gerontology, the concept of vulnerability is explored, which can be defined as the elderly ones with a multidimensional quality of life affected, with an increased risk of loss of autonomy and progression of dependence [1, 2].

Despite the growing number of publications analyzing this topic, it can be seen that there is still insufficient knowledge about understanding the vulnerability of the elderly [1, 3]. Identifying vulnerable older people and understanding their causes and consequences is a key task of medical and social policy in developing protocols and measures to improve the quality of life of vulnerable older people.

The main goal of this proposed article is focused on the analysis of the concept of vulnerability and the existing methods used to detect it in the elderly. The main objectives are to highlight the main aspects of the contemporary vision on vulnerability in the literature, the causes of occurrence, risk factors and the impact on the quality of life of the elderly and society in general.

In order to realize the goal of the study, various specialized books, publications from the GoogleSearch database, Hinari, Pub Med, etc. were studied, including the pages of the official sites of the European Geriatric Society, Italian, French, etc. for the identification of scientific journals dedicated to vulnerability, by using key-words "fragility syndrome", "vulnerability", "vulnerability in the elderly", "VES-13" etc. Also, the bibliography of the selected publications was studied. The information was systematized, highlighting the main aspects of the contemporary vision in works of the last 5 years. According to the search engine, 31 publications were selected (clinical trials and literature review).

Results

Vulnerability is a state of instability, caused by a reduction in adaptation reserves, with a high risk of dependence, hospitalization, mortality, and an imbalance between somatic, mental and social elements, so the concept of vulnerability is a state of dependence, with an increased risk of addiction and the development of fragility syndrome. The main predisposing factors of vulnerability are: age over 85yrs, loss of autonomy for daily activities and instrumetal daily activities (according to Katz and Lawton scores), inadequate social support (especially in case of loneliness, widowhood, poverty), cognitive function affected by the installation of disorders cognitive impairment (according to mini-Mental Status Examination (MMSE) and/or MoCa tests), decreased nutritional reserves or affected nutritional status (determined by Body Mass Index (BMI) and/or Mini-Nutritional Assessment (MNA) test), physical inactivity, postural instability, depression (according to Hamilton score and/or mini-Geriatric Depression Scale), sensory disturbances, comorbidities, iatrogenic pathology [4-6].

In geriatrics, the term "vulnerability" implies a multidimensional aspect, including age-related changes, multimorbidity, including consequences of enduring diseases, functional disability, socio-economic and cognitive problems in the elderly (fig. 1).

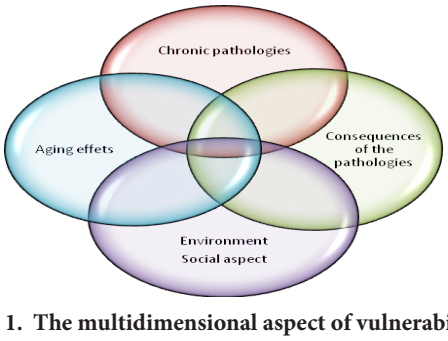

Fig. 1. The multidimensional aspect of vulnerability in the elderly

According to the results of the studies, the identification of people over the age of 65 and who are in a situation of vulnerability, at risk of functional decline and disability is an important and significant step towards building and prioritizing appropriate care for this group of patients [1, 2]. Through the analysis of the literature, the vulnerability of the elderly results from different conditions, which are correlated with each other, biological, social and genetic factors stand out. Elements such as: biological aging, chronic pathology with deteriorating health, inability of health services, frequent hospitalizations and female gender were identified as precursors to the studied concept. There are 4 major risk factors, which may contribute to the development of vulnerability to the elderly:

- ✓ Functional dependence.
- ✓ Loss of autonomy.
- ✓ Social precariousness.
- ✓ Limited access to care.

The attributes, antecedents and consequences of the concept were identified, presented in figure 2, based on the studied literature.

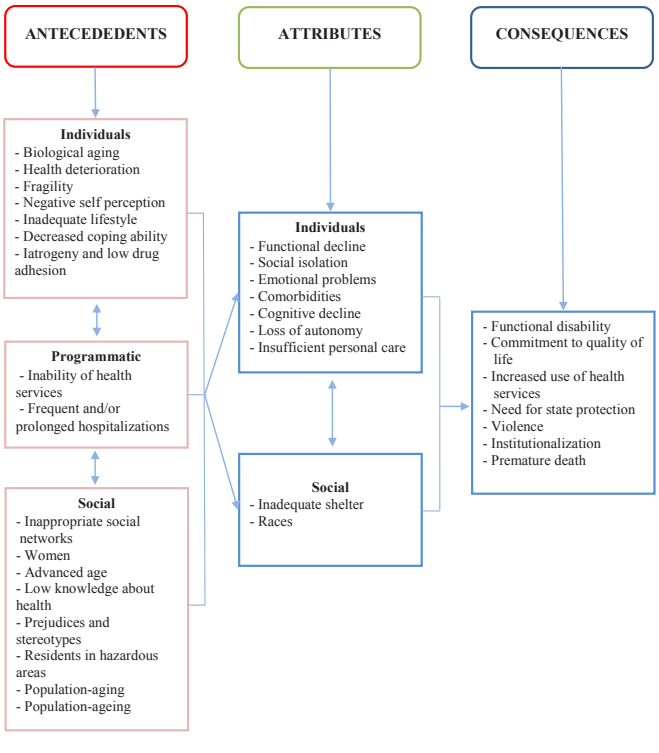

Fig. 2. Background, attributes and consequences of the concept of vulnerability in the elderly, identified from a review of the integrative literature, 2018

There are studies in the literature that describe screening tools for the functional decline and quality of life of vulnerable elderly people [7, 8]. One of these is the Vulnerable Elders Survey-13 (VES-13), a tool used to predict the onset of functional disability, institutionalization and death of the elderly, included for the first time in the 2014 Health Guide for the Elderly of the Brazilian Ministry of Health, called Protocol for the identification of vulnerable elderly people – VES-13

[9]. Another tool is Comprehensive Geriatric Assessment that involves functional, cognitive and emotional status assessment scores (ADL, IADL, Tinetti, MMSE, Hamilton). In addition, there are other tests and scores that may be used in some cases, such as: Fried Criteria – evaluating fragility syndrome, SPPB, SARC-F, TUG, TRST, CIRS-G, QLQ-C30, ICOPE.

Vulnerability assessment measures are important for identifying older people at increased risk of deteriorating health, which is an important target for interdisciplinary intervention. Identifying vulnerable people over the age of 60, at high risk of functional and cognitive decline, is an important and significant step towards building and prioritizing appropriate care for these patients [1, 3]. There are studies in the literature that describe screening tools for the functional decline and quality of life of the elderly (fig. 3) [4, 5].

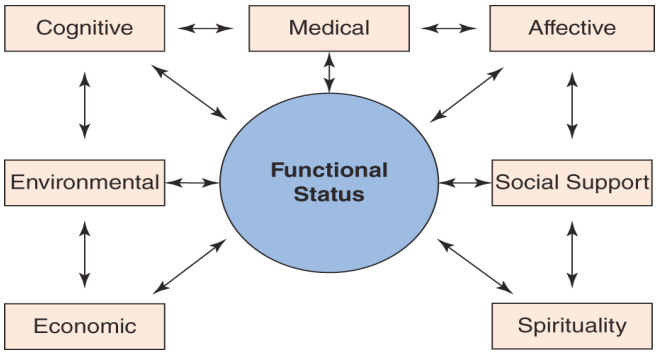

Fig. 3. Multidimensional assessment of vulnerability in the elderly (complex geriatric assessment, ICOPE)

Vulnerable people are those whose autonomy and functionality are impaired, according to this, we can consider that the elderly are those who face chronic diseases, functional decline and loss of social role, being at risk of becoming vulnerable. However, older people do not age uniformly and are vulnerable, for this purpose it is necessary to highlight the risk factors associated with vulnerability in the elderly and the need for intervention to prevent or reduce the degree of vulnerability.

Functional dependence. Functional dependence is defined by the inability to perform daily physical or instrumental activities (ADL, IADL). In Switzerland, 10% of people over 65 and 20% of those aged 75 to 94 need help with at least one point in the baseline ADL score (e.g. such as a morning toilet) [3]. The most common pathologies associated with functional decline are: cardiovascular pathology, cerebrovascular disease, stroke, hip fractures, osteoarthritis and cognitive impairment [1]. Functional dependence creates dependence on others, especially on a caregiver.

Loss of autonomy. Autonomy is the ability to decide and function on one's own [5]. Loss of autonomy makes the person vulnerable, depriving him of the power to act alone and imposing decisions made by others. Loss of discernment, in particular, deprives the person of the right to autonomy, especially in the case of dementia and major depression, associated with increased risk of loss of decision-making, depending on the severity of cognitive impairment [2, 6]. Loss of autonomy in the absence of cognitive deficits can be caused by chronic pathology, which in addition to deep isolation, dependence on others, fear of death and loss of life partner can all affect the patient's autonomy [7].

Social insecurity. Research on the elderly in Switzerland has highlighted two main areas of social precariousness: social isolation and financial problems. Social isolation is associated with poor life quality, impaired physical and cognitive status [8]. In the elderly, physical inactivity is a major risk factor for social isolation and increases the risk by 60% within two years [9]. In Switzerland, almost 5% of the population is over 80 years old, this figure is expected to increase dramatically in the next few years due to the aging process. In this age group, almost 42% of men and 87% of women experience social isolation, mainly due to widowhood. One consequence is that 24% of people over the age of 80 years receive medical and social support, at least once a week, and the help of neighbors [10]. Poverty and social isolation, beyond their impact on health, however, remain factors associated with an increased risk for vulnerability, especially when combined with other risk factors.

A number of studies (Brazil, USA, etc.) revealed a high prevalence of vulnerability in the elderly according to the VES-13 score, it was estimated in respondents aged> 65 years, between 40-50% of cases were vulnerable people, with a score ≥ 3 points. The vulnerability of the elderly results from different conditions, correlated with each other, especially biological, social and genetic factors. There was a functional decline between 13 and 24% of cases, especially in the elderly over 75 years, and an association with health problems, mobility and low autonomy in over 50% of cases, with a poor quality of life and increased risk of institutionalization.

Starting from studies in the world gerontological literature, in terms of the socio-demographic characteristics of vulnerability, most of the selected scientific papers were published on the continent of South America (No 12; 34.3%), especially in Brazil, where they have conducted 11 studies on the research of this concept, research on vulnerability among the elderly has been started since 2000, predominantly in 2015, 2014 and 2010 (14.0%), followed by the USA and Europe, highlighting the increased interest of researchers in terms of improving knowledge in this area during last years. In Brazil, two studies were highlighted, which included VES-13, the first in 2014, with 368 respondents, over 65 years, average age 71.4 years, 84% had ≥3 comorbidities, predominantly sensory disorders (21.1%) and hypertension (19.23%). When assessing the degree of physical vulnerability, 52.2% of the elderly were vulnerable, according to VES-13≥3p. Of these, 72.4% were women, between 70 and 79 years (41.1%). There was a decrease in performance of basic and instrumental activities of daily life ($p<0.001$). Another quantitative, cross-sectional study was conducted in Distrito Federal, Brazil, on a group of 956 respondents over 60 years, of which 32.4% had VES-13 scores ≥ 3 p. clas-

sified as vulnerable. Some variables showed a positive correlation with the VES-13 score, in the case of hypertension (p = 0.035), diabetes (p = 0.027), to moderate, in the case of depression (p <0.001), urinary incontinence (p <0.001) and falls (p <0.001). It was also observed that a 1% increase in the income of the elderly led to a reduction of 0.27 points (p <0.001) in the VES-13 score, reducing the vulnerability. At the same time, another cross-sectional, descriptive and analytical study, between January 2016 and December 2017, on a number of 1062 respondents, of which 57.3% were women, the average age of 69 years (± 7.8). A total of 427 individuals (40.2%) were vulnerable and 635 (59.8%) were not vulnerable according to the VES-13 score. A total of 635 (59.8%) elderly people were classified as robust, 176 (16.6%) at risk of fragility and 251 (23.6%) fragile. According to the results in most studies, women and those over 75 years of age were considered in the most vulnerable category [10]. At the same time, a study conducted in the USA revealed a VES-13> 3points, 32% of respondents were appreciated as vulnerable, in this group the risk of death is 4.2 times higher and functional decline aprox. 2 years, compared to those who scored <3 p. [11]. A pilot study included the elderly with oncological pathology, patients with prostate cancer aged >70 years, the vulnerability of this category of elderly was found. Out of 50 participating patients, 50% were identified as vulnerable according to VES-13 (score ≥ 3), 60% of patients had deficiencies in ≥2 tests in the CGA, with deficiencies in several areas. Patients with VES-13 scores ≥3 p., according to the evaluation of daily activities showed reduced physical performance, comorbidities and cognitive impairment [11, 12]. In the case of the Caucasian population, the study included 864 respondents (mean age 80.9 ± 8.2 years, 615 women) who were hospitalized in the Geriatric Hospital, functional vulnerability presented 91.2% with a VES-13 score of ≥3 p. In the case of the Irish population, the assessment of the proportion of vulnerability was assessed as identical to the US sample (32.1% compared to 32.3% USA), according to VES-13.

One of the first aspects that is compromised in the aging process is the performance of activities of daily living, mainly because it involves tasks that require greater physical and cognitive integrity, often related to the social participation of the individual, such as shopping, answering the phone and using means of transport. Developed studies have identified routine activities to which older people have reported the greatest difficulties, such as: difficulty of shopping, doing household activities, performing light tasks, such as managing finances and performing the toilet alone [12, 13]. It should be noted that elderly people with a functional decline are vulnerable to the increased risk of hospitalization, institutionalization and early death, with a 4.2 times higher risk of loss of autonomy and death in the first 2 years after the initial assessment [14].

Studies suggest that older people are vulnerable due to decreased physical and/or mental health. Factors such as deteriorating health, sensory disturbances, cognitive impairments, psychological decline, recurrent episodes of falls and fragility have been strongly linked to the vulnerability of the elderly. Such conditions favor frequent referrals to health services. A significant proportion of vulnerable elderly people need long-term medical care [15, 16]. Another important aspect highlighted in the literature was gender, as the highest rates of vulnerability and morbidity are attested in women [12, 17, 18]. A Canadian study of older people living in the community showed that life expectancy among women was negatively affected by social problems, such as family and social isolation, economic problems and loneliness [19]. Depression most affected the vulnerability score, followed by urinary incontinence and hearing loss.

Vulnerability involves multiple etiology in the progression of functional decline, the interrelationship between comorbidities such as: heart disease, diabetes, hypertension, Parkinson's disease, and social determinants aggravate this phenomenon [20]. In the case of vulnerable elderly, research shows that social involvement decreases, against the background of a functional and cognitive decline, and a higher frequency of chronic and infectious diseases against which the decrease of interest and empathy towards society appears.There is a need for care adopted for the needs of the elderly, regular assessments of their health, assessment of physical and cognitive status. Performance in instrumental and basic activities of daily living are a widely accepted and recognized parameter for assessing functional capacity, which can be defined as the concrete possibility to manifest the physical and mental abilities necessary for an independent and autonomous life. Multiple comorbidities are a risk factor for functional decline. It was estimated that vulnerability is strongly associated with psychological and physical changes [21, 22].

Biological aging is a continuous process that causes irreversible changes on the body and has a negative impact on the functionality of the individual, sarcopenia is highlighted as a slow, progressive and seemingly inevitable process of loss of strength and muscle mass. It is considered one of the most important physiological changes that occur with the aging process, as it is responsible for reducing functional status and consequently reduced mobility. In the context of this study of the literature, data were identified that vulnerable elderly people have difficulties and/or inability to perform certain mobility-related activities, such as flexion/extension or kneeling, lifting or carrying a weight of aprx. 5 kg, raising the arm above shoulder level, writing or handling with small objects. Low mobility is the most affected area with repercussions on the health of the elderly, associated with endurance and reduced muscle strength, disability and dependence in performing daily activities [23, 24]. One of the first aspects that is compromised in the aging process is the performance of daily activities, mainly those involving tasks that require greater physical and cognitive integrity, often related to the social participation of the individual, such as shopping, answering the phone and use the means of transport. In vulnerability studies, the elderly reported difficulties in performing various tasks: shopping, managing their own finances and doing household work [25, 26].

A survey conducted in the Netherlands showed that the decline in the performance of instrumental activities varies in the elderly population over 65, between 13% and 24%, resulting in a burden for society as a whole and the economy [27]. Regarding the prevalence of dependence for the basic activities of daily living, it was observed that most elderly people had difficulty bathing without help from a caregiver, which was statistically correlated with vulnerability, indicating a significant degree of functional dependence [25, 27].

Losses caused by functional decline can be prevented or minimized if the older adult is encouraged to initiate a specific physical activity program, such as strength training, especially for the lower limbs and under the supervision of suitably qualified professionals. Moreover, the recognition and identification of functional changes can promote early intervention and prevention of disabilities, especially among the elderly [23, 25].

Studies demonstrate the multidimensionality of the studied concept, emphasizing the particularities of vulnerability during aging, there is a need for additional studies in the context of gerontology, because it has significant influence on life quality [1]. Although it is used frequently, the concept is complex and subjective, as there is no agreement in the literature on this phenomenon, thus showing different generic and incipient definitions that often do not take into account the specifics of the elderly, socio-cultural, economic and political interactions with biological processes throughout the life. Therefore, it is important to highlight the need for further studies to understand this concept, allowing its clarification, for its subsequent applicability in practice related to the needs of the elderly.

The degree of vulnerability is subject to a series of combinations of elements present physically, cognitively and socially. Aging is a natural biological process, which over the years, brings many changes, and produces structural and behavioral effects that directly affect the bio-psycho-social aspect and influences the quality of life [16, 28]. Vulnerability assessment measures are important for identifying elderly people at high risk of deteriorating health, which is an important target for interdisciplinary intervention. Vulnerability is determined by gender, age over 75 years and the presence of chronic diseases, these being the priority groups for the elaboration of intervention strategies to improve health condition for elderly people. Elderly patients have unique medical and social problems, especially in the context of long-term care, which depends on available family and social care, as well as the possibilities for diagnosis and the presence of comorbidities, however, the negative effects of senility can be minimized with measures aimed at comprehensive health care. The findings highlighted by the critical analysis of the literature provided relevant theoretical materials, highlighting the particularities of vulnerability in the aging process, with specific regional, gender and age characteristics. Elucidation of characteristics of vulnerability allows theoretically and practically to develop the effective care strategies for the elderly. Public policies aimed at promoting the physical and mental health of the elderly may help reduce the vulnerability for this category of population.

The growing elderly population needs adequate health services by providing comprehensive care. It is very important to recognize vulnerability and to have a multidisciplinary team, in order to promote comprehensive interventions for this category of the elderly [1, 3].The aging process involves an increased risk for the development of vulnerability, because senescence is a process characterized by a multitude of changes that influence the health of the elderly, then the quality of life of the individual and society in general.

Conclusions

Vulnerability assessment measures are important for identifying older people at high risk of deteriorating health, which is an important target for interdisciplinary intervention.

Vulnerability is determined by the gender, age over 75 years and the presence of chronic diseases, these being the priority groups for the development of intervention strategies for health of the elderly.

Elderly patients have unique medical and social problems, especially in the context of long-term care, which depends on the available family and social care, as well as the possibilities for diagnosis and the presence of comorbidities.

References

1. Carmo ME, Guizardi FL. The concept of vulnerability and its meanings for public policies in health and social welfare. Cad Saúde Pública. 2018;34(3):e00101417. doi: 10.1590/0102-311X00101417.
2. Barbosa KTF, Costa KNFM, Pontes MLF, Batista PSS, Oliveira FMRL, Fernandes MGM. Aging and individual vulnerability: a panorama of older adults attended by the Family Health strategy. Texto Contexto Enferm. 2017;26(2):e2700015. https://doi.org/10.1590/0104-07072017002700015.
3. Rodrigues NO, Neri AL. Vulnerabilidade social, individual e programática em idosos da comunidade: dados do estudo FIBRA, Campinas, SP, Brasil [Social, individual and programmatic vulnerability among the elderly in the community: data from the FIBRA Study conducted in Campinas, São Paulo, Brazil]. Ciênc Saúde Colet. 2012;17(8):2129-39. Portuguese. https://doi.org/10.1590/S1413-81232012000800023.
4. Arveux I, Faivre G, Lenfant L, et al. Le sujet âgé fragile [The fragile elderly subject]. Rev Gériatr. 2002;27:569-81. French.
5. Rockwood K, Fox RA, Stolee P, Robertson D, Beattie BL. Frailty in elderly people: an evolving concept. CMAJ. 1994;150(4):489-95.
6. Gonthier R. Le concept de fragilité: pourquoi est-il essentiel? [The concept of fragility: why is it essential?]. Rev Gériatr. 2000;25(3):135-8. French.
7. Ayres JRCM, Calazans GJ, Saletti HCJ, França IJ. Risco, vulnerabilidade e práticas de prevenção e promoção da saúde [Risk, vulnerability and health prevention and promotion practices]. In: Akerman M, Campos GWS, Carvalho YM, Drumond MJ, Minayo MCSH, editors. Tratado de Saúde Coletiva [Collective Health Treaty]. 2nd ed. Rio de Janeiro: Editora Fiocruz; 2012. p. 375-418. Portuguese.
8. Talmelli LFS, Gratão ACM, Rodrigues RAP. Modelo híbrido para desenvolvimento de conceito em Enfermagem utilizando revisão integrative e análise de conteúdo [Hybrid model for concept development in Nursing using integrative review and content analysis]. Saúde Transform Soc. 2016;7(1):9-15. Portuguese.
9. Agu FC. Healthy aging reports: a conceptual and ethical analysis of vulnerability and independency. SAGE Open. 2013. https://doi.org/10.1177/2158244013491413.

10. Amancio TG, Oliveira MLC, Amancio VS. Factors influencing the condition of vulnerability among the elderly. Rev Bras Geriatr Gerontol. 2019;22(2):e180159. https://doi.org/10.1590/1981-22562019022.180159.
11. Mohile SG, Bylow K, Dale W, Dignam J, Martin K, Petrylak DP, Stadler WM, Rodin M. A pilot study of the vulnerable elders survey-13 compared with the comprehensive geriatric assessment for identifying disability in older patients with prostate cancer who receive androgen ablation. Cancer. 2007 Feb 15;109(4):802-10. doi: 10.1002/cncr.22495.
12. Barbosa KTF, Fernandes MGM, Oliveira FMRL, Tibúrcio ABCB, Alves ABR, Ramos CEB. Vulnerabilidade física entre idosos: diferencias por sexo [Physical vulnerability in elderly: gender differences]. Cultura Cuid. 2015;19(42):90-100. https://doi.org/10.14198/cuid.2015.42.09.
13. Maia FOM., Duarte YAO, Secoli, SR, Santos JLF, Lebrão ML. Cross-cultural adaptation of the Vulnerable Elders Survey-13(VES-13): helping in the identification of vulnerable older people. Rev Esc Enferm USP. 2012;46(Spec):116-22. doi: 10.1590/s0080-62342012000700017.
14. Pulignano G, Del Sindaco D, Di Lenarda A, et al. Usefulness of frailty profile for targeting older heart failure patients in disease management programs: a cost-effectiveness, pilot study. J Cardiovasc Med. 2010;11(10):739-47. doi: 10.2459/JCM.0b013e328339d981.
15. Brocklehurst H, Laurenson M. A concept analysis examining the vulnerability of older people. British J Nurs. 2008;17(21):1354-7. doi: 10.12968/bjon.2008.17.21.31738.
16. Sarvimäki A, Stenbock-Hult B. The meaning of vulnerability to older persons. Nurs Ethics. 2016;23(4):372-83. doi: 10.1177/0969733014564908.
17. McGee HM, O'Hanlon A, Barker M, et al. Vulnerable older people in the Community: relationship between the Vulnerable Elders Survey and health service use. J Am Geriatr Soc. 2008;56(1):8-15. doi: 10.1111/j.1532-5415.2007.01540.x.
18. Bell SP, Schnelle J, Nwosu SK, et al. Development of a multivariable model to predict vulnerability in older American patients hospitalised with cardiovascular disease. BMJ Open. 2015;5(8):e008122. doi: 10.1136/bmjopen-2015-008122.
19. Armstrong JJ, Andrew MK, Mitnitski A, et al. Social vulnerability and survival across levels of frailty in the Honolulu-Asia Aging Study. Age Ageing. 2015;44(4):709-12. doi: 10.1093/ageing/afv016.
20. Rubio AE, Lazaro AA, Martinez TT, Magallón B. Enfermedades crónicas y deterioro funcional para las actividades de la vida diaria em población mayor no institucionalizada [Chronic diseases and functional deterioration in activities of daily living in community-dwelling elders]. Rev Española Geriatr Gerontol. 2009;44(5):244-50. Spanish. doi: 10.1016/j.regg.2009.03.011.
21. Wallace LMK, Theou O, Pena F, et al. Social vulnerability as a predictor of mortality and disability: cross-country differences in the survey of health, aging, and retirement in Europe (SHARE). Aging Clin Exp Res. 2015;27(3):365-72. doi: 10.1007/s40520-014-0271-6.
22. Seidel D, Crilly N, Matthews FE, et al. Patterns of functional loss among older people: a prospective analysis. Hum Factors. 2009;51(5):669-80. doi: 10.1177/0018720809353597.
23. Garcia PA., Dias JMD, Dias RC, et al. A study on the relationship between muscle function, functional mobility and level of physical activity in community-dwelling elderly. Rev Bras Fisioter. 2011;15(1):15-22.
24. Van Iersel MB, Munneke M, Esselink RA, et al. Gait velocity and the Timed-Up and-Go Test were sensitive to changes in mobility in frail elderly patients. J Clin Epidemiol. 2008;61(2):186-91. doi: 10.1016/j.jclinepi.2007.04.016.
25. Ursine PGS, Cordeiro HÁ, Moraes CL. Prevalence of housebound elderly people in the urban region of Belo Horizonte (Minas Gerais, Brazil). Ciên Saúde Colet. 2011;16(6):2953-62. doi: 10.1590/s1413-81232011000600033.
26. Del Duca GF, Silva MC, Hallal PC. Disability relating to basic and instrumental activities of daily living among elderly subjects. Rev Saúde Pública. 2009;43(5):796-805. doi: 10.1590/s0034-89102009010600001.
27. Suijker JJ, Buurman BM, van Rijn M, et al. A simple validated questionnaire predicted functional decline in community-dwelling older persons: prospective cohort studies. J Clin Epidemiol. 2014;67(10):1121-30. doi: 10.1016/j.jclinepi.2014.05.014.
28. Cahir C, Moriarty F, Teljeur C, Fahey T, Bennett K. Potentially inappropriate prescribing and vulnerability and hospitalization in older community-dwelling patients. Ann Pharmacother. 2014;48(12):1546-54. doi: 10.1177/1060028014552821.

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AP wrote the first manuscript; GS conducted literature review; VF conceptualized the idea; VO revised critically the manuscript and completed the final text. All the authors approved the final version of the manuscript.

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Hereditary thrombophilia and adverse pregnancy outcomes

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Abstract

Background: Multiple studies have found a relatively increased risk of placenta-mediated pregnancy complications in women with congenital thrombophilia, especially early recurrent pregnancy loss, fetal loss, early-onset preeclampsia, intrauterine growth restriction, and premature abruption of normally positioned placenta. However, the extent of the association and the absolute risk are very modest, but they significantly increase in pregnant women with severe obstetric complications.
Conclusions: There is convincing evidence that deficiency of natural anticoagulants (antithrombin, protein C, protein S) is a risk factor for late fetal loss. Factor V Leiden G1691A gene mutation and prothrombin G20210A gene mutation are associated with a double risk for early and unexplained recurrent pregnancy loss and for non-recurrent late fetal loss. The association of congenital thrombophilia with preeclampsia is much more uncertain, being probably limited factor V Leiden G1691A gene mutation and more severe cases of preeclampsia. Fewer data are available on intrauterine growth restriction (IUGR) and premature abruption of the normally positioned placenta. There is insufficient evidence to suggest an association of other forms of congenital thrombophilia with adverse pregnancy outcomes. In addition, genetic and epidemiological research suggests that placenta-mediated pregnancy complications are of polygenic multifactorial etiology, with a risk determined by the interaction of multiple genetic variants and other risk factors.
Key words: pregnancy complications, hereditary thrombophilia, recurrent pregnancy loss.

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Introduction

Congenital thrombophilia, in general agreement with the literature, in addition to its established role in the pathogenesis of venous thromboembolism (VTE) increases the risk of gestational vascular complications and adverse pregnancy outcomes. The role of congenital thrombophilia in gestational vascular disorders and adverse pregnancy outcomes (miscarriage, fetal loss, preeclampsia, intrauterine growth restriction and premature abruption of the normally positioned placenta) has been evaluated in several studies and the results are inhomogeneous and contradictory [1-3]. The evaluation of a large number of studies in the literature on congenital thrombophilia in pregnancy does not assign an overall incidence of risk, indicating only an absolutely low risk in pregnant women and in the fetus during pregnancy. It is still difficult to establish the exact quantitative data of the risk for certain adverse events in the presence of these mutations [4, 5]. In addition, the size of estimated risks varies, due to the heterogeneity of different study designs [6].

Major forms of hereditary thrombophilia, currently recognized as independent risk factors for VTE, include procoagulant abnormalities – factor V Leiden G1691A (FVL) gene mutation of homozygous or heterozygous type, homozygous or heterozygous prothrombin G20210A gene mutation, endogenous deficiency of natural anticoagulants – antithrombin (AT), protein C and protein S, and mutation of the methylenetetrahydrofolate reductase (MTHFR) C677T gene. The most common forms of congenital thrombophilia are the FVL G1691A gene mutation and the prothrombin G20210A gene mutation of the heterozygous type, the other types of congenital thrombophilia (AT deficiency, protein C and protein S deficiency) have a higher thrombogenic potential, but are less common [4, 7-10].

The data below summarize the prevalence of the most important types of congenital thrombophilia, including recently detected, a brief review of the available evidence for each congenital thrombophilia in relation to adverse pregnancy outcomes and the risk of VTE.

FVL G1691A gene mutation. The risk of developing VTE increases 2-7 times in people with heterozygous FVL G1691A gene mutation and 40-80 times in people with homozygous FVL G1691A gene mutation [7]. About 40-44% of women with VTE, in pregnancy or postpartum, are carriers of the FVL G1691A gene mutation and most are heterozygous [4, 11].

Data on the relationship between the FVL G1691A gene mutation and fetal loss are inconsistent. In general, the FVL

G1691A gene mutation contributes to a small increase in the risk of miscarriage and recurrent pregnancy loss (RPL) [1, 7, 12, 13]. In terms of other parameters of obstetric morbidity, a recent meta-analysis suggests that there is a significant increase in the frequency of obstetric complications in general, prenatal mortality (stillbirth), severe preeclampsia, intrauterine growth restriction (IUGR), newborns small for gestational age and premature abruption of the normally positioned placenta (PANPP) in carriers of the FVL G1691A gene mutation [7, 12].

Two recent and comprehensive literature reviews, which included prospective, retrospective case-control and cohort studies with a moderate heterogeneity, established that carriers of the FVL G1691A gene mutation had a relatively high risk of RPL (1.52- 2.02 times higher). However, the absolute risk of pregnancy loss in women with the FVL G1691A gene mutation is low (4.2%) [14].

There is no significant association between the FVL G1691A gene mutation and preeclampsia, between the FVL G1691A gene mutation and IUGR or newborns small for gestational age [14]. The association between PANPP and the FVL G1691A gene mutation is also insufficient [8].

However, the association with severe preeclampsia of the FVL G1691A gene mutation was assessed in a meta-analysis of case-control studies and found a strong association; the Odds Ratio (OR) ranges from 2.04 to 3.0 [13]. A systematic literature review and a recent meta-analysis, estimating 42 case-control and cohort studies, published in 2016, found a significant association of the FVL G1691A gene mutation with newborns small for gestational age (OR 1.40) and the lack of association of the FVL G1691A gene mutation with premature birth [15].

Prothrombin G20210A gene mutation. The risk of VTE in carriers of prothrombin G20210A gene mutation is 2-5 times higher, and in combination with the FVL G1691A gene mutation – 20 times higher [7]. The heterozygous type of prothrombin G20210A gene mutation has a 3-8-fold higher risk, and the homozygous type – 18-80-fold higher risk of thromboembolic events [9].

A systematic literature review found an OR of 2.49 for early pregnancy loss, 2.7 for early RPL, 2.66 for late pregnancy loss, 2.54 for preeclampsia, 2.92 in newborns small for gestational age and 7.71 for PANPP in pregnant women with the prothrombin G20210A gene mutation, compared to pregnant women who do not have this form of congenital thrombophilia [13].

Data cumulated from several studies indicate a significant association between the prothrombin G20210A gene mutation and RPL, total and severe preeclampsia. Although the relationship between the prothrombin G20210A gene mutation and VTE during pregnancy is clear, the results of some studies do not confirm the impact of this mutation on obstetric complications: early RPL, preeclampsia, including early or severe onset preeclampsia, PANPP, IUGR, and newborns small for gestational age [1, 7, 8, 12, 14].

Protein C deficiency. In general, studies have not found a clear association between protein C deficiency and RPL [1, 4, 7], but in the case of the association of protein S and protein C deficiency, there is a correlation with an increased incidence of stillbirth [4]. There are no studies that have found an association between protein C deficiency and early pregnancy loss, IUGR or PANPP. Protein C deficiency has been associated with an increased risk of miscarriage in the second trimester, preeclampsia, and stillbirth [8].

Protein S deficiency. A literature review, published in 2002, evaluating 3-5 relevant studies, found an increased risk of preeclampsia (12.7 times higher) with an absolute risk of 12.3% and an increase in stillbirths (16.2 times higher) with an absolute risk of 6% in pregnant women with protein S deficiency. There are no studies that have found an association between protein S deficiency and early pregnancy loss, IUGR or PANPP [8].

Given the low prevalence and limited data, definitive conclusions on the effect of protein C and protein S on RPL and other pregnancy complications cannot be reached and further investigations are needed [1, 3].

AT deficiency was the first hereditary thrombophilia identified, which significantly increases the risk of VTE. Being the most thrombogenic, it has the highest risk of VTE among hereditary thrombophilias and often requires long-term anticoagulant therapy. The lifetime VTE risk accounts for 70-90%, being particularly high in pregnancy, postpartum period and after major surgery [7].

Data on the role of AT deficiency in fetal loss (RPL, late loss) are contradictory. Some studies have found that women with AT-III deficiency have an increased risk of embryonic death and fetal death compared to the general population [7, 8]. The results obtained in other studies suggest an association between AT deficiency and pregnancy loss, but the definitive causal relationship is not **yet** established. AT-III deficiency is rarely associated with severe preeclampsia, IUGR or PANPP, but this could be «false negative» due to the low prevalence of this form of thrombophilia [7].

MTHFR C677T gene mutation. Hyperhomocysteinemia is an independent risk factor for VTE. Although several studies have not found a firm relationship between the homozygous MTHFR C677T gene mutation or elevated homocysteine levels with adverse pregnancy outcomes [1, 7], a recent meta-analysis revealed an association of the MTHFR C677T gene mutation with severe preeclampsia [12].

Another meta-analysis, which included 26 case-control studies with 2120 cases and 2949 controls (1997-2005), and some recent studies found that the MTHFR C677T gene mutation is a genetic risk factor of unexplained RPL (two or more consecutive miscarriages) only in the Chinese and Iranian population, but not in the population of European countries [16, 17].

Two recent meta-analyses, which included 27 and 37 studies (1997-2011 and 1997-2012) with 2427 and 3559 cases, 2120 and 5097 controls (published in 2012 and 2013), identified a significant association between the MTHFR C677T gene mutation and unexplained RPL (two or three and more consecutive pregnancy losses) in the East Asian subgroup (Chinese, Japanese and Korean population) and in

the mixed subgroup (Indian, Brazilian, Bahraini, Mexican and Egyptian population), but not in the Caucasian population, including the population of European origin. In addition, the study found a genetic heterogeneity between the ethnic groups [18, 19].

Three recent meta-analyses, published in 2014 and 2015, which included a substantial number of cases and controls, revealed statistically significant results of the relationship between the MTHFR C677T gene mutation and hypertension in the population, hypertension in pregnancy and preeclampsia, in particular among Asians and Caucasians. The authors consider that this polymorphism is an independent risk factor for hypertension and preeclampsia [20, 21].

The results of the Hordaland Homocysteine Study, conducted among 5883 women aged 40-42, with 14492 pregnancies, and published in 2004, revealed that maternal polymorphism MTHFR C677T is associated with increased pregnancy complications in general, increased risk of PANPP and, possibly with IUGR or newborns small for gestational age [22].

Therefore, all types of congenital thrombophilia are associated with an increased risk of VTE during pregnancy. Several studies confirmed the increased risk of obstetric complications in pregnant women with congenital thrombophilia, but despite the increased relative risk, the absolute risk of VTE and negative pregnancy outcomes is reduced. Moreover, the risk estimation largely depends on the type of hereditary thrombophilia analyzed, the methodological criteria applied for patient selection, the ethnic groups included in the study, the differences in sensitivity and specificity of laboratory methods of thrombophilia detection and the polygenic multifactorial etiology of pregnancy complications.

There is convincing evidence that deficiency of natural anticoagulants (AT, protein C, protein S) is a risk factor for late fetal loss. The FVL G1691A gene mutation and the prothrombin G20210A gene mutation are associated with a double risk for unexplained early RPL and non-recurrent late fetal loss. The association of congenital thrombophilia with preeclampsia is much more uncertain, being probably limited to the mutation of the FVL G1691A gene and more severe cases of preeclampsia. Fewer data are available about IUGR and PANPP. The absolute risk of VTE and negative pregnancy outcomes increase significantly in case of a combination of two or more forms of hereditary thrombophilia.

Contemporary technologies have recently contributed to the discovery of new forms of hereditary thrombophilia: factor V A4070G gene mutation, factor XIII G103T gene mutation, MTHFR A1298C gene mutation, methionine synthase (MTR) A2756G gene mutation, methionine synthase reductase (MTR) A66G gene mutation, plasminogen activator inhibitor (PAI-1) gene mutation, cystathionine beta-synthetase (CBS) 844ins68pb gene mutation. However, information on these polymorphisms is limited, and their role in the development of unexplained RPL and other obstetric complications is still controversial [23, 24].

The factor V A4070G gene mutation has a pathological role, causing a moderate resistance to activated protein C and interacting with the FVL G1691A gene mutation to produce a more resistant phenotype to activated protein C. As no significant increase in the prevalence was identified, the presence of the factor V A4070G gene mutation is unlikely to be an independent risk factor for pregnancy-associated complications. It is controversial whether this mutation is associated with an increased thrombotic risk in the absence of the FVL G1691A gene mutation [9, 25].

The heterozygous MTHFR A1298C gene mutation has a strong association and is a genetic risk factor for RPL [17, 26] and PANPP [27]. The association of RPL with MTHFR A1298C polymorphism is found in previously published studies and in a meta-analysis of 5 studies, published in 2013 [26, 28].

However, recent meta-analyses and case-control studies did not identify any association between the MTHFR A1298C gene mutation and unexplained RPL (two or three and more consecutive pregnancy losses) [18, 29, 30], preeclampsia [20], IUGR or newborns small for gestational age [22]. In contrast, this mutation may have a protective effect by raising serum folic acid levels and decreasing homocysteine levels in the serum [31, 32], and may significantly reduce the risk of pregnancy complications in asymptomatic nulliparous pregnant women associated with MTHFR C677T polymorphism [33], although these results require confirmation.

The CBS 844ins68 gene mutation is not a genetic risk factor for venous thrombosis [34], although the results of some studies suggest possible effects of this polymorphism on the risk of idiopathic miscarriage, but it does not have a significant certainty [35].

MTR A2756G gene mutation. The role of the MTR A2756G gene polymorphism in the development of hyperhomocysteinemia is still controversial. Some studies did not find any significant risk of hyperhomocysteinemia in people with the MTR A2756G gene mutation. The effect of reducing homocysteine levels is synergistic in people with both mutations (CBS 844ins68 gene mutation and MTR A2756G gene mutation), and homocysteine levels are much lower than in people with one of these polymorphisms [36]. Other studies have shown that the MTR A2756G polymorphism independently increases the risk of hyperhomocysteinemia [31, 32].

The methionine synthase-reductase gene mutation (MTRR A66G) does not affect folic acid levels or the incidence of folate deficiency, has no effect on plasma concentration of total homocysteine, folate or vitamin B_{12} in pregnant women [37] or the Chinese population [31, 32]. However, some studies have shown that the MTRR A66G gene polymorphism and folic acid deficiency have been significantly associated with high serum levels of total homocysteine [38].

The interactions between gene polymorphisms regulating folic acid metabolism and homocysteine have significant interactive effects leading to a dramatic increase in the risk of folate deficiency with the rise of serum homocysteine concentration. Furthermore, patients with two or more risk genotypes (MTHFR C677T, MTHFR A1298C, MTR A2756G, MTRR A66G gene mutations) have higher folate deficiency rates, especially those with the combination of

MTHFR C677T and MTRR A66G, between MTHFR C677T and MTHFR A1298C or all four risk genotypes [31, 32].

Glycoprotein IIb / IIIa mutation. Although information on the role of glycoprotein IIb / IIIa mutation in the development of pregnancy complications is limited, some studies have found a significant association of glycoprotein IIIa gene mutation with RPL [28], and PLA2 gene polymorphism is associated with miscarriage between the 10th and the 20th week of pregnancy [9].

β-fibrinogen gene G455A mutation. Elevated fibrinogen levels are associated with an increased risk (4-fold higher) of developing VTE, possibly through increased blood viscosity and platelet aggregation [7, 9]. The relationship between β-fibrinogen G-455A polymorphism and RPL is controversial. Some studies have found a significant association of β-fibrinogen gene G-455A mutation with RPL and may predict an increased risk of repeated pregnancy loss [28, 39]. However, a recent meta-analysis, published in 2015, did not reveal any statistically significant association between β-fibrinogen G-455A polymorphism and RPL, including after the separate analysis of the Asian and Caucasian population [40].

PAI-1 gene mutation. A recent, comprehensive up-to-date meta-analysis, which examined a total of 3561 cases and 5693 controls, revealed a statistically significant correlation of PAI-1 4G/5G gene polymorphism with VTE in the general population. This association does not depend on ethnicity – the increased risk of VTE has been found in Asians and Caucasians, especially in people with the association of other genetic thrombophilic disorders [41].

A recent systematic analysis and meta-analysis, published in 2015, which included 18 case-control studies (conducted in 12 countries between 2003 and 2014) with a total of 3684 cases and 2208 controls, found a significant association between PAI-1 4G/5G polymorphism and RPL risk (two or more consecutive pregnancy losses in the first two trimesters). However, there was a statistically significant heterogeneity between the included studies [42].

Another recent meta-analysis, published in 2015, which included 22 case-control and cohort studies (2003–2014) with a total of 4306 cases and 3076 controls, also found a significant association between PAI-1 4G/5G polymorphism and RPL risk. The race-based analysis found that PAI-1 4G/5G polymorphism was significantly associated with an increased risk of RPL in Caucasians (OR=2.23; 95% CI 1.44-3.46; P = 0.0003) and no significant association was identified in Asians (OR=1.47; 95% CI 0.84-2.59; P = 0.18) [43].

However, several prospective case-control and cohort studies did not find any positive association of PAI-1 4G/5G polymorphism (isolated or in combination with other forms of congenital thrombophilia) with an increased risk of RPL and other adverse effects of pregnancy, although in most studies the small sample size limits the interpretation of these results [44-47].

Factor XIII V34L gene mutation. The association of V34L polymorphism of coagulation factor XIII with an increased risk of RPL is still debatable and controversial. According to the results of case-control studies, the factor XIII V34L gene mutation of heterozygous type and, to a lesser extent, of homozygous type, is significantly associated with unexplained RPL and can be considered a risk factor for RPL, being associated with the early development of RPL [48]. A recent meta-analysis, published in 2015, also demonstrated a close association of the factor XIII V34L gene mutation with RPL [40].

However, some studies have not found any association of the factor XIII V34L mutation (isolated or in combination with other forms of congenital thrombophilia) with RPL. However, the small sample size and the inclusion in studies of different ethnic groups limited the interpretation of the results [45, 49].

Therefore, discrepancies in the relationship between different forms of hereditary thrombophilia and placenta-mediated pregnancy complications can be explained by the small sample size, the difference between the ethnic groups, and the geographical variation of different cohort studies. Large case-control, prospective, and population-based studies are needed to confirm or to disprove the association of more recently discovered hereditary thrombophilia with adverse pregnancy outcomes.

The success achieved in the last decades in the field of molecular medicine, biology and medical genetics has contributed to the evaluation from fundamentally new perspectives of the pathogenesis of many obstetric complications, which increase fetal, infant and maternal mortality. RPL or recurrent miscarriage, pregnancy stopped in evolution, premature birth, intrauterine fetal death, PANPP, IUGR, severe preeclampsia, eclampsia, thromboembolism are still very current problems for modern obstetrics. The discovery of different forms of acquired and hereditary thrombophilia elucidated much more widely the pathogenesis of multiple diseases, including in general obstetrics [7, 50].

Although the contribution of congenital thrombophilia to negative pregnancy outcomes is controversial, a number of complications (fetal loss, preeclampsia, PANPP, IUGR) have been associated with congenital thrombophilia [51]. The association between pregnancy complications and different forms of congenital thrombophilia, both quantitatively and qualitatively, is briefly described below.

Recurrent miscarriage. The term miscarriage is often used to define pregnancy loss from conception to 20 weeks of gestation. Subsequently, the loss of pregnancy is called the death of the fetus or stillbirth. Early pregnancy loss is defined as the lack of fetal cardiac activity up to 12 weeks of gestation, and late pregnancy loss – the lack of fetal cardiac activity after 12 weeks of gestation [52, 53].

Common definitions of RPL, also known as repeated miscarriage, include the following: 1) two or more miscarriages in the first trimester (up to 12 weeks of gestation), documented by ultrasound or histopathological examination, 2) three or more consecutive pregnancy losses up to 10–12 weeks of gestation, which are not necessarily intrauterine [1, 50, 54].

The hemostatic system plays an important role in the normal development and success of pregnancy, the implantation process, the invasion and normal development of the

trophoblast and placentation. The implantation of the fertilized egg in the decidua establishes a contact between the fetus, the placenta and the maternal circulation which is crucial for the pregnancy success. Prothrombotic changes and thrombosis can interfere with these processes, contributing to severe consequences, including miscarriage, and may account for many unexplained cases of previous RPL [3, 13, 52, 53].

While the role of acquired thrombophilia has been accepted as an etiological factor for RPL, the contribution of specific inherited thrombophilic gene polymorphisms is controversial. About 52% of pregnant women with severe obstetric complications (severe preeclampsia, IUGR, PANPP, stillbirth) had at least one of the three main thrombophilic mutations (FVL G1691A gene mutation, prothrombin G20210A gene mutation, and MTHFR C677T gene mutation) [55]. Among women with congenital thrombophilia (FVL G1691A gene mutation, protein C deficiency, protein S deficiency, AT deficiency, combined defects), fetal loss was found in 29.4% of cases [56].

A group of thrombogenic gene mutations, especially different combinations thereof: FVL G1691A, factor V H1299R (R2), factor II prothrombin G20210A, factor XIII V34L, β-fibrinogen G-455A, PAI-1 4G/5G, glycopropein IIIa L33P, MTHFR C677T and MTHFR A1298C can identify people at risk of RPL. Individual examination revealed that 3 of these mutations (PAI-1 4G / 5G, p = 0.009; factor XIII V34L, p <0.0001 and homozygous MTHFR C667T, p=0.0001) correlated statistically significantly with RPL, compared to the control group. The other 6 mutant genes, cumulatively, also correlated statistically significantly with RPL (p <0.0001) [57].

Several studies (retrospective, prospective, case-control, cohort, cross-sectional, clinical) and contemporary meta-analyses found no association or found a very weak association with an absolutely very low risk between different forms of congenital thrombophilia or their combinations and RPL [1, 7, 11, 12, 14]. The most recent and well-designed systematic review and meta-analysis of the association of congenital thrombophilia and placenta-mediated pregnancy complications revealed an absolutely increased, but small, and statistically significant risk of late RPL in women with the FVL G1691A gene mutation [14].

A growing body of evidence (meta-analyses, cross-sectional studies, case-control studies) suggests a significant association of late pregnancy loss and unexplained early RPL with various types of congenital thrombophilia (FVL G1691A gene mutation, prothrombin G20210A gene mutation, MTHFR C677T gene mutation, MTHFR A1298C gene mutation, β-fibrinogen G-455A gene mutation, protein C deficiency, protein S deficiency). Robust evidence for such an association shows the FVL G1691A gene mutation, the prothrombin G20210A gene mutation and hyperhomocysteinemia [1, 3, 7, 9, 11, 12, 58-61].

In three systematic analyses and meta-analyses, which included case-control studies, prospective cohort studies, cross-sectional and randomized controlled trials, only the heterozygous FVL G1691A gene mutation was associated with early and late RPL and late non-recurrent fetal loss, the heterozygous prothrombin G20210A gene mutation – with early RPL and late non-recurrent fetal loss, and protein S deficiency – with RPL and late non-recurrent fetal loss. Protein C deficiency and AT deficiency have not been associated with fetal loss [13, 62, 63].

A systematic literature review with 16 case-control studies for analysis of FVL G1691A gene mutation and 7 case-control studies for analysis of prothrombin G20210A gene mutation, which defined RPL as two or more pregnancy losses in the first or second trimester of pregnancy, found a double risk of RPL in women with congenital thrombophilia compared to women without thrombophilia. The authors concluded that hereditary thrombophilia may be an unrecognized cause of RPL [61].

A meta-analysis, which included 31 population-based studies, showed that the magnitude of the correlation between congenital thrombophilia and fetal loss varies depending on the time of fetal loss and the type of thrombophilia. Only some forms of congenital thrombophilia are associated with fetal loss. In the first trimester, RPL was associated with the FVL G1691A gene mutation and prothrombin G20210A gene mutation, and late non-recurrent fetal loss was associated with the FVL G1691A gene mutation, the prothrombin G20210A gene mutation, and protein S deficiency. The FVL G1691A gene mutation was more strongly associated with late pregnancy loss than with early pregnancy loss, and in the case of exclusion of other potential causes of fetal loss. The MTHFR C677T gene mutation, protein C deficiency, and AT deficiency have not been significantly associated with fetal loss [62].

A large prospective case-control study (European Prospective Cohort on Thrombophilia - EPCOT) enrolled 1384 women (843 women with thrombophilia and 541 control women). The frequency of miscarriage (fetal loss before 28 weeks of gestation) and stillbirth (fetal loss after 28 weeks of gestation) was assessed together and separately. The study found a statistically significant association of pregnancy loss in pregnant women with congenital thrombophilia, and stillbirth especially in women with combined defects or AT deficiency. Women with congenital thrombophilia (AT deficiency, protein C deficiency and protein S deficiency or FVL G1691A gene mutation) have a 3.6-fold higher risk of stillbirth and a 1.3-fold higher risk of miscarriage. Women with combined birth defects had the highest risk for stillbirth (14.3), compared with 5.2 for AT deficiency, 2.3 for protein C deficiency, 3.3 for protein S deficiency and 2.0 for the FVL G1691A gene mutation. The results obtained have important implications for treatment and provide a justification for clinical trials of thromboprophylaxis in women with RPL [2, 56, 64].

In general, the data described above suggest a clear relationship between congenital thrombophilia and negative pregnancy outcomes [65, 66]. However, heterogeneous small research groups, differences in study design, ethnicity, and geographical location may impact the results, limiting the statistical power of the research, the validity of the conclusions and the practical importance of the phenomena stud-

ied [10, 65]. For these reasons, some scientists believe that the evidence for the association of congenital thrombophilia with pregnancy loss should be interpreted with caution, as it is still weak and unconvincing, lacking the systematic integration of classification and co-factors [10, 65, 67, 68].

Women with combined thrombophilia, especially with congenital thrombophilia associated with acquired thrombophilia or two and more congenital thrombophilias, have the highest risk of early and late RPL [1, 7, 13, 56, 61, 69]. A meta-analysis and some case-control studies confirmed that the FVL G1691A gene mutation is a weak, but well-established risk factor for unexplained RPL (two or more consecutive events) and for one or more late fetal losses with a risk increase of 2-7 times. A significantly increased risk (2.5-fold for early RPL and 2.3-fold for non-recurrent late fetal loss) was also determined in pregnant women with prothrombin G20210A gene mutation. A twofold increase in the risk of fetal loss among pregnant women with AT deficiency, protein C deficiency, protein S deficiency, and FVL G1691A gene mutation has been reported in several studies [70].

A large population study evaluating 32.683 non-thrombotic primigravida women revealed an association of the heterozygous FVL G1691A gene mutation and prothrombin G20210A gene mutation with a significant increased risk of miscarriage (3.2 and 2.4, respectively). The relationship was significant only in the group of women who had a miscarriage from the 10th week of gestation (3.5 and 2.6, respectively) [51, 70, 71].

In recent years, the association of RPL with hypofibrinolytic gene polymorphisms (coagulation factor XIII V34L, PAI-1 4G / 4G, PAI-1 4G / 5G and ACE I / D gene mutations) has become increasingly evident [43, 72, 73]. Homozygous PAI-1 4G / 5G and MTHFR C677T gene mutations in women with RPL and the FVL G1691A, factor V H1299R, heterozygous ACE I / D gene mutations in both parents play a crucial role in RPL and should be considered risk factors. In addition, RPL is determined by thrombophilic gene mutations in both parents (not just maternal gene mutations) [74]. However, a meta-analysis evaluating 11 studies with a total of 1545 women with RPL and 960 normal women, did not identify any associations of PAI-1 4G/5G gene mutation with idiopathic RPL, including more than two or three recurrent miscarriages, in Caucasian and non-Caucasian population, but a high heterogeneity was identified in the included studies [69].

Therefore, in most cases, the evolution of pregnancy in pregnant women with congenital thrombophilia is devoid of events. However, there is an argument that women with any type of thromboembolic defect, especially with a combination of defects, have a higher prevalence of complications in pregnancy [37]. Although the results of published studies are contradictory, most comments suggest an association of RPL with FVL G1691A gene mutation and prothrombin G20210A gene mutation. These results vary in different populations, reflecting an interaction of several genetic and acquired risk factors [3, 51].

Although solid randomized data on this subject are missing, the analysis of existing results revealed that there is no relationship between congenital thrombophilia and RPL, or hereditary thrombophilia has a low impact on placenta-mediated complications, probably explained by the study design differences, the relatively low prevalence of some forms of congenital thrombophilia and the small number of study participants, differences in inclusion and exclusion criteria, the inclusion of heterogeneous patient populations in different studies and the application of various definitions of complications that did not allow the researchers to identify a significant increase in the risk of RPL. The associations obtained in some studies may be caused by the high frequency of these mutations in the population, which does not reflect the causal relationship [6].

Preeclampsia is an increasingly common, but poorly understood, multisystemic inflammatory syndrome of pregnancy, associated with abnormal placental development and homeostasis disorders leading to inadequate maternal-fetal circulation, caused by endothelial dysfunction, vasoconstriction, placental ischemia, hypercoagulation with uteroplacental thrombosis [1, 11, 75].

Congenital thrombophilia may partially contribute to the development and severity of preeclampsia due to disorders of the hemostatic system: hypercoagulability with vasculopathy and secondary thrombosis disrupt the perfusion of the intervillous space, contributing to the development of placental infarction, IUGR, PANPP, premature birth and intrauterine fetal death [2, 13, 56, 76].

Some case-control studies and prospective cohort studies (unicenter and multicenter) found the association of total and severe preeclampsia with thrombophilia (congenital and / or acquired). Thrombophilia exacerbates perinatal outcomes in pregnant women with severe preeclampsia: the risk of premature birth (before 32 weeks of gestation), IUGR, and perinatal mortality are significantly higher [3, 77-79].

Data cumulated from several studies and meta-analyses found a significant association of FVL G1691A gene mutation with total preeclampsia (twofold increased risk) and severe preeclampsia (threefold increased risk), prothrombin G20210A gene mutation with total, severe and mild preeclampsia [3, 6, 77, 80-83]. Preeclampsia (total and severe) is the only complication in which a significant and consistent association with homozygosity of MTHFR C677T gene mutation has been found. The presence of a very modest association with mild preeclampsia cannot be excluded, which is very difficult to detect even in significant groups of women [13, 20, 65, 77, 84].

A systematic literature review, published in 2002, found an increased risk of preeclampsia / eclampsia in pregnant women with heterozygous FVL G1691A gene mutation, heterozygous prothrombin G20210A gene mutation, homozygous MTHFR C677T gene mutation, protein C deficiency, protein S deficiency or activated protein C resistance. Unfortunately, the analysis included many small sample studies, which reduced the overall statistical power and no definitive conclusions could be drawn [51, 85].

Two recent extensive meta-analyses, published in 2014 (111 studies with 15094 cases and 21633 controls) and in 2015 (54 studies with 7398 cases and 11222 controls), thus

guaranteeing the statistical power of meta-analyses and obtaining more accurate estimates, revealed an association of MTHFR C677T gene polymorphism with an increased risk of preeclampsia, especially among Asian and Caucasian population [21, 51].

No relationship was found between the deficiency of natural anticoagulants (protein S, protein C and AT) with preeclampsia, as the number of subjects with such defects was too small to allow definitive conclusions to be drawn [3, 65, 77], although some studies revealed a significant association between protein C and preeclampsia [79].

However, a meta-analysis, published in 2003, and the most recent and well-designed systematic review and meta-analysis of prospective cohort studies did not observe any consistent and significant effect of the FVL G1691A gene mutation and prothrombin G20210A gene mutation on the risk of preeclampsia, although a modest effect cannot be ruled out [14, 65, 86]. While studies published in 1996-2000 demonstrated a significant association of the FVL G1691A gene mutation with preeclampsia, studies published in 2001-2002 did not provide such convincing evidence, except for a small increased risk attributed to the FVL G1691A gene mutation for preeclampsia. At the same time, it must be acknowledged that the meta-analysis cannot exclude the possibility of associating the FVL G1691A gene mutation with severe forms of preeclampsia [86].

Therefore, the association of preeclampsia with congenital thrombophilia is much more controversial. Currently, there is insufficient evidence to demonstrate the association of any form of congenital thrombophilia with preeclampsia. Several recent studies and meta-analyses suggest that there is an association between congenital thrombophilia and the development of severe preeclampsia but not mild one. Severe preeclampsia is mainly associated with the FVL G1691A gene mutation, hyperhomocysteinemia, protein S and protein C deficiency, AT-III deficiency, prothrombin G20210A gene mutation, and MTHFR C677T gene mutation [1, 11].

Intrauterine growth restriction is most commonly defined as a fetal weight, estimated at the ultrasound examination, for gestational age less than the 10th percentile, and severe IUGR – an assessed fetal weight less than the 5th percentile [10, 11].

The association between congenital thrombophilia and IUGR or newborns small for gestational age (<2500 g) has not been consistently demonstrated in various studies and this relationship is highly controversial. The results vary from study to study, depending on the size of the study sample and the selection criteria used. However, a definitively determined causal relationship was not found, although an association was confirmed in women with severe IUGR, but not in cases with mild IUGR [1, 11, 12, 87, 88].

Some systematic literature analyses and meta-analyses noticed a significant association between the presence in mothers of the homozygous FVL G1691A gene mutation or the heterozygous prothrombin G20210A gene mutation with IUGR [8, 13, 17, 52, 90]. None of other congenital prothrombotic defects have been associated with IUGR [3, 13].

A systematic literature review, published in 2002, found an increased risk of IUGR in pregnant women with heterozygous prothrombin G20210A gene mutation, homozygous MTHFR C677T gene mutation, protein S deficiency or anticardiolipin antibody IgG isotype. However, these analyses are not definitive due to the small sample size included in the studies and the large confidence interval, which reduced the impact of the findings [51, 85]. A prospective cohort study, published in 2015, confirmed that the presence of the homozygous MTHFR C677T gene mutation could increase the risk of developing IUGR [89].

Four studies – a family-based case-control study (published in 2002) [88], a large prospective cohort study (published in 2007) [91], a genetic study and a meta-analysis of prospective cohort studies (published in 2008) [81], a meta-analysis of case-control studies and prospective cohort studies (published in 2009) [92] – contradict this association and did not reveal an increased risk of IUGR in pregnant women with polymorphisms of congenital thrombophilia.

Premature abruption of the normally positioned placenta. In general, there is insufficient evidence to establish a link between congenital thrombophilia and PANPP, although a potential association with thrombophilia is suggested by a number of studies. The absence of a consistent association, demonstrated between any type of congenital thrombophilia and PANPP, is possibly caused by the low incidence of these events [1, 11].

A systematic literature review, published in 2002, found an increased risk of PANPP in pregnant women with homozygous and heterozygous FVL G1691A gene mutation, heterozygous prothrombin G20210A gene mutation, homocysteinemia and activated protein C resistance [85].

Some recent systematic literature reviews found a significant risk of PANPP in pregnant women with heterozygous prothrombin G20210A gene mutation, followed by heterozygous FVL G1691A gene mutation and hyperhomocysteinemia [3, 6, 13, 51]. A cohort study, published in 2015, confirmed that the presence of FVL G1691A gene mutation could increase the risk of PANPP [89].

However, these results were questioned in a meta-analysis of case-control studies, published in 2008, and a meta-analysis of prospective cohort studies, published in 2010, which did not find any significant risk of premature abruption of the normally positioned placenta in women with FVL G1691A gene mutation or G20210A prothrombin gene mutation or MTHFR C677T gene mutation, except for some evidence of possible genetic causes [3, 14, 27].

Therefore, there is a small amount of robust evidence associated with negative pregnancy outcomes and congenital thrombophilia. The FVL G1691A gene mutation is associated with RPL and late pregnancy loss, prothrombin G20210A gene mutation is associated with RPL and late pregnancy loss, protein S deficiency is associated with late pregnancy loss. There is insufficient evidence to suggest an association of other forms of congenital thrombophilia with pregnancy complications. Due to the poor design quality of case-control studies and prospective cohort studies, there was often an increased relative risk of these complications, especially early RPL, fetal loss and preeclampsia, in women with con-

genital thrombophilia. However, the magnitude of the association and the absolute risk remain very modest, suggesting that congenital thrombophilia is more of a contributing factor than a primary cause. The existence of a thrombophilic genetic defect does not exclude other risk factors, especially considering the multicausal etiology of obstetric complications in pregnancy. The literature analysis according to the severity of adverse pregnancy outcomes illustrates that the risk associated with congenital thrombophilia increases significantly in pregnant women with severe obstetric complications (severe preeclampsia, eclampsia, PANPP, IUGR less than the 5th percentile or unexplained stillbirth) [3, 12, 89].

Conclusions

1. Prospective cross-sectional, case-control and cohort studies have shown that hereditary thrombophilia is more prevalent in the group of women with recurrent pregnancy loss, late miscarriage, early-onset preeclampsia, premature abruption of the normally positioned placenta, and intrauterine growth restriction.

2. There is convincing evidence that deficiency of natural anticoagulants (antithrombin, protein C, protein S) is a risk factor for late fetal loss. The factor V Leiden G1691A gene mutation and prothrombin G20210A gene mutation are associated with a double risk of recurrent early and unexplained miscarriage as well as non-recurrent late fetal loss. The association of congenital thrombophilia with preeclampsia is limited to the factor V Leiden G1691A gene mutation and more severe cases of preeclampsia. There is insufficient evidence to suggest an association of other forms of congenital thrombophilia with adverse pregnancy outcomes.

3. The risk associated with congenital thrombophilia increases significantly in pregnant women with two or more forms of hereditary thrombophilia and in pregnant women with severe placenta-mediated pregnancy complications (severe preeclampsia, eclampsia, premature abruption of normally positioned placenta, intrauterine growth restriction with weight below the 5th percentile or unexplained stillbirth).

4. Due to the poor design quality of case-control studies and prospective cohort studies, there has often been an increased relative risk of pregnancy complications associated with congenital thrombophilia, especially early RPL, fetal loss and preeclampsia, but the extent of the association and the absolute risk remain very modest. Prospective, multicenter studies are needed on large samples of patients, which would validate the role of thrombophilia in the development of obstetric diseases.

References

1. Pritchard AM, Hendrix PW, Paidas MJ. Hereditary thrombophilia and recurrent pregnancy loss. Clin Obstet Gynecol. 2016;59(3):487-497. doi: 10.1097/GRF.0000000000000226.
2. Leaf RK, Connors JM. The role of anticoagulants in the prevention of pregnancy complications. Clin Appl Thromb Hemost. 2017;23(2):116-123. doi: 10.1177/1076029615615972.
3. Liatsikos S, Tsikouras P, Manav B, et al. Inherited thrombophilia and reproductive disorders. J Turk Ger Gynecol Assoc. 2016;17(1):45-50. doi: 10.5152/jtgga.2016.15212.
4. Dobbenga-Rhodes Y. Shedding light on inherited thrombophilias: the impact on pregnancy. J Perinat Neonatal Nurs. 2016.;30(1):36-44. doi: 10.1097/JPN.0000000000000146.
5. Alijotas-Reig J, Ferrer-Oliveras R, Esteve-Valverde E, et al. Inherited thrombophilia in women with poor aPL-related obstetric history: prevalence and outcomes. Survey of 208 cases from the European Registry on Obstetric Antiphospholipid Syndrome cohort. Am J Reprod Immunol. 2016;76(2):164-171. doi: 10.1111/aji.12534.
6. Robertson L, Wu O, Greer I. Thrombophilia and adverse pregnancy outcome. Curr Opin Obstet Gynecol. 2004;16(6):453-458. doi: 10.1097/00001703-200412000-00003.
7. Tranquilli AL, editor. Thrombophilia. InTech; 2011. 226 p. doi: 10.5772/1329.
8. Davenport WB, Kutteh WH. Inherited thrombophilias and adverse pregnancy outcomes: a review of screening patterns and recommendations. Obstet Gynecol Clin North Am. 2014;41(1):133-144. doi: 10.1016/j.ogc.2013.10.005.
9. Kozma K, Jurca C, Bembea M. Factorii genetici ai trombofiliilor ereditare şi implicarea lor în avortul spontan [The genetic factors of hereditary thrombophilia and their implication in spontaneous abortion]. Rom J Med Pract. 2015;10(2):94-101. Romanian.
10. Duffett L, Rodger M. LMWH to prevent placenta-mediated pregnancy complications: an update. Br J Haematol. 2015;168(5):619-638. doi: 10.1111/bjh.13209.
11. Louis-Jacques A, Maggio L, Romero S. Prenatal screening for thrombophilias: indications and controversies, an update. Clin Lab Med. 2016;36(2):421-434. doi: 10.1016/j.cll.2016.01.016.
12. Simcox L, Ormesher L, Tower C, et al. Thrombophilia and Pregnancy Complications. Int J Mol Sci. 2015;16(12):28418-28428. doi: 10.3390/ijms161226104.
13. Robertson L, Wu O, Langhorne P, et al. Thrombophilia in pregnancy: a systematic review. Br J Haematol. 2006;132(2):171-196. doi: 10.1111/j.1365-2141.2005.05847.x.
14. Rodger M, Betancourt M, Clark P, et al. The association of factor V leiden and prothrombin gene mutation and placenta-mediated pregnancy complications: a systematic review and meta-analysis of prospective cohort studies. PLoS Med. 2010;7(6):e1000292. doi: 10.1371/journal.pmed.1000292.
15. Hemsworth EM, O'Reilly AM, Allen VM, et al. Association between factor v leiden mutation, small for gestational age, and preterm birth: a systematic review and meta-analysis. J Obstet Gynaecol Can. 2016;38(10):897-908. doi: 10.1016/j.jogc.2016.08.001.
16. Ren A, Wang J. Methylenetetrahydrofolate reductase C677T polymorphism and the risk of unexplained recurrent pregnancy loss: a meta-analysis. Fertil Steril. 2006;86(6):1716-1722. doi: 10.1016/j.fertnstert.2006.05.052.
17. Farahmand K, Totonchi M, Hashemi M, et al. Thrombophilic genes alterations as risk factor for recurrent pregnancy loss. J Matern Fetal Neonatal Med. 2016;29(8):1269-1273. doi: 10.3109/14767058.2015.1044431.
18. Cao Y, Xu J, Zhang Z, et al. Association study between methylenetetrahydrofolate reductase polymorphisms and unexplained recurrent pregnancy loss: a meta-analysis. Gene. 2013;514(2):105-111. doi: 10.1016/j.gene.2012.10.091.
19. Wu X, Zhao L, Zhu H, et al. Association between the MTHFR C677T polymorphism and recurrent pregnancy loss: a meta-analysis. Genet Test Mol Biomarkers. 2012;16(7):806-811. doi: 10.1089/gtmb.2011.0318.
20. Yang B, Fan S, Zhi X, et al. Associations of MTHFR gene polymorphisms with hypertension and hypertension in pregnancy: a meta-analysis from 114 studies with 15411 cases and 21970 controls. PLoS One. 2014;9(2):e87497. doi: 10.1371/journal.pone.0087497.
21. Wu X, Yang K, Tang X, et al. Folate metabolism gene polymorphisms MTHFR C677T and A1298C and risk for preeclampsia: a meta-analysis. J Assist Reprod Genet. 2015;32(5):797-805. doi: 10.1007/s10815-014-0408-8.
22. Nurk E, Tell G, Refsum H, et al. Associations between maternal methylenetetrahydrofolate reductase polymorphisms and adverse outcomes of pregnancy: the Hordaland Homocysteine Study. Am J Med. 2004;117(1):26-31. doi: 10.1016/j.amjmed.2004.01.019.
23. Aracic N, Roje D, Drmic Hofman I, et al. Low molecular weight heparin treatment and impact of inherited thrombophilia type in pregnancies with previous adverse outcome. J Matern Fetal Neonatal Med. 2015;28(3):306-310. doi: 10.3109/14767058.2014.916268.

24. Liguori R, Quaranta S, Di Fiore R, et al. A novel polymorphism in the PAI-1 gene promoter enhances gene expression. A novel pro-thrombotic risk factor? Thromb Res. 2014;134(6):1229-1233. doi: 10.1016/j.thromres.2014.09.021.
25. Pecheniuk NM, Morris CP, Walsh TP, et al. The factor V HR2 haplotype: prevalence and association of the A4070G and A6755G polymorphisms. Blood Coagul Fibrinolysis. 2001;12(3):201-206. doi: 10.1097/00001721-200104000-00006.
26. Nair R, Khanna A, Singh R, et al. Association of maternal and fetal MTHFR A1298C polymorphism with the risk of pregnancy loss: a study of an Indian population and a meta-analysis. Fertil Steril. 2013;99(5):1311-1318.e4. doi: 10.1016/j.fertnstert.2012.12.027.
27. Zdoukopoulos N, Zintzaras E. Genetic risk factors for placental abruption: a HuGE review and meta-analysis. Epidemiology. 2008;19(2):309-323. doi: 10.1097/EDE.0b013e3181635694.
28. Jeddi-Tehrani M, Torabi R, Zarnani A, et al. Analysis of plasminogen activator inhibitor-1, integrin beta3, beta fibrinogen, and methylenetetrahydrofolate reductase polymorphisms in Iranian women with recurrent pregnancy loss. Am J Reprod Immunol. 2011;66(2):149-156. doi: 10.1111/j.1600-0897.2010.00974.x.
29. Yousefian E, Kardi M, Allahveisi A. Methylenetetrahydrofolate reductase C677T and A1298C polymorphism in Iranian women with idiopathic recurrent pregnancy losses. Iran Red Crescent Med J. 2014;16(7):e16763. doi: 10.5812/ircmj.16763.
30. Poursadegh Zonouzi A, Chaparzadeh N, Asghari Estiar M, et al. Methylenetetrahydrofolate reductase C677T and A1298C mutations in women with recurrent spontaneous abortions in the Northwest of Iran. ISRN Obstet Gynecol. 2012;2012:945486. doi: 10.5402/2012/945486.
31. Li WX, Dai SX, Zheng JJ, et al. Homocysteine metabolism gene polymorphisms (MTHFR C677T, MTHFR A1298C, MTR A2756G and MTRR A66G) jointly elevate the risk of folate deficiency. Nutrients. 2015;7(8):6670-6687. doi: 10.3390/nu7085303.
32. Li W, Cheng F, Zhang A, et al. Folate deficiency and gene polymorphisms of MTHFR, MTR and MTRR elevate the hyperhomocysteinemia risk. Clin Lab. 2017;63(3):523-533. doi: 10.7754/Clin.Lab.2016.160917.
33. Said JM, Higgins JR, Moses EK, et al. Inherited thrombophilia polymorphisms and pregnancy outcomes in nulliparous women. Obstet Gynecol. 2010;115(1):5-13. doi: 10.1097/AOG.0b013e3181c68907.
34. Franco R, Maffei F, Lourenço D, et al. The frequency of 844ins68 mutation in the cystathionine beta-synthase gene is not increased in patients with venous thrombosis. Haematologica. 1998;83(11):1006-1008.
35. Popp R, Crişan T, Rotar I, et al. Cystathionine β-synthase 844ins68 genetic polymorphism in spontaneous abortion susceptibility. Appl Med Inform. 2011;29(4):34-40.
36. Yakub M, Moti N, Parveen S, et al. Polymorphisms in MTHFR, MS and CBS genes and homocysteine levels in a Pakistani population. PLoS One. 2012;7(3):e33222. doi: 10.1371/journal.pone.0033222.
37. Barbosa PR, Stabler SP, Machado AL, et al. Association between decreased vitamin levels and MTHFR, MTR and MTRR gene polymorphisms as determinants for elevated total homocysteine concentrations in pregnant women. Eur J Clin Nutr. 2008;62(8):1010-1021. doi: 10.1038/sj.ejcn.1602810.
38. Song CS, Song WB, Bao JY, et al. Association between decreased plasma folate levels and MTHFR C677T, and MTRR A66G gene polymorphisms as determinants for elevated total homocysteine concentrations in pregnant women. Hered Genet Curr Res. 2018;7(1):1000193. doi: 10.4172/2161-1041.1000193.
39. Torabi R, Zarei S, Zeraati H, et al. Combination of thrombophilic gene polymorphisms as a cause of increased risk of recurrent pregnancy loss. J Reprod Infertil. 2012;13(2):89-94.
40. Li J, Wu H, Chen Y, et al. Genetic association between FXIII and β-fibrinogen genes and women with recurrent spontaneous abortion: a meta-analysis. J Assist Reprod Genet. 2015;32(5):817-825. doi: 10.1007/s10815-015-0471-9.
41. Wang J, Wang C, Chen N, et al. Association between the plasminogen activator inhibitor-1 4G/5G polymorphism and risk of venous thromboembolism: a meta-analysis. Thromb Res. 2014;134(6):1241-1248. doi: 10.1016/j.thromres.2014.09.035.
42. Chen H, Nie S, Lu M. Association between plasminogen activator inhibitor-1 gene polymorphisms and recurrent pregnancy loss: a systematic review and meta-analysis. Am J Reprod Immunol. 2015;73(4):292-300. doi: 10.1111/aji.12321.
43. Li X, Liu Y, Zhang R, et al. Meta-analysis of the association between plasminogen activator inhibitor-1 4G/5G polymorphism and recurrent pregnancy loss. Med Sci Monit. 2015;(21):1051-1056. doi: 10.12659/MSM.892898.
44. Magdoud K, Herbepin V, Touraine R, et al. Plasminogen activator inhibitor 1 4G/5G and -844G/A variants in idiopathic recurrent pregnancy loss. Am J Reprod Immunol. 2013;70(3):246-252. doi: 10.1111/aji.12116.
45. Lino F, Traina E, Barreto J, et al. Thrombophilic mutations and polymorphisms, alone or in combination, and recurrent spontaneous abortion. Clin Appl Thromb Hemost. 2015;21(4):365-372. doi: 10.1177/1076029613520465.
46. Barlik M, Seremak-Mrozikiewicz A, Drews K, et al. Correlation between factor VII and PAI-1 genetic variants and recurrent miscarriage. Ginekol Pol. 2016;87(7):504-509. doi: 10.5603/GP.2016.0034.
47. Salazar Garcia M, Sung N, Mullenix T, et al. Plasminogen activator inhibitor-1 4G/5G polymorphism is associated with reproductive failure: metabolic, hormonal, and immune profiles. Am J Reprod Immunol. 2016;76(1):70-81. doi: 10.1111/aji.12516.
48. Mehdi S, Khosravi A, Pakravesh J, et al. Factor XIII Val34Leu polymorphism and risk of recurrent pregnancy loss in Iranian population: a case-control study. Frontier Biol. 2016;11(6):471-475. doi: 10.1007/s11515-016-1419-x.
49. Bagheri M, Rad I, Omrani M, et al. The Val34Leu genetic variation in the A subunit of coagulation factor XIII in recurrent spontaneous abortion. Syst Biol Reprod Med. 2011;57(5):261-264. doi: 10.3109/19396368.2011.576308.
50. Huchon C, Deffieux X, Beucher G, et al. Pregnancy loss: French clinical practice guidelines. Eur J Obstet Gynecol Reprod Biol. 2016;201:18-26. doi: 10.1016/j.ejogrb.2016.02.015.
51. Battinelli E, Marshall A, Connors J. The role of thrombophilia in pregnancy. Thrombosis. 2013;2013:516420. doi: 10.1155/2013/516420.
52. de Jong P, Goddijn M, Middeldorp S. Antithrombotic therapy for pregnancy loss. Hum Reprod Update. 2013;19(6):656-673. doi: 10.1093/humupd/dmt019.
53. Garrido-Gimenez C, Alijotas-Reig J. Recurrent miscarriage: causes, evaluation and management. Postgrad Med J. 2015;91(1073):151-162. doi: 10.1136/postgradmedj-2014-132672.
54. Kaiser J, Branch D. Recurrent pregnancy loss: generally accepted causes and their management. Clin Obstet Gynecol. 2016;59(3):464-473. doi: 10.1097/GRF.0000000000000214.
55. Kupferminc M, Eldor A, Steinman N, et al. Increased frequency of genetic thrombophilia in women with complications of pregnancy. N Engl J Med. 1999;340(1):9-13. doi: 10.1056/NEJM199901073400102.
56. Preston FE, Rosendaal FR, Walker ID, et al. Increased fetal loss in women with heritable thrombophilia. Lancet. 1996;348(9032):913-916. doi: 10.1016/s0140-6736(96)04125-6.
57. Goodman CS, Coulam CB, Jeyendran RS, et al. Which thrombophilic gene mutations are risk factors for recurrent pregnancy loss? Am J Reprod Immunol. 2006;56(4):230-236. doi: 10.1111/j.1600-0897.2006.00419.x.
58. Lee GS, Park JC, Rhee JH, et al. Etiologic characteristics and index pregnancy outcomes of recurrent pregnancy losses in Korean women. Obstet Gynecol Sci. 2016;59(5):379-387. doi: 10.5468/ogs.2016.59.5.379.
59. Patil R, Ghosh K, Vora S, et al. Inherited and acquired thrombophilia in Indian women experiencing unexplained recurrent pregnancy loss. Blood Cells Mol Dis. 2015;55(3):200-205. doi: 10.1016/j.bcmd.2015.06.008.
60. Osman OM, Abulata NN. Inherited thrombophilia and early recurrent pregnancy loss among Egyptian women. Open J Obstet Gynecol. 2015;5(5):251-258.
61. Kovalevsky G, Gracia C, Berlin J, et al. Evaluation of the association between hereditary thrombophilias and recurrent pregnancy loss: a meta-analysis. Arch Intern Med. 2004;164(5):558-563. doi: 10.1001/archinte.164.5.558.
62. Rey E, Kahn S, David M, et al. Thrombophilic disorders and fetal loss: a meta-analysis. Lancet. 2003;361(9361):901-908. doi: 10.1016/S0140-6736(03)12771-7.
63. Gao H, Tao F. Prothrombin G20210A mutation is associated with recurrent pregnancy loss: a systematic review and meta-analysis update. Thromb Res. 2015;135(2):339-346. doi: 10.1016/j.thromres.2014.12.001.

64. Vossen C, Preston F, Conard J, et al. Hereditary thrombophilia and fetal loss: a prospective follow-up study. J Thromb Haemost. 2004;2(4):592-596. doi: 10.1111/j.1538-7836.2004.00662.x.
65. Greer I, Brenner B, Gris J. Antithrombotic treatment for pregnancy complications: which path for the journey to precision medicine? Br J Haematol. 2014;165(5):585-599. doi: 10.1111/bjh.12813.
66. Silan F, Mosse I, Gonchar A, et al. Comparison of the thrombophilic gene polymorphisms and recurrent pregnancy loss: Results on combined gene effect of FV Leiden, FVR2, FXIII, MTHFR (A1298C and C677T), PAI-1 4G/5G and ACE I/D genes in RPL Women from Minsk/Belarus and Canakkale - Sivas/Turkey. Biomed Genet Genomics. 2016;1(4):87-93. doi: 10.15761/BGG.1000117.
67. Bennett S, Bagot C, Arya R. Pregnancy loss and thrombophilia: the elusive link. Br J Haematol. 2012;157(5):529-542. doi: 10.1111/j.1365-2141.2012.09112.x.
68. Rodger M, Paidas M. Do thrombophilias cause placenta-mediated pregnancy complications? Semin Thromb Hemost. 2007;33(6):597-603. doi: 10.1055/s-2007-985756.
69. Su MT, Lin SH, Chen YC, et al. Genetic association studies of ACE and PAI-1 genes in women with recurrent pregnancy loss: a systematic review and meta-analysis. Thromb Haemost. 2013;109(1):8-15. doi: 10.1160/TH12-08-0584.
70. De Stefano V, Rossi E, Za T. Inherited thrombophilia and obstetric complications. Haematol Rep. 2005;1(10):18-21.
71. Lissalde-Lavigne G, Fabbro-Peray P, Cochery-Nouvellon E, et al. Factor V Leiden and prothrombin G20210A polymorphisms as risk factors for miscarriage during a first intended pregnancy: the matched case-control 'NOHA first' study. J Thromb Haemost. 2005;3(10):2178-2184. doi: 10.1111/j.1538-7836.2005.01581.x.
72. Dossenbach-Glaninger A, van Trotsenburg M, Schneider B, et al. ACE I/D polymorphism and recurrent first trimester pregnancy loss: interaction with SERPINE1 4G/5G and F13 Val34Leu polymorphisms. Br J Haematol. 2008;141(2):269-271. doi: 10.1111/j.1365-2141.2008.07058.x.
73. Shakarami F, Akbari M, Zare Karizi S. Association of plasminogen activator inhibitor-1 and angiotensin converting enzyme polymorphisms with recurrent pregnancy loss in Iranian women. Iran J Reprod Med. 2015;13(10):627-632.
74. Ozdemir O, Yenicesu G, Silan F, et al. Recurrent pregnancy loss and its relation to combined parental thrombophilic gene mutations. Genet. Test. Mol. Biomarkers. 2012;16(4):279-286. doi: 10.1089/gtmb.2011.0191.
75. Wilson ML, Goodwin TM, Pan VL, et al. Molecular epidemiology of preeclampsia. Obstet Gynecol Surv. 2003;58(1):39-66. doi: 10.1097/00006254-200301000-00022.
76. Giannubilo S, Landi B, Ciavattini A. Preeclampsia: what could happen in a subsequent pregnancy? Obstet Gynecol Surv. 2014;69(12):747-762. doi: 10.1097/OGX.0000000000000126.
77. Mello G, Parretti E, Marozio L, et al. Thrombophilia is significantly associated with severe preeclampsia: results of a large-scale, case-controlled study. Hypertension. 2005;46(6):1270-1274. doi: 10.1161/01.HYP.0000188979.74172.4d.
78. Kupferminc M, Fait G, Many A, et al. Severe preeclampsia and high frequency of genetic thrombophilic mutations. Obstet Gynecol. 2000;96(1):45-49. doi: 10.1016/s0029-7844(00)00861-9.
79. Saghafi N, Mohammadzadeh Vatanchi A, Tara F, et al. Evaluation of selected thrombotic factors among pregnant women with preeclampsia and normal pregnant women. Iran J Reprod Med. 2014;12(12):793-798.
80. Fong F, Sahemey M, Hamedi G, et al. Maternal genotype and severe preeclampsia: a HuGE review. Am J Epidemiol. 2014;180(4):335-345. doi: 10.1093/aje/kwu151.
81. Dudding T, Heron J, Thakkinstian A, et al. Factor V Leiden is associated with pre-eclampsia but not with fetal growth restriction: a genetic association study and meta-analysis. J Thromb Haemost. 2008;6(11):1868-1875. doi: 10.1111/j.1538-7836.2008.03134.x.
82. Wang X, Bai T, Liu S, et al. Association between thrombophilia gene polymorphisms and preeclampsia: a meta-analysis. PLoS One. 2014;9(6):e100789. doi: 10.1371/journal.pone.0100789.
83. Lin J, August P. Genetic thrombophilias and preeclampsia: a meta-analysis. Obstet Gynecol. 2005;105(1):182-192. doi: 10.1097/01.AOG.0000146250.85561.e9.
84. Niu W, You Y, Qi Y. Strong association of methylenetetrahydrofolate reductase gene C677T polymorphism with hypertension and hypertension-in-pregnancy in Chinese women: a meta-analysis. J Hum Hypertens. 2012;26(4):259-267. doi: 10.1038/jhh.2011.11.
85. Alfirevic Z, Roberts D, Martlew V. How strong is the association between maternal thrombophilia and adverse pregnancy outcome? A systematic review. Eur J Obstet Gynecol Reprod Biol. 2002;101(1):6-14. doi: 10.1016/s0301-2115(01)00496-1.
86. Kosmas I, Tatsioni A, Ioannidis J. Association of Leiden mutation in factor V gene with hypertension in pregnancy and pre-eclampsia: a meta-analysis. J Hypertens. 2003;21(7):1221-1228. doi: 10.1097/00004872-200307000-00002.
87. McCowan L, Craigie S, Taylor R, et al. Inherited thrombophilias are not increased in «idiopathic» small-for-gestational-age pregnancies. Am J Obstet Gynecol. 2003;188(4):981-985. doi: 10.1067/mob.2003.218.
88. Infante-Rivard C, Rivard G, Yotov W, et al. Absence of association of thrombophilia polymorphisms with intrauterine growth restriction. N Engl J Med. 2002;347(1):19-25. doi: 10.1056/NEJM200207043470105.
89. Livrinova V, Lega M, Dimcheva A, et al. Factor V Leiden, prothrombin and MTHFR Mutation in patients with preeclamsia, intrauterine growth restriction and placental abruption. Open Access Maced J Med Sci. 2015;3(4):590-594. doi: 10.3889/oamjms.2015.099.
90. Coriu L, Copaciu E, Tulbure D, et al. Inherited thrombophilia in pregnant women with intrauterine growth restriction. Maedica (Bucharest). 2014;9(4):351-3
91. Clark P, Walker I, Govan L, et al. The GOAL study: a prospective examination of the impact of factor V Leiden and ABO(H) blood groups on haemorrhagic and thrombotic pregnancy outcomes. Br J Haematol. 2008;140(2):236-240. doi: 10.1111/j.1365-2141.2007.06902.x.
92. Facco F, You W, Grobman W. Genetic thrombophilias and intrauterine growth restriction: a meta-analysis. Obstet Gynecol. 2009;113(6):1206-1216. doi: 10.1097/AOG.0b013e3181a6e96a.

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Pregnancy in multiple sclerosis: from scientific aspects to practical

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Abstract

Background: Multiple sclerosis (MS) is a disease that affects young people of reproductive age (20-40 years old), predominantly women. Therefore, almost every patient has questions about pregnancy and breastfeeding. Family planning is one of the key issues in the choice of treatment tactics. Despite the growing number of therapeutic options for individualized treatment, it is still a question how to manage women with MS who become pregnant while taking disease-modifying drugs or want to become pregnant after starting this treatment.
Conclusions: Women with MS should not be discouraged from pregnancy due to their illness. It is necessary to proactively discuss pregnancy planning with all women with MS of childbearing age. Based on available data, interferon beta and glatiramer acetate appear to be most suitable for use up until the time of confirmed pregnancy. A large amount of data (more than 1000 cases) obtained from registries shows that use of interferon beta before conception and during pregnancy suggests no evidence of increase in the rate of congenital anomalies or spontaneous abortions. For women with persistent high disease activity, pulsed immune reconstitution therapy gives additional opportunity for family planning after the last dose. The choice between available options for pulsed immune reconstitution therapy should be based on efficacy balanced against the risks.
Key words: multiple sclerosis, pregnancy, disease-modifying treatment.

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Introduction

Multiple sclerosis (MS) is most often diagnosed between the ages of 20-40, the age at which many people may be thinking of starting or extending their family. Recent analysis of the 3rd edition of the Atlas of MS as an open-source global compendium of data regarding the epidemiology of MS showed that globally females are twice as likely to have MS as males and this is consistent with both prior editions of the Atlas. However, the ratio of women to men is as high as 4:1 in some countries, and in others this ratio has doubled since 2013 [1]. This disproportional increase of MS incidence in females, particularly notable in the Middle East, is explained by some investigators as a result of improved diagnostic methods and a better access to MRI [2, 3].

In a survey of 5949 patients, registered in the NARCOMS (North American Research Committee on Multiple Sclerosis) database, 79% of patients did not become pregnant after a diagnosis of MS. In 34.5% of these patients, the choice concerned MS-related issues [4]. A survey on 271 female patients with MS in Switzerland undertaken to help understand the effects of family planning on their treatment decisions revealed their concerns about: disease course during pregnancy (44%), disease course after pregnancy (61%), the health of the unborn child (47%), possibility for breastfeeding (21%), taking care of the child after birth (45%), the availability of MS therapy options (35%) [5]. It is important to provide a framework to discuss concerns regarding pregnancy and MS, as well as the uncertainty about the course of disease during pregnancy, postpartum and in the long-term.

Management of women with MS is a complex task that should cover pre-pregnancy guidance, care during pregnancy, delivery and anesthetic options, postpartum and specific advice concerning currently approved disease-modifying drugs [6].

Despite the growing number of therapeutic options for individualized treatment, it is still a question how to manage MS in women who become pregnant while taking disease-modifying drugs or want to become pregnant after starting this treatment. It is therefore important to proactively discuss family planning and pregnancy, especially when considering disease-modifying treatments. As the complexity and spectrum of available disease-modifying drugs increase, in many countries MS pregnancy data is being gathered in nationwide registries.

This literature review that includes data from national registries demonstrates the modern approach to the management of women with MS with reproductive potential depending on the disease activity and the stage of family planning.

Discussion

Today it is known that MS is not a hereditary disease, and does not significantly affect the ability of conception, and the likelihood of adverse pregnancy outcomes such as the risk of spontaneous abortions, stillbirth, cesarean delivery, premature birth, or birth defects [4]. However, pregnancy planning should not coincide with time of diagnosis, to allow for evaluation of disease activity and course. It is recommended to ensure disease control before pregnancy, depending on disease activity with stabilization for at least 1 year before pregnancy. Proactive counselling and formulation of a pregnancy and disease management plan with at least annual revision may help to avoid the situation when following discussion with their health care practitioner (HCP), women with MS defer treatment because they wish to have children in the future [6-8].

Women with MS should follow standard advice for all pregnant women: ensure good sleep, hygiene, and diet, avoid alcohol and smoking, take recommended routine prenatal supplements including folic acid [4, 6, 7]. Along with the absence of a significant impact of MS on child health, women with MS considering pregnancy should be counseled upon the unpredictable course of MS, the effect of MS on the course of pregnancy, on how the pregnancy may affect disease. The potential teratogenic risk associated with disease modifying drugs (DMDs) and the increased risk of vitamin D deficiency associated with MS should also be discussed within pre-pregnancy consultation [6, 9, 10].

Although MS has no significant impact on the ability of women to conceive, about 10% of all women have difficulties getting or staying pregnant and may choose to use assisted reproductive technology (ART) [4]. In most studies, women stopped taking DMDs prior to initiating ART, leaving them at a higher risk of relapse. Additionally patients with MS may experience an increase in relapse rate following the use of ART, which may be the result of stress and hormone alterations associated with ART. Distress is also associated with *in-vitro* fertilization failure [7, 11].

While women are pregnant, they may experience fewer MS relapses, especially in the third trimester [12-14]. However, in patients with highly active disease, pregnancy may not be sufficient to control disease activity [6]. About 25% of women experience a relapse within 40 weeks of pregnancy and nearly 30% within the first 3 months after giving birth. These data are mainly derived from studies involving untreated women during and after pregnancy. A risk factor for increased disease activity after childbirth is relapse during the year before and during pregnancy [15]. It is important to note that pregnancy has no effect on the progression of disability in MS [16]. In this regard, it is important to continue therapy, but most DMDs are contraindicated during pregnancy (tab. 1).

Pregnancy may often complicate the diagnosis of new disease activity. Clinical presentation of MS can be similar to common pregnancy-related symptoms, which makes it difficult to attribute any symptoms to the disease process if they develop during an established pregnancy [17]. Although pregnant women may experience fewer relapses, pregnancy may exacerbate other MS symptoms. One of the most common pregnancy-related symptoms similar to presentation of MS is fatigue. Pregnant women may find that they do not sleep well, and maternal iron deficiency may also lead to anaemia and fatigue. Current evidence suggests that women with MS have a higher risk of anaemia during pregnancy when compared with healthy individuals [17]. In some countries (in the U.S.) amantadine is used as the only approved treatment for fatigue, but there are no adequate studies in pregnant women regarding the use of this medication. In pregnancy, increasing weight may diminish mobility which can mimic reduced mobility in MS. Spasticity and gait abnormalities may worsen with pregnancy. Pressure from the uterus on the bladder may increase frequency of urination resembling bladder and bowel dysfunction. Vision changes due to pregnancy-induced hypertension as well may seem to the patient as a manifestation of MS, therefore other possible causes of vision problems should also be excluded.

Indicators of new onset of MS symptoms may include sensory changes, paralysis, muscle weakness or spasm, cognitive changes. Pregnant women with MS who notice worsening of pre-existing conditions or a new onset of distinct MS-related symptoms should undergo neurological examination. The neurological condition of all pregnant patients with MS should be ideally checked every 3 months including screening for depression and anxiety given that many women with MS may experience a higher incidence of depression during pregnancy [17]. Existing comorbidities can also contribute to general deterioration. Urinary tract infections may be more frequent in pregnant women with MS when compared with pregnant healthy controls [6, 17]. To date there is a current lack of data on the safety of treatments for some comorbidities in pregnant women. Regarding treatment of severe relapses during pregnancy warranting treatment published literature reports on corticosteroids, which may be considered depending on corticosteroid type, period of pregnancy, and duration of treatment and dosage. During the second and third trimesters, short courses (3–5 days) of prednisolone or methylprednisolone may be considered. Corticosteroid administration should be avoided during the first trimester of pregnancy due to the potential, but rare, risk of teratogenic effects (e.g. cleft palate). Plasma exchange may be taken into account for severe relapses that are not responsive to corticosteroid treatment [6, 7, 18].

For women who experience relapse warranting imaging, there are no conclusive data to show that MRI exposure up to 3T is associated with foetal harm, but gadolinium should be avoided [6, 7, 18]. Regarding additional foetal scans in women with MS, Association of British Neurologists (ABN) guidelines state that it is not required [6].

Today it is known that after childbirth, the level of hor-

mones and cytokines returns to normal and the activity of the disease increases. This gradual normalization decreases the immunosuppressive effect of pregnancy and leads to an increase in MS activity. Additionally, this can also be facilitated by the stressful effect of childbirth itself and the increasing stress in a woman associated with childcare.

Global trends show that the waiting time before resuming therapy after childbirth can last up to 100 days, which is often associated with the inability to use DMD during breastfeeding, although therapy is extremely important to reduce disease activity and slow the progression of disability. According to Hellwig K. et al., 2012 about 30% of women experience a relapse within the first 3 months after giving birth. This data was obtained from patients who were not taking DMDs [15].

Some of the implications of stopping DMDs use which must be considered are: loss of disease-control benefits after stopping DMDs requiring continuous administration, potential rebound disease, further monitoring, and elimination periods of up to 48 months for some medications. Recommended preconception washout periods for DMDs vary greatly (tab. 1).

International American Academy of Neurology (AAN) 2018 and ABN 2019 guidelines state that DMD choice may be influenced by plans for pregnancy and during prepregnancy counselling, HCPs should consider the safety of all drug treatments during pregnancy when prescribing to women of childbearing age [6, 32].

According to ECTRIMS/EAN 2018 guidelines the use of interferon beta or glatiramer acetate until pregnancy is confirmed may be considered for women with a high risk of disease re-activation; treatment continuation may be considered in specific cases. In women with persistent high disease activity who do not wish to delay pregnancy, treatment with some DMDs during pregnancy could be an option after discussion of the potential implications [33].

Based on available data, interferon beta and glatiramer acetate appear to be most suitable for use up until the time of confirmed pregnancy. However, a systematic review concluded that further research was necessary for glatiramer acetate as studies were small. Natalizumab is a large molecule that is unlikely to cross the placental barrier to any great extent during early pregnancy. If natalizumab is administered in the last quarter of pregnancy, concentrations may be higher in infants than in mothers which can result in reversible hematological abnormalities such as thrombocytopenia and anemia. Natalizumab must be discontinued at a maximum of 3 months prior to pregnancy. After consideration of the individual risk-benefit, natalizumab may be given until pregnancy is confirmed or, in rare cases, the benefit of continuing natalizumab during the entire pregnancy may outweigh the risk of recurring severe disease activity but only after careful risk-benefit consideration. It is recommended that a pediatrician be in attendance during delivery and that blood cell count, liver enzymes, haptoglobin and bilirubin be controlled in the newborn [16].

To date, emerging data continue to change family planning for people with MS given the increased identification of long-term developmental effects on children exposed to DMDs.

Data from global registries demonstrates that there is no evidence of an adverse effect of interferon beta-1a on pregnancy outcomes or on the frequency of live births of children with congenital abnormalities. Both indicators, as well as the body weight of newborns, are in accordance with indicators in the general population. Based on over 1000 pregnancy outcomes, obtained from registers and post-registration observations, EMA approved respective changes in label.

The European Interferon-beta Pregnancy Registry is a prospective collection of pregnancy data from interferon beta-treated women from 31 European countries. Between 2009 and 2017, the registry collected 948 pregnancy reports with a known pregnancy outcome. This is one of the largest prospective cohort studies conducted to date, providing data on pregnancy outcomes in women with MS who received interferon beta. Of the known outcomes, 794 were live births. Overall, 82.0% (777/948) of pregnancies resulted in live birth without congenital anomaly. When comparing IFN-beta-exposed pregnancies with the general population, the prevalence of spontaneous abortions (10.7% vs 10–21%) and congenital anomalies in live births (2.1% vs 2.1–4.1%) were found to be within reported ranges. The data gathered from these pregnancy cases suggest no evidence that IFN-beta exposure before conception and/or during pregnancy adversely increases the rate of congenital anomalies or spontaneous abortions [34].

The German MS Pregnancy Registry is a nationwide collection of data on pregnancy outcomes for women diagnosed with relapsing-remitting MS, conducted from 2008 to 2013. It was shown that the proportion of live births, average body weight and length at birth, as well as the time of onset of labor in patients with MS who took interferon beta (n = 251) were comparable to those in the group untreated with DMDs (n = 194) [35].

The Scandinavian registry includes data on pregnancies in Finland and Sweden from 1996 to 2014 (n = 3054). According to its results, there was no evidence of adverse pregnancy outcomes in connection with the use of interferon beta. The incidence of spontaneous abortions is comparable in the interferon beta-1a cohort and in the cohort without DMD treatment (8.1% vs 11.1% respectively). The proportion of cases of ectopic pregnancy in women who received interferon beta-1a was 1.6% vs 2.9% in the group without DMD treatment. The weight of the live births without congenital abnormalities in the groups receiving and not receiving therapy was similar [36].

The database analysis by Sandberg-Wollheim M. et al., 2011 presents prospective and retrospective reports on the safety of Rebif (interferon beta 1-a), including post-market observational data and isolated reports from clinical trials. Prospective data (n = 425) indicate no increase in the incidence of spontaneous abortions in women who received Rebif (11.5%) compared to the general population (up to 20%) [37].

Table 1. Recommended contraception period (months after last dose) use during pregnancy and breastfeeding according to EU Summary of Product Characteristics (SmPC) of DMD

DMD	Contraception requirements/ washout period	Use during pregnancy/reproductive toxicity data	Use during breastfeeding
Alemtuzumab [19]	Contraception use recommended for 4 months following each course of treatment	Alemtuzumab may cross the placental barrier and thus be potentially dangerous to the fetus. Animal studies have demonstrated reproductive toxicity. It is not known whether alemtuzumab can harm the fetus when given to pregnant women or whether it can alter reproductive capacity. Alemtuzumab should be administered during pregnancy only if the potential benefit justifies the potential risk to the fetus.	Breast-feeding should be suspended during each course of treatment and for 4 months following the last infusion of each treatment course.
Cladribine tablets [20]	Contraception use recommended for 6 months after last dose, ensuring that a barrier method is included during treatment and 1 month after last dose	Animal studies have shown reproductive toxicity. Cladribine is contraindicated in pregnant women	Breast-feeding should be discontinued during treatment with cladribine and for 1 week after the last dose
Dimethyl fumarate [21]	None given	Animal studies have shown reproductive toxicity. Dimethyl fumarate is not recommended during pregnancy and in women of childbearing potential not using appropriate contraception	A decision must be made whether to discontinue breast-feeding or to discontinue dimethyl fumarate therapy
Glatiramer acetate [22]	None given	Studies in animals have not shown reproductive toxicity. Current data on the use of glatiramer acetate 20-40 mg/ml in pregnant women indicate no malformative or feto/neonatal toxicity. To date, no relevant epidemiological data are available. As a precautionary measure, it is preferable to avoid the use of glatiramer acetate during pregnancy unless the benefit to the mother outweighs the risk to the foetus.	A decision must be made whether to discontinue breast-feeding or to discontinue/abstain from glatiramer acetate therapy taking into account the benefit of breast-feeding for the child and the benefit of therapy for the woman.
Fingolimod [23]	Contraception use recommended for at least 2 months after last dose	Based on human experience, post-marketing data suggest that the use of fingolimod is associated with a 2-fold increased risk of major congenital malformations when administered during pregnancy compared with the rate observed in the general population (2-3%; EUROCAT). Consequently, fingolimod is contraindicated during pregnancy.	Due to the potential for serious adverse reactions to fingolimod in nursing infants, women receiving fingolimod should not breastfeed.
Interferons [24-26]	None given	A considerable amount of data (more than 1000 pregnancy outcomes) from registries and post-marketing experience does not show a higher risk of major congenital anomalies after exposure to interferon beta during preconception or the first trimester of pregnancy. However, the extent of exposure during the first trimester is questionable, given that data were gathered when interferon beta use was contraindicated during pregnancy, and treatment was presumably stopped when pregnancy was detected and/or confirmed. Evidence regarding exposure during the second and third trimester is very limited. Based on animal studies, there is a possibly higher risk for spontaneous abortion. The risk of spontaneous abortions in pregnant women exposed to interferon beta cannot be appropriately evaluated based on the data that are available, but the evidence does not suggest an increased risk thus far. If clinically needed, the use of interferon beta may be considered during pregnancy.	Limited information available on the transfer of interferon beta-1a into breast milk, together with the chemical/physiological characteristics of interferon beta, suggests that levels of interferon beta-1a excreted in human milk are negligible. No harmful effects on the breastfed newborn/infant are anticipated. Interferon beta can be used during breast-feeding.

Mitoxantrone [27]	Contraception use recommended for at least 4 months for women and at least 6 months for men after last dose	There are very limited data on the use of mitoxantrone in pregnant women. Mitoxantrone was not teratogenic in animal studies at doses below human exposure but caused reproductive toxicity. Mitoxantrone is considered a potential human teratogen because of its mechanism of action and the developmental effects demonstrated by related agents. For this reason, the use of mitoxantrone to treat MS is contraindicated for pregnant women.	Because of the potential for serious adverse reactions in infants from mitoxantrone, breast-feeding is contraindicated and must be discontinued before starting treatment.
Natalizumab [28]	None given	If a woman becomes pregnant while taking natalizumab, discontinuation of treatment should be considered. A benefit-risk evaluation of the use of natalizumab during pregnancy should take into account the patient's clinical condition and the possible return of disease activity after stopping the treatment. Animal studies have demonstrated reproductive toxicity. Data from clinical trials, a prospective pregnancy registry, post-marketing cases and available literature do not suggest an effect of this medicinal product exposure on pregnancy outcomes. The completed prospective natalizumab pregnancy registry contained 355 cases and their outcomes. There were 316 live births, 29 of which were reported to have birth defects. Sixteen of the 29 were considered major defects. The rate of defects corresponds to the ones reported in other pregnancy registries involving MS patients. There is no evidence of a specific pattern of birth defects with this drug. Cases from published literature reported transient mild to moderate thrombocytopenia and anaemia observed in infants born to women exposed to natalizumab in their third trimester of pregnancy. Therefore, it is recommended that newborns of women exposed to this drug during the third trimester of pregnancy are screened for potential haematological complications.	Breast-feeding should be discontinued during treatment with natalizumab.
Ocrelizumab [29]	Contraception use recommended for 12 months after last dose	There is a limited amount of data from the use of ocrelizumab in pregnant women. Postponing vaccination with live or live-attenuated vaccines should be considered for neonates and infants born to mothers who have been exposed to ocrelizumab in utero. No B cell count data have been collected in neonates and infants exposed to ocrelizumab and the potential duration of B-cell depletion in neonates and infants is unknown. Animal studies (embryo-fetal toxicity) do not indicate teratogenic effects. B-cell depletion in utero was detected. Reproductive toxicity was observed in pre- and post-natal development studies. Ocrelizumab should be avoided during pregnancy unless the potential benefit to the mother outweighs the potential risk to the foetus.	Women should be advised to discontinue breast-feeding during ocrelizumab therapy.
Teriflunomide [30, 31]	Recommend accelerated elimination to achieve <0.02 mg/L if pregnancy occurs/prior to stopping contraceptive use	Studies in animals have shown reproductive toxi-city. Teriflunomide may cause serious birth defects when administered during pregnancy. Teriflunomide is contraindicated in pregnancy.	Teriflunomide is contraindicated during breast-feeding

There is limited information on the penetration of interferon beta-1a into breast milk. It is known that interferon beta-1a is not able to easily get into breast milk, since it is a large polar molecule (22500 Da) closely bound up with T-lymphocytes and other immune cells. Newborns can receive 0.0006% of the maternal dose of interferon beta-1a in breast milk. In addition, interferon beta-1a is poorly absorbed when taken orally, so it is not expected to get into the bloodstream of a newborn when breastfeeding [38, 39]. Latest EU label permit to take interferon beta-1a during breastfeeding

period. Whereas in the case of glatiramer acetate preparations, a choice should be made between lactation and their intake, weighing the benefits for the child from breast feeding and the benefits of the mother from treatment.

There`s a lack of treatment options for women with persistent high disease activity who do not wish to delay pregnancy. However, with the advent of pulsed immune reconstitution therapies represented with alemtuzumab and cladribine there`s additional opportunity for family planning. Whereas drugs used for maintenance or escalation therapy do not maintain their beneficial effect after cessation of therapy, these new highly effective therapies can show prolonged treatment effects after a short treatment course [40]. Despite that mitoxantrone could be used not only as induction, but as pulsed immune reconstitution therapy, there are no data supporting its use as pulsed immune reconstitution therapy. Moreover, due to frequent severe adverse events, mitoxantrone is not recommended for routine use in patients with RRMS [40].

It is possible to become pregnant and breast-feed after the last administration of the drug in 4 months for alemtuzumab or in 6 months for cladribine.

Long-term remission is seen in most of patients after treatment with alemtuzumab and cladribine. After one full course of alemtuzumab 58.0–68.5% of patients did not need additional cycles for 5 years. In the 5-year extension of the CARE-MS I study 31.5% of the patients received additional alemtuzumab cycles: 22.1% received one additional cycle, 8.0% two additional cycles and 1.4% received three additional cycles [40].

After cladribine 3.5 mg/kg in the 2-year randomized placebo-controlled study, 75.1% of patients remained relapse-free in years 3 and 4 without further treatment. Primary interim analysis of CLASSIC-MS study which is the median 10 years' follow-up of patients from the cladribine tablets clinical development program showed that 73.5% of patients did not require further DMD treatment until ≥4 years after last dose of cladribine tablets [41].

The main safety risks of pulsed immune reconstitution therapy include reactivation of latent infections such as tuberculosis, and risk of herpes zoster which is associated with severe lymphopenia. As a reflection of the mechanism of action of cladribine, lymphopenia was more frequent in the cladribine groups (combined cladribine group 27% versus 1.8% in placebo) in the CLARITY trial. Overall, cladribine tablets were well tolerated and showed a favorable safety profile across all studies. For alemtuzumab several rare infections such as Listeria meningitis have been reported. The main disadvantage in alemtuzumab-treated patients is the risk of secondary immune-mediated disorders (including thyroid disease in >40% of patients), which, however, has not been reported with cladribine. Also, unlike cladribine, long-term monitoring is required after the last administration of alemtuzumab. If pulsed immune reconstitution therapy has been decided, the choice between alemtuzumab and cladribine should be based on efficacy balanced against the risks [40].

Conclusions

Perceptions regarding pregnancy in women with MS and risks of DMD treatment are changing. Emerging data will continue to inform practical and evidence-based guidance for pregnancy planning in MS. Having MS should not in itself limit family planning.

The possibility of pregnancy should be considered when prescribing DMDs to all women of childbearing age. It is important to provide a framework to discuss concerns regarding pregnancy and MS and to consider uncertainty about long-term disease activity.

Treatment with DMDs should not be delayed. First-line injectable treatments, such as glatiramer acetate and beta interferon, may be continued during pregnancy. In patients with high disease activity pulsed immune reconstitution therapy can be considered before pregnancy, which is not recommended for 4 months following alemtuzumab and for 6 months following treatment with cladribine. After this time-frame there are no contraindications.

References

1. Walton C, King R, Rechtman L, et al. Rising prevalence of multiple sclerosis worldwide: Insights from the Atlas of MS, third edition. Mult Scler. 2020;26(14):1816-1821. doi: 10.1177/1352458520970841.
2. Hawkes CH, Giovannoni G, Lechner-Scott J, Levy M. Is the incidence of multiple sclerosis really increasing? Mult Scler Relat Disord. 2020;45:102527. https://doi.org/10.1016/j.msard.2020.102527.
3. Sumelahti M-L, Holmberg M, Murtonen A, Huhtala H, Elovaara I. Increasing incidence in relapsing-remitting MS and high rates among young women in Finland: a thirty-year follow-up. Mult Scler Int. 2014;2014:186950. http://dx.doi.org/10.1155/2014/186950.
4. Coyle PK. Management of women with multiple sclerosis through pregnancy and after childbirth. Ther Adv Neurol Disord. 2016;9(3):198-210. doi: 10.1177/1756285616631897.
5. Kamm CP, Muehl S, Mircsof D, et al. Role of family planning in women with multiple sclerosis in Switzerland: results of the women with multiple sclerosis patient survey. Front Neurol. 2018;9:821. https://doi.org/10.3389/fneur.2018.00821.
6. Dobson R, Dassan P, Roberts M, et al. UK consensus on pregnancy in multiple sclerosis: 'Association of British Neurologists' guidelines. Pract Neurol. 2019;19(2):106-114. doi: 10.1136/practneurol-2018-002060.
7. Amato MP, Bertolotto A, Brunelli R, et al. Management of pregnancy-related issues in multiple sclerosis patients: the need for an interdisciplinary approach. Neurol Sci. 2017;38(10):1849-58. doi: 10.1007/s10072-017-3081-8.
8. Hughes SE, Spelman S, Gray OM, et al. Predictors and dynamics of postpartum relapses in women with multiple sclerosis. Mult Scler. 2014;20(6):739-46. doi: 10.1177/1352458513507816.
9. Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG. Multiple sclerosis. N Engl J Med. 2000;343(13):938-52. doi: 10.1056/NEJM200009283431307.
10. Rasmussen PV, Magyari M, Yoon Moberg J, et al. Patient awareness about family planning represents a major knowledge gap in multiple sclerosis. Mult Scler Relat Disord. 2018;24:129-34. doi: 10.1016/j.msard.2018.06.006.
11. Hellwig K, Brzosko B, Airas L, Smith EK. Fertility and assisted reproductive techniques in women with MS. In: Houtchens MK, Sadovnick AD, editors. Health issues in women with multiple sclerosis. Wiwn: Springer; 2017. p. 9-17.
12. Confavreux C, Hutchinson M, Hours MM, et al. Rate of pregnancy-related relapse in multiple sclerosis. N Engl J Med. 1998;339(5):285-91. doi: 10.1056/NEJM199807303390501.
13. Vukusic S, Hutchinson M, Hours M, et al. Pregnancy and multiple sclerosis (the PRIMS study): clinical predictors of post-partum relapse. Brain. 2004;127(Pt 6):1353-60. doi: 10.1093/brain/awh152.

14. Houtchens MK, Edwards NC, Schneider G, et al. Pregnancy rates and outcomes in women with and without MS in the United States. Neurology. 2018;91(17):e1559-69. doi: 10.1212/WNL.0000000000006384.
15. Hellwig K, Haghikia A, Rockhoff M, Gold R. Multiple sclerosis and pregnancy: experience from a nationwide database in Germany. Ther Adv Neur Disord. 2012;5(5):247-53. doi: 10.1177/1756285612453192.
16. Hellwig K. Pregnancy in multiple sclerosis. Eur Neurol. 2014;72(1):39-42. doi: 10.1159/000367640.
17. Stuart M, Bergstrom L. Pregnancy and multiple sclerosis. J Midwifery Women's Health. 2011;56(1):41-7. doi: 10.1111/j.1542-2011.2010.00008.x.
18. Kaplan TB. Management of demyelinating disorders in pregnancy. Neurol Clin. 2019;37(1):17-30. doi: 10.1016/j.ncl.2018.09.007.
19. European Medicines Agency (EMA). Summary of Product Characteristics (SmPC): Lemtrada [Internet]. Amsterdam: EMA; © 1995-2021 [cited 2021 Jan 6]. Available from: https://www.ema.europa.eu/en/documents/product-information/lemtrada-epar-product-information_en.pdf
20. European Medicines Agency (EMA). Summary of Product Characteristics (SmPC): Mavenclad [Internet]. Amsterdam: EMA; © 1995-2021 [cited 2021 Feb 16]. Available from: https://www.ema.europa.eu/en/documents/product-information/mavenclad-epar-product-information_en.pdf
21. European Medicines Agency (EMA). Summary of Product Characteristics (SmPC): Tecfidera [Internet]. Amsterdam: EMA; © 1995-2021 [cited 2020 Nov 16]. Available from: https://www.ema.europa.eu/en/documents/product-information/tecfidera-epar-product-information_en.pdf
22. European Medicines Agency (EMA). Summary of Product Characteristics (SmPC): Copaxone 40 mg [Internet]. Amsterdam: EMA; © 1995-2021 [cited 2020 Sep 26]. Available from: https://mri.cts-mrp.eu/human/downloads/DE_H_5283_004_FinalPI_2of2.pdf
23. European Medicines Agency (EMA). Summary of Product Characteristics (SmPC): Gilenya [Internet]. Amsterdam: EMA; © 1995-2021 [cited 2020 Dec 1]. Available from: https://www.ema.europa.eu/en/documents/product-information/gilenya-epar-product-information_en.pdf
24. European Medicines Agency (EMA). Summary of Product Characteristics (SmPC): Rebif [Internet]. Amsterdam: EMA; © 1995-2021 [cited 2020 Dec 1]. Available from: https://www.ema.europa.eu/en/documents/product-information/rebif-epar-product-information_en.pdf
25. European Medicines Agency (EMA). Summary of Product Characteristics (SmPC): Betaferon [Internet]. Amsterdam: EMA; © 1995-2021 [cited 2020 Dec 1]. Available from: https://www.ema.europa.eu/en/documents/product-information/betaferon-epar-product-information_en.pdf
26. European Medicines Agency (EMA). Summary of Product Characteristics (SmPC): Avonex [Internet]. Amsterdam: EMA; © 1995-2021 [cited 2020 Sep 12]. Available from: https://www.ema.europa.eu/en/documents/product-information/avonex-epar-product-information_en.pdf
27. European Medicines Agency (EMA). Summary of Product Characteristics (SmPC): Novantrone and associated names [Internet]. Amsterdam: EMA; © 1995-2021 [cited 2020 Nov 2]. Available from: https://www.ema.europa.eu/en/documents/referral/novantrone-article-30-referral-annex-iii_en.pdf
28. European Medicines Agency (EMA). Summary of Product Characteristics (SmPC): Tysabri [Internet]. Amsterdam: EMA; © 1995-2021 [cited 2021 Apr 12]. Available from: https://www.ema.europa.eu/en/documents/product-information/tysabri-epar-product-information_en.pdf
29. European Medicines Agency (EMA). Summary of Product Characteristics (SmPC): Ocrevus [Internet]. Amsterdam: EMA; © 1995-2021 [cited 2021 Apr 12]. Available from: https://www.ema.europa.eu/en/documents/product-information/ocrevus-epar-product-information_en.pdf
30. European Medicines Agency (EMA). Summary of Product Characteristics (SmPC): Aubagio [Internet]. Amsterdam: EMA; © 1995-2021 [cited 2021 Jan 22]. Available from: https://www.ema.europa.eu/en/documents/product-information/aubagio-epar-product-information_en.pdf
31. Oh J, O'Connor P. An update of teriflunomide for treatment of multiple sclerosis. Ther Clin Risk Manag. 2013;9:177-90. doi: 10.2147/TCRM.S30947.
32. Rae-Grant A, Day GS, Marrie RA, et al. Practice guideline recommendations summary: Disease-modifying therapies for adults with multiple sclerosis: Report of the Guideline Development, Dissemination, and Implementation Subcommittee of the American Academy of Neurology. Neurology. 2018;90(17):777-88. doi: 10.1212/WNL.0000000000005347.
33. Montalban X, Gold R, Thompson AJ, et al. ECTRIMS/EAN Guideline on the pharmacological treatment of people with multiple sclerosis. Mult Scler. 2018;24(2):96-120. doi: 10.1177/1352458517751049.
34. Hellwig K, Geissbuehler Y, Sabidó M, et al. Pregnancy outcomes in interferon-beta-exposed patients with multiple sclerosis: results from the European Interferon-beta Pregnancy Registry. J Neurol. 2020;67(6):1715-1723. doi: 10.1007/s00415-020-09762-y.
35. Thiel S, Langer-Gould A, Rockhoff M, et al. Interferon-beta exposure during first trimester is safe in women with multiple sclerosis – A prospective cohort study from the German Multiple Sclerosis and Pregnancy Registry. Mult Scler. 2016;22(6):801-9. doi: 10.1177/1352458516634872.
36. Hellwig K, Geissbuehler Y, Sabidó M, et al. Pregnancy and infant outcomes with interferon beta: data from the European Interferon Beta Pregnancy Registry and Population Based Registries in Finland and Sweden. Mult Scler Relat Dis. 2018;26:P240. doi: 10.1016/j.msard.2018.10.036.
37. Sandberg-Wollheim M, Alteri E, Moraga MS, Kornmann G. Pregnancy outcomes in multiple sclerosis following subcutaneous interferon beta-1a therapy. Mult Scler. 2011;17(4):423-30. doi: 10.1177/1352458510394610.
38. Hale TW, Siddiqui AA, Baker TE. Transfer of interferon β-1a into human breastmilk. Breastfeed Med. 2012;7(2):123-5. doi: 10.1089/bfm.2011.0044.
39. Almas S, Vance J, Baker T, Hale T. Management of multiple sclerosis in the breastfeeding mother. Mult Scler Int. 2016;2016:6527458. doi: 10.1155/2016/6527458.
40. Sorensen PS, Sellebjerg F. Pulsed immune reconstitution therapy in multiple sclerosis. Ther Adv Neurol Disord. 2019;12:1-16. doi: 10.1177/1756286419836913.
41. Giovannoni G, Leist T, Aydemir A, Verdun Di Cantogno E. CLASSIC-MS: Long-term efficacy and real-world treatment patterns for patients receiving Cladribine tablets – Interim data with 8-14 years follow-up. In: MSVirtual2020: 8th Joint ACTRIMS-ECTRIMS Meeting; 2020 Sep 25-26; Washington.

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AB conducted literature review, wrote the manuscript, revised the final text; AMB conducted literature review; OO wrote the manuscript; MS wrote the manuscript; MG revised the final text; VL conducted literature review, wrote the manuscript, revised the final text. All the authors approved the final version of the manuscript.

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Academician Eugen Gladun – on the 85th anniversary
27.04.1936–30.05.2014

These days academician Eugen Gladun would have turned 85 years old. A man of a special moral cleanliness who worked selflessly, being demanding of himself and of his colleagues, always showing professional competence, erudition, and managerial skills.

Eugen Gladun was born on April 27, 1936 in the village of Nesfoia, Cernauti region, in a family of teachers. After graduating from high school, he became a student at the State Institute of Medicine in Chisinau, and in 1959 he successfully graduated from the Faculty of General Medicine. Eugen Gladun started his activity as an obstetrician-gynecologist at the district hospital of Briceni. In 1961 he was recommended for PhD studies at the Department of Obstetrics and Gynecology of Chisinau State Institute of Medicine. As a disciple of Professor A. Cocerghinschi, he defended his PhD thesis in medical sciences in 1964. He was later hired as an assistant, then a lecturer at the same Department of Obstetrics and Gynecology of CSIM.

In 1984, Eugen Gladun completed his Doctor Habilitated dissertation in medical sciences, with a well-known Russian scientist from St. Petersburg as his consultant – Professor A. Slepyh, also a disciple of Professor A. Cocerghinschi.

As the chief of the department, Professor E. Gladun conducted substantial pedagogical and scientific activity, and was rightly the head of a new school of obstetricians-gynecologists, which successfully combined the traditions of great forerunners with the new trends and directions of scientific research.

E. Gladun was the academic adviser of 26 PhD theses and 8 Doctor Habilitated theses in medical sciences. He published 7 monographs on the most important scientific directions of the specialty. More than 200 scientific publications, 70 inventions and rationalization proposals have been developed by Professor E. Gladun. He organized scientific congresses and conferences and, in his turn, participated in many international congresses and symposia.

The scientific works of Professor E. Gladun and his disciples are known in the Moldovan scientific community and abroad. This led to his being elected a member of the International Association of Obstetrics and Gynecology and the European Association of Obstetrics and Gynecology.

E. Gladun had also accomplished important organizational tasks. In a firm and uncompromising manner, Professor Gladun founded the Institute for Scientific Research in the Field of Maternal and Child Health (currently the Institute of Mother and Child), which he led with great skill for 15 years.

Following the legislative elections in the Republic of Moldova in 1998, Eugen Gladun was elected deputy in the Parliament of the Republic of Moldova, and later was appointed Minister of Health. In this position, he showed civic boldness and superior organizational talent. It was during this time that the healthcare system reforms began, thus preventing an imminent crisis. In 2000, E. Gladun was elected a corresponding member of the Academy of Sciences of Moldova.

In the period of 55 years of activity, he proved to be a scientist by vocation through his studies and a researcher with phenomenal intuition through his works, providing an excellent model for his successors in the field.

For special merits in the medical and didactic activity, the academician was awarded the Emeritus title, being then designated as a laureate of the State Prize. Moreover, the scientist was decorated with *Civic Merit* medal, and the highest state distinction of the Republic of Moldova – *Order of the Republic* – was bestowed on him.

A true activist for the reform and improvement of the obstetrical-gynecological service of the Republic of Moldova, Eugen Gladun was an illustrious scientist of local medicine, a generator of new ideas in medical practice and a leader in science who helped people better understand the role of knowledge and creativity in the evolution of society and civilization as a whole.

Academician Eugen Gladun will remain in the hearts and memory of colleagues and disciples as an outstanding health professional with extensive clinical experience and a native talent to explain clearly while answering collaborators and students' questions, which made him beloved by all who were around him.

Emil Ceban, MD, PhD, Professor
Rector of *Nicolae Testemitanu* State University
of Medicine and Pharmacy of the Republic of Moldova

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